ADVANCES IN GEROSCIENCE:
IMPACT ON HEALTHSPAN AND CHRONIC DISEASE
BETHESDA, MD – OCTOBER 30-31, 2013

Felipe Sierra, PhD: Good morning everybody. Thanks for being here. My name is Felipe Sierra. I'm the Director at the Division of Aging Biology at the National Institute on Aging; and I'm actually the head of the Trans-NIH Geroscience Interest Group who's in charge of this meeting.

I'd like to welcome you to this very first ever meeting of the geroscience subject, which is a field that spans the basic biology of aging and the diseases related to aging or chronic diseases of the elderly.

I would like to start by thanking all my Trans-NIH partners. There's 20 institutes involved in this effort. A lot of people have put a lot of work into getting this up to where we are now, and we all realize that this is not the end. It's just the beginning of what we are trying to do.

I also would like to thank our partners, the Alliance for Aging Research and the Gerontological Society of America who have been very, very helpful in preparing all of this, especially during the government shutdown. So they kept working while we were home.

Of course, I also want to thank the many corporations that you see in the slide who have provided the funds for this. I should stress that no further funds have been used for this activity. This is all coming from these donors, so a big thank you to them.

Before I start, a couple of practical things, I want to bring your attention to this hashtag that's in your folders. This Hashtag Aging Summit, we want you to tweet, those of you of an age that you know how to tweet. We want as much social activity as possible, social media, press coverage, etc. And there will be press around. If they ask you questions, just talk to them. It's good to talk to these people.

Also, I've been asked officially to let everybody know that the event is being recorded for future videocast. It's not being videocast live, but it will be posted afterwards; so everybody should know that and be aware.

So we have a very fantastic program but also very tight program. There's going to be a lot of things going on in a very short time, so without further ado, I would like to introduce Dr. Francis Collins, who's the Director of the NIH and who gives some welcoming remarks.

Dr. Collins is a physician-geneticist who is known for his discoveries of diseased genes and his leadership of the Human Genome Project. He was a director of the National Human Genome Research Institute at the NIH from 1993 to 2008. And he has many accolades. He's an elected member of the Institute of Medicine and the National Academy of Sciences. Many, many awards such as the Presidential Medal of Freedom in 2007, the National Medal of Science in 2009. And in 2010, he was a corecipient of the very prestigious Albany Medical Center Prize in Medicine and Biomedical Research. I could go on and on with Dr. Francis Collins' accomplishments, but we don't have time for that. So, Dr. Collins.

Welcoming Remarks

Francis Collins, MD, PhD: Thanks, Felipe. Thank you, Felipe. Good morning everyone. It's wonderful to have a chance to issue some words of welcome here and what I think is going to be a remarkable meeting with a scientific agenda that is broad and deep and will, I think, provide a lot of interaction possibilities between people who have attended this meeting.

We're all very glad the government is open so that we can actually gather here in the Natcher Building in the Kirschstein Auditorium, instead of having terrible consequences occur to this meeting, as happened to some of the meetings that were held in the first half of October. We sincerely hope that kind of really

devastating event, namely a government shutdown, will not happen again for another 100 years. If it does, we will be prepared for it next time, but I sure hope we don't have to be.

I do want to thank all those who work so hard to create this possibility of this remarkable meeting. Felipe, especially, but also others who work with him, Kevin Howcroft and Ron Kohanski, have been part of the leadership effort.

And maybe I should ask those who are a part of the Trans-NIH Geroscience Interest Group to stand up, so you can see who they are, the folks that have contributed their time and effort to building this remarkable meeting and other things that go with it. Yes. Thank you all.

So I think the existence of this focus on geroscience is indicative of the way in which our ideas about health and disease have been evolving over the course of the last decade or so. Perhaps more traditionally we focused on individual disease risks, whether it's cardiovascular disease or cancer or Alzheimer's, but increasingly we're discovering, on the basis of molecular information, on the basis of more careful study of the process of aging, that it is not a good idea to consider such issues in isolation; but that we would be better served in gatherings such as this to appreciate the interactions between those disease risks.

And, of course, also, the other thing that I think we're increasingly appreciating is that it serves us well not only to focus on disease but to focus on health and to understand more about healthy aging and how that can occur and why in some instances it doesn't by contrast, will add to our understanding of how to improve the health of our nation and of the world.

Certainly for me coming out of the field of genomics, one of the exciting areas that we are all looking forward to is learning more about what is it that seems to allow exceptional longevity. Much of that, of course, is going to be environmental, but some of it appears to be hereditary. If we had the complete data sets that we dream of, of both of those kinds of data on people who live to exceptionally long years of life, what would that teach us about the ways in which we could offer longevity to greater numbers of individuals. And we're on our path toward seeing that achieved.

Again, perhaps in the past, this notion of focusing on health has been a little bit less of an emphasis. Focus on disease has been the emphasis. I think we're seeing a very appropriate balancing act now start to appear.

From my own perspective, we have lots to learn also by studying specific examples where aging seems to be accelerated. Last year when I spoke to this group, I had the opportunity to talk about research going on in my own laboratory about Hutchinson-Gilford progeria, one of the more dramatic forms of premature aging, where the lab was able to discover the cause ten years ago and in just four years get to a therapeutic trial using farnesyl transferase inhibitors.

When I spoke last year, we had not yet published the result of that. That publication came shortly after that, showing that there does appear to have been benefit to these children, at least in the form of reduction in the progress of cardiovascular disease.

I don't know how many of you had a chance to see the HBO special last week called "Life According to Sam," which focused on progeria, narrated by Sam Berns, a 17-year-old boy with this disease who I've known since he was 3. Really remarkable piece in terms of how it laid out the consequences of what this disease means to this boy and his parents. But also a very good teaching exercise about how research happens, how you go from a basic science discovery to an idea about therapy, how you conduct a clinical trial, and so on. A very useful thing, I think, to point people to who are trying to understand the research process.

And there will certainly be at this meeting reflections on some of these models that teach us general properties of aging. It might not surprise you that we are now engaged in progeria and testing rapamycin

in a mouse model of the disease. There will be lots of rapamycin conversation, I'm quite sure, over the course of the next day and a half at this meeting because of the potential here for showing a path towards longevity that teaches us something about mTOR and ways in which it plays a role in this process.

I do want to ask you also to pay attention to something which we at NIH are particularly concerned about right now and which was featured in a cover story of *The Economist* last week and that's the issue of reproducibility. It has come, I think, to many of our attention that when you look carefully at what happens after a preclinical study is conducted that then leads to the possibility of moving into a clinical trial, that things don't always go the way you would like. And, in fact, publications have suggested that a substantial fraction of preclinical studies, particularly involving animal models, can't really be reproduced when the company that aims to try to move that into human patients does that work and finds that they are unable to show that this result can be replicated in a different laboratory, in a different way.

And there are all kinds of technical reasons why that may sometimes happen, but I think it's become quite clear that we, the biomedical research community, need to pay close attention to this issue and that perhaps some of the rigor that ought to be included in preclinical studies has not always been there.

I give a lot of credit to the National Institute of Aging in terms of the way in which they focused on this issue as far as interventions that might actually result in increased longevity because you can imagine in a mouse model why that is a challenge to be sure that you have gotten the data right. And having set up a system to be sure that such claims are tested in a very reproducible way has been, I think, a good example of what we should probably be doing even more of.

I just raise this as an issue that I think all of us should be looking closely at to be sure that in the pressure, and it's an extreme pressure now with budgets being so tight, to do research and get the results published, that the appropriate attention is paid to the rigor of the study and that the conclusions can then be backed up and replicated in another laboratory. Something to watch over.

Well, again, I want to welcome you. Primarily, that's my main goal here and to set the table very briefly for what I think is going to be a remarkable day and a half of reflections on the ways in which these studies of the aging process can intersect with each other. I hope all of you will take full advantage of the opportunities to make new friends, develop new collaborations. This is a field, geroscience, that is very much in wonderful ferment; and by being here, you're part of that.

And I personally am delighted to see this kind of intersection occurring and wish you all the best in making the most of this remarkable meeting. Thank you very much.

Session I: Keynote Presentations

T. Kevin Howcroft, PhD: All right, good morning. My name is Kevin Howcroft. I'm a program director at the National Cancer Institute and Chair of the Summit Planning Committee. And this opening session, well we have three keynote presentations by leaders in geroscience; and I'll ask that audience members who have questions to approach the speakers during the 30-minute break after the keynote session out in the lobby area.

Our first keynote address is by Dr. Chris Murray. Dr. Murray is a physician and health economist. He earned his medical degree from Harvard University and a PhD in international health economics from Oxford University. Currently, Dr. Murray is Professor of Global Health and Director of the Institute for Health Metrics and Evaluation at the University of Washington. Dr. Murray will present efforts by a consortia of investigators around the world that he has led to quantify the magnitude of health loss due to chronic disease. Chris?

The Global Burden of Disease

Christopher Murray, MD, PhD: Well, good morning. I'm not sure I qualify as a leader in geroscience, but I will tell you about the Global Burden of Disease Study, which I hope will be something slightly different from what you'll be hearing during the rest of this important meeting.

So let me tell you about the Global Burden of Disease, 2010 study, and some of the continued work of this consortium to try to understand patterns of health around the world. First off, what is this study? This study actually dates back to the early '90s. It's a continuation of a body of work that became, initiated actually by the World Bank in 1991, to fill a gap in information on epidemiology of different diseases around the world. And the way we like to think of it now is that it's a systematic, scientific effort to assess the magnitude of health loss around the world on a country-specific basis over time by age and by sex, using approaches that at least facilitate comparison.

Currently, we cover a mutually exclusive and collectively exhaustive set of 291 diseases and injuries. And for each disease and injury, there's often more than one clinical sequela that we examine. So we track over 1,000 clinical outcomes, and we also look at 67 behaviors or environmental exposures or metabolic risks that explain some of the patterns of disease and injury that we see around the world.

The study has been largely funded, but not exclusively funded, by the Bill and Melinda Gates Foundation. There was a dedicated issue of the journal of *The Lancet* last December, and there continues to be quite a large number of papers coming out from this consortium, including our paper on stroke that was out over the weekend.

So what I'm going to present is the work of a large body of scientists from around the world. In fact, nearly 500 from over 300 institutions in 50 countries. And this consortium continues to grow, as I'll mention at the end of this presentation.

Now I will use some terminology, which may not be familiar to you; so let me give you some brief definitions to facilitate this presentation. When we say "health loss," we are actually comparing the state of functional health and death rates in a population compared to some ideal, which in our case is that everybody lives in full health with a life expectancy of 86 years. The number 86 is picked because that's the synthetic life table, based on the lowest observed age-specific death rates around the world today.

We then compute years of life lost, as the number of deaths at each age times the difference between the life, by using the life expectancy in this reference norm. So if you die at birth, you've lost 86 years. If you die in your 80s, you've lost a smaller number.

We also compute the nonfatal component of health, which will turn out to be rather important; and that is years lived with disability. Years lived with disability is really quite simple. It's the prevalence of 1,000 plus clinical sequela times a severity weight for each outcome. The severity weights are based on surveys of the general population around the world in representative countries.

So it's quite literally just prevalence times the severity weight and then summed across these different conditions. And our overall metric of health, called DALYs or disability-adjusted life years, is just the arithmetic sum of premature mortality and years lived with disability. Just to note that unlike the US use of the word disability, in the GBD the term disability really only refers to any short- or long-term health loss.

Now here's a schematic of the study, and the reason this is important is to highlight that the approach to measurement here is rather protocolized. That for every type of data source that we can identify, there's a particular set of tests around validity and comparability that are undertaken; and then there's attempts to fill in both missing information, as well as to deal with inconsistent information using a range of Bayesian statistical methods. The study falls into four large blocks of analysis: analysis around death rates in the top left; analysis around causes of death in the top right; analysis around 1,000 plus sequela and comorbidities of those 1,000 plus sequela; and then the analysis of risk factors on the bottom left.

Behind each box is even more detail, and for each type of data source, there's a series of biases that we try to identify and correct for. I am not going to go through each of these steps, but just to highlight if you're interested, there is a lot of documentation available about each component here in this process.

Now, one of the things that I think is interesting about the work to date has been the use of data visualization to help both the investigators in this study identify patterns of disease, but also to let others explore what's a very large database of results. So in all there's over 1 billion findings, if you think about the matrix of age, sex, country, time, disease, and various versions of uncertainty. And what we've created is a series of online tools that you can access at any point that I'm now going to show you some of the key results from the study.

So let me switch to those tools. I am using a version of these that is a website mirrored onto my hard drive, not knowing what sort of Internet access we have. But everything I'm going to show you is available online.

So what I'd like to do is start with some basic patterns around the world, and I think some of these are probably pretty relevant to the discussion here in this conference around geroscience. So starting with the most simple way of thinking about health, and that's just looking at death. This diagram shows death rates at the global level or death numbers, excuse me, at the global level by age. And in this study, we break down deaths under age 5 into four groups – early neonatal, late neonatal, postneonatal, and ages 1 to 4. Then five-year age groups out to 80+. And you may say, well, 80+ is a rather large group to not track in more detail, and that's actually one of the important limitations that we see in data around the world, that very often countries are still not reporting detailed age groups over 80.

But, nevertheless, from 1990 to 2010, I'm going to show you the change in the number of deaths; but this diagram also shows the composition of death in each age group by 21 broad-cause groups. So, for example, yellow here is diarrhea/pneumonia. The dark blue here is cardiovascular disease. The light blue is cancers. The salmon color here is the chronic respiratory conditions. And you can see some of the other smaller causes as well.

So from 1990 to 2010, due to both changing death rates and demographic change, you see a pretty profound shift in the pattern of global mortality, with a reduction in child deaths from about 12 million down to about 7 million and a huge increase in the number of deaths globally over age 80. So those demographic shifts are visible even at this very macro view. And when we look at country-specific results, in some places those shifts are really even dramatically more profound in a place like Bangladesh due to both mortality and fertility change. It's really quite extraordinary.

Now I mentioned that we use multiple metrics for outcome, so here at the global level, let me shift from looking at deaths to years of life lost. That's going to put much more emphasis on deaths at young age and in young adults, and you'll see that quite visibly. Let me just change the axis here to be focused on 2010.

And we see a lot of premature years lost still due to deaths under age 5; considerable premature death in terms of years of life lost at younger adult ages from things like HIV, TB; road traffic accidents in purple; a range of injuries; and then, of course, the premature mortality from heart disease, cancer, diabetes in green, and a range of other causes over age 60 and on into 80 plus.

One of the more important insights from this study is that when we look at the disability component, and that is, again, prevalence times severity, and some across diseases, we see a completely different age pattern and a different set of causes that dominate. So this is years lived with disability at the global level again where the dominant causes of years lived with disability are things like mental and behavioral disorders, musculoskeletal disorders, a grab bag of other noncommunicable, which includes vision loss, hearing loss, and congenital and anemia, and then contributions from malnutrition, from chronic respiratory conditions.

But, interestingly, the contribution, even at older ages, to disability from cardiovascular disease and cancer is comparatively smaller than the contribution from mental health, musculoskeletal disorders, and that vision loss/hearing loss category. You put all this together and you get the complexity that we know as patterns of health around the world with multiple simultaneous agendas, an agenda around premature death in children, an agenda around premature mortality in young adults and disability. And then, of course, the agenda around the range of conditions at older ages.

So that's one way of looking at the results. I want to show you another way, again, using these tools to understand some of the range of patterns we see around countries in the world.

So this is age-standardized death rates for the G20 countries, because that gives you a nice range of countries across the world, to which I've added Niger as the country with some of the worst levels of health in the world in West Africa.

And you can see in 2010 an enormous variation in age-standardized death rates, with Japan in this group at the lowest. The US quite a bit higher than Japan in terms of age-standardized death rates. But, of course, dramatically lower than countries like Indonesia, Russia, India, or South Africa.

But when we shift to looking at years of life lost, you see an even bigger range of age-standardized rates across countries. And when we shift to looking at years lived with disability, we see this rather interesting finding that while there's this enormous variation across countries in premature mortality and death rates, there is much less variation across countries in age-standardized disability rates.

In fact, what we're observing is that as premature mortality goes down, we see minimal change in the prevalence of disability. Maybe from 1990 to 2010 in most high income countries a 5% or 6% decline. And the combination of aging of the population, the clear relationship between prevalence of disability with age means that the volume of disability in terms of experience by populations goes up steadily, even if the age-standardized rates are very slowly going down. And that's such a marked contrast to the trends in mortality that we're seeing the burden of disease progressively shift towards disability as a key factor and not premature mortality.

So there's a range of ways for us to explore these patterns, so let me try another visual tool to help us think about disease patterns and their transition. I'm now back in 1990. I'm showing you something called a tree map. It's a square pie chart. So just like a pie chart, the size of the box is the same as the size of a wedge.

And this is showing visually the 21 broad cause groups, the noncommunicable causes are in blue; the communicable, maternal/neonatal causes, those more associated with poverty, in red; and injuries in green. And globally the epidemiological transition, driven by changing disease rates, mostly around mortality and changing demography, means from 1990 to 2010 you had this progressive shift towards noncommunicable diseases at the global level.

But, of course, if we look by country, that shift is more dramatic in many regards. I'm going to add a map now on the bottom, and if we shift to looking at noncommunicable disease in the bottom figure and shift to looking at these in terms of percent, what we see is the percent of burden measured by disability-adjusted life years due to the noncommunicable causes.

And as you would expect in a place like the US here, 85% of the burden is due to NCDs. In a place like China, it's not far behind now; and China's an example of a country where the epidemiological transition has moved at an extraordinary pace, so the disease profile in China as a country, and particularly China in the east of the country is quite similar now to the United States.

A place like India, the epidemiological transition is in a halfway point, with about 45% of the burden from noncommunicable diseases, 45% from the communicable causes, and injuries making up the rest. And,

of course, in West Africa, we still have a disease pattern that's really dominated by the infectious causes of both childhood mortality and young adult mortality.

So that's the sort of range of the epidemiological transition. Another way to see that, and I think gets to some of the geroscience agenda, is with these arrow diagrams where we can look at the rank list, with uncertainty by the way in the little brackets, of causes or leading causes of premature mortality and disability in 1990 connected to the causes in 2010. NCDs are blue; the communicable, maternal, and neonatal causes are red; and injuries are green. And at the global scale, you can see lots of shifts going on with ischemic heart disease becoming the world's number one cause, but pneumonia is still number two, and stroke number three, and then a cluster of diarrhea, HIV/AIDS, and malaria at the top. But then you start to see things like low back pain or COPD in these causes.

This rank list at the global level reflects both rates and demography. So the changes, these percent increases over here, the 30% increase in the burden of ischemic heart disease is both changes in age-standardized rates and shift to older ages. We can shift to looking at age-standardized rates and remove the effects of demographic change, and there what you see is that ischemic heart disease, although going up globally in terms of numbers, the rates are actually going down by 18% from 1990 to 2010; pneumonia going down by about 50%, stroke going down by 23%.

But there's a handful of things where there's little change, low back pain; some things with increases, like malaria or road injury; major depression no real change; diabetes going up by 11% in terms of age-standardized rates; and things like some other causes we go lower in the list like dementia. If we look at the United States, what we're going to see is, in terms of age-standardized rates, big increases for drug use disorders. As an example, big increases for alcohol use, Alzheimer's. And if we went lower in the cause list, we would see big increases for Parkinson's as well. So there's a lot of detail about what's happening around the world by specific causes that's accessible and speaks to both some general patterns we observe in the world and the regional specificity that we see in the data.

Another important dimension of the study is to go beyond the ICD list of diseases and injuries and look at some of the underlying exposures that may account for the patterns of disease that we see around the world. So, again, using this metric of disability-adjusted life years, I'm going to change the X axis to percent, here's the leading risk factors we see globally. And I think you can get a sense of the epidemiological transition if we go back to 1990 that shows up in these risk factors.

Back in 1990, the leading global risk was malnutrition in children, followed by diet, more generally in terms of its contribution to adults. Indoor air pollution, number three. Smoking, number four, high blood pressure and then suboptimal breastfeeding. And over the 20-year period, you see big shifts towards the individual behavioral risks that underlie the NCD patterns we see in the world with now diet, high blood pressure, smoking at the top. And high Body Mass Index coming in here as the sixth risk; but if we look, for example, at developed countries as a grouping, what we'll see is that obesity is actually coming in as number four.

So there's a lot of detail on this risk transition, and it opens up many opportunities for us to understand where one might intervene on risk factors to have broad effects on health outcomes. And our findings match some of the things that, for example, came out of the IOM study on the US that things like tobacco and obesity do account for a substantial fraction of some of the variation in lifespan that we observe across high income countries as an example.

So let me show you the specific results now for the US related to the Global Burden of Disease work is our drive towards subnational detail. And I'll give you a little bit more update in a moment, when I go back to PowerPoint. But I want to show you some of these findings on lifespan that have come from drilling down in some of the countries that we examined, namely the United States.

So here's another online tool, and this reflects assessment of life expectancy by locality in the United States or namely by county. And for those who are interested, this is using some small area Bayesian methods to generate full life tables for each county over time for each year; and I'm going to play a little

movie of life expectancy in the United States for women from 1985 to 2010. So change in life expectancy over a generation.

Good news is that parts of the Upper Midwest, California, parts of New England are seeing steady increases in lifespan for women; and you can see that in the patches of blue. Parts of Florida as well. But what is intriguing for understanding lifespan in the United States is that although there are these communities with quite substantial gains in a generation, up to seven years improvement for women, that there is actually a whole host of populations where there's been very little change for women.

In fact, if I just change this map to look at change in life expectancy over that 25-year period, you see all the places in red and the darker yellow there's actually been no change in female lifespan. So there are these populations with substantial increases, but there's also a host of some 900 counties in the United States with no statistically significant improvement in lifespan.

And, of course, connecting this up to the rest of the burden of disease work, the work hasn't been completed. Actually, only on some of the risk factors for trying to tease out what are the diseases, injuries, and risk factors that might account for this unusual heterogeneity in progress and lifespan in the United States.

Okay, on to some of the other aspects of the study and where we are going in the future. So somewhat to our surprise, when we published these results last December, we were immediately approached by the UK government and asked if they could use the results from the Global Burden of Disease to benchmark progress of the UK on key outcomes – some cancer outcomes, cardiovascular disease, and a range of risk factors – against countries in Western Europe. And the surprising thing to us was that why hadn't this happened before, and it comes back to this fundamental issue around comparability. That as reported, cause of death data is actually hard to compare because of national variation in coding practice. And there's a tremendous amount of work behind the scenes in the Global Burden of Disease Study to try to harmonize and standardize cause of death coding and reporting.

And so having this somewhat cleaned up data set facilitated for them this benchmarking exercise, and that spawned a series of benchmarking exercises that we hadn't actually foreseen. So the UK one turned rather quickly into a study published in *The Lancet* in March, and that was both from our consortium and from people in the Department of Health in the UK. That then led to some identification where the UK was doing rather poorly, even on areas where they had invested heavily, and led Jeremy Hunt, the Secretary of State for Health, to issue a call to action on the day the paper came out. And probably more interestingly or importantly from a scientific point of view, the commitment to measure using the same definitions and approaches, the burden of disease in the UK for 12 subnational units – Wales, Northern Ireland, Scotland, and 9 regions of England. And that's work that's underway.

A similar process happened in China with an analysis of the Burden of Disease results and also a publication from that and a commitment by the government, China CDC, and some key surveillance groups in China and some investigators at Peking University Medical College to collaborate on producing provincial level and eventually county level, we believe, analyses of health outcomes in China. And that's actually rather exciting because it's such a large place. There actually turns out to be much better data than we knew about, and there's some really interesting focal patterns of disease outcomes that are starting to emerge.

We had a similar analysis here in the US. The GBD investigators put together an analysis of the state of the US health using this database. It was published in *JAMA* and timed to an event at the White House, hosted by Michele Obama. And there was also the release of that subnational results around the county life expectancy at this event at the White House. And so there's been quite a bit of interest in further analysis, we hope, at the subnational level using the same case definitions and approaches from the Burden of Disease work.

This drive or interest in subnational work is continuing to grow, so the government of Mexico and the National Institute of Public Health in Mexico will produce subnational assessments for Mexico. Same for

Australia and the same for Indonesia, and several other countries are starting to discuss, such as India, whether this is feasible.

So let me move on to ending this presentation with where this body of work is going. We view the analysis, the scientific publications, and particularly the online tools as a global public good. And we're very happy that we have funding for some of the core costs from the Bill and Melinda Gates Foundation to produce annual updates of this body of work. The first will be bringing the assessment through to the year 2013 and will be published next year, and that will include these subnational studies for the UK, China, and Mexico.

We're also expanding the scope of this analysis, and one will be adding a forecasting element. We had a set of forecasts that were produced in the 1990s for the first Global Burden of Disease study at the regional level, and we will now be producing country forecasts. That'll probably be something that'll take another couple of years to finalize the methods and produce results. So look for 2015 for those sort of findings.

One of the early results from that sort of work is that we think that lifespans will be much longer than what, for example, the Social Security administration of this country thinks. So that has some profound implications for the financial viability of things like Social Security. Perhaps not a surprise because we have historically underestimated progress in improving lifespan in high income countries.

Second direction of scope is to track health expenditure at the national level for the same disease and injury categories because I think it'll be very interesting, eventually, to be able to look at change in spending versus change in outcome at a disease-specific level and identify where there has been quote "value for money" in the sense that spending on let's say an outcome like diabetes you've seen the greatest improvement.

We hope very much to continue this vein of work that I mentioned on the county level outcomes, and we would like to undertake and are trying to apply for funding from a variety of sources to do the full burden of disease analysis at the local level in the United States. And so that's part of work that we hope will be done and something planned for the future.

So one thing I want to show before closing for those who I know have in their mind the question, well, where is the data from? And so I want to point out that we are trying rather consistently to make available both these results from the analysis but also the raw data that goes into it. So online, you can look up all the mortality data, the underlying primary mortality data. For example, here's the United States. This is the vital registration data. You can look at different age groups and see the raw data that's used in the analysis.

But you can also do that for cause of death data. So if you go to another tool that's online and look at cause of death, you will see the raw data that's available for each country on each cause of death. And we don't yet have these tools online for the thousand clinical sequela, but next May we hope that we will be able to put up available visual databases like this that show you the metadata for every study that's used in this analysis, whether it's from the published literature or from unpublished analyses where we're given permission to show them.

So thank you very much for your attention.

Ronald A. Kohanski, PhD: Good morning. My name is Ron Kohanski. I'm a division deputy director at the National Institute on Aging and with Felipe cofounder of the Geroscience Interest Group.

The next keynote address is by Dr. Brian Kennedy on the current status of basic research on aging. Dr. Kennedy received his PhD in biology from the Massachusetts Institute of Technology, was a faculty member of the Department of Biochemistry at the University of Washington, and is currently President and Chief Executive Officer of the Buck Institute for Aging Research. Dr. Kennedy?

Current Status on Basic Research on Aging

Brian Kennedy: Well it's a great privilege to be here, to be able to give this talk, and what I'm going to try to do today is sum up a lot of aging research and geroscience research that's gone on from many labs around the United States and the world and give you sort of a baseline feel for where we are today in terms of how aging happens, what we think can be done about it, and what might happen if we do that. So let me just jump right into it.

This is the Buck Institute, and these are the statistics that I want to start with. With Dr. Murray's speech, I usually use his data; so I couldn't use that, so I'm just going to stick to one slide here. And the point is that in 2050, 2 billion of the 9 billion people on Earth are going to be over the age of 60. And in just a few years from now, there's going to be more people over the age of 65 than under 5. And the reason I point this out is that this is not just a quality of life issue. It's not just about diseases of old people. This is a major economic issue that's facing the world. If these people that are over 65 have chronic diseases, can't work, and have high healthcare costs, there's not going to be enough people working to generate a big enough economy to pay for them. So we have a major economic issue on our hands.

We're not the only ones to think about that. In fact, many governments are thinking about it. And the way they're thinking about it is interesting. So they'll consider things like healthy aging versus sick aging, but a lot of the work is trying to figure out how to change social programs and social policy to adapt to having older people in the population. So how do we deal with personal income, independence? What about at the social level, fiscal sustainability. Are pensions too high or are they too low? If people are living longer, can they afford it? Should we change retirement age? And you can go down this list. The only reason I really point this out is that the world is already thinking about this problem, although they're not thinking about it really from the perspective of aging research.

And if you look at institutes like the United Nations or the World Health Organization, they're suggesting what countries should do to take care of the burden of older populations. And everything from reducing pension benefits to reducing healthcare cost, extending work lives, and on down the list.

Now one thing you'll notice in here, there's no column that says, "Keep people healthy longer" or "do something about the diseases of aging." And we think there should be. If you do something like extend work lives, if people are not healthy and they're not functioning highly, then it's not going to help much to extend the retirement age and make people work longer.

And people come to me all the time, and they say, "Well, if you're successful and we're healthy longer, we're going to have to work longer. And what I tell them is, "No, you're going to have to work longer, and we're just hoping we can keep you healthy while you're doing it" because those kinds of changes are going to happen throughout the world. Of the 20 countries that were recently surveyed, 13 of them are currently thinking about raising retirement age, for instance.

So let's come back to aging for a minute. This is a little bit of an oversimplified slide, but I think it illustrates the point of how the world has changed. This is a rough estimate of global life expectancy since 10,000 years ago, and you can see that it was holding quite steady, based on the data we have, around 25. Not quite as accurate as your data, but I'm doing my best, around 25 years of age, until very recently, within the last 300 years or so when life expectancy has gone up over 60 and much higher than that in many countries.

And the point of this, there's really two issues at hand here. One is we've never lived in a world like this before. We didn't evolve to live in a world where we were living to 80 years of age, and the environmental conditions that we have now are nothing like what we adapted to live in. And so a world where 20% of the people are over the age of 65 was not envisioned by 4 billions years of evolution, and it's a new world we have to figure out how to adjust to.

Now why did this happen? Is it because aging researchers have been so great over the last 300 years and have slowed down aging? Well, no, unfortunately, it's not. It's really because we're not dying of nonage-related diseases, as you've heard in the last lecture. And this is a slide from Tom Kirkwood that I stole from him, and it really illustrates what happens to laboratory animals.

If you take mice, for instance, and put them in the lab, they live to be about three years of age; and they stay healthy for quite a while. If you put them in the wild, you're not finding three-year-old mice. You're finding mice that die at four, five, six months. They're barely living long enough to produce progeny for the next generation.

And I would argue that this is what's happened in the human population. We've gone from living in the wild to living in a protected laboratory environment where we have, most of us have enough food to eat; we're not dying as much from infectious disease or predation or childbirth or anything like that, and so we're living longer. We're living in the lab now.

And another way of looking at this is here, again from Tom Kirkwood, is to think about it this way. We have a very limited amount of resources to devote to living, and our main goal is at the far right side here, which is to produce progeny. That's fitness. That's what we're selected for. And we allocate those resources to maximize fitness, and we might allocate them to growth or maintenance of ourselves, which is good for aging, or storing materials or reproduction, and we're going to choose how to allocate these resources if we're a species to best optimize fitness. We're not optimizing for aging. And so that's two-fold. I think one of the things, it explains why the diseases of aging happen; and they come on after reproductive years end. Well we're not selected to stay healthy during that window of time.

But it also suggests there's a lot of malleability in the process, that it's possible to change aging rates. And we actually know that. There are plenty of long-lived species out there. Here's a naked mole rat, which you'll probably hear more about that lives to 30 years of age, 10 times longer than mice, which it's the same size as a mouse. It has more or less the same metabolism. It does a lot of things similarly to a mouse, but it lives to 30 years of age; and there's many, much longer-lived species out there. So nature can figure out a way to keep animals healthy for a long period of time if it optimizes fitness.

Okay, so I'm going to come to three questions today. What are the major causes of aging, can we slow the rate of aging, and will that impact the chronic diseases of aging?

And the first one I'm just going to spend one slide on because, really, this is what you're going to hear a lot about over the next two days. And so it would be silly for me to tell you in a very 10,000 foot level what you're going to hear at ground level from many scientists over the next couple days.

I'll just point out that we really don't know in detail the molecular mechanisms that drive the aging process yet, but it's probably some combination of these things. Now I really grouped them into three categories: damage accumulation, so reactive oxygen species; damage to mitochondria, DNA damage, or protein misfolding and aggregation that occur in cells with age. We know this happens in multiple tissues in the body and multiple organs. We know it drives some of the age-related disease processes, and it probably contributes directly to aging.

There's also a large body of research that's gone on suggesting that loss of homeostasis is important. We see changes in epigenetic reprogramming with aging. That's a relatively new finding. Adult stem cell pools are not able to replenish damaged tissue during the aging process. This is something over the last ten years that's emerged and may be a major force driving aging.

Changes in metabolism driving some of the age-related diseases like diabetes and then changes in cell signaling pathways that occur during the aging process. And then, finally, this group here, antagonistic pleiotropy. I'll just define that, or these would be things that are important for fitness, pathways that you have that keep you healthy when you're young but may have a cost when you get older.

I've tentatively put cell senescence in here. You'll hear a lot about cell senescence. This is the loss of proliferative potential that cells have in cell culture and also in your body during aging. It's a complicated story, but I think it's probably one of the driving factors in aging. Shortening of telomeres. You hear about that. And also inflammation. Again, acute inflammation in response to injury or crisis is important. Chronic elevated inflammation is probably driving some components of the aging process.

And so my guess is it's some combination of these things, some of which are more important in different tissues that are really in compilation driving what we call aging and contributing to age-related diseases.

Can we slow the rate of aging? Well, we're not the first people to think about this, it turns out. One example is Qin Shi Huang, who was a very successful emperor. At the end of the Warring States Period, he unified China, started the Great Wall, Terracotta Army, many great successes. He decided that immortality would be a good thing for him and had many of his chemists and doctors working on how he should stay alive forever, and they chose mercury. And every day he took mercury. One day they made a miscalculation, and he took a little too much mercury; and let's put it this way, he didn't achieve immortality. So that was a little bit of an unsuccessful approach. I think we've been a little more successful in recent years.

And at the start of this is really dietary restriction. Here's one piece of data. This is now eight decades old in mice and in rats. And reducing the amount of calories from an ad libitum diet or a diet where they can have as much as they want down to levels that are just above malnutrition extends lifespan in many species of rodents, mice, and rats, and a whole range of different organisms.

And this is really the first evidence, I think, that you can change aging; but it's also evidence that the environment has a big impact on aging, and I want to come back to that a couple times during this talk.

And I also want to point out that the real strengths of the aging model organisms over the last three decades or so that have driven advances in the field, and what's really happened is there's two kinds of species that we really focused on. These nonvertebrates like yeast and worms and flies here, they have great advantages. They age very, very quickly. You can do aging studies within two weeks to two months. They're quite cost effective. You can have billions of worms in your lab, and you can do very nonbiased genetic studies where you can survey the whole genome and say which mutations affect aging.

When that happens, you can then find these genes and then test them in more complicated organisms like mice and rats and primates and even hopefully humans. The problem here, of course, is that they age slowly. This is a four decade experiment. They're extremely expensive, and so you can't do those genome-wide surveys as easily. So by combining these two sets of species, you can really get an advantage.

You can develop hypotheses here in these species that are easy, but we don't know how closely they're related to human aging, and then test them here where we have a good feel that there's going to be conserve factors with human aging.

And, really, there's three pathways that have emerged as the most prominent in the aging field, and I'm going to take you through each of those. But what I want to tell you is that that's a very limited set. There's probably many pathways, tens of different pathways at least that affect aging; and I'm just going to highlight the ones that have been studied the most.

First off, the insulin IGF signaling has been linked to aging in almost all of the species we study. Now here's some of the first data from Cynthia Kenyon's lab showing that mutation in the insulin receptor, DAF-2 dramatically extends lifespan in worms. And multiple points in this pathway, all the way from insulin signaling through into FOXO regulation, you can get pretty dramatic changes in aging.

This is probably related to human aging. This is just one piece of data from Yousin Suh in Nir Barzilai's lab where they found that there are functionally significant insulin-like growth factor receptor mutations in centenarians. So these very long-lived populations, a subset of these individuals had mutations that have reduced IGF receptor activity.

So, conservation, all the way from worms to humans. The sirtuin pathway is something that's really emerged as a driving factor in many of the diseases of aging. We actually published the first paper in yeast a long time ago now showing that mutations that affect sirtuins can extend lifespan. That was tested more directly for Sir2, this protein deacetylase that you'll hear something about in the next two days. There's been data in worms and flies and a whole range of data suggesting that SIRT1, the human ortholog of Sir2 can affect disease processes across really a wide range of tissues. And so this is a very active field of endeavor in aging research or geroscience.

And then, finally, I want to talk about the TOR pathway, a more recent entry, but I think very relevant to aging. Reduced TOR signaling affects aging in yeast, in worms, in flies, in mice. There's starting to begin to be some data in centenarians, and I think that this is something we need to focus on in some detail. This is, actually, something my lab works on too.

I'd point out just a couple things. For one, this is a signaling pathway, this kinase here, TOR, that's highly responsive to nutrients. And so it may feed into this dietary restriction response. The more calories you have, the higher TOR signaling you have; and you devote your efforts to growth and reproduction as opposed to maintenance. If you reduce TOR signaling, you turn on a number of pathways that make you stress resistant and likely to live longer.

And second of all, that this TOR pathway is intimately connected to insulin IGF signaling as well; and so I say two different pathways, but they may really be very similar. There's also been data suggesting that sirtuins may be regulating this pathway. So whether we're looking at three different ways of modulating aging or three overlapping ways of modulating aging is still something that I think is an open question in the field.

And one of the reasons I highlight this pathway is that there's a drug, rapamycin that is a very specific inhibitor of TOR; and that allowed the National Institute of Aging Intervention Testing Program to test rapamycin in mice to see if a small molecule can affect aging.

This is the first one that really had a robust effect. They, for reasons I won't go into, gave the mice rapamycin at 600 days of age. That's relatively old for a mouse, and it caused a 15% lifespan extension in females and a 10% lifespan extension in males. So there's a couple of points I want to make about this.

First, it's really driven a lot of research in biology and in medicine because since this paper in 2009, if you just search rapamycin and aging, there's about 430 papers that have come out. And so that's giving you a feel for how important these kinds of discoveries are, that small molecules can really impact the aging process.

Now, the question really is you can make animals live longer. Are they healthy longer? And I'm going to spend some time on that. The first thing I want to do is just show you movies that Randy Strong at University of Texas, Health Science Center in San Antonio showed us.

These are the last two mice from the control group that at 38 months of age in this NI Intervention Testing Program study. You can see that this one's having trouble getting around. It's not grooming very well. It's not using its back legs well. Randy told me this one has a tumor. And these mice that have the rapamycin are still doing pretty well. They're getting around the cage, they're being inquisitive, they look like younger mice.

Now they don't look like four-month-old mice. This is not a panacea, but these mice are actually doing quite a lot better at the same time frame with this compound.

Now, is this the only compound that's going to affect aging? No. It turns out that in worms now and in yeast, there are hundreds of genes that impact aging; and I suspect that's going to be the case in mammals as well. And as the NI Intervention Testing Program goes on, they're finding other compounds. And this paper is, sorry for a bit of a typo here. This paper is in press at *Aging Cell*, and it shows another compound, acarbose, extends lifespan more in males than females. It's interesting because rapamycin has an effect more in females than males. This molecule has more of an effect in males, and you can see it's quite a robust extension of lifespan in the male setting.

So this inhibits digestion of polysaccharides and inhibits uptake of sugars from the GI tract. It's already used to treat diabetics; and, again, it may be impacting aging and not just diabetes.

This is another drug. There's going to be a lot of these coming out, and so this, I think, has the potential to really change how we think about research because the question's going to be do molecules like this impact diseases of aging; and can they be used for prevention or therapy?

So where do we stand? Can we extend lifespan and health span? I think the answer is clearly yes, either from environment or genetics or drugs in these nonvertebrate species. I think it's clearly yes in mice at this point. In primates, we have a lot less data. There's been one dietary restriction study showing an extension of lifespan and another one that didn't show lifespan extension but showed some health span benefits or the animals would stay healthy longer. We don't know, but I'm sure there're genetic mutations that will affect aging in primates, although we haven't identified any yet. And then there have been no studies that I know of on small molecules.

This is my graduate advisor. Can we do anything to help him? I would argue that environment, yes, that both exercise and nutrition are probably impacting aging. Genetics from the centenarian studies, at least in those very long-lived populations, there are genetic events that impact aging. And maybe some of these drugs that we're already talking about, already taking like nonsteroidal anti-inflammatory drugs, statins, maybe they're already impacting aging to a small degree. We don't know that yet, but I think it's worth at least putting maybe here.

So that's where the field stands, and the question is, if we can impact longevity or health span, will that cause a beneficial effect on the chronic diseases of aging?

So this is from Felipe. This shows the leading causes of mortality in the US. The data is probably not quite as good as the data you just heard, but it illustrates the point cardiovascular disease, cancer, stroke, lung diseases, all of these things, they have one thing in common; and that's the biggest risk factor is aging.

We don't think about that. When we think about cardiovascular disease, you hear things like cholesterol is the biggest risk factor. But there's a 20-fold increase in the rate of cardiovascular disease as you get older, and we could make this argument about a whole range of different diseases that are increasing in prevalence in the US and throughout the world.

And I think this is really the mantra of our institute at the Buck, is how is it that aging enables all these diseases to happen? Of course, it's not the only risk factor. There are multiple independent factors driving disease, but we think it's the most important common one.

And we also think it has a functional impact because when you have these mutations that extend lifespan, they tend to prevent many of these diseases in the animal models. So we think it's a novel approach to go after, to say what is it about aging that enables disease; and can we do something about that?

Now, I just want to touch a little bit on the links between different diseases, and this is data from a study in England, I think, where they looked at relative disease risk for people who exercise. So they put people in different quintiles, whether they don't exercise much at all or they exercise a lot. And the main point of this was to show that when you exercise, it reduces risk of death from cardiovascular disease. And that happened, and it went down pretty dramatically.

But the interesting thing they found is that the control group who didn't have cardiovascular disease, the rate of mortality went down just as dramatically, suggesting that exercise is impacting the other chronic diseases of aging as well.

Now you can look at hundreds of these kinds of studies and find flaws or things that make you worry about each individual study, but when you put them all together, I think it's pretty clear that the environment can, and how you behave impacts the rate at which you age.

And so if we look at what can be achieved now, and I want to take a step back because I ask people in the public a lot whether they want to live longer, and a lot of times they say, "No, I don't want to do that." And I say, "Why? Why is that?" And they say, "Well, my 80-year-old grandmother is taking 25 pills a day, she's sick, she's in pain. Why do I want to live longer like that?" And what they're imagining is something like this is going to happen. So this is when you're born, you're relatively healthy, and you die at some point. And before that, you have an onset of morbidity, of chronic diseases of aging which impact your ability to live life the way you want to live it.

And what people imagine is that these mutations that extend lifespan are doing this. They're keeping you sick longer. But that's not what they're doing, at least I believe in the mouse studies. They're keeping the animals healthy longer, and there's still a period of decline.

Now, ultimately, I think what we'd like to achieve is this, where we have compressed morbidity. Animals or people live longer and they have a much shorter period of chronic disease or morbidity. I don't know that this is achievable yet from the genetic studies and small molecule studies, but I think we've gotten to this stage. At least in a mammal we've shown that this can happen.

And if you look at the changing demographics of the world, there's a huge economic benefit to keeping people healthy longer right now, even if there's still a period of decline afterwards. And so I think it's reasonable to set this as a goal and maybe hope that we can some day get to this.

But, of course, you know the problem. The problem is that the world thinks about drug development, pharmaceutical companies, and that's targeted treating disease. And so if you look at where pharmaceutical companies focus their effort, it's on disease treatment, to a lesser extent but still somewhat disease prevention. And the idea of giving a drug to a healthy person to keep them healthy longer still hasn't resonated for all sorts of reasons, including regulatory ones.

But if you look at the promise of aging research, it's almost the inverse, that we think it's going to be feasible to keep people healthy longer looking at these pathways. I think it'll be useful for disease prevention.

The question though is if you talk about treatment, waiting till people get really sick and trying to make them better again, will these pathways that impact aging do anything or not? And this, I think, is the challenge we really have to surmount if we're thinking about taking the research we're doing and having an impact on the human population. How do we work with these two inverse pyramids? At what level to actually bring some of the basic research findings that we have into a translatable form that is useful for human population.

Here's another way to think about it. We're doing basic aging research and identifying the pathways that affect aging. And I've argued that it will extend health span already. Will it prevent disease and will it treat chronic diseases? And so I just want to spend one or two slides talking about each of these things,

starting with prevention. And I'm going to use rapamycin as a case study, not because I think that's the only way to do this but because we know the most about it right now.

And this is a study that we did where we took mice at 24 months of age, and we gave them rapamycin for 3 months and did a whole lot of transcriptomics, biochemistry, metabolomics on these mice. We also looked at cardiac function, and I'm not going to show you the details of this study, just to say that in many of the measures, like ejection fraction in the heart or fractional shortening, which measures left ventricular function and these two measure output, we not only found that the mice were protected – here's the decline in function in the control mice in these two groups – not only were the mice treated with rapamycin protected over this 3-month window, it actually got better. The mice at 27 months of age were doing better than the 24-month mice before they started the assay. And it also dramatically suppressed left ventricular hypertrophy.

This is just one study on rapamycin my many different labs, and there's a lot of data like this out there. It's suggesting that rapamycin is going to be beneficial for a range of chronic diseases of aging, not all of them. It actually accelerates other diseases of aging like cataracts, so it's not a panacea, and it may not be the right drug. But I think it illustrates the point that the things that slow aging are likely to prevent at least a subset of diseases of aging.

What about treating chronic disease? Well, those of you who know about rapamycin know that it was found 40 years ago or more, and it's been tested by pharma companies over a whole range of different disease indications. And at least in animal models, there's good data for rapamycin in neurodegenerative disease now and Alzheimer's, Huntington's, Parkinson's. It's already in the clinic for some rare forms of cancer. At one point there was a hope that it was going to be much more useful in cancer than it's turned out to be, but it is protective in some kidney cancers and others.

There's data in stroke. There's quite a bit of animal-related data on cardiovascular disease. If you are interested in Type 2 diabetes, I'm happy to talk about it. I'm not going into it today. So it behaves like a molecular that would slow aging, might be expected to behave.

And, in fact, there have been a wide range of clinical trials with rapamycin. Over 2,000 ongoing trials with either rapamycin or first-generation analogs have been tested now across a whole range of different disease indications. And some have been effective. There's a problem, of course, with side effects of rapamycin. I don't want to go into it in detail, just to illustrate that people have already used this drug in treatment; and it is effective in some cases and not in others.

This is what we think is happening from our research and many others in the field, is that the events that are driving aging are related to the events that are driving disease. And so when we identify these pathways that are impacting the aging process like TOR or SIRT1, they're also the things that go wrong during disease. So my current hypothesis is that aging is about damage, it's about changes in signaling, it's about all kinds of events that I told you about before – cell senescence, stem cell dysfunction.

One of the things that happens when these events go wrong is that they cause secondary consequences. We think one of those is elevated TOR signaling, and the reason we say that is if you look across a whole range of different tissues with aging, the TOR pathway gets aberrantly turned up; and the benefits of rapamycin may be that it's just suppressing that, that it's blocking that secondary consequence of aging.

SIRT1, another enzyme I told you about that's important for aging, goes down in many tissues with aging. And the molecules that activate SIRT1 may be suppressing that decline. And so I think these pathways are really pointing us to things that may be secondary consequences of the damaging events that cause aging. But they overlap dramatically with the events related to disease.

We've looked at TOR signaling in a range of different disease states now, and it tends to go up in cardiovascular disease. There's data on brain diseases. So it's very similar to aging. And so for treatment, it may be possible to work backwards. Use aging to identify these pathways, and then work

backwards to figure out which diseases these therapies that restore these pathways might be beneficial for.

So I do believe that these pathways are going to be relevant to treating chronic disease as well as preventing it. But it's certainly a process that needs to be studied in more detail.

Okay, so what you're going to hear over the next two days are focused discussions around many of the pathways that we think are driving aging. I've already mentioned them: inflammation, the stress and adaptation to stress that occurs during the aging process over time, changes in epigenetics, changes in metabolism, the onset of macromolecular damage with age, protein folding and proteostasis, and then stem cells.

And I think that you're going to hear compelling cases that each of these processes are contributing to what we call aging, and they're also driving different diseases of aging. And it's my hope that having these kinds of discussions are really going to stimulate everyone here to think about this question and what it is about aging that's driving disease and how is that an opportunity to look at these diseases from a new perspective and maybe develop new therapeutic approaches to treat them.

So with that, I want to stop and just thank you for the time and to give the talk, and hopefully I've set the stage and this will be a great two-day meeting. Thank you.

Dr. Sierra: In case you've forgotten, I'm Felipe Sierra. I'm not going to introduce myself further. The next and last keynote speaker for this morning will be Dr. Linda Fried, who's the Dean of the Mailman School of Public Health at Columbia University in New York. Dr. Fried received her MD from Rush Medical College in Chicago and her masters in public health from Johns Hopkins. Her talk will be on "Frailty and Relationships to Disease in the Elderly." Dr. Fried.

Frailty and Relationships to Disease in the Elderly

Linda Fried, MD, MPH: Well, good morning. It's a pleasure and an honor to be here today, and I was asked to frame out a particular perhaps subquestion of what Brian was just talking about; and I hope by honing in on frailty and relationships to disease in the elderly that it will exemplify some of what was just discussed.

Recently an article in the *Journal of the American Medical Directors Association* published the results of a consensus conference held last year, delegates from six major international European and US societies, which agreed that physical frailty should be considered an important medical syndrome, defined as a medical syndrome with multiple causes and contributors, characterized by diminished strength, endurance, and reduced physiologic function that increases an individual's vulnerability for developing increased dependency and/or death. Now, you could say this a little more colloquially, as one of our colleagues has done, that we're talking about a syndrome of shrinking, slowing, weakness with low activity and low energy.

What I'd like to do is summarize the thinking behind that statement, show you some evidence as to what is emerging in a syndrome with a now recognizable phenotype, what is emerging as the potential underlying physiology and biology, and then conclude by linking that to a number of questions about chronic disease.

So I'll start from a theory born of geriatric medicine that, in fact, frailty has a characteristic phenotype, which constitutes, essentially, a vicious cycle very much, at least in appearance, related to energy dysregulation. So sarcopenia we know is a hallmark of the clinical presentation of people who are frail, and we know many things about pair-wise associations, even 15 or 20 years ago when I certainly started thinking about this. We know that loss of muscle mass with aging predicts declines in strength and power and exercise tolerance, that all of them together predict slowed walking speed and other motor performance and further declines in physical activity. And we know that, from a variety of studies, that at

least in a subset of older people, there is a mismatch between the level of activity and the level of nutrition such that even in the face of low levels of physical activity, in the subset, you see chronic undernutrition. And then you see the beginnings of a very vicious cycle.

Now a question, of course, that certainly started my own thinking was whether this clinical appearance was somehow related to energy dysregulation associated with aging. And I'll try and show you some emerging thoughts about that.

But to get there, of course, we had to start with some thing that was standardizable; and with colleagues in the audience, certainly one of whom will be speaking today, we developed a standardized approach of saying that a syndrome, a clinical syndrome has to have a constellation of signs and symptoms that in combination, not by themselves, are recognizable and that point to a distinct underlying pathophysiology.

And the vicious cycle that I just showed you can be boiled down to five different potential key manifestations, and we hypothesized initially that we might be able to identify, following the definition of a syndrome, an underlying pathophysiology if we looked at the people who had multiple of these.

And we have gone on over the years to provide measures for that, and based on a huge amount of validation studies, identified people with three, four, or five of these criteria as being frail, with none of them nonfrail, and identifying intermediate state with one or two of those five. Pretty simplistic approach but with a very, I think, reasonably well developed underlying biologic set of hypotheses.

And what did we learn? Well, we learned that if you look at people with three, four, or five of these criteria in the Cardiovascular Health Study, a major multicenter national study of people, men and women 65 and older, that the prevalence rises with increasing age, that it's consistently higher in women compared to men, and that by the upper age groups, the oldest old, at least a quarter of community-dwelling older adults manifest this type of frailty.

And we also have shown through many studies of our own and others that this is a very highly vulnerable subset who are clinically at risk for a host of outcomes: mortality, falls, disability, and dependency, delayed and incomplete recovery from illness, and at highly elevated risk of adverse outcomes associated with hospitalization and surgery. Notably, outcomes that we associate with many chronic diseases as well.

So to sum across many studies, we have found that frailty as a phenotype of aging is a marker of elevated vulnerability for these outcomes and that there's a dose response association, that the more manifestations of frailty that are present, the greater the likelihood of those outcomes, and the lower the time frame to experiencing them.

And we also have found that this is a chronic progressive process. In 90% of older adults who have become frail, the initial presentation, shown by Qian-Li Xue, is muscle weakness, slowing down, and low physical activity and that any of these manifestations predict the development of the rest over even a three-year period. And there is compelling evidence that the early stages are most likely responsive to intervention, while the end stages appear much less so. And what I'd like to show you is that there is emerging evidence of a distinctive underlying biology of multisystem dysregulation that is very tightly related to this phenotype.

But, first, I'll digress for the sake of this conference to say that there is also strong evidence from many investigators that this frailty syndrome is associated with a range of chronic diseases quite consistently. Cardiovascular disease, both clinical and subclinical, COPD, anemia, cancers, all the things that were on the prior two speakers' list of prime suspects, plus HIV, late life depression. And that inflammatory diseases, in particular, are tightly associated with the presence of frailty and that multiple inflammatory diseases present in combination exacerbate that risk of frailty quite significantly.

Here's one piece of data from a study by Anne Newman from the Cardiovascular Health Study looking at mean maximal internal carotid wall thickness, a subclinical measure of cardiovascular disease, strongly a marker of atherosclerotic plaque. And what you can see in the left-hand crosshatched bar is that as you go from, if you're looking at people with no clinical diagnosis of cardiovascular disease and you're looking at the mean wall thickness, that the proportion with the mean wall thickness increases as you go from not frail to intermediate or prefrail to frail so that there is an association with subclinical disease in the absence of clinical disease, as well as here in the gray bar with cardiovascular disease. I should say almost no association.

So what's going on below the water line? If you have a presentation that has some clinical significance in terms of predicting vulnerability for adverse outcomes, is there a specificity to this syndrome in terms of underlying pathophysiology; and how might that be related to chronic disease itself?

So I'm going to summarize what I'm quickly going to present, which is that, in fact, that many physiologic systems are now understood to be dysregulated in the presence of this frailty phenotype, that the relationship between the number of systems dysregulated and the likelihood of frailty is nonlinear, and that there is strong emerging evidence that frailty is actually an emergent property of a dysregulated complex adaptive system.

So let's start at the phenotype, which I presented to you before, understanding it's associated with vulnerability for these adverse outcomes, and ask some questions about the physiologic dysregulation.

And here's a summary of the systems that have been shown to be abnormal or dysregulated and associated with frailty, starting, of course, with sarcopenia as a hallmark of frailty, inflammation, decreased heart rate variability, altered clotting processes, anemia, a number of altered hormones, including the IGF-1 and insulin resistance that Dr. Kennedy was referring to, and a host of micronutrient protein and energy deficiencies.

One example from work by Jeremy Walston, again from the Cardiovascular Health Study, simply and clearly shows that looking at C-reactive protein as a marker of inflammation, that the mean CRP level rises in association going from left to right with frailty status from not frail to intermediate to frail. And here another marker, measure of inflammation, high IL-6, the proportion in a different study population, the Women's Health & Aging Studies, with high IL-6 again increases in a step-wise fashion going from the green, nonfrail; to yellow, prefrail; to red, frail. But very interestingly, two other measures of low IGF-1 in the middle and low DAGAS on the left, proceed in association with frailty in the same step-wise parallel dose response relationship.

Looking at nutrient deficiencies, work by Richard Semba has shown that as the number of micronutrient deficiencies present rise, so does the incidence of frailty. And this is in two population-based studies: The Women's Health & Aging Studies, I and II, of highly disabled and nondisabled older women, ages 70 to 79.

And if you look within what you might think of as a physiologic module of systems, namely hormonal systems, and just do a simple count of the number of hormones at abnormal levels in older women, in association with frailty status, and you look particularly at the orange and the red, you see that those, or the yellow and the red, you see that those with two systems abnormal, two hormones at abnormal levels, or three or more at abnormal levels in the red, again, increase in step-wise relationship with rising frailty status.

If you roll that all together across modules of different physiologic systems, you find that a simple count of the number of systems abnormal in association with frailty, and again this is from the Women's Health & Aging Studies, I and II, and I point you to the yellow and red, shows that as the number, as you go from left to right, from nonfrail in the first group to prefrail in the middle group, to frail in the right-hand group, that the proportion with four or five or more systems abnormal rises quite dramatically.

In that same set of analyses, we showed that, in fact, that count of the number of systems abnormal across many different modular physiologic systems actually is associated with a prevalence of frailty in a nonlinear relationship.

So summarizing across many, many studies, what we're seeing increasingly is strong evidence that for the dysregulation of the nonlinear complex adaptive system that maintains a resilient and robust human organism and, not surprising to this audience, there is mounting evidence that the biologic changes of aging and perhaps a diverse range of them are likely to be drivers of this multisystem dysregulation at the physiologic level.

So what have we seen? That in this picture of multisystem dysregulation, we're seeing dysfunction of a number of modules of physiologic systems, modules which are connected through physiological networking, mutual regulation, and redundancy consistent with a dynamical complex adaptive system. That the result could well be thought to be loss of homeostatic regulation and an underlying decline in reserves with which our resilient bodies maintain that regulation; and that this appearance may well relate to both the phenotype of frailty and vulnerability to stressors.

Now what might be going on conceptually? Well, of course, we have biologic changes with aging at the molecular genetic level and many changes that we will be discussing over the next three days. And, of course, these changes we know drive lots of alterations at the physiologic level.

These alterations at the physiologic level also mutually regulate each other in a tight redundant complex network essential to homeostasis. And if we saw a depletion of this tight network within and across circles, we, of course, in a healthy organism, perhaps a younger organism, know that we rely on many complex and compensatory mechanisms if any one of them is not functioning adequately. And feed forward and feed backward loops that are extremely tightly maintained in a healthy organism.

And that is true, of course, at many levels of our function. And if these compensatory mechanisms, if these regulatory relationships are diminished, we could be well threatening our ability to maintain homeostatic reserves and regulations.

What might that look like for the person? This is a wonderful slide from Qian-Li Xue and Ravi Varadhan. But if you had a human being who was hospitalized, the hospitalization and the illness or a stressor, they're healthy, they bounce back pretty quickly. But if, in fact, there is some dysregulation of their homeostatic reserves, the time to bounce back may well be greater. And if it proceeds and gets wider and wider, that trough, the person is in great trouble in terms of bouncing back.

So perhaps, in fact, frailty is the emergent property of a dysregulated complex adaptive system. Do we have any evidence for that? Well, I'll show you a little bit and then move on to chronic diseases.

Here's data led by Ravi Varadhan in which he looked at mean diurnal profiles of cortisol over a 24-hour period in the older women in the Women's Health & Aging Study II and showed that in the nonfrail, you saw intact diurnal variation. In those who were frail on the solid line, you saw the same first thing in the morning, salivary cortisol as those who were nonfrail. But through the day, a consistently higher level of cortisol over 7 measures.

But that's in steady state. What would happen, theoretically, in a complex dynamical system? You might see not much difference in a steady state; but if, in fact, regulatory networks are compromised, you might see less ability in a stressed state to respond to a stressor in people who are frail by this definition.

So I'll show you a couple of experiments that have been recently reported and one that is in preparation to try and test this hypothesis, looking at whether challenge tests of frail, prefrail, and nonfrail old, old women might reveal evidence as to dysregulated, underlying biology associated with this phenotype. And I'm going to show you a few examples, particularly targeted to helping us think about energy dysregulation, although not all.

As I mentioned, the Women's Health & Aging Study II is a prospective observational cohort study of women 70 to 79 years of age in 1994, selected to be representative of the two-thirds least disabled. The data I'm showing you are at a point of 15 years of follow-up, and this study was funded, as is all this research that I'm showing you, by the NIH, primarily the National Institute on Aging.

The theory that we were evaluating in these challenge tests of old, old women was that physiologic dysregulation and frailty would be more notable in a stressed state and would be manifested as delayed exaggerated or prolonged responses to the stressor and delayed recovery to baseline in the frail compared to the nonfrail.

We administered, actually, eight different challenge tests over time. I'm going to mention several of them very quickly, so that you can see the patterns across the responses. These are safe, clinically used tests: glucose tolerance test, ACTH stimulation test, magnetic resonance spectroscopy of the tibialis anterior with a 30-second isometric plantar flexion exercise and a simple flu shot looking at antibody response.

And here are a few findings, some of the highlights. This is the results of a flu shot challenge in frail and nonfrail older adults. There were men as well as women in this particular substudy. This was conducted by Sean Lang. And what he found was that the rates of seroconversion, the percent who seroconverted in response to influenza immunization, a clinically significant response of four-fold or higher increase in H1 titers, was almost nonexistent in the frail; observable in H3N2, 5%; no response at a clinically significant level for H1N1 or B in the frail, while reasonable response in the nonfrail.

Here's the results of glucose tolerance tests performed by Rita Kalyani and Ann Coppola showing, not surprising to us, that the fasting blood glucose in these 85- to 95-year-old women was no difference by, really not different by frailty status, with the red being frail, green prefail, black nonfrail. But you can see that blood measures over time show after 30 minutes that the frail elevation in mean glucose level was quite much greater in the frail compared to the prefrail and the nonfrail and that the time to recovery was well beyond what we even thought to measure. You see the same thing on the right-hand side in terms of mean insulin level. So exaggerated response, prolonged elevation, delayed recovery.

Notably, in addition to the fact that in the fail versus nonfrail, there was no difference in the fasting glucose level. The glucose levels for the frail were almost 70 milligrams per deciliter higher, quite significant at 120 minutes. And in addition, although I don't have time to show you, in other measures we found profound elevations in glucose raising hormones and lowered glucose lowering hormones in the same testing in the frail compared to the nonfrail. So the whole system appears dysregulated.

Also notable in thinking about disease, this study was done in women who had no clinical history of diabetes. And excluding those women in this subset of old, old women who had no history of clinical diabetes, only 27% of them had a normal fasting glucose and a normal GTT. Forty-eight percent had prediabetes, and 25% had undiagnosed diabetes. And most of that was due to the abnormal glucose tolerance test.

So dysregulation across this whole pathway of hormones regulating insulin resistance and energy metabolism. And, of course, in the top, potentially driving inflammation.

A final study done by Ravi Varadhan. A magnetic resonance spectroscopy evaluation of these old, old women looking at their tibialis anterior and conducting 30-second isometric exercise in the scanner to look at the time to recovery of phosphocreatine; and what you can see is that these old, old women who were frail, which is on the right, had much delayed time to recovery of phosphocreatine compared to women who were not frail.

Now, the only other study that I'm aware of that has looked at this was in IL-10-frail mice compared to normal mice and a study by Akki. And in a steady state, not under stress conditions; and what they found was quite suggestively parallel in terms of lower phosphocreatine levels at rest in the IL-10 mice and then much lower synthesis of ATP in the IL-10 mice as well.

So where are we? Well, what we're seeing is that there are some nodules of a complex adaptive system that are themselves dysregulated, that there's evidence for problematic mutual regulation. That, in fact, the phenotype emerges when the severity of this is past, perhaps, a certain threshold and that this is related clinically to a compromised ability to adapt to stressors and high risk of adverse clinical outcomes.

Is this due to our initial core hypothesis that this was driven by dysregulated energetics associated with aging, which could lead to a generalized phenomenon and phenotype? Well, there is mounting evidence to support that. I don't have time to show it, but it certainly is a subject for discussion at this meeting.

But let's think about what I just showed you and conclusion in terms of the implications for the potential relationships of frailty, both as a phenotype and as a biologic process and chronic diseases.

So, there is mounting evidence that the frailty syndrome may well be aging-specific and aging-related, but it may also be a final common pathway, particularly for catabolic diseases kicked off at a certain level of severity of those diseases. This is one potential mechanism for the association of frailty with chronic disease.

A second is, and there is some evidence now to support this, that, actually, the coexistence of frailty and chronic disease in the same person is associated with elevated risk for shared outcomes like disability, like mortality. And we have seen that in studies in HIV/AIDS, in congestive heart failure where the risk of disability and mortality are at least additive between that chronic disease and frailty. And, in addition, it's very clear that people hospitalized or admitted for surgery, if they go in frail have worse outcomes than those who do not go in frail.

In addition, there may well be, as Brian Kennedy talked about, shared risk factors for frailty and chronic diseases. I'm particularly thinking off the cuff, off the bat about inflammation and insulin resistance. There's a nice figure from Alfalalo suggesting that in terms of cardiovascular disease, many aspects of what we'll be discussing over the next few days in terms of the basic biology of aging may well, of course, stimulate chronic, low grade inflammation with aging, which is clearly a precipitant of frailty and, for example, of cardiovascular disease and shared outcomes.

In addition, there are shared behavioral predictors of frailty and chronic disease. Low physical activity, smoking, and low, poor dietary intake are all predictors of frailty. We know that at this point. Same predictors for many chronic diseases.

So the aging-related changes of frailty, the basic biologic and physiologic changes could well, as Dr. Kennedy suggested, be driving disease development.

One of the intriguing questions that is worth thinking about, I think, is where does actual dysregulation end and disease begin? The data that I showed you on glucose dysregulation suggested that in the women who did not, in these old, old women who didn't have clinically known diabetes, only 20% of them were normal in terms of their response to the oral glucose tolerance tests, particularly. Is that disease? Is that dysregulation of aging?

So we have many potential relationships of frailty with clinical disease, which could even be thought of as end organ manifestations of shared processes, whether they're at the physiologic level or the biologic level.

So I'll end in a way similar to where Dr. Kennedy ended. There are many biologic drivers of aging that all of you in the audience work on. Is there one in particular that is driving this phenomenon of frailty or is this a combination of multiple biologic drivers stimulating the multisystem dysregulation we see at a physiologic level?

So where are we? Well, we know that frailty appears to be a clinical syndrome, that it predicts terrible outcomes in the short term, that there are many systems dysregulated in parallel, many physiologic

systems associated with frailty, and that the association is nonlinear, and perhaps is the phenomenon of complex systems unraveled, if you will. And that there's mounting evidence of underlying biologic alterations predicting this particular phenotype and possibly these physiologic dysregulations.

The relationship with disease is a very exciting question to be pursuing, and I hope today that I've been able to suggest some potential mechanisms to be thinking about in terms of that.

Overall, does this matter? I think that's the rhetorical question, given that we're all here to think about these issues. But if you think particularly about frailty, there are a number of illuminating insights, I think, about opportunities to focus on health span and prevention, as Dr. Kennedy just laid out so compellingly.

If you think about frailty, if I think about frailty as the emergent property of a dysregulated complex system, that would certainly suggest to me that the opportunities for prevention do not casually lie in tweaking one or another dysregulated abnormal system. That the dynamics have to be either, that are addressed, either much more basic or much more multisystem; and physical activity is a prime example of that.

So thank you. I hope that this has set up some discussions for the day. I do want to point out that all of the data that I just showed you, except for the mouse data, are data from population-based studies, which I think will provide in the future much of the basic science of these kinds of very complicated questions. Thank you. Thank you.

Dr. Sierra: All right, we're going to have a 30-minute break now. We're going to reconvene at 9:45, I believe, for the inflammation session. Thank you.

Break
Session II: Inflammation

Alison Deckhut Augustine, PhD: Good morning, everyone. Could we please take our seats so we could get started. Thank you.

Okay, I'm just going to start talking because this part's not so important.

So good morning, and welcome to the session on inflammation. My name is Alison Deckhut Augustine, PhD, and I'm from the National Institute of Allergy and Infectious Diseases. Dr. Grace Shen and myself, Grace is from the National Eye Institute. We co-organized this session with help from our other colleagues at NIH who are Francesca Macchiarini from NIAID, Evan Hadley from National Institution on Aging, and Janice Allen from the National Institute of Environmental Health Sciences. And just as a footnote for Grace, her name was accidently left off the Organizing Committee, but she is part of the Organizing Committee and actually the co-lead.

So our co-chairs for this session on inflammation are Claudio Franceschi and Judith Campisi. Dr. Franceschi is Professor of the Department of Experimental Pathology of the University of Bologna, and he will give an introductory overview of the topic for this session.

Then Dr. Campisi is a professor at the Buck Institute for Research on Aging, as well as a senior scientist at the Lawrence Berkeley National Laboratory. Dr. Campisi will direct the discussion which follows the last speaker, and she will also give her summary of the session. So with that, I'd like to invite Dr. Franceschi to please come up and give the introduction.

Introduction

Claudio Franceschi, MD: Okay, it's a great pleasure and honor to be here, and I would like to thank all the organizers. Okay, this is a busy slide but gives you three messages. First of all, some years ago I

proposed the name “inflamaging” for the purpose of chronic inflammation that is characteristic, persistent, sterile, that is characteristic of aging.

And please give a look to the subtitle, “An Evolutionary Perspective of Immunosenescence” because this is the end result of my studies on the evolution of the immune system and on the central role of innate immunity in macrophages.

The second message is that as many previous speakers mentioned, inflammation, and I will use the term inflamaging from now on, is shared by the most important diseases of aging, which all have a greater inflammatory component and the pathogenesis.

The third message is that inflamaging is a multisystemic inflammation, and several organs and systems contribute. The gut microbiota, the adipose tissue, the immune system, of course, but also the liver, the mast cells, and probably also the brain as it will be mentioned by other speakers. So we need systems biology or systems medicine approach to tackle inflamaging.

So I will summarize. There are a lot of open questions, as you can imagine, but I will try to summarize some of the most important characteristics that Judith Campisi and myself identified.

Inflamaging, I think, is triggered by a variety of danger signals, which can be either exogenous like cytomegalovirus or HIV virus, bacteria of the gut microbiota and elsewhere, and its product or endogenous. And this is very important. Self-danger signals, senescent cells that Judith has explored since about ten years; and they have an inflammatory phenotype. Damaged cells, dysfunctional organelles such as the mitochondrial, cell debris, self-cell debris because every day every second a lot of cells are destroyed, alter the modified proteins and N glycans and DNA, which is recognized as a bacterial DNA. ATP, reactive oxygen species, age, theramides.

Thus, I think that garbage disposal play a major role in triggering inflammation and inflamaging; and I propose to you this new word, garbaging, which should deliver the message. All these potential stimulants are physiologically produced, even in young humans. And it’s tempting to speculate that the health status, the physiological health status is maintained and assured by a physiological inflammatory tone as it was predicted about a century ago by the famous immunologist Metchnikoff as an essential ingredient to maintain body homeostasis.

Within this perspective, inflamaging would represent the progressive increase with age of such an inflammatory tone fostered by increasing exposure to exogenous danger agents, the type previously mentioned, and increased generation of endogenous danger signals because of the increased number of senescent cells, increasing number of cell debris, damage and dysfunctional mitochondrial altered molecules as I mentioned before.

So, but due to the increased generation of endogenous danger signals, there is decreased garbage disposal and you will hear about during these days, including the decreased efficiency of the UPS proteasome, ubiquitin-proteasome systems, autophagy and mitophagy as other people will mention. And, finally, there are two major players, the increased activation of NF-kappa-B and inflammasome.

I would like to propose to you that inflamaging is a fire, and that this fire propagate in the body. So I have a propagating view of the aging process, and there is evidence that the aging phenotype is maintained by cell-autonomous mechanism. Mainly responsible are the aged microenvironment, the niche, and the macroenvironment, all the stuff which are circulating in the plasma or blood of all the animals and humans.

So inflamaging can propagate from cells to cells and from organ to organ, and there are data suggesting that exosomes in the nanovesicles can propagate micro-RNAs and other stuff that can play a role, also cytokines.

Local and systemic inflammation trigger, sustain, and reinforce each other in a vicious circle, so it's very difficult to identify which came first.

In the cancer field, the propagation by standard cells of DNA damage, of DNA damage response and inflammation is very well known and has been conceptualized as part of inflammation.

I would like also to give, to speculate and to give you the idea that how important can be inflamaging because I think that the reason the demographic revolution, the doubling of life expectancy in the last 50 years could be at least in part the result of a decreased rate of inflamaging due to a life-long decreased production of or exposure to garbage and their signals because of hydrogenized environment, sanitation where there are less microbes, less cellular and molecular damages as a consequence of less physically and I would stress emotionally stressful lifestyle which can also cause inflammation. And, particularly, this was particularly important because happened also, included the pregnant women and the newborns and children.

And you know the devastating effect in the literature that's been described when a fetus is exposed to high inflammatory environment in the uterus, and there can be long-term effects, mainly probably due to epigenetic effect; and, of course, there is a decreased inflammatory memory.

Conversely in poorer countries, the rate of inflamaging is still much faster than that observed in rich countries but are led by much higher and precocious incidence of age-related diseases. And I think that this can explain, at least in part, the data shown by the previous, the first speaker.

Finally, garbaging and inflamaging can be major therapeutic targets because of the identification of the major sources of inflamaging in the last 20 years has paved the way for a variety of possible interventions aimed at eliminating or neutralizing the unnecessary, excessive inflammatory stimuli. We can work on the gut microbiota. We can slow down the rate of inflamaging and of its causes, and we can postpone/avoid the onset of major age-related phenotypes.

There are some data that will be shown later on in the session that the elimination of senescent cells, for example, has as a consequence the rejuvenation of the entire body. And there will be also presentation that the inhibition of inflamaging activation can be another major target. But there are other that I mention.

I think that I finished, and I think I can introduce Luigi Ferrucci. It's a great pleasure for me because when I know Luigi Ferrucci since when he was in Italy at the National Institute on Aging, and I was the Scientific Director of that institute; and Luigi was a young, very promising doctor, geriatrician.

Now he has become Scientific Director of the Intramural Research Program at NIA, and so I'm very particularly pleased to introduce you.

How do inflammatory processes associated with chronic disease differ from those due to acute insults/disease?

Luigi Ferrucci, MD, PhD: Thank you, Claudio, for the introduction and the really pleasant memory of that time.

I'm going to try to give you briefly an introduction on the proinflammatory state of aging, what Claudio has named the inflamaging phenomenon. And I want to start by saying what inflammation is and why we're talking about inflammation now. We're talking about somewhat of a different inflammation.

So this is what is happening in a normal inflammatory process, which is by its nature an acute process where you have a cause and there is the normal inflammatory reaction as a response to eliminate this infection, trauma, or toxic activity.

And the inflammatory process is witnessed in the circulation by elevated level of proinflammatory markers. And one of the most well-known of these inflammatory markers is IL-6, and interleukin 6 will be mentioned and probably by all the speakers this morning.

And later on, there is a reaction of acute phase reactive proteins, C-reactive protein is the most known, and is now also used in clinical practice. But this reaction is meant to eliminate this damage. And when the process, the response can be successful, the level of IL-6 and CRP tends to go down; and you have a beautiful healing and elimination of the cause of inflammation.

Unfortunately, what is happening with chronic dysregulation and the so-called inflamaging is something a little bit different. And I think that this is really what makes an enormous different one than we talk about inflammation in terms of inflammatory response when we talk about the chronic proinflammatory state of aging.

So IL-6 or C-reactive protein tends to go up at some point. We don't really understand when this is happening, but it may remain elevated for a very, very long time. And the chronically elevated level of these inflammatory markers is epidemiologically associated with a number of bad consequences.

And I'm going to show you data from one of the studies, the InCHIANTI study showing that even when you select a very, very healthy population, the level of many inflammatory markers increase with aging. And so there is something intrinsically connected to aging that upregulates the chronic inflammatory process.

This is a very healthy population without any cardiovascular disease, without any cardiovascular risk factor, without any dysnutritional or chronic inflammatory disease. So this is not caused by disease but something that we don't really understand.

So is that inflamaging, is this chronic elevation or inflammatory marker important? It's incredibly important. There are a number of studies that have shown, and this is one of the first studies, showing that if you take a population of older people that have absolutely no disability and no mobility problem and you measure IL-6 or other inflammatory markers, you see that above a certain threshold, because between different studies, vary between 2.5 and 3.5 key picogram for milliliter, the risk of developing disability increases linearly. And this is one of the most powerful biomarker predictor of disability. I want to stress this point because I think it's very important in talking about aging and older people.

This transition from being somebody who's able to do everything that they can in life, walking out, shopping, enjoying fully their life, and the transition to a condition where they need help just to move from their bed, there is an enormous gap in quality of life that goes from being completely mobile and having mobility disability. And we have now a biomarker that predicts strongly this risk, over and beyond everything we know. And the biomarker that we have used so far in the handling of disease.

The mechanism for elevated chronic level of inflammatory biomarkers and the consequence of elevated inflammatory biomarkers had been identified by epidemiological studies; but the underlying mechanism that connect these phenomenon are absolutely not known.

I'm going to try to walk you through a dichotomy of possible interpretation. So, you start normally in inflammatory reaction with the harmless stimulus. There could be some damaged cell, some irritant chemicals, and/or pathogen that caused the garbaging that Claudio was actually talking about this morning. It's a very beautiful definition.

And what's happening is that you'll have an inflammatory response, which is really the attempt to remove damaged cells, irritant, and pathogens. And when this is occurring, all the energy and all the attention is dedicated to the removal of this aggression. You'll have to, the inflammatory reaction is meant to fight this aggression. It's dedicated, all the army, all the energy, all the supplies to this task.

And, in fact, there is really the shutdown on many of the surrounding cellular mechanism. For example, we know that there is reduced food absorption during inflammation. There is a stimulated glycogenolysis at the level of the mast cell. There is very, very little growing of the mast cell with downregulation and reduction in the level of IGF-1. In the bones, there is demonstrated osteoclast, downregulated osteoclasting at the level of the bone marrow. The entire marrow activity is reduced, but in particular the activities of the erythropoietin is biologically downregulated so that even though their erythropoietin are right to the bone marrow, does not stimulate the production of erythrocytes at the level of atherosclerosis. There is stimulation of atherosclerosis and inhibition on the T_____ reactivity at the level of the brain in animal models, been demonstrated that when there is chronic inflammation, all the stimulus to neurogenesis, including exercise, tend to be a lot less effective.

So everything that is building, everything that requires use of energy is kind of shut down when there is this defense to this acute aggression. And this actually, in some way, makes lots of sense because when somebody is fighting you, what you want to do is to win the battle. If you win the battle, then you have time to build. You have time to repair. You have time to maintain. If you don't win the battle, you're going to be dead, so it doesn't really matter whether you have done those repairs or not.

And so the problem here is that in the normal reaction, as I was explaining before, when this is effective, you eliminate the cause of inflammation and you'll have switch off of inflammation and then you have the healing process.

The problem with aging is that we never go to this part, and I think that we need to at least have two alternative hypotheses. Either we never remove the cause of inflammation, so there is something that is continuing, stimulating the inflammatory response that is always there. And the other alternative is that there is some intrinsic property of the immune system, for example, a dysregulation of NF-kappa-B is one of the possible hypothesis that maintain activated inflammation in spite of the fact that inflammation is no longer required.

This is not trivial. It's become incredibly important because if inflammation is fighting something, downregulating inflammation may not be a good idea. If dysregulation occurs intrinsically in the immune system, that acting on dysregulating and downregulating inflammation, pharmacologically could be a bright idea and might have very good consequences.

I see that I don't have very much time, but I was just going to go with you through an example of how, when we talk about inflammation and we make the equivalence between inflammation and high inflammatory markers, this could be an excessive simplification.

For example, in the muscle, if you have a trauma or muscle damage, the first thing you do is the infiltration of polymorphonucleates. And then a number of process that tend to remove the debris. But when this happens, then you have the switching of the immune system in the repair mode. And so the immune system does not only fight and important to fight the aggression, but it's also important to rebuild when the aggression has been removed.

And so if we don't have this new phase of inflammation where the downregulation occurs and the new building phase occurs, there may be very important metabolic processes. Thank you very much.

Judith Campisi, PhD: So our next speaker is Jim Kirkland. Jim got his MD and PhD from the University of Toronto. He's now a professor at the Mayo Clinic, and he's director of the Kogod Center for Aging Research at the Mayo.

How do local and systemic sources of chronic inflammation contribute to chronic disease processes?

Jim Kirkland, MD, PhD: So I was asked to give a tent style talk that's data free, and basically what I'm going to do is ask five questions. I'm going to steal the thunder from the talk by saying the answers to the questions are either yes or both.

So I've got two introductory slides there. The first question is this. Circulating proinflammatory molecules are predictors of age-related morbidity, but are they important drivers or markers of many age-related diseases in humans? So I'll just start off by summarizing the previous talks, and that's that there's chronic sterile, low-grade inflammation that tends to increase in many older individuals to different extents and is also associated with many of the major or pretty well all of the major age-related chronic diseases.

And this inflammation is associated with or may drive or be driven by cellular senescence and macromolecular damage. These things are all highly interrelated.

We have to remember that we're not just dealing with cytokines in these situations. There are other kinds of proteins, and there are a lot of nonprotein actors that may be important and are understudied, including various lipid-related molecules, ceramides, prostanoids, and things, bradykines and other neurological mediators. And sometimes we're talking about things that may have systemic effects, not just through getting into the circulation, but also through effects on the nervous system, afferent and efferent loops. So there's a lot of work that needs to be done to sort some of these things out.

Now with respect to this particular question, are they important drivers or markers of many age-related diseases in humans? The simple answer is, "Yes, in many cases. Yes, plus no in some cases. And no in others." So there's a whole diversity.

One of the ones that's yes plus no is a situation where there's a division between effects of the kinds of consequences of a disease state, and that's myelofibrosis. So this disease is often associated with an IL-6-producing translocation. Sometimes, about half the time it's associated just with high circulating IL-6 levels without the translocation.

It turns out from some very nice work that Ellho Taferi did that if you use JAK1/2 inhibitors to block effects and production of IL-6 in these patients, in a big study that was published in the *New England Journal*, you do not affect the underlying disease state as manifest by time to developing leukemia or clonal myeloproliferation assays of bone barrow biopsies. But there are tremendous effects on constitutional symptoms.

So these patients who normally have a profound IL-6 syndrome with the kinds of things that Dr. Fried talked about with weight loss and cachexia and severe depression and decreased movement and cognitive function, all the rest of it, those things are dramatically reversed by giving JAK1/2 inhibitors. But the rate of progression of the underlying disease itself is not affected.

So this is an example of a yes and no situation. Other processes involve production of factors that act very locally in localized diseases that tend to be age-related. So in arthritis, for example, or various other kinds of situations, there can be autocrine or paracrine factors that are released that may not get into the circulation but are important to drivers and are related to both aging processes and the diseases. And then most of these diseases there is some kind of escape into the circulation.

The second question is damaged molecules or cells, senescent cells, or commensal flora are among stimuli that can initiate age-related inflammation. So to what extent do distinct components of inflammatory signatures of each stimulus affect phenotypic outcomes or age-related disease? And, again, the answer is to a large extent, to some extent, or to no extent? So there's a lot of variation.

Some of the explanation for this is that inflammation varies. The nature of inflammation varies from inducer to inducer. So in the case of the senescence-associated secretory phenotype, for example, which is one of the things which can underlie inflammation, particularly IL-6 production. Simin Meydani,

for example, showed that most of the circulating IL-6, which is associated with frailty syndrome actually comes from fat tissue. We'll talk about that in a moment.

But the SASP appears to differ amongst cell types, quite profoundly. For example, IL-6, one of the major mediators of the SASP, is not increased in corneal epithelial cells. So there are exceptions and there are differences amongst tissues. With respect to fat tissue, if you take human fat cell progenitors from different regions, which are a very potentially proinflammatory cell type, their baseline characteristics differ substantially, depending on developmental gene expression profiles in these resident progenitors in different fat depots.

So you can take preadipocytes from abdominal regions, from thigh regions, and they're totally different, for example, with respect to homeotic gene expression signatures and Wnt signaling elements. And these things appear to drive, to some extent, in some studies that are underway and some of which are about to be published, the kind of inflammatory mediator expression that varies amongst cells from, originating from different depots at baseline.

It turns out that when you make these cells senescent, you know, given that they're different in their secretory profiles at baseline, their SASP is very different. So there appear to be differences, depending on the tissue that's inflamed and the nature of the induction of inflammation that results in potential differences with respect to phenotypes. And I talked before about myelofibrosis.

So the third question is do stimulus-specific inflammatory mediators act primarily to distance, that is systemically, or only locally? And the answer is both. So with respect to this question, a lot of the cytokines that are produced, and other inflammatory mediators, tend to have very biphasic effects that depend on concentration. And there are concentration gradients as you go away from the source of inflammation.

For example, TNF alpha at low concentrations can be a very strong driver of proliferation of a variety of different cell types. At higher concentrations, it inhibits proliferation, inhibits differentiation, and can cause apoptosis. So there is a concentration dependency and sort of a gradient effect that can occur that's complex and that we don't completely understand with respect to either aging phenomena or age-related diseases.

Some of these mediators are degraded locally very quickly. For example, TNF-alpha. Others make it into the circulation and might have a paracrine effect, and yet others are purely autocrine like IL-1-alpha and tend not to even make it outside the cell.

So given that some of these things are stimulus-specific, given that some are up to a greater extent than others with respect to age-related diseases and they have biphasic effects and they may or may not escape into the circulation. You'd expect the answer to this question to be both.

Next question is what are the roles of local versus systemic production of proinflammatory cytokines in driving phenotypes and pathologies associated with aging? And, again, the answer is both can be important. It depends on the disease state that you're looking at. It depends on the particular cytokines or other inflammatory mediators that are involved. And we also have to bear in mind that there are inflammatory mediators, as I mentioned before, like bradykines or substance P that tend to be produced in age-related proinflammatory states and disease-related proinflammatory states that act through the nervous system as well.

Some of these inducers get into the circulation in sort of low quantities but then have a major effect on another organ. For example, the liver with respect to production of CRP or ferritin or other kinds of outcomes that can get produced by small quantities of local inflammatory factors escaping into the circulation and driving liver production. Some of these factors also act locally with respect to stem cell and progenitor niches.

The final question is, is there an age-related decline in protective, local, or circulating factors that are present in tissue from younger individuals? I think this is a very potentially exciting area. I think we'll hear more about this later. But it looks like there are TGF-beta-related factors, other local factors that, in parabiosis studies and other kinds of experiments appear to be protective that are, in some cases, anti-inflammatory and are produced by cells from younger individuals.

So in the long run, we need more work to understand the answers to these questions. They're important questions. We need to understand more about differences amongst tissues in cytokines and inflammatory mediator production.

We need to bear in mind that we're not dealing with just cytokines or even proteins. There are other things going on. And, most importantly, we need to come to the mechanism-based interventions. So it does appear that doing things like eliminating senescent cells, for example, which appear to be the major source of IL-6 from fat tissue do reduce frailty in experiments that were done at Mayo.

And it appears that rapamycin partly acts through modulating this secretory phenotype. JAK inhibitors do. And they have impact on certain kinds of age-related disease outcomes like nutritional state, fat tissue growth, and so forth. So there may be interventions coming along. Thank you very much.

Dr. Campisi: Thank you, Jim. So our next speaker is Jayakrishna Ambati, and he got his MD from SUNI Brooklyn, and he is now professor at the University of Kentucky; and he's going to talk to us primarily about inflammation in the eye.

What are the core inflammatory components or pathways that cause inflammatory damage across a spectrum of chronic diseases?

Jayakrishna Ambati, MD: Good morning everyone. It's a great pleasure and privilege to be here today, and I was asked to share my thoughts on commonalities and differences in inflammatory drivers of chronic diseases. And so I thought I would do so by framing the question in the context of four of the great diseases of aging, and this audience, no doubt, is very familiar with at least three of them. There is a tremendous human and economic toll that's exactly by conditions like Alzheimer's and atherosclerosis and arthritis associated with aging. But perhaps less well appreciated is the equivalent kind of toll that's exacted by a condition called age-related macular degeneration whose worldwide prevalence today exceeds that of all solid tumors combined and rivals that of Alzheimer's disease.

And while all these conditions have multifaceted and often nebulous etiologies, they're all characterized by an age-dependent, degenerative, and destructive set of processes that result often in a common hallmark, a common molecular hallmark that is accumulation and inadequate elimination of what's been termed self-debris.

And these toxic accumulations over time initiate an inflammatory response that at the outset can be quite beneficial in promoting health and longevity of both the tissue and the organism. But as you've heard earlier today from a variety of speakers, for a set of unknown reasons, that inflammatory cascade is often not turned off and persists; and it's in that context that it tends to become detrimental to anatomy and function.

Now self-debris can take many forms. They can be polypeptides, lipids, sugars, and as we've shown recently, they can even be noncoding or repetitive RNAs. And despite the diversity of these self-debris substances, they often tend to result in similar functional pathologies.

And one reason for that might be the discovery over the past few years of a common node called the inflammasome, which is an immune platform that can integrate by funneling these very heterogeneous signals and start transducing a set of signals that initiate and potentiate cellular damage and dysfunction by means of cytokine production, for example, ultimately resulting in cellular dysfunction and death.

Now the inflammasome, of which the NLRP3 inflammasome is the best studied example, can be thought of as a molecular machine that churns out powerful cytokines like interleukin 1-beta and IL-18. And this particular inflammasome has really emerged in recent years as a very attractive therapeutic target in a variety of these disease states that we're talking about today. Really convergent data sets from multiple groups have implicated the NLRP3 inflammasome in a starring role really, both in human tissues derived from patients with this condition and in studies showing that antagonizing or blocking the inflammasome or its mediators can have really powerful beneficial effects in relevant animal models of this condition. So I think in coming years we'll see a lot of pharmacological activity surrounding NLRP3, MyD88, and the interleukins and so on.

Now, of course, there are dramatic differences amongst these disease states. One of those likes in the cellular phenotype that drives these conditions, and they can be dramatically different. For example, in some of these conditions, it's really degeneration of the cell and tissue that drives the dysfunction, whereas in other conditions, as you see in the column on the right, it's actually cellular proliferation that does the damage. And the actor, the cellular actors can be quite different. And so I think it's important to capture some of these really nontrivial subtle differences as we study these conditions.

Now a recurring theme in all these diseases is, of course, immune dysfunction; but there too what arm of immunity are we talking about. In certain states, adaptive immunity can be important. Innate immunity's often the driver in other disease states, and so-called resident neural immunity fuels the fire in conditions like Alzheimer's or age-related macular degeneration. And again here too, these are not really crystal clear, crisp distinctions. It can be a lot of overlap, particularly during various stages of the same disease in the same individual.

And angiogenesis or blood vessel growth is increasingly recognized as a key pathological hallmark in many of these disease states. And most often it tends to be pathological in driving specifically conditions like the neovascular form of macular degeneration or degenerative arthritis. But here too there are states even within the same condition. For example, in the atrophic form of macular degeneration, it can be the absence or the insufficiency of blood vessels in the choroid that supplies the retina that can fuel the fire as it were. And there are also conflicting data sets about angiogenesis and its benefit or its pathological implications in conditions like atherosclerosis.

So as we move forward, really, I think in trying to understand and capture the various inflammatory drivers and subsystems that regulate the contribution of inflammation, I think I'd like to observe, at least, that the eye has emerged, has really proven to become a really spectacular platform for discovery and innovation in terms of inflammation in aging diseases.

So I think it's pretty well known now that a decade ago that the first really strong data sets from the GWAS study showing complement factor H being statistically associated with age-related macular degeneration came from this platform being studied. And now we actually have the reality of artificial silicone retinal viewing devices for patients with end stage retinal diseases.

The eye was also the first place where innovative therapeutics like small interfering RNAs and aptamers were introduced in humans and also, coincidently and importantly, where the first in vivo, off-target, immunological and proinflammatory activities of these particular drugs were discovered.

And one of the reasons that the eye has emerged as a prime platform for testing a lot of these kinds of devices and drugs is because it's really a very nicely compartmentalized system that permits attractive pharmacokinetics and very attractive self-limitation of adverse effects.

Now the eye being very accessible and transparent for much of its structure permits really an unparalleled view of pathology in motion. So what you see here, for example, is this cellular debris accumulating in the central retina of a person; and this can be followed longitudinally over time. Here is a view of new blood vessels that are invading the retina in a patient with macular degeneration.

And the eye has really proven to be the frontier for cutting edge imaging tools that permit really histologically, approaching resolution images of this self-debris and vasogenetic edema that results from proinflammatory conditions. So I think the eye can teach us a lot, not just about ocular diseases, but about inflammatory conditions in general. And so I'd like to leave you with that thought. Thank you very much.

Dr. Campisi: So our next speaker is Deep Dixit, and he got his PhD from the University of Hannover, and he's now Professor at Yale University.

Can age-associated chronic inflammation be adaptive or beneficial or is it always pathogenic?

V. Deep Dixit: Great. I trained at Intramural Program of the National Institute on Aging, so it is really a great honor for me to be here at NIH and to share the stage with leaders in the field.

So I was asked to initiate a discussion on this topic, "Can age-associated chronic inflammation be adaptive or beneficial or is it always pathogenic?" I'm afraid I do not have any answers, perhaps more questions as we move forward.

Of course, we all agree on this, so point being that inflammation here is a major risk factor for several of these chronic diseases. And one would argue that if one is to compress morbidity, as Dr. Kennedy very elegantly explained in his talk, that perhaps it suggests that many of the organs in the body may be aging at perhaps similar rate if one is to understand those processes. But it's clearly not the case, so I'm going to touch a little bit on the adaptive immune system and the innate immune system.

So this is a picture of a young thymus. This is a magnetic resonance image of somebody who is around 24 years of age. Metabolically healthy. This is where the thymus is located, right above the aortic arch; and this small fat nodule in this thymus suggesting that majority of this is still functional. And, as most of you know, thymus is critical for establishing the T-cell repertoire. This is the place where T-cell specificities are created. Okay?

When we are young, we have a preponderance of naïve T-cells; and this is the reason why we also have a diverse T-cell repertoire diversity. And the consequence of overall this process is that our immune system is functioning, at least adaptive immune system is functioning really well. And what is happening is very, very puzzling, but extremely conserved throughout the vertebrates; and this is a picture of somebody who is metabolically totally healthy. And what you're looking at is this white mass that you see is hyperintense region that is basically a thymus that is full of lipid.

So this is a person actually not old, somebody who may have gotten ____1 at the age of about 40, probably the same age. So he's not an old person. And what is happening is that as we get older, the number of T-cells in the body do not change. The system is highly adaptive. And as we heard today, that most of us never lived beyond the age of 40, 45 until we reached the year 2000.

But as we get older, the flavor of T-cells change. We have less of the naïve T-cells and there's a homeostatic expansion of the effective memory T-cells, the cells that have previously seen the antigen.

And as a result of that, there is a restriction of T-cell receptor repertoire diversity; and we heard from Dr. Fried how frail individuals are much more prone to getting influenza. And there are many reasons for that. One of the reasons is that adaptive immune system is degenerating with age. And this is happening very early on.

So the question remains, is the stage set for us to be inflamed as we're getting older? Is it adaptive? Is it beneficial? You decide.

Other important component is innate immune system which is contributing to inflammation. As you heard from Luigi, the phrase that he coined, inflamaging.

But one of the limiting factors in this is that all our knowledge of inflammation comes from classical host defense literature where inflammation is required for protection against infections. And so none of the classical signs of inflammation exist in aging – redness, fever, swelling, pain, all that, right? But like Charles Janeway conceptualized inflammation, it is broken down into three major components. You have to have inducers, and you have to have immune sensors, and then the mediators. And inducers, which can also be called danger signals which Polly Matzinger, not very far from here in NIAID coined. So for these signals to exist, you have to have immune sensors to sense it; and then you have the effect.

And the most important ones of those are, of course, microbes, infections. But there are also several sterile danger signals. And as we heard that aging is associated with this damage that continues, now we know several of those are elevated with age. Luigi here has shown that with age uric acid is one of those factors. Several others are increased with age. They are sterile. They are of nonmicrobial origin.

And for these signals to be present, we have pattern recognition receptors, Toll-like receptors. And you heard from Dr. Ambati about the NOD-like receptors. And one of the components of that is an inflammasome. So these things can sense all these danger signals, all these factors that are present here, these inducers leading to an inflammatory response; and all these mediators and effectors, including IL-6, IL-18, they get induced in this process.

So then, of course, the question emerges, how much of this is a beneficial response? Clearly, inflammation has an extremely important beneficial response to injury and infection. You get inflammation, tissue repair and survival. And as we heard, with the possibility in the theory of antagonist pleiotropy, that the protection against infections and survival through the reproductive age is perhaps the primary function of the immune system in terms of its defense.

Of course, there's a cost associated with inflammation that, as we all know, in young, of course, infection immune response is a highly emergent intensive event. The cost in young is at least anorexia and sickness. But we recover from those things and we survive.

In older individuals, as we know, the mechanisms of these process are still fairly unknown; and it contributes to sepsis and death in older individuals.

But, again, this concept of inflammation comes from classical immunological work with infections. This is, perhaps, very different than what we are encountering with aging. So this is just a thought, and there is very few data to actually, right now, to really prove any of this. But what we do know is that there are certain metabolic danger signals that increase with age. And they're listed, some of them.

And they can emerge from several sources. Mitochondria dysfunction, perhaps, as we'll hear later in the day from latent infections. From alterations in the gut microbiota. There's more and more evidence that's emerging with that. And these things eventually are supposed to lead to chronic low-grade inflammation. And all these things that we see, well this is all nice and good, but the point remains is that we have actually very little causality and very little causal data in the field about how inflammation is actually causally linked to aging. And as you all know, there are very few of these anti-inflammatory or the classical anti-inflammatories have resulted in lifespan extension or are that effective in these scenarios.

So Dr. Ambati already explained that to you with regard to the inflammasome, but there is some data that is emerging now suggesting that NLRP3 inflammasome is, perhaps, quite important in aging. And what this complex does, as Dr. Ambati explained, is it senses these various danger signals or these sterile factors. This includes bacterial pathogens associated in molecular patterns.

And there are two main inflammasomes that have recently emerged, NLRP3 and there is also a noncanonical inflammasome called caspase-11 because this inflammasome is also triggered. It actually

senses LPS independently of Toll-like receptor 4. So there has been a lot of studies that suggest that there may be metabolic endotoxemia, that there may be LPS leaking from the gut and perhaps contributing to the inflammation.

So we had looked into this. So now the data are fairly clear, which is that it is primarily the NLRP3, canonical inflammasome with these three component parts that get assembled when they see these danger signals leading to the activation of caspase-1 in aging, at least in mouse models.

And that the noncanonical inflammasome, the caspase-11 inflammasome, that senses LPS which could be important in metabolic endotoxemia is really not engaged. And what that does is as you get the activation of caspase-1, caspase-1 has multiple substrates in the body. Two major ones are IL-1-beta and IL-18. So IL-1-beta and IL-18 is kept in a macrophage in a pro form. So these two cytokines are released, pro IL-18 and pro IL-1-beta only when the caspase-1 is present. It cleaves those things.

And so we looked into the downstream, which one of these two cytokines are important because therapeutically it is an important question. And the data would suggest that when you ablate the NLRP3 inflammasome, you can actually attenuate component, several parts that are associated with inflamaging, which is associated with bone loss but adipose tissue inflammation, glucose intolerance, and frailty. So this is actually first causal evidence in my view that we can show the activation of this complex can lead to several of these inflammation-associated conditions.

Important thing which potentially is important for therapeutics is that if you block IL-1-beta, at least in mice, in this case genetically, only partial of this downstream effects of NLRP3 are effective. So if you block NLRP3, sorry, IL-1-beta, you get only partial improvements in CNS, inflammation, and cognitive decline in frailty. So I'll leave you with this thought, and we'll probably have a good round discussion after this. Thank you.

Dr. Campisi: So our last, but not least, speaker of this session is Russ Tracy; and he is a professor at the University of Vermont and director of several translational units within the university.

Are there interventions that can alter the dynamics of inflammation and prevent/limit chronic disease?

Russ Tracy, PhD: Okay, great. Thanks very much, Judy, and thanks for the invitation to speak. I too was asked to address a particular question. "Are there interventions that can alter the dynamics of inflammation and prevent/limit chronic disease?" The answer, of course, is yes. And there are many ways to think about this, so I've chosen to take one approach that might be a little new to some of you, thinking it might prove interesting.

And that is based on the work we've been doing recently in HIV-positive communities. There's a competing meeting in Baltimore at this same hour on HIV and aging, which pushes the notion that HIV is actually accelerated aging. And some of the concepts may prove useful to us in thinking about interventions. So that's my goal today.

So the virus kills CD4 cells where they live, and they don't live in blood particularly. They live in lymphoid tissue. So it kills them in places like the thymus and the spleen and bone marrow and lymph nodes and in gut-associated lymphoid tissue which breaks down some of the barrier function leading to microbial translocation.

So you have a significant problem right off the bat in that you have microbial products in excessive amounts getting into the blood stream and other places. That plus what looks to be like an antiviral interferon-mediated response in the liver, causes a global protein synthesis decline. That leads to changes in coagulation factors, which leads to, in essence, a procoagulant state.

So you have LPS stimulating through endotoxemia, and you have a change in coagulation, leading to a procoagulant state. And so the first point I'd like to make is that when we think about inflammation, we don't limit it to innate and adaptive immunity. We include coagulation in the triad. Now, there's many, many other components, as has already been pointed out by speakers today; but those are the three that we tend to think about and try to make measures of when we do studies of inflammation.

So we can then think about interactions of these pathways, and this is a complicated slide. My point here really is to talk about how things like tissue factor expression and leading to hypercoagulation can lead to cardiovascular disease. The same can be true going back the other way. We're talking about microbial translocation, leading to changes in liver function. the same can be true going back the other way to a certain extent. And we get these alterations in association with a variety of initiators and other risk factors, leading to age-associated diseases and one we've studied the most is cardiovascular disease.

So that's the kind of overarching design. I've tried to summarize it in a slide like this where a position that we and many others have been talking about for 20 years now is that nonimmune stimulators really around low level wound healing frequently, like alcoholic liver damage or smoke-related lung damage, these are inflammatory conditions. We like to point out they lead to both changes in innate immunity and coagulation. Both of these can contribute to something like atherosclerotic progression. And if there's enough of that development, that can, itself, become one of the stimulators, so that erosion of plaque leads to the activation of the coagulation system which then leads to more inflammation and more stimulation, so you get into this cycle of activity.

What we've been thinking about more recently is what about immune stimulators, and that's where the HIV work has led us to contemplate about this. And viruses like HIV, but CMV have become particularly important. We've recently published that CMV biasing towards Th1 cells is associated with increased atherosclerosis, for example, and that that kind of change in terms of specific immune functions, like biasing and helper function, is something of interest to us. But in today's discussion, it's that all of this activity, whether it's virally driven, bacterially driven, or it's autoimmune driven, takes, as was just shown really nicely by Dr. Dixit, naïve cells converts them to memory effector cells; and you end up becoming increasingly immunosenescent.

And so one of the things we've been exploring in population studies is that increasing immunosenescence leads to increased inflammation, in part because you're getting more effector memory cells functioning. But also in part because you're losing the ability to deal with invasion appropriately. So that puts increasing burden on innate immunity. It puts increasing infectious disease burden, because you're inefficient in removing, and it gives you increased cancer risk due to loss of immune surveillance, something that's very obvious in the HIV community; and we hope to be exploring more in non-HIV older populations soon.

So that's our overarching design. If we think about interventions then, some key ideas come up. Oh, yeah, one other point is can we substitute all chronic diseases for atherosclerosis? And we have a paper in publication where low naïve cells, not only do they predict atherosclerosis in healthy populations, they predict Type 2 diabetes as well, we think through the same mechanism. That it's increasing inflammation due to inefficient adaptive immunity.

So, some key questions to consider then if you think about interventions in that overall context. When to intervene. Early versus late. That's somewhat equivalent to prevention versus treatment. And it may be different for behavioral modification versus pharmacotherapy. Linda Fried talked about exercise as almost always being a good thing to do. Stopping smoking is almost always a good thing to do. Losing some weight. So these are very effective interventions, that we've known for 50 to 100 years in some cases, can affect health. And we know now they can affect health through the systems we're discussing.

So we have added information about it. There are many powerful anti-inflammatory therapies that have long-term consequences about which we're going to have a tough time knowing because the randomized controlled trials aren't fundable. We need to do them for 40 or 50 years, and no one is going to allow us to do that.

So at the end of the day we're going to use biomarkers in Phase II/III type studies to try and assess what the long-term function might be. Evan spent a lot of time getting the study NIA funded, calorie functioning, and that's one approach to do this sort of work. I think there should be many more approaches like that.

It seems like, interestingly, these powerful therapies may, in many cases, be better tolerated, however, in young than in old. In the example I use as anti-TNF therapy where it's a wonderful addition to the armamentarium in juvenile arthritis and inflammatory bowel disease. But you put it in older folks with heart failure, where you know part of the problem is TNF signaling and apoptotic and cardiomyocytes; and they actually failed in clinical trials. And we can argue about doses and approaches and regimens, but it's clearly much better tolerated in young people than in old people. So we're going to have some questions to answer as we approach that, and other key questions are the how to intervene because we have the classic problem that almost every speak has talked about. And I won't belabor it, but the same system that's causing the pathophysiology we need for normal physiology. So how do we intervene on this and yet keep it functional and active?

Behavioral modification. I'm trying to, in the next five or so, to give a structure to how we might think about interventions. And it's going to be both behavior and pharmacotherapy. And so this one that would reduce proinflammatory risk factors, well stop smoking. There's a great one. Pharmacotherapy to reduce proinflammatory risk factors. Well, how about valacyclovir for CMV infection? If this is going to turn out to be an important user-upper of the adaptive immune system, thereby leading to inflammation, well maybe we should start approaching a very common, ubiquitous infection that affects many, many people. And looking at the age of the folks in this room, about 50 to 60% of this population is CMV seropositive. It's chewing up 3% in NHANES, a very nice study, chewing 3% of all your T-cells. Of all the things your T-cells have to work on, 3% are targeted to CMV peptides. This is a terrible use of immune landscape and something we may want to go try and do something about.

Behavioral modification reduced inflammation response, exercise a great one. Pharmacotherapy to reduce inflammation response, aspirins or canakinumab or other strong cytokine interventions may, in fact, play a role there.

And then therapies to reduce the need for inflammation. Well, one that's sort of still *Star Trekie* is let's look at those thymuses in those old folks, and is there something we can do about it? Can we get thymic, a replacement to actually function properly and reboost the immune system again? And I heard some evidence about other types of inhibitors, removing SASP cells and other things that might work as well.

So my last slide. What intervention might make the most sense? Well, everybody in this room can probably add to this slide. There's large numbers of targets, so I offer no evidence. I have a comprehensive list. This is the one, a way again of maybe structuring the thinking that we have found useful in a recent review article. I took out the HIV-specific part of this, but the rest of this, I think, is reasonable.

Getting rid of excess copathogen burden might be one approach. And particularly looking in the elderly at microbial translocation increases and LPS in plasma does go up with age, and you're getting increased bacterial translocation as you age. And there's, sevelamer and others may play a role in being able to sequester it or keep it from translocating. If you have poor T-cell function, there are possibilities for increasing that functionality without actually increasing the number of T-cells, increase the function of the ones that you have. Lymphoid and tissue fibrosis is a big problem; and there are antifibrotic agents like pirfenidone and others that might be interesting to explore. More systemic chronic inflammation, there's lots of potential candidates. I won't go through all of that.

Hypercoagulation. We unsuccessfully recently tried to argue for a dabigatran trial in - a low dose dabigatran trial in HIV-positive people as an anti-inflammatory drug because it calms down the coagulation system, playing on the interactions of these systems. And maybe there's some interest in that.

Cellular aging, I'm sure we'll hear lots more about this, and then attacking metabolic dysregulation. Again, we've already heard something about that. I'll stop there, and thank you for your attention.

Wrap-Up

Dr. Campisi: So thank you to all the speakers, and now we're going to have an open question and answer period. We'll welcome questions from the audience. I'll just very briefly summarize some of the main points of this session, the first being that inflammation really is the glue that holds together basic aging processes that occur and the diseases of aging. And this, of course, is the main focus of this entire summit is how to understand how aging impacts these multiple diseases that we call collectively the diseases of aging. And it seems that inflammation is really one of these glues.

It's, obviously, not going to be a simple problem to solve because we need the inflammatory response to survive; and so we're going to have to think about clever ways of dampening the pathophysiological functions of inflammation and maintaining the normal functions that are essential for fighting off infection and for dealing with trauma.

Question and Answer Session

So I think what we'll do now is open the question and answer period up to the audience. What I would ask is please use the microphones in the aisle to address your questions to the panel. And please state your name and your origin so that the audience can know who you are.

We have a question. Yes, go ahead.

David Gobel: This is for Dr. Tracy. I'm Dave Gobel, Methuselah Foundation. I came from my mother. Dr. Tracy, I'm very interested in the hypercoagulation. Perhaps it's well known to everyone in the audience, but not to me. Your slide mentioned alcohol insult and injury, subclinical. Could you talk about that a little bit and how much it contributes to the overall chronic element of inflammation.

Dr. Tracy: First of all, I think there are many such insults. Many were mentioned already this morning. Type A personality and that sort of thing, it contributes as well. So there's lots of choices around those.

I think it was also mentioned this morning, and I'd echo it, that we don't honestly know from an amount standpoint how much, but epidemiologically we can find many associations of such things with poor outcomes, even when we try to control for all the other ones.

So my belief is that it's not insignificant. It's certainly in our hypothesis that it's not insignificant. It's something we're pursuing rapidly in as many venues as we can.

To talk about coagulation just super briefly, D-dimer is a biomarker that many in the audience might be familiar with. You get it when you make a little fibrin and then lyse the fibrin with some plasmin. So you get a very specific marker of how much thrombin you've generated, how much fibrin you've generated, and how much fibrinolysis you've done. That's a marker that is never zero in anyone. We're always doing some of this, and it increases with age.

And it's a very strong predictor of bad outcomes in older people. In the Cardiovascular Health Study that Linda talked about, D-dimer is a very strong predictor of all-cause mortality, not just clot-based mortality. So that's also true, by the way, in HIV populations.

So there's something that it's signaling, that this increasing coagulation is signaling in both the elderly and other infirmed populations that we don't completely understand; but we think it's tied to the broad system that these guys gave you wonderful slides about already this morning that covers lots of bases and gives us signals across a wide range of physiology.

Dr. Franceschi: I would like to add just a little bit because some years ago we started the study of centenarians, and one of the major observations was that all the major coagulation factors are increased in the centenarians but they are free of most of the related diseases. So we called this the paradox of centenarians, but paradox means that we didn't understand what was going on.

But I would like to draw your attention that probably is important not only to look at the classical factors, but to some other protective factors which despite the increase of classical coagulation markers can protect these people which are a model of extreme longevity.

Dr. Campisi: Yeah, try to keep your comments short because I see we have a line of people who want questions.

Dr. Tracy: But to Claudio's point, I'll just point out that the amount of the coagulation factors is less important than the balance. And, for example, interferon-mediated global protein synthetic drops by hepatocytes leads to lower levels of all the coagulation factors; but that leads to an imbalance that, between pro and anticoagulants, that makes you procoagulant. And that's what we've been pursuing, have modeling to prove, and may, in fact, be something of interest in the centenarians.

Dr. Campisi: Question.

Zoltan Ungvari, MD, PhD: I'm Zoltan Ungvari from the Reynolds Oklahoma Center on Aging; and, first of all, I would like to congratulate the organizer. It was a really exciting session. And I just would like to draw your attention to a cell type which is only present in each organ affected by inflammation. This is the vascular, microvascular endothelial cell. You have 5 kilogram of endothelial cell in your body, and the age-related alteration of this cell type, namely the proinflammatory phenotype shift in gene expression profile of this cell type would affect all the organs affected by age-related inflammatory diseases. So any comment on that?

Dr. Franceschi: Yes, there are some micro-RNAs which are specific. We did some studies on HUVEC and we showed that there is this miR-146a, which is highly increased when this HUVEC becomes senescent. And I think that you did, that Judy did the same type of observation. So you are right, that the endothelial cells are a very important target.

Dr. Kirkland: Also, HUVECs and endothelial cells seem to be more susceptible to senescence than many other cell types more readily. And when you look at senescent cells in tissues, they tend to follow blood vessels.

Dr. Ungvari: Just a comment back, I'm sorry. HUVEC, not a really good model of aging research because that's made of umbilical endothelial cells. But you are right. I think if you look at other endothelial cells, such as cellular microvascular endothelials or coronary endothelials, you see this proinflammatory profile.

Dr. Franceschi: We studied not only HUVEC, but also the precursors in the blood.

Dr. Campisi: Yes, a question?

Judith Frydman, PhD: Yes, my name is Judith Frydman. I'm from Stanford University, and I have a question to the general panel. So we heard a lot about inflammation rising with aging and that inhibiting inflammation can be good or bad and maybe as a therapy could be a little bit traumatic if it also helps fight disease and infectious disease.

So what we didn't hear a lot about is what are the molecular mechanisms by which inflammation can be deleterious? And is it senescence, is oxidative damage to proteins or membranes? And perhaps these would be better avenues to target therapeutically the bad parts of inflammation without leaving elderly people without immune response or a protective response.

Dr. Campisi: Well remember that during inflammation the whole idea of calling the immune system to the site of the wound or the trauma is to fight off a pathogen, to kill. And so the cells that respond are designed to make toxic products that can be damaging to the surrounding tissue, with the idea of killing an invading pathogen.

So I don't know. Somebody have some good ideas on how to target those toxic products or keep them more specific for a pathogen as opposed to a cellular target?

Speaker: One thing would be to eliminate senescent cells periodically in people while they're healthy.

Dr. Campisi: If they are the main source.

Speaker: If they are. But it does appear, at least in mouse models, that they contribute substantially.

Dr. Ambati: So one of the advantages in the eye is that some of the operative systems are slightly different in that in contrast to the kinds of diseases that Mishall has been looking at, for example, or others on this panel.

It's actually amateur cells, nonprofessional immune cells in the eye that are actually doing this damage. So, for example, in the context of age-related macular degeneration, it's not really invading macrophages or invading neutrophils or invading anything that are doing the damage. It's actually resident retinal pigment epithelial cells that are actually assuming this amateur role of an inflammatory cell and transducing these NLRP3 signals.

So in that particular instance, because of the confined nature, both from a spatial perspective, as well as from a confined nature of the cellular perspective, you can actually target specifically say NLRP3 or downstream interleukins or downstream adapter proteins like MyD88 that wouldn't have an overall detrimental effect by blocking the organism's ability to combat infection.

Dr. Campisi: Thanks.

Dr. Ferrucci: I want to add something really quickly. I think that one of the issues, how do you preserve the phasic nature of inflammation? You have an inflammatory response, and do not forget that in the repair, part of the inflammation, you know, downregulating those growth factors, are going to create degenerative problem in the affected tissues.

And so how do we affect inflammation without avoiding to kill also those positive responses, and I think that the problem is really understanding what is the mechanism. Is the mechanism something that is intrinsically downregulated or dysregulated in the immune system and them we need to find that mechanism or is the immune system responding to something that is potentially damaging? And so downregulated that inflammatory response is going to have consequence because we are basically removing a protection. And I think until we understand that dualistic hypothesis, it's very difficult to know what is the best intervention.

Dr. Campisi: Very quickly.

Dr. Franceschi: Sorry to talk again about the centenarians, but they are people who live very long; and they are successful in a sense.

Centenarians are inflamed. They have very high level of IL-6 and of many other inflammatory compounds. So this is another paradox, but they have also a lot of anti-inflammatory compounds around. There is an increased cortisol, there are increased TGF-beta that we showed some years ago. IL-10 is, we don't know. Adiponectin is very high. So is the balance between inflammatory and anti-inflammatory compounds which probably eventually matters to have a pathological phenotype.

Dr. Campisi: Sounds like personalized medicine. Yes, question?

Janko Nikolich-Zugich: Janko Nikolich-Zugich, University of Arizona. So we've heard a lot of really great ideas, a lot of interesting interventions, some correlations which are tantalizing about the predictors. What we are not quite so close to is quantifying the precise sources of inflammation in the body and really understanding the relative relations of what were between some of these possible sources. So in terms of the cells producing IL-6, you know, one of the questions that – and I would like to hear from the panel about what you think, where we stand in terms of quantifying it and how do we move forward, which is, I think, really a grand challenge.

Specifically, I would like to hear from the SASPologists on the panel where, how many SASP cells do we have around; and if you take them away, who else is producing IL-6? Are other cells making IL-6 in that case, and what are the relative balances, and how do we really get to the grips between what's that and what's microbiota-induced? And if you take, for instance, in the last speaker's talk, if we take away the memory cells that maybe trigger happy in terms of the T-cell site, are the innate immune cells producing all of the inflammation? Again, a little bit more discussion along those lines.

Dr. Campisi: So I think the answer to your question is right now we have the tools to do some of the experiments you suggest. You know, we can eliminate senescent cells and ask what's left? We can eliminate effector T-cells and ask what's left. Those experiments simply have not yet been done, but I think they're important questions. And we're on the cusp of understanding, I think, what the contributions are of each.

Speaker: At least in fat tissue it appears most of the IL-6, which is most of the IL-6 that rodents have, according to Simin Madani, comes from the stromal vascular fraction of fat tissue. When we fact sort from the INK-ATTAC animals where their p16-positive senescent cells are GFP-positive, when they're fact sorted, the bulk, overwhelming bulk of IL-6 is concentrated within the senescent cell fraction, at least if you believe that increased p16 is a decent marker of it with GFP. That was in the INK-ATTAC paper.

Dr. Ferrucci: I want to add two facts. One is when we looked at gene expression in humans, we don't see that the FNA for IL-6 is upregulated. So the IL-6 that we see, it does not come from the circulating cells. That is one thing that we can say.

The second thing is that if you look at IL-6, not at baseline in the morning, in the fasting state as everybody does, but you look at the area under the curve of IL-6 during the day, you know, there is absolutely no correlation between the baseline level and the area under the curve during the day with large, large variability between individuals.

So, yes, there may be basing induction of IL-6, but should be an inducible mechanism that really create the variability of exposure to IL-6. And that's why I think that senescence is probably one part, but that doesn't explain all of it.

Dr. Dixit: Just a quick comment regarding the potential sources. So like Judy mentioned, I mean I think we are not there yet to really have the answer at this point of time. One thing that is sometimes lost is that, like Jim was mentioning, if you look at the stromal vascular fraction of the adipose tissue, so when you have your floating adipocytes on top and these cells in the bottom, close to 70% of those cells are of hematopoietic origin. Okay? Almost every hematopoietic lineage cell is present there. And there are proinflammatory cells. Diane Matthews has shown that there are regulatory cells in the context of obesity, in the context of aging.

We really do not have, at this point of time, solid data to conclude either way. And I hope as the new data emerge, we will be in a better position to answer those questions.

Dr. Franceschi: Very briefly also, we are publishing a paper where we show that the mitochondrial DNA, circulating mitochondrial DNA is increasing with age and is functional. So it's proinflammatory. So these are many other sources of say garbage that we have not looked properly.

Speaker: There are, but, you know, we keep having concepts without being able to quantifiably say, okay, well this one's really important as opposed to this one is really minor. So that's the frustrating part about it.

Dr. Tracy: Just a quick thought on senescent cells. I don't know much about nonlymphocyte senescent cells. But among lymphocytes, that you get CD28-negative senescent cells as a clone reactivates over and over. Something occurs where the normal fate of those cells gets avoided by a few so they don't apoptose out, and they don't become memory cells. They become senescent cells.

And I don't know what—

Speaker: And I don't know in this case, because if you look at those cells in vivo, if you look at CD28-negative cells in vivo, plenty of them turn over, even though, if you give them a T-cell receptor-specific stimulus, they will not divide in vitro. Plenty of them turn over in vivo. So calling them senescent is really dangerous and really, really difficult in the absence of precisely defining them.

Dr. Tracy: Agreed, and so we'll call them CD28-negative cells. But the ones in tissues that are resident seem to be the ones that produce something most similar to what the other types of senescent cells in resident, in tissue seem to be producing.

And so the question then is what causes reactivation of the same clones of cells over and over, and that's where we get to something like CMV or HIV or other herpes virus, reactivating viruses that—

Speaker: Which is, unfortunately, another place where we can't really—

Dr. Campisi: Let's move on and hear from another. Yup, go ahead.

Richard Faragher: Richard Faragher, University of Brighton. I think that was a lovely session. It covered a lot of ground, and I'd like an answer which I think is probably going to be quick from the panel. Of the two sort of broad approaches that are coming through of removing senescent cells or trying to modulate the inflammatory milieu that they're contributing to, which do the panel think is most likely to be successful over the sort of medium time frame? The reason I say this is because I seem to be one of the first accidental SASPologists or anti-SASPologists because my lab reported the observation that IL-6 doesn't go up with senescent keratocytes. And that is a physiological deficit in the eye because there you need an IL-6 response to clear things like bacterial keratitis. So you can see advantages and disadvantages to anti-inflammatory treatment in that argument when it's body wide, whereas going after the cells themselves is attractive to me, but it's obviously much more difficult to achieve on a short time frame. If you had to do one, which would you plan for?

Dr. Campisi: Well, which SASP factor would you target in the circulation?

Richard Faragher: No, I was just saying would you try and generally—? So would you try and use a general anti-inflammatory approach or would you go after the senescent cells themselves?

Dr. Campisi: Well, I don't think that there's any evidence that the general anti-inflammatory approaches are as global as we would like to think they are. So, yeah, so Jim probably has some ideas on this. I would say targeting senescent cells, you know, we're not there yet to do it pharmacologically; but certainly it can be done genetically now. And we can test whether it's efficacious.

Richard Faragher: Okay, yeah.

Speaker: I agree. It's interesting to test. There are emerging pharmacologic approaches for doing that. We'll see how it all works out.

Speaker: What we have don't work. I mean the general inflammatory markers, administrating an anti-inflammatory drug will not downregulate the amount of circulating cytokines. So that, at least, doesn't work.

Speaker: Yeah.

Dr. Kirkland: You know, you mentioned about senescent keratocytes, and certainly the eye is a very different beast in terms of immune privilege and whatnot. But one of the things we have learned from the half a dozen ongoing and completed anticomplement trials in macular degeneration is that these people actually, contrary to expectation, don't develop any local or systemic infections and they're not prophylaxed for it.

The same thing with a lot of intravitreous corticosteroid injection. Typically people don't get it. So at least in the context of the eye, we may be quite lucky in terms of being able to use these agents without fear of losing the ability to combat infection.

Dr. Campisi: Next question, to the right. Yes.

Constantine Kotsanis, MD: I'm Dr. Kotsanis, as represented at the Kotsanis Institute. The question is to Dr. Tracy. In your excellent lecture, you mentioned one of the ways to improve immunity is thymic replacement. Could you please expand on that, what recommendations do you have?

Dr. Tracy: So my major recommendation is don't try it tomorrow, but there are clearly efforts in the past and ongoing now looking at progenitor cell replacement, for example. What you try to do in a pancreas with beta cell replacement, you can try to do in a thymus if you could figure out how to do it.

So I hope to, I preface my comment as it's sort of still science fiction, at least to my knowledge about it.

Dr. Kotsanis: It's getting away from science fiction. It's not as science fictiony. There's a whole contingency of really bright people who are studying thymus and looking at how various mechanisms are responsible for degeneration of thymus that happens and multiple causes for that. There are problems in the progenitors that come from bone marrow. There are problems in the thymic stromal, and I think that a whole session needs to be devoted to that, the point being that there is really at this point of time no evidence in humans. There is some data in mouse models suggesting that certain growth factors are efficacious.

For example, KGF or FGF-7, which is actually in trials in humans was accidentally found to increase T-cell production from thymus because it rebuilds the epithelium. So patients that undergo mucositis after hematopoietic stem cell transplantation, they were given kappavence. And there are trials ongoing at Memorial Sloan-Kettering, several other places, if you give LHRH agonists, you provide over a continuous period of time, what that does is it shuts off your HPG axis. And in mice, it's shown to be efficacious in rebuilding the thymus; and Richard Boyd has shown some data. So look up online. You'll find it's not science fiction.

Speaker: Yeah, can someone comment on the German Thymus Society? Anybody from the European friends. Maybe they know that, anything about that?

Dr. Campisi: From the what? Can you repeat that?

Speaker: The German Thymus Society.

Dr. Campisi: Anyone? No, sorry. Next question.

Michael Yaffe, PhD: Mike Yaffe from MIT and Harvard Medical School. A question based on Russ's presentation about inflammation and coagulation.

My read of the literature is that centenarians are equally procoagulable, so it's not like they have somehow managed to live a long time by not activating the coagulation cascade.

But I wonder from an epidemiological point of view, has anybody looked at the crosstalk between these systems? If you look at elevated cytokine levels and procoagulant activity with a multiregression model, can you predict who's going to get age-related degenerative conditions and who isn't? And along those lines, if we look at the natural experiment of people with Factor V Leiden who are procoagulable. Do they have an increased incidence of age-related degenerative conditions?

Dr. Ferrucci: Well as far as I know, there is very little studies that collect enough information on both coagulation and inflammatory markers. And the few studies that have done it have shown an association with cardiovascular disease and that both coagulation and inflammation contribute independently on the prediction. But, of course, there is no change over time in inflammatory marker and in coagulation that I know about. So that is really the big question, and I don't know whether you have more.

Dr. Tracy: Sure. So there's been a lot of epidemiologic exploration of both sides of the things you're discussing. And as Luigi says, both the level and the rate of increase of coagulation factors and systemic inflammatory mediators, both predict bad outcomes.

The prediction takes on an interesting turn. In younger people, middle aged, the prediction is primarily around cardiovascular disease. Now that could be what do we assess in those populations. What's going to happen frequently enough for us to see?

But when you get into the elderly, the prediction changes from cardiovascular disease to all-cause mortality. Something like IL-6 is a much stronger predictor of all-cause mortality than it is of MI, for example, in an elderly population, as is D-dimer as a coagulation marker as I mentioned before.

At a molecular level, there's a lot of crosstalk, and it's well documented, Chuck Esmond's work and a variety of other folks show that many of the same enzymes participate not only in activation of monocytes and secretion of cytokines in virus mediation. Nigel Mackman just had a paper showing that thrombin signaling through its receptor is critical for proper antiviral interferon response. So the upregulation of coagulation in a viral infection may, in fact, not simply be a bad epiphenomenon that leads to blood clots in H1N1 people in the hospital, for example, but it may actually be part of the normal evolution of the system so you have increased thrombin to generate appropriate interferon response. So we're getting into a really interesting time I think as we look at this, but that's what I can tell you.

Speaker: And all-cause mortality and Factor V Leiden patients, is it increased? Anybody know?

Dr. Tracy: We have looked at it in elderly populations. I'm not sure we ever did the analysis to be honest with you. I think we've looked at it mainly around blood clotting, but the argument would be, "Yeah, you'd need a feedback loop to get to inflammation for the all-cause talk." And, you know, whereas if it's going up for a similar reason, if both sides, the feedback's important; but you're also getting independent increases, that would be more powerful.

Dr. Campisi: Question? Yes, on the right.

Penny Dacks, PhD: Penny Dacks, Alzheimer's Drug Discovery Foundation. My question is about the models that are used to predict efficacy. So I think in the last year there was a very high profile paper suggesting that the animal models that have been used to develop drugs for more acute, chronic acute inflammation like a sepsis, for example, have really not been predictive of clinical efficacy in people. And so when it comes to systemic inflammation, there's several aspects of my question.

One, is there evidence that the animal models are more predictive? Two, do you think there's strategies, maybe, to protect against this kind of thing, maybe by using a diversity of animal models rather than sticking with just a couple animal models. And, three, whether or not there might be a purchase for really testing those animal models and, for example, gene expression patterns for their similarities to humans.

Dr. Campisi: Anyone? I think I agree with you, not enough attention is paid to, on human mouse or human rat or human-animal model differences. On the other hand, I would also say that that particular example was a very specific example of where there was evidence actually that the mouse models were failing, and that was largely ignored.

But I think for the most part, I would say what we do need is to pay much more attention to when the mouse fails us and when it does not. And there are, at least in certain aspects of human disease, fairly successful interventions in the mouse to make it more human-like. And the more we understand those differences, the more we can so-called humanize the mouse to make it a better model for people. The problem with other animal models is if they're big, they're expensive. And if they're long-lived, they're also expensive.

Dr. Dacks: True, though even going to the statistician with short-lived animal models, I wonder if choosing a diversity and making sure that the effects are consistent across them, if that would also increase transitability. I'm not sure.

Dr. Campisi: Exactly, and I think Rich Miller will maybe talk a little bit about mixing up genetic backgrounds so we're not dealing with idiosyncratic genetic backgrounds, which is another problem that some of us deal with. Does anyone else care to comment?

Dr. Ferrucci: Yes. Another problem is that in most of the experiment that I know, for example, the gut microbiota of mice has not been assessed properly. So I think that this is a measure variables because it can vary from lab to lab. We were discussing with Richard at 6:00, so we were not particularly. But the problem is there. So there are a lot of variations between laboratories, and I think that to control for the gut microbiota of the effect of diet can be a measured target to standardize the models.

Dr. Campisi: And make sure it's more human-like.

Dr. Dixit: Just to add on the point of gut microbiota, animal models, and we heard today, in the morning, from Dr. Francis Collins, as to the importance of having studies that are repeatable across laboratories if we have to more forward.

One of the problems is, with regard to gut microbiota, is that all these mouse models where we are crossing cre-flox this and that, and it is very important, many people actually still compare their knockouts with wild types that do not come from _____ crosses. Sometimes they even come from different facilities. I mean I've seen papers published like that. And so this is completely wrong. It shouldn't be done.

Regarding the alterations in microbiota, there are so many influences to that. Diet is one, but another major one is the water. So, for example, if you are in a facility in New Haven, for example, you get the city water, which is chlorinated, just you and I drink. But many facilities then hyperchlorinate it. Then you can imagine if you are doing an aging study, and you are killing mice, hyperchlorinated water over a period of two years. And then the biofilms that develop on those places that mice drink. So you're looking at a point, an artificial system; and sometimes those things are leading to discrepancies in the literature.

Dr. Campisi: Well, I think the main issue though is how do we make the mouse, our animal models more human-like? And then the other problem is variability between labs.

I think we need to move on. We've still got quite a few people.

Juan Manuel Falcon, PhD: My name is Juan Manuel Falcon. I came from CIC bioGUNE in the north of Spain. And I would like to know your opinion about the role that exosomes could play during the inflammation and also in the aging context. Thanks.

Dr. Ferrucci: Well, this is a very hot topic; and most of the, well, at least part or what we are measuring in the blood is, in reality, is in exosomes or in vesicles, in nanovesicles. So I think that we have to give a look to what we have published to know where the stuff that we measured were in the blood as such; but most probably, they were vacuolated by exosomes or other stuff. So we need the new type of experiment where we isolate with proper technique the exosomes, and we do experiment where we can use exosome from old animals to see if they have an effect on young cells and vice versa because—

And then in the field of cancer, there is a lot of literature. So I think that this is a very hot topic for everybody. For example, the mitochondrial DNA probably is within exosome because the enzymes would destroy it very quickly. So I think it's a very important point, yeah.

Dr. Campisi: It's important. Yes? On the right.

Dean Jones, PhD: Yes, hi. Dean Jones from Emory University. First of all, just fabulous presentations and discussion. The question I have relates to this unaffordable 40-year-experiment. The reality is each and every one of us is a 40-year or more experiment. We are uncontrolled, and we have all sorts of variable aspects. But yet our healthcare systems, whether it be a Mayo or Kaiser Permanente or others will, in fact, be following us for our lifetime.

So the question is do you have any suggestions on measurements, affordable measurements that could be made today that would be potentially useful 20 years or 40 years down the road for us to address these health issues?

Dr. Ferrucci: Well that's a very interesting question and certainly timely because there are a number of countries that are actually creating large cohorts that will be followed over time to try to make discoveries instead on targeted experiment by looking at large population and mining data. So what are the essential measures that we can do?

I suspect that IL-6 at this point is not ready for being translated into clinical studies but will certainly be ready to be studied in large population to see whether change in inflammatory markers are associated with important outcome, even when we look at the general population. And there will be, you know, certainly an important idea.

One problem with that approach is that we're really discovering that those static measures that we use are really revealing a little part of the exposure to this risk factor. And so there is some limited values of doing those static measures, but certainly my suggestion will be that IL-6 is ready for that use.

Dr. Campisi: Any other suggestions? Russ? Quickly, quickly.

Dr. Tracy: Russ and quickly.

Dr. Campisi: I'm timing.

Dr. Tracy: The assessments of the immune system, from my perspective, is one of the most important things we could do; and there's a variety of measures that we could add, but that would have to be worked out. But if we could get a simple assessment, maybe provocative in some way, that's easy to do and simple, tie it to flu, as Linda was saying. You know, tie it to a flu vaccination but in a careful, controlled way, something like that that would build up information on populations over time. That might be a useful thing to do.

Dr. Ferrucci: I know that, sorry. Just a quick comment. I know that the word "biomarker" is not popular here in the states. But in Europe we did a huge study called MARK-AGE in which we tested more than 300 potential biomarkers in people from 35 to 75. And the results are just coming out. So I think that there can be a revival of the biomarkers to check.

So I think that in this sense, Europe and US could make something like this, an event like this on biomarkers. But I think that the failure that I know was in previous studies here in US has some deleterious, long-term effects, I think.

Dr. Campisi: It was a little premature. Yes.

Nicolas Musi: Yes, Nic Musi from the Barshop Institute in San Antonio. So we've been interested, similar to many other groups looking at the role of the microbiome on inflammation in the context of aging and other diseases. And we've been looking at the role of metabolic endotoxemia and other bacterial products.

And one serious limitation is that there's no standardization in how to measure endotoxins in blood. So I think that before the field can move forward into specific topic, something has to be done in order to standardize a measure of endotoxins in the blood, perhaps the NIH or CDC can put together a working group and sort out that problem.

Dr. Franceschi: I agree totally, even if I'm working in Italy.

Dr. Campisi: Thank you, yes?

Jeffery Kelly, PhD: Hi, I'm Jeff Kelly from Scripps. So I work on an aging, associated familiar aggregation disease that, unlike Alzheimer's, affects the peripheral and autonomic nervous systems as well as the heart. And I would say now there's really compelling genetic and pharmacologic evidence that aggregation drives these diseases. But if you talk to the physicians who treat these patients, the common sort of observation is that a patient has, even though they carry these mutations their whole life, they often have an inflammatory event that seems to initiate the disease.

And then it seems to then become a chronic, inflammatory disease. So I'd love to get, it's really hard for us in this field to think about how to think about this, so I guess that's my question. How does one think about this initiating inflammatory event that then now transforms this carrier to a disease subject?

Dr. Campisi: There's going to be an entire session on proteostasis and the whole idea of how aggregated proteins can drive—

Dr. Kelly: Yes, I know. I'm a speaker in that session, but I'm almost certain nobody in that session is going to talk about inflammation.

Dr. Campisi: Maybe you can slip it into—

Dr. Ferrucci: Well, I think that is actually, this is a key question because we all suspect that there is an inflammatory event that generalize inflammation, that start that process of inflammation or chronic inflammation or proinflammatory state of aging that we all are describing from the phenotypical point of view.

There are animal models that are quite interesting from this perspective, but I don't think there is any study in humans. And, for example, if you create inflammation in a joint in a mouse, in a young mouse, you have no reactivity at the level of the brain. In an old mouse, you have hyper production of IL-1 and reactivity at the level of astrocyte and microglia.

So there is some sort of hyper array activity of the peripheral tissue to an inflammatory response to something that has been caused locally. And I think that this is one of the issues that we need to study more because it is possible that inflammation starts somewhere else but then becomes, almost, an automatic phenomena that cannot be reversed.

Dr. Franceschi: Inflammation is a fire.

Dr. Ferrucci: It's _____ actually.

Dr. Campisi: I think the NIH is worried that we're all going to become calorically restricted if we don't break for lunch. I've been getting signals for the last five minutes. Arlan, do you want to say something?

Arlan Richardson: Yes. This was something Brian brought up. It's related to the question on mouse models. And one of the things that people don't, in other words, Judy was talking about humanizing the mice; and certainly we've done that with cancer, ALS, and other diseases.

The problem is, is that those mice always get the disease when they're very young. So they're looking at a, it would be looking at cancer in teenagers or something like that when you're doing it and the same way with Alzheimer's. So I think that one of the explanations that these therapies don't translate is that we're looking at it in a different milieu. And we just brought up the point that Luigi made was that inflammation has a different effect in young animals versus old animals. And so you would expect, when you're inducing atherosclerosis or Alzheimer's or whatever it is, you would have a different phenotype and maybe different therapies would work in a young animal and not an old animal.

Dr. Campisi: I guess it's voluntary at this point. Those of you who want to calorically restrict and those of you who want to eat.

Grace Shen, PhD: Hi, I'm Grace Shen from NEI, and I just want to thank the co-chairs, the panelists, and the audience for a very engaging first section for this meeting. And the lunch time now, we are breaking for lunch; and it's upstairs in the cafeteria. If you already have purchased a ticket, you get the lunch there; and there's also food to be purchased upstairs.

We'll start promptly at 1 PM on the second session of Adaptation to Stress.

Lunch
Session III: Adaptation to Stress

Julia Rowland, PhD: Okay, we'll get started. Good afternoon and welcome to this afternoon's session on Adaptation to Stress. I'm Julia Rowland, and I have the privilege of directing the Office of Cancer Survivorship within the Division of Cancer Control and Population Sciences at the National Cancer Institute. And I have been joined to plan this special session by my esteemed NIH colleagues, Dr. George Niederehe, from the National Institute of Mental Health and Partap Khalsa from the National Center for Complementary and Alternative Medicine, fellow members of the Geroscience Interest Group.

Our co-chairs for this session, as you can see, are Drs. Elisa Epel and Gordon Lithgow. In your program is much more detail. If we read everybody's bios, we'd be all afternoon and through tomorrow, and probably the weekend. So these are really brief introductions. And apologies for that, but that keeps us moving.

So Dr. Epel is Professor in Department of Psychiatry at the University of California, San Francisco, and a leader of the new UCSF Center on Obesity Assessment Study and Treatment. And Dr. Lithgow is Professor and Director of the Interdisciplinary Research Consortium on Geroscience at the Buck Institute for Research on Aging. I would also point out that it's Dr. Lithgow who developed the definition of geroscience, now published in Wikipedia that, of course, we adopted for our interest group. He's going to

be directing the discussion which follows the last speaker in this session, providing a summary of the session and concluding remarks.

And we will get started with Dr. Epel who is going to give us the opening topic overview presentation. So, Dr. Epel.

Introduction

Elissa Epel, PhD: Thank you so much, Julia. Thank you. Very happy to be here. So, now for something a little bit different. I'm going to give you a brief introduction to the good and bad of stress. And this is going to be a little different because we're going to be switching between worms and people, so you've got to listen carefully so you don't make the wrong conclusions.

So when we think about what regulates aging biology, we know how important it is to study aging in context to know that genes unfold in environments in different rates. And stress has come to the forefront as a factor that can alter the rate of aging. It can make some people age faster in cat years. And/or maybe it can slow down aging. So let me tell you first about a dramatic solid finding in the basic science of aging.

So when model organisms are exposed to adversity, short bursts, when there's time to recover, they develop stress response which is often characterized by these increases in these heat shock factors or heat shock proteins. And this promotes stress resistance, multiplex stress resistance. The organism is now able to recover more quickly from all different types of stressors. And this is somehow tied to longevity. So this is really the question if we can understand more how stress resistance might promote longevity and harness this. So in other words, what doesn't kill you can make you stronger in these specific models.

So let me just summarize this in a little more detail; and if there are any inaccuracies, don't blame Gordon who provided the slide.

So what we know is that a range of stressors — you know, biotics, osmolarity, heat shock, nutritional fluctuations, metal exposure, inflammation, oxidative stress — all of these can alter the rate of aging. With kind of a hormetic stress or short-term stress, you can see more of a longevity phenotype and with what we call toxic stress, which means the stress response overwhelms the reparative processes, you see damage and early death.

And so there are several pathways that are good candidate pathways to help us understand this stress resistance model, like the insulin-like growth factors, changes or improvements in intracellular damage, and repair signaling, getting the garbage out, and metabolic control.

So we definitely need to understand how this works more in these basic organisms and how this is tied to longevity. But we also, at the same time, need to be looking at this in a translational way and ask the question about how this relates to human aging.

So we know that stress resistance in basic research is tied into different pathways of aging, and we still need to explore exactly how. And then really the big question for our symposium today is how does this relate to humans? So we can take human cells out and look in vitro, and, yes, of course, there's hormesis — they respond to physiological stressors. But for humans, stress is a very important word because we are bombarded by common daily stressors as well as severe traumas that leave imprints on our health.

So stepping back and just thinking about well if stress is so important, wouldn't we see that in the epidemiological data? One of the most consistent findings about human diseases of aging — and now I'm talking about all diseases, not just one disease — is that people with lower incomes and lower

education develop these diseases early. This is robust, this is across diseases, this is what some people think is premature aging.

And so the common theory, one of the common models for understanding this is through stress pathways. Does the psychological burden of chronic financial strain, coping every day without resources, etc., lead to accelerated aging? So this might be, this idea, this question, can psychological stress lead to accelerated biological aging, that might seem like a vast canyon or a huge leap, if you're not very familiar with the stress literature.

So let me give you some very brief background. What do we know about psychological stress in humans? So in the short run, acute stress changes a tremendous number of both physiological responses in regulatory systems as well as intracellular mechanisms. So what we know most about is the immune system, since that's what we can look at easily. And we know that short-term stress can lead to immune enhancement; long-term stress can lead to immune suppression. And you're going to hear about that, one of the most elegant models, in a minute by Firdaus Dhabhar.

We also know that under acute stress, we release inflammation. Let me say that again. Stress causes an increase in inflammation an hour later. It doesn't go down quickly. Chronic stress causes systemic subtle increases in inflammation beyond aging. We know this with caregivers, we know this with depression. So I can't emphasize that enough. This question about stress and aging is really not such a mystery, at least in terms of the inflammatory pathway.

You're going to hear about another new emerging model of how stress gets under the skin. We call it social signal transduction developed by Steve Cole and now a model that's been replicated in many human models of stress. And this is an elegant way of understanding how the stress response turns into signals that create a pattern of gene expression that looks, I think you'll agree, very pro-aging.

So I will end there and let our speakers fill in the details. But my point is that stress has effects from molecular to systemic. Physiological effects we can easily measure, but also into generational effects. And we won't go into that today, but I think to really understand human aging, we don't start with a blank slate. We are born programmed for certain rates of aging based on what we know now from animal models as well as human cohort studies. That there are prenatal programming effects that are profound.

So we're going to first hear more about good and bad stress from Firdaus, when stress is linked to disease processes versus more of a salutary response. Then we'll hear from Gretchen how we can use experimental style models to really understand this stress resistance more, down to the cellular molecular level. We'll hear from Richard Miller what we all want to know, how we can harness stress resistance, our deep understanding of this to slow aging, and that's real aging and, I assume, independent of disease. How does exercise affect physiology, disease, and possibly even stress? And then, lastly, how stress affects the genomic gene expression profiles.

So we'll first hear from Firdaus who's from Stanford University and has been stressing out rats.

Under what conditions is stress linked to disease processes?

Firdaus Dhabhar, PhD: I want to begin by thanking the organizers for putting together a fantastic meeting, as well as my session chairs for giving me a chance to share some thoughts with you, and you all, of course, for being here.

Here is a poster from the National Library of Medicine that says, "Stress is a loaded gun. If left untreated, it can kill you just as surely as a bullet." It says, "Don't wait for the gun to go off. Get help today." National Library of Medicine conveniently located across the street from here.

And what we want to consider here is that while this situation can, indeed, be true, you want to keep in mind that Mother Nature gave us the stress response to help us survive, not to kill us.

So when we talk about stress, we mean a constellation of events that begins with a stressor, the stimulus or a stressor that precipitates a reaction in the brain — this is your stress perception — that then results in the activation of fight-or-flight systems in the body. This is your biological stress response. And in order for stress to have any kind of effect on brain, body, or cell, you absolutely need the biological stress response without which it doesn't really matter what's going on stress wise.

We find it very useful to define stress into two broad categories. So we specifically define short-term or acute stress as stresses that last for minutes to hours in duration, and we define long-term or chronic stress as that which lasts for weeks to months to years in duration.

Okay, so if a predator walked into this room, a cheetah, for example, or a lion, and roared, it would pretty much stimulate a fight-or-flight response in most of us as we figure out how to help ourselves and help others get out of harm's way. The response consists, among others, of increased heart rate, increased hormones like adrenaline, noradrenaline, cortisol, and cytokines, recently discovered to be released in the blood stream — Elissa alluded to this — under purely psychologically stressful conditions. And maybe we can talk about this some more in the discussion if there are questions.

But something like I'm doing right now, giving a talk in this charged, time-dependent situation, also activates a similar biological response. I was surprised to see that maybe Elissa had a little bit of fight-or-flight going too.

Now Elissa loves running. She loves running, she does it really well; but every time she does it, guess what, the same biological response is activated. And getting even a little bit more exotic than that, intensely excitatory stimuli such as approaching someone for a first kiss, especially if the person doing the approaching is romantically challenged, also will activate the stress response. In fact, sexual intercourse is one of the most potent activators of this response. So Mother Nature gave us this response. It's activated under conditions where we need protection, where we need to perform, and also in instances that might involve pleasure.

So how does the immune system react during short-term stress? And I'm going to summarize many, many years worth of work in this one slide. And essentially the immune cells are like the body's soldiers, and they reside in the body's barracks, for example, organs like the spleen or in the body's boulevards, which is the blood vessels.

Very early on, after the beginning of a stress response, within about 2 to 15 minutes of the beginning of a stress response, you have a large scale mobilization of these soldiers or immune cells so that they leave their barracks, rush out of their barracks, and enter the boulevards of the blood vessels of the body. And this is registered as an increase in absolute numbers of cells in the blood stream early on during stress. So if this is your baseline, cell numbers increase within about 2 to 15 minutes.

As the stress response progresses, you now have a decrease. The decrease can also go below baseline. And this decrease in the blood stream, decrease in absolute numbers of cells in the blood stream does not represent a net loss or destruction of cells from the body, but it represents a redistribution. So the stress response is sending the body's soldiers now from the boulevards onto potential battlefields where there may be trouble, such as wounding or pathogen entry. And a great example of such potential battlefields is the skin.

So what the stress response has done is increase the numbers of defenders in sites of potential trouble where the immune system might be about to experience trouble. What the response also does is it increases the fire power of these immune cells so that if, indeed, there is an immunological challenge or an immune activation that takes place, then the immune cells that are there are better able to perform their function. Now if there is a site of ongoing inflammation and the fight-or-flight stress response is superimposed under those conditions, this response will also direct greater immune cell traffic to the site of inflammation.

So then the question is if immune cells rush to the skin during stress, does this enhance skin immune function? What does this mean functionally? There are many, many different studies that we in our group and others have done; and the answer is yes. So I'll give you a few examples.

If you couple this adaptive immunological fight-or-flight response, a leukocyte redistribution response, during surgery, some patients mount this adaptive response during surgery; other patients, in this case humans, do not. What you find is that patients who do mount the adaptive response show greater postsurgical recovery. And this is a study that Elissa and I did together with our colleagues at Yale.

If you couple the immunological fight-or-flight response with a vaccination, you significantly enhance both innate as well as adaptive immune responses. So we did the preclinical work in this, and then others independently in one single step replicated our work in humans. So you couple exercise with a vaccine or a psychological stressor with a vaccine, that coupling enhances the vaccine response. So, again, these studies we did preclinically; they've been replicated clinically; and the last bit is preclinical now.

So we have a model of UV-induced squamous cell carcinoma. It's a completely naturalistic model for emergence, progression, and naturalistic regression of tumors. And if during the time course of tumor development, we now pulse the system with this fight-or-flight stress response, generating the immune fight-or-flight stress response, you significantly enhance antitumor immunity and induce tumor regression.

So what are mechanisms of stress-induced immuno-enhancement? You have increased leukocyte traffic and infiltration, you have increased cytokine gene expression — and I'm just going to rapidly summarize, but there are publications on these that I can refer you to. We have increased macrophage and dendritic cell activation and traffic to draining lymph nodes, we have increased mammary T-cell formation in situations where adaptive immunity is coupled with a stressor, and all these changes are driven by the principle three stress hormones epinephrine, norepinephrine, and cortisol.

Now short-term stress can not only enhance immune function, but it also enhances cognitive and physical performance. And whether or not this happens depends on the genotype. So some alleles of genes like the COMT genes have a variant that is more efficient at clearing dopamine from the prefrontal cortex, other variants are not. And depending on what that does, you have different levels of performance under stress conditions or not. But even if you have a gene that's sort of less effective under stress conditions, through training you could actually harness it to harness your protective fight-or-flight response even for performance, in this case a mental performance.

A cognitive reappraisal — simply knowing that all stress is not all bad; anxiety under testing conditions might actually help you — can actually enable people to do better. And a challenge versus threat state in terms of what a person find themselves in is also a determinant of how this can affect cognitive and physical performance.

All right, so what about bad stress? How does an adaptive system, a fight-or-flight system become maladaptive? And the answer appears to lie in the transition from those acute short-term responses with rapid shutdown to long-term or chronic stress. So short-term stress lasting from minutes to hours can be beneficial. Long-term stress has numerous deleterious effects on brain and body. So it has been known to increase susceptibility to viral and bacterial infection, some types of cancers, increased susceptibility to proinflammatory and autoimmune disease, suppress wound healing and protective vaccine-generated responses, and increase depression and related mental health disorders.

So what are the mechanisms that mediate the deleterious effects of chronic stress; and I would say aging in this case, circadian rhythm disruption is one mechanism. You have overall suppression of baseline immune cell numbers. You have a decrease in the fight-or-flight response stimulated by acute stress all in the chronic stress background. You have decreased leukocyte telomere length. So this is work that Elissa did. So that suggests increase in immunological senescence and maybe biosenescence in other cell types. You have a suppression of protective cell-mediated immune responses, but at the same time you have enhancement of low grade chronic inflammation, the kind we heard about in the session just before lunch; and in some cases, enhancement of Type 2 cytokines and their actions, increases in

oxidative stress. And all of this, in some cases, can be compounded by increases in health aversive behaviors, especially on the part of humans.

So, therefore, there are many benefits of minimizing of bad stress responses and optimizing good stress responses. And, overall, we've put together the concept of a stress spectrum to try and sort of figure out how all this might, or at least conceptualize how all this might come together. So at one end of the spectrum you have your good stress, this is your fight-or-flight stress response. At the other end of the spectrum, you have bad stress, depression stress-related disorders. And in between, you have the zone of low or no stress. So this is your resting zone.

What you want for good health is to minimize the bad stress, maximize your zone of low or no stress, and optimize your fight-or-flight stress response. So how do you get there? There are different mechanisms, but sleep, nutrition, and exercise appear to be the principle three. Then there are things like giving and receiving social support, living a compassionate lifestyle, training in some cases, cognitive reappraisal like I mentioned, and sort of having a genuine gratitude for whatever your circumstances are.

And then there may be individualized programs. In this case, it's whatever floats your boat. So meditation, yoga, walking, hiking, levitation for some, dance, music, gardening, photography, art, fishing, and so on; but whatever works for you, you would know it. And the point is that your psychological and physiological resilience mechanisms help you to stay within this side of the stress spectrum. And if you do that, it's good for immunity, good for cognitive and physical performance, good for overall health. So our goals are harness the biology of good stress to promote health and well being; reduce or eliminate bad stress as much as possible; and maximize the zone of health, healing, and resilience.

And a big thank you, again, to my collaborators, students, as well as our funding agencies. Thank you.

Dr. Epel: So now I'd like to introduce, we're going to get to hear from Gretchen Darlington from Baylor College of Medicine who has done seminal work in models of aging through the IGF pathway. Thank you, Gretchen.

What experimental models may be useful in defining the impact of stress at a molecular and cellular level?

Gretchen Darlington, PhD: Thank you, Elissa. In preparing our talks, we were all asked to consider what we know, what we think we know, and what we'd like to know; with the last category being the major one, of course. And we certainly know a lot about these two models, laboratory-based models, of delayed aging. From these models, we've learned a great deal about the basic mechanisms of aging. And although we have much less information about stress mechanisms, I think that we have great potential for experimentation into the impact of stress in these particular models.

So I think you've seen something similar to this. Both nutrient restriction and growth hormone signaling deficiency work in terms of extending lifespan in all these four species. And the strategy that the community in basic mechanisms of aging has used has been a very powerful one. Broad genetic screens are done in the lower organisms to identify genes that impact aging. That gets translated to the mouse, which I would suggest comes between worms and humans, and the ability to create genetic mutations with point mutations with surgical precision in the mouse, to test them in a tissue-specific manner, and to Arlan's point, to be able to turn things on or off at various stages in life with mouse models I think has been very powerful. And, certainly, mouse experiments are a lot more expensive than those in lower organisms, but I think they give us an opportunity to see much more in terms of detail of the basic mechanisms.

So Brian showed you earlier a typical survival curve under nutrient restriction in the mouse. The greater the restriction, the longer the maximal lifespan. In essence, if you're a mouse, the less you eat the longer you'll live, up to a point, and then you fall into malnutrition.

But I think the really interesting and important observation here is that there is a corresponding delay in the onset of mortality, and morbidity during this period is greatly reduced by nutrient restriction. And during that period we have reduced incidence of many age-associated disorders including cancer, cardiovascular and kidney disease, cataracts. There are a host of other characteristics of the functional decline in aging that are improved during this period under nutrient restriction.

Then turning to the genetic model of growth hormone signaling deficiency, you can see the homology of many of the genes immediately downstream from the insulin-like peptides and their receptors. In fact, mutations have been made in all of these genes in the mouse, and they extend lifespan to varying degrees. Quite considerable differences, depending on the gene that has been mutated, with the growth hormone deficiency itself being one of the more potent mediators of lifespan extension. And at this point there have been 25 or 30 genetic mutations that have been created, generated in order to study the activity of each of those genes in delaying aging.

So here are two examples of single gene mutations that lead to extended lifespan and extended healthspan. And the survival curves for these two mutants and the mutants of the, obviously, growth hormone deficient, they're the small ones, the survival curves look for all the world like the nutrient restriction curves with marked delay of onset of morbidity as well as mortality. And, again, there's a reduction in tumor burden; cognition is retained until later ages; and muscle loss, for example, is delayed.

Now these models do not represent a model of frailty. The mutant animals are not decrepit. They're not hiding in the corner of the cage waiting to meet their maker. They are quite active. And here they are trying on their Halloween costumes looking forward to the treats because these animals can eat as much as they want and still live a longer and healthier life.

Now the NI is not particularly interested in making mice live longer. So I'll try to give you what will be fill-in a bit, but it'll be a very superficial summary of what is known in nonhuman primates and even in the human population.

Brian's slide this morning indicated that extended longevity in primates was a big maybe. There have been two independent, ongoing studies — they're continuing — on long-term calorie restriction. And although increased maximal lifespan is not projected in one of the studies, is in other, both studies have shown a very significant decrease in the onset of cancer. And there was a trend for the onset of other age-related disorders to be delayed, more so in one study than another.

And so at this point, whether and how nutrient restriction may work in nonhuman primates is somewhat open. But being an optimist, I think that with the continuation of the studies, a better definition and review of the study design, we will be able to say that nutrient restriction is working similarly in many respects to what we've seen in the mouse.

Nutrient restriction in humans, very difficult studies to carry out. There have been some short-term studies, 6 to 12 months, that were carried out in overweight individuals and then individuals who have had longer term caloric restriction which has been self-imposed have also been examined. And in both instances, there was an improvement in common risk factors for cardiovascular disease, glucose tolerance, and insulin action was improved and a reduction in weight and fat mass, as you might imagine. However, in the individuals with longer term caloric restriction, bone density and muscle mass were lower; and the participants complained of lethargy, feeling cold, and feeling hungry — surprise, surprise.

So what about growth hormone signaling? Well, we have no information about that in primates, in nonhuman primates. And in humans, all the physicians in this room know that growth hormone deficiency can be a very serious medical problem, particularly in infancy. However, there have been some very

interesting studies of genetic variants in genetic islet populations. And you heard from Brian this morning about the IGF-1 receptor variants and the increased incidence of that variant in the centenarian population.

There's another example which was a study carried out in a population in which there was an increased incidence of a mutation in the growth hormone receptor. And in that population, the incidence of cancer in the mutant or affected individuals was extremely low, around 1%, whereas in their relatives and controls, it was as high as 17% and many of those cancers were listed as the cause of death. The prevalence of diabetes was also markedly different, 5% to 6% incidence in the related population, but not a single case among the affected individuals.

So in the category of what we think we know, of the many individual genes that have been shown to impact aging, we think we know a few of the major downstream targets. And these are thought to be operating in energy metabolism and detoxification. And I've listed five pathways instead of three. This is probably artificial. These pathways interact and overlap. Some of them have been shown by genetic analysis to extend lifespan. Others just have a high association with lifespan extension, such as xenobiotic metabolism.

So what would we like to know? Well we'd like to know whether nutrient restriction and growth hormone deficiency is protective against the many stresses. And I have to say that there have been some studies done in the mouse but mostly of an acute nature. We have very little to no information about psychological stress; and with the increasing sophistication of behavioral studies in the mouse, I think that's an opportunity.

We also would like to know more about chronic exposures as opposed to acute exposures. Conversely, what would an examination of aging human populations with and without stressors reveal? And really what I was thinking here was something that Dr. Fried talked about this morning. Can we look at the basic pathways that we know to be important in extension of lifespan and extension of healthspan and what is happening at that level in various populations? And we need better readouts for that in the future.

And, finally, the last question which we all are asking is, are these critical molecular changes druggable? And I think the detailed analysis of basic pathways in the mouse will give us points of intervention for mimetics of caloric restriction and growth hormone signaling deficiency.

And the next speaker will address the prospects and progress in that area. I will yield the floor to Dr. Miller and thank you for your attention.

Dr. Epel: Richard Miller from University of Michigan who has made some discoveries linking stress resistance to aging. Thank you, Richard.

Cellular Stress Resistance: Clues to (Real) AntiAging Medicines?

Richard Miller, MD, PhD: So my goal today is to help get to this situation. It's now routinely easy in mice to do this and it's not yet possible in people, but I'd like to see that change. This is today what a normal person looks like at age 70. And if we could accomplish in people what is now absolutely routine in mice, this is what a normal person would look like at the age of 114.

The things I'd like you to understand, the things I'd like you to sort of build into your bones, your philosophy is that it's not that hard to slow aging in lab animals and the effect is ten times larger than a cure for cancer. When you do this, you get animals that are not just alive a long time, they're healthy for a long time; and it's my personal belief — I hope this is not too controversial — that it might be nice to figure out how these things work and then to seek safe, effective, drugs that slow aging in people.

I'm going to talk about nutrition, I'm going to talk about diet, about genes, and I'm going to talk about drugs. This is the nutrition slide. There are at least three different ways, some partially overlapping, to do this in rats and mice: calorie restriction, Gretchen has mentioned, most of you have heard of this. Methionine restriction has been shown to work since 1993 in rats. You get an effect that's just as big as calorie restriction and it turns out there's a window of vulnerability in the first three weeks of life. If you limit their nutrition for three weeks, you get a long-term permanent slowing down of the aging process.

I won't go into the details of that. This is not that kind of a session, but I want to emphasize that these animals are not just alive, they are healthy. They slow cancer, they slow arthritis, they slow kidney disease, all of these things listed on the slide and many more. I have this fantasy of some day having a chance to talk to Dr. Collins and say, "We've got a secret trick that slows cancer." It actually reduces cancer incidence rates by 90%. It has a side effect, it also slows arthritis. And another side effect, it slows memory loss and loss of muscle strength, etc., etc. Why aren't we spending an awful lot of our effort on studying this?

The one slide I will permit myself to show you on function comes from Roger McCarter. You put an exercise wheel in the cage of the animals. If they are control animals, they lose interest in about six months. If they are caloric restricted animals, they are running 5 kilometers a day at an age where all the controls are dead. So these animals are quite vigorous, quite healthy.

JL Shinskey published this in *Science* in 1990. A 50-year-old woman has 31 years of average lifespan remaining. She'll die at the age of 81. I am in favor of a cure for cancer. I think that would be nice or a cure for heart disease, but it must be admitted that they don't get her very much. They get her 2.6 and 2.7 years of extra life respectively. The red bar is what we get routinely by caloric restriction, by methionine restriction in rats, and by some of these mutations. I should think that this system would be one that you'd really want to learn how that works.

The nice thing about the mutants is that they are pointers to new drugs because the mutants it's known what they code for, it is not too hard to imagine ways to accomplish pharmacologically what that mutation has accomplished for you. And you can see this is a Snell dwarf mouse and when the last control mouse is dead, sort of the human equivalent age of 100 or 110, we still have about 80% of those alive and healthy.

I won't give you the long list of mutants that can do this. You can see the number of publications on individual mutants that extend mouse longevity continues to go up linearly and there are now, as Gretchen mentioned, well over 30 of these. I will point out that some of them should ring little bells about where to look for drugs. The one I'm fond of because we've had an inflammation session is MIF. MIF is a proinflammatory cytokine migration inhibition factor. You knock it out in a mouse, you get a 15% to 20% lifespan increase. And there are pharmacological companies that are already spending an awful lot more than my budget when trying to figure out how to design drugs to block MIF activity. PAPP-A knockout, Cheryl Conover's mutant, also has similar advantages.

And these mice also slow cancer, slow arthritis, etc., etc., etc. I had a slide here which Felipe insisted I could not show you in which Roger Alban has documented the Snell dwarf mutation also slows Huntington's disease. But if you want to ask a question about it later, we can talk about that some.

We're also working on drugs. So rapamycin has been mentioned already several times. This is developed by the Interventions Testing Program that the NIA funds. We just last week had in *Aging Cell* a paper accepted showing that acarbose and NDGA also have potent effects. The effect of acarbose on the males is just as good as rapamycin. Some of these have mechanisms that are easily going to be investigatable in primate as well as in humans. All four of those drugs are available either in Europe or in America.

The only thing I want to say about them given the limited timeframe here is that they have interesting sex specific effects. Acarbose has a huge effect in male mice. And although it's a significant benefit for female mice, it's a small benefit. Rapamycin has a benefit in both sexes but for reasons we're just

working out now the benefit seems to be bigger in females. This gives us lots of ideas about how they might be working and ways in which to adjust the strength of the response.

I think that the work that is being funded now to look at drugs that slow the aging process is sort of like this kind of a toolkit. This is what the Cro-Magnons were working on in Europe 35,000 years ago. Fifty to 100 years from now, we'll have a toolkit that looks like this. We won't be dumping something as crude as rapamycin onto a batch of mice, but we will have tailored drugs aimed at specific cell types, at specific intervals in the lifespan. We'll be able to do something refined and, hopefully, more effective with much higher benefit to cost ratio.

So connecting this to stress and aging, I want to discriminate between facts, on this slide, and I what I suspect may be true, which will be on the next slide. The first fact to mention is that in the worms — and Gordon Lithgow is really one of the first people to notice this — longevity mutants resist multiple stresses. They resist oxidation and heavy metals and UV light and DNA damaging agents. And I think that's really important because the implication is that it is the stress resistance that leads to the longevity effect.

Those worms with high stress protein, Tom Johnson's group showed this, live longer. And it had been shown by Mark Tager and others that if you take an individual worm and just heat it up for ten minutes, that also gives a lifespan benefit. So all of these put together suggest, at least in the worms, that augmenting the stress resistance pathway leads to long-term effects, beneficial effects on longevity.

Getting this into mammals has been proceeding patchwork, bits and pieces. It's been known for many years, decades now, that caloric restricted mice are resistant to many different kinds of toxins that they might eat. And some cells from many of these slow aging mice are resistant to toxins as well giving you a malleable system to evaluate.

The guesses, the implications from that which I wouldn't pretend are proven, is that the links between stress, aging, and the sort of "insuliny" stuff that comes in quotation marks, meaning it might be IGF-1, those links evolve very early prior to the split between worms, flies, and mammals. So you can study them in flies and worms and have a pretty good guess that it may all work in mammals.

The other key implication here is that multiple stresses are on the same control knob. We've seen slide after slide already today where there are 5 to 10 to 20 different kinds of stress pathways or inflammatory pathways are bad things. The point of this study is that they may all have a single rheostat that turns them up and down together related to stress biology. And I'm not talking about psychological stress or worried about the cheetah or about my next grant review. I'm talking about the kind of stress that affects cells.

I'd really like to know which cells from long lived mice resist stressors are important for this. Is it the vascular endothelial cells? Is it some brain cell? Is it some part of the immune system? Is it the liver? The answers to that are going to be very important. They're easy to work, relatively easy to work out in worms and will be really important following the lead of the worm biologist to try to get that kind of information in mammals. And I'd particularly like to know whether drugs that increase lifespan do so by means of stress resistance and, conversely, whether if we found drugs that increase stress resistance, would they be a good bet to increase healthy lifespan?

Now that we have this panoply of tools, some diets, some drugs, and some mutations that can lead to extended longevity, it puts us for the first time in a position to ask, what do they have in common? We have some guesses. People have suggested heat shock proteins. I think ATF4 is likely to be a major player. Others have talked about xenobiotic markers. We now have the tools to test those guesses and to try to identify the molecular and cellular pathways that are always or almost always slowed down in a slow aging mammal and then use those to focus our attention as we try to develop drugs that effectively slow aging enough to preserve health for 100 or 110 or 120 or more years.

That's the material I wanted to present, and I'm grateful for the invitation to come and talk to you today. Thank you.

Dr. Epel: So now we're going to hear from Nathan LeBrasseur of the Mayo Clinic, and he's going to talk to us about exercise. And we're all going to want to get up and run out after this.

How does exercise affect stress and stress-related diseases?

Nathan LeBrasseur: Well thank you very much. It's an absolute pleasure to be here. and I feel a little bit upstaged by the fact that we now are thinking about cheetahs and sex instead of the glitz and glamour of exercise. But, nonetheless, I will give my talk.

I want to make a few very important points. We talk about the role of exercise in affecting stress and stress-related diseases, but it's really important to recognize that exercise is a robust and multifactorial stress. It's composed of mechanical, metabolic, neural, and hormonal stimuli that we're just starting to appreciate and understand how they work.

The second important point I want to make is that the stresses of exercise mediate adaptations not just in the skeletal muscle and in the cardiovascular system. I think we've been simple minded in recognizing that these systems have robust adaptations to exercise, but, indeed, organs such as the brain, the pancreas, and the liver, adipose tissue, bone, also adapt to exercise. So here we're looking at an intervention that has a multitude of effects.

Finally, it's important to note— and I'm trying to sell you on the point — that the adaptations in these different organ systems to exercise make them resilient to a host of age-related diseases and increase our resiliency or lower our sensitivity to a host of stresses.

So having said that, I've really changed the title of my talk to "Exercise and Aging: Believe the Hype." But I would be remiss not to mention the obvious, and this was alluded to by the morning speakers, Dr. Fried and Murray in particular, as well as Dr. Kennedy, in terms of what we're really facing today is not a problem of too much exercise or how to administer exercise, but really this incredible challenge to health and well being of physical inactivity. And I would propose to you that we need to view sitting as this generation's smoking. Sitting is clearly the most prominent and one of the most robust risk factors for a host of chronological conditions.

Today physical activity has been relatively engineered out of life when we think about it. In fact, the most robust thing that many of us do during the day is to transfer from our iPad to our iPhone to our iMac and things of that nature. So we've been very effective at engineering physical activity out of life. And just to kind of tell the story and the power of physical inactivity, I thought it was about time today to give a case study.

So I want to tell you about my buddy, Bob, who's 56 years old, is a great guy but spends a lot of his time at work and at home sitting and being sedentary. And what I mean by sedentary life is not just a lack of moderate or vigorous physical activity, but the fact that he has very low or no habitual physical activity. And the problem with that is that it's often coupled with poor nutritional habits. If there's one threat to society more than inactivity, it's nutrient excess both in the quality and the quantity of foods that we're consuming and these are leading to this overwhelming epidemic of obesity in the United States, which is linked to inflammation and a host of the chronic diseases that have been talked about earlier today.

And the problem with obesity is not just so much body weight, but the fact that these lipids are spilling over into other organs such as liver and skeletal muscle creating toxic intermediates that are damaging these organs. And Bob is noticing through his regular physician visits that his fasting glucose levels are elevating, his LDL to HDL ratio is not going in the right direction, his blood pressure is rising, and his inflammatory state is also increasing. And, as a result, Bob is really at risk for a host of chronic conditions

that have already been alluded to today and, in particular, cardiovascular disease, diabetes, cancer, and even cognitive impairment.

So Bob's doing okay, right? He's trying to manage some of his problems with medication, though the compliance is a bit of an issue. His high blood pressure medications don't make him feel any better so he's not so good about taking it and there's some side effects. His beta blocker makes him tired and a little bit depressed and tired. And on top of that, as we heard earlier, is that the stress of life is adding to these things and is quite shocking. It was beautifully displayed by Firdaus how really psychological stress drives a lot of these same triggers and mechanisms in our body that other forms of physical stress drive.

And because of Bob's state of health, he's particularly prone to the negative effects of stress and he mounts these inflammatory and oxidative stress responses in multiple organs because of it, and these kind of perpetuate the cycle. So when we look at this kind of mess that Bob is in and this vicious cycle and we try to identify one quick fix, to me, what really is a resounding possibility is the fact that we need to address the sedentary lifestyle. And Bob is asking, "Am I just aging?" Is this just what is a feature of aging in terms of all these side effects that we're displaying on this screen? And, as a scientist, we've asked this morning already many times, is this really a model of accelerated aging? And these are important questions to ask.

So Bob had a bit of a come to Jesus moment — figuratively, not literally. I don't mean to offend anybody — and said that he was going to change his physical activity levels, both by increasing his habitual physical activity and introducing some moderate physical activity into his daily life. So in doing so, Bob really found a number of positive effects. There was a recent report even highlighted in *The New York Times* that those who are taking part in regular interval exercise training have improvements in terms of their energy intake. So there's lower quantity and improved quality.

He sees improvement in his body composition but I would argue that body weight should not be your primary metric for improvements in health. But if we were to do more analysis of Bob, we would see that his organ health has also improved in terms of his composition and quality.

Bob is witnessing lowering risk factors, including glucose and his lipid levels. His blood pressure is improving, he has lower inflammation, and he's avoiding some of the conditions that we're most concerned about, age-related conditions including cardiovascular disease, cancer, type 2 diabetes, and mild cognitive impairment.

As a result of his improved health, of course, he can take fewer medications, he has less notable side effects, and importantly and what's quite striking to me — and I wouldn't have appreciated this unless I was going to give this talk today — is the fact that he is more tolerant of every-day life stressors. So whether he is in that sandwich generation where he's taking care of his kids as well as his parents or the financial stresses of life or work-life balance, he is more resilient and tolerant for those stressors.

So exercise really is showing a powerful effect here. And I think what we're faced with today is not whether or not exercise is good for you, but how, who, and what benefits from exercise? And I think there's a number of very interesting questions. And one is how is exercise working? And there's a number of different reports that have come out recently that demonstrate that exercise is clearly affecting the basic mechanisms of why we age.

So Mark Tornopolsky recently published a very beautiful paper in *PNAS* showing that in the PolG progeroid mouse that exercise was able to completely prevent all the different conditions of aging that this mouse experiences by generating new mitochondrium, improving mitochondrial function and mitochondrial DNA abundance.

In addition to that, exercise seems to drive hormesis and oxidative stress. So we're a little bit guilty of always pleading that if a little bit of something is good, more must be better; and if a lot of something is bad, less must be good. And here is a story of the fact that this U-shaped curve where we need a little bit

of oxidative stress to mediate the effects of exercise and cause positive effects on a number of adaptations to exercise.

Beth Levines' group showed nicely that exercise is a robust stimulus for autophagy restoring normal protein homeostasis. There's a volume of work out there today showing the role of exercise on inducing heat shock proteins and restoring proteostasis and affecting the ribosome.

Recent work in, and our group has been really interested in the role of exercise on the abundance and accumulation of senescent cells as we age and in response to different dietary interventions. That looks quite promising but still preliminary.

The role of exercise in inflammation is one that's quite controversial. There's clear data in large epidemiological studies that those who are more physically active have lower levels of at least CRP. The data on IL-6 and TNF alpha receptors is less clear but somewhat promising. In part, these are because the confusion here is due to the fact that really the right studies have not been done quite yet.

The other interesting area that we're particularly interested, as are a number of other labs, is this concept of myokines. So how does muscle communicate with other organs and tissues? There is now a growing list of muscle secreted proteins that clearly impact the health of bone, the liver, the brain; and this is an exciting area for investigation.

But we're still stuck with the basic questions as well about who benefits from exercise? In part, we'd like to argue that we need to really be active from cradle to grave. I think there's a lot of arguments for that. But also this question about prevention, preventing age-related changes and these different cellular processes versus treating those derangements in cellular processes. Where is exercise most effective?

We think one area that's particularly interesting in late life is in this context of frailty. So can we use prehabilitation strategies to improve resiliency to elective procedures such as surgeries in older persons and how effective is that.

Just to kind of make another comment about the prevention versus treatment, the work by Mark Tornopolsky, again, I think it's just something that really needs to be highlighted here. However, that was really a story of prevention as well where exercise was introduced earlier in life. Whether or not, if it was introduced later in life, it would be effective at preventing age-related conditions is not yet known.

And, finally, what? What do we need to do? What is active enough? We don't quite understand what the critical threshold is in terms of the duration, the intensity, and the frequency of exercise. Is habitual physical activity just good enough? If Bob just parks a little bit farther away at work, uses the stairs more regularly, is that sufficient to kind of ramp up his system and improve his resiliency and lower his sensitivity to a host of stressors?

We're still working hard in this space. Exercise has a bit of a stigma to it so we try to use the term physical activity a little bit more regularly.

You know, really the goal here is to understand, again, what role physical inactivity plays in accelerating the process of aging and how effective is exercise as intervention to prevent them. And, hopefully, at one point, we will come up with strategies — it's a bit pie in the sky — but to harness some of the effects of exercise. We clearly won't get all of them in a pill; but it's possible we think, to capture some of them in that form and, hopefully, have a powerful impact on a host of age-related conditions.

So with that I say, "Go Bob, go!" and look forward to your questions during the discussion.

Dr. Epel: Thank you so much, Nathan. And lastly we're going to hear from Steve Cole of UCLA who has studied from *in vitro* models to population biology showing how psychological and social stress affects patterns of gene expression. Thanks, Steve.

How does the human genomes response to stress regulate aging?

Steve Cole: So, as you're all aware, we in America actually excel at sort of leading our lives efficiently. We get them over with about 5% faster than any comparable nation that has a sort of similar social and economic composition. And we do that not because we somehow are turning ourselves over faster, at least relative to these other folks, or sort of collectively reaching our Hayflick limit sooner. In fact, what we do instead is we excel at contracting chronic illnesses of the sort that you heard about earlier today.

So things like atherosclerosis, neurodegenerative diseases, metastatic cancer are the major architects of our relative what you might call efficiency in lifespan. This isn't particularly new. This has been going on for decades. There's probably something about American circumstances that contribute to this. But what is new, and you're all probably intimately familiar with, is our ability as scientists to understand the molecular mechanisms of these dynamics, particularly in the last maybe 15 years or so with the human genome sequencing and the subsequent infrastructure that's given us.

We now, for just about any highly prevalent disease, can show you a portrait, like this, describing what we know about the particular genes that organize together in programs to carry out the production of disease. Usually not on purpose. Usually these are inadvertent, well-meaning responses that the genome is programmed to carry out by evolution which was described earlier, work great for us in the Pleistocene; and in our strange new world, not so productive.

But, nonetheless, if death were a molecular biologist, he actually would have a fairly nice recipe for how to generate disease in a relatively generic way, actually kind of independent of the specific diseases and across a wide variety of different tissue systems. So death, the molecular biologist, would in calling forth some kind of recipe for disease often say, "Let's upregulate the expression of genes involved in inflammation," as we've heard a lot about today, "and let's at the same time push down the expression of the genes that actually carry out the fundamental physiological processes of the cells and tissues. And so, in this particular case, of a heat plot from a leukocyte gene expression profile. We might, for example, see death ordering up the reduction in the production of expression of genes involved in adaptive immune responses or even innate immune responses.

So we now have a fairly clear portrait of these kinds of dynamics and we can ask about how this kind of architecture of disease intersects with the architecture of the human genome's response to environmental conditions. And, in fact, that's what I'm showing you in that heat plot there. Although I've shown it as kind of the recipe for disease, it's actually a representation of data from basically transcriptomes from leukocytes sampled from people that confront one of the most robust epidemiologic risk factors we have for all-cause mortality.

And I think if this risk factor were the kind of physical, chemical insult that our intuitions often sort of threaten us most with, things like benzene in the drinking water or smog in the air or radon in our hallways, that this particular profile of transcriptional response to environmental stress wouldn't be particularly surprising.

But what is more surprising is that this particular transcriptional response was structured apparently primarily by the extent to which the people in the first few rows of that heat plot simply feel disconnected from the rest of humanity. So it's loneliness that's the risk factor in this epidemiologic study and why lonely people should be turning on inflammatory genes and suppressing antibody mediating genes and innate antiviral responses is a little bit of a puzzle. We'll talk about that in a moment. But I don't want to leave you with the impression that this is a terribly specific or quirky puzzle. In fact, we see similar kinds of transcriptomic responses across different types of tissue environments in response to a wide variety of different adverse psychological and social conditions. So this seems to be something of a generic transcriptional response to adversity.

We understand, without going into too much detail in the limited time here, conceptually, quite well how this happens. We know, first, that these effects are causal. This isn't some kind of effect of disease on gene expression profiles and social behavior as a consequence. We know that because we can

randomize animals to different social experiences and start to see the production of these different gene expression profiles and the remodeling of tissue in ways that facilitate disease.

We also know, conceptually, that the reason that these different types of adversity can push these conserved gene expression profiles is predominantly because they all have the capacity to evoke responses in the central nervous system through what you might call generic stress circuitry, activating central nervous system structures that program basically responses in a wide variety of tissue systems via exactly the kinds of neuroeffector molecules that Firdaus talked about earlier — catecholamines from the sympathetic nervous system, glucocorticoids from the hypothalamic pituitary adrenal access. And these systems, the neuroeffector molecules, engage receptors that in turn activate or sometimes repress transcription factors and are ultimately the orchestrators of these changes in gene expression.

We also understand a fair amount on the particulars. Not just that generic story I told you about there, but what happens in response to what particular kinds of psychological or social exposure, for instance, in people confronting chronic adversity that, in general, they think they can overcome, but they're just feeling challenged? Often show repetitive long-term chronic activation of the sympathetic nervous system, releasing catecholamines in a wide variety of tissue environments which interact with particularly adrenergic receptors on the surface of cells. And they have a particular stereotyped impact on the human transcriptome. So, for example, from immune cells, they tend to upregulate the transcription of proinflammatory genes while simultaneously pushing down the transcription of genes involved in antibody production in Type 1 interferon responses.

If you are confronting severe overwhelming threat for an extended period of time, you may get a different response involving glucocorticoids, which has a different impact on the genome. So, for example, it tends to suppress both proinflammatory gene expression and these adaptive immune responses. So different types of adverse experience have different biochemical representations in the body and they, therefore, evoke different transcriptional responses.

We know these effects also aren't limited to just changing transcription from tan existing portfolio of cells. You can actually call forth different cells from the body as a function of this if it protracted enough. If they become sort of a lifestyle of threat and adversity.

So the way adverse environments seem to call forth, for instance, more myeloid lineage cells from the hematopoietic system is by via the nervous system's ability to evoke sympathetic nervous system signaling and the innervation of hematopoietic stem cell niches by the sympathetic nervous system. In the bone marrow, what you end up with is, for example, a program that essentially advises hematopoietic stem cells to lighten up on the production of lymphoid lineage immune cells and start pumping out more myeloid lineage immune cells. So you now have more neutrophils and monocytes and subsequently macrophages and tissue, dendritic cells circulating through the body. And they can then interact with tissue damage initiated elsewhere in the body in ways that tend to accelerate these disease processes.

So here's one example. Let's say we've got an initiated breast cancer, and we can kind of follow that up over time as that primary tumor spins off metastases into the rest of the body. If we simply add stress to this kind of system, even relatively modest amounts of stress for not terrifically long periods of time, you can have a mouse that generates somewhere on the order of about 10 to 30-fold more distant metastasis as a function of that experienced adversity. No difference in the growth of the primary tumor, which is in this breast cancer model you can see in that quadrant right there. So it's not like the stress is giving you cancer. This is a case where the cancer comes from being alive. It comes from making a copying mistake in your DNA or one of the other ways that we can initiate a tumor.

What this stress does is it basically pumps myeloid lineage cells into the primary tumor and kicks off a variety of different programs that essentially allows the tumor cells to escape and colonize distant tissue. We know this is mediated by the myeloid cells because we can block these dynamics with drugs that block, for instance, colony stimulating factor 1. We can also block them at the level of the fight-or-flight stress response with drugs that block engagement of adrenergic receptors.

So that's one example of how you can evoke disease from what is otherwise a kind of protective and reasonable response from the bone marrow. If you're confronting long-term adversity, go ahead and upregulate the cells that should be defending you against tissue injury, bacterial infections associated with that. But, inadvertently, you've now got patrolling the body a bunch of cells that when they go into the brain can facilitate neurodegeneration. In the vasculature, they can contribute to atherosclerosis. In lungs, they aggravate your infections there. Polarize your responses to all kinds of diseases.

So what this really is is just a situation where we've seen that the acquired wisdom of the human genome says, "You should change your gene expression profile in response to certain experiences of life." That made sense in the Pleistocene when there was a good binding between the different types of pathogens we confronted and the different experiences we had in daily experience. That is not so much the case anymore, so now we have bodies that adapt to adversity by changing their structure. And in the same way this tree has adapted to a force constantly pushing on it and, therefore, becomes vulnerable to gravity, our genomes have adapted to a world that sees this association between experience and microbes and changes as a result. And now we've hooked those experiences up to nonphysical stressors.

So that's one example of how the human genome's response to stress can regulate aging.

Discussion and Wrap-up

Gordon Lithgow, PhD: Thank you for a great set of talks. About 20 years ago when it became apparent that longevity mutants in the nematode *Caenorhabditis elegans* were resistant to multiple forms of stresses, I thought it would be a good idea to go off to a stress conference and find out what stress was. And I wandered from room to room. And in one room, there was a talk about heat shock induction of molecular chaperone genes in *E.coli*. End in the very next room there was a talk about health outcomes as a result of the psychosocial stresses of the Serbian-Croat wars. I had no idea why these people were at the same conference; absolutely no idea. I had no idea whether they talked to each other, whether they went to each other's sessions or not.

Twenty years later, I think we're beginning to see signs of why that might have been a good idea. Now the connections between stress and aging we're working on hard. Molecular mechanisms are being uncovered. You'll hear more about this tomorrow.

The mechanisms, the connections between psychosocial stress and age-related disease, now really open up the possibility that we are looking at some sort of continuum. Discuss. Aubrey.

Aubrey De Grey, PhD: So I thought wonderful session. Also I think in reference to the morning sessions, I'd like to ask a question to all the panelists about cross-species comparisons and contrasts. So as we've heard, there seems to be enormous conservation of the genetic basis, of health extension and life extension across from worms through to mice through to humans, and also, of course, of the types of stimulus that evoke that health extension and life extension.

But the magnitude of the response so far is, of course, very much less of the proportion of lifespan in longer lived species over species that have three orders of magnitude difference in natural lifespan. We have less than one order of magnitude, absolute extension, you know, maybe six or eight months in worms and certainly not six or eight years even in humans.

So it seems to me that we need to consider the possibility that even though the master control genes that are giving us any kind of response in all these species seem to be so conserved, nevertheless the downstream effector pathways that actually determine the magnitude of that response are simply more feeble in long-lived species perhaps due to less selection for them or something.

And I'm wondering whether the panelists have any ideas for how we might actually test that hypothesis and, thereby, test the plausibility of the idea that Rich mentioned at the beginning of his talk, of actually getting the same proportion of increase in lifespan in humans that we can so routinely do in mice.

Dr. Lithgow: Rich.

Dr. Miller: Sure, dogs. Test the drugs in dogs. Pet dogs at five years, you start with five-year-old dogs you'll know whether the drugs work or not. The dog owners will take care of them. They'll appreciate the free vet care. And if the dogs live a long time, they'll be very happy. You check the dog after a year. If it's sick, you stop the drug. No problem.

Dr. Lithgow: I just maybe want to challenge the premise of the question. With the simple invertebrates, we've had the opportunity to look at many, many alleles and do many, many experiments. That opportunity hasn't been true for the rodent studies, so maybe there are longer lived rodent mutants out there if only we had the money to look for them. This side.

Naresh Chand, DVM, PhD: My name is Naresh Chand. I am from NIDA, National Institute on Drug Abuse. I have interest in food addiction and obesity. I was a former food addict myself, growing disproportionately. Extension of life is one thing. Importantly, its quality of life must bear just more to my mind than the longevity of life. I thought anybody should express on it.

Dr. Miller: I'd like to express an opinion about that. Over and over again people have been talking about quality of life and quantity of life as though they were on a seesaw, that you got one by giving up the other. But all the evidence so far points in exactly the opposite direction. All of the interventions that we have in mice, at least so far, that increase quantity of life do that by postponing a vast range of diseases and areas of disability and dysfunction as is perfectly logical that that's how you would increase lifespan. It's by increasing the amount of time that individuals are healthy. The tempting idea to say, "Well you get one or the other," is against the evidence and doesn't make a powerful pro facie case either.

Dr. Lithgow: This side.

Kevin Becker, PhD: I'm Kevin Becker from NAA/IRP. I have a question that's a peculiar question that could have been asked in this morning's session and it's related to what you said Gordon just now, that I've more and more in the work that I do, you know, I do genomics and other stuff, we have 25,000 words for genes and we have 10,000 words for links now and microRNAs. But we have very few phenotypic descriptors for these general terms like stress or inflammation.

It would be very useful to have an ontology, a specific ontology or hierarchical ontology of types of stress or types of inflammation. So even when it gets into the title of a paper and you search for those papers, you could specify what they're talking about and it would be extremely useful to specify phenotypic words.

Dr. Epel: I couldn't agree more and I think that there's an initiative coming out of NIA for more of a scientific ontology of stress because the word stress doesn't mean anything alone. It's kind of a common catch-all term and so we might think that heat shock-induced longevity is relevant to psychological stress. And unless we break down the word stress and have a good ontology, we can't even talk and compare them.

But I just want to point out that I think you probably see now after hearing some of the talks that psychological stress is only one tiny piece of the phenomena. Biological stress follows psychological stress and affects the cell. So I hope that point was made. And, I'll stop there.

Dr. Darlington: I just wanted to add that ideally we'd like to have a definition of stress that you could point to various molecular changes. So and kind of my hope and the point of my talk was if we could go back to the major pathways that we know extend lifespan and, as Rich points out, extend healthspan, and ask

whether populations of depressed individuals really have a change in those pathways that impact mTOR and sirtuins and so forth, that we'll get a better definition of what stress means under each circumstance.

Dr. Lithgow: Steve, do you think we need an ontology when we've got gene expression profiles?

Dr. Cole: We probably do, although I do have to agree that the word stress now means so many different things that it becomes an invitation to confusion than an opportunity for clarity. And so I think often we strive to eradicate that term from our papers. Not because we don't believe in the phenomenon but because precisely that it's so confusing. So we really do a lot of pharmacology to say, "We're talking about beta adrenergic signaling." You can think of it as stress. That's probably what it means in the ecology of human life; but as a scientific term, it means so many different things that it starts to mean almost nothing. So more specificity.

Nir Barzilai: Yeah, Nathan, it's for you and maybe for Gordon too. You know, our friends here from the NIDDK — and, by the way, I'm Nir Barzilai from Einstein. Our friends from the NIDDK/NIHLB are looking at this program and they're probably wondering where is oxidative stress in this program? And I wanted to bring it up because maybe we have better view of what it means in our field and it's certainly very important in their fields.

But exercise is one of those examples that, if anything, you're going to really induce huge oxidative stress and yet all the outcomes are good. So could we talk for a second on oxidative stress? Not that you talked about it, but maybe that's the opportunity to get something about that.

Dr. LeBrasseur: Yeah, I probably spoke too quickly when I did mention it in that last slide in terms of the different stimuli related to exercise. Clearly, exercise is a very robust activator of oxidative stress and oxidative stress is, in fact, necessary to mediate some of the adaptations that exercise confers.

Again, I think there's a case where we assume that oxidative stress as a damaging agent needs to be wiped out from the system but, clearly, it plays an important role in mediating some of the effects of exercise. And what as we exercise is we not only induce oxidative stress but we also induce the systems and regulatory networks that counteract it. So oxidative stress, the temporal nature and rise in different stressors associated with exercise are temporally regulated in a way that they go away. And when those stressors are chronic whether it's psychological or physical, that's when we think it's quite damaging.

Dr. Lithgow: Have very similar situation in invertebrates where small amounts of oxidative stress appear to lead to longevity where, of course, large amounts lead to premature aging and death. It's a mixed bag right now based on genetic modifications of oxidative stress response, enzymes, and regulators as to what's going on. But I suspect when we get down to when we start talking chemistry, that's the chemistry we're going to be talking about.

Dr. Miller: Can I add just one comment very briefly? That is that the worm studies, and now much more slowly the mouse cell studies, argue that these can be under coordinated control. That at least mutants that lead to augmented resistance to oxidative stress also often lead to resistance to a wide range of stressors. And figuring out how that works is going to be a very important cutting edge kind of intervention science.

Dr. Lithgow: Yes.

Dr. Dhabhar: So I want to sort of follow up on sort of just raising caution not to sort of reductionize stress too much because I think that some systems ought to be studied as multifactorial physiologic systems. So sometimes we may reduce stress to like an adrenergic receptor because it's convenient to study it that way, but what you're doing is ignoring everything else that goes on when an organism is stressed.

And one important distinction would be an organismal level stress and a cellular level stress and then you define it. And that's what we do in our papers. We sort of define what we are talking about. The

difference that we have found in terms of the duration of that biological stress response is critical. I'm not saying it's the only way of interpreting the system, but when you look at it that way, short versus long term, you really see a break up of the different effects of the stressor.

So while we do need an ontology and while we do need sort of clarification and discussion of definitions, getting too much down the pathway of saying, "Oh stress is so confusing, so nebulous," might actually harm us in the long run by not enabling us to study it in its entirety which we might need to do to really got to the bottom of it.

Dr. Kohanski: Ron Kohanski from NIA. This question is based on Dr. Dhabhar's slide on the barracks, boulevards, battle fields. So you have an increase in cell number with the duration of stress and then a fall off. At some point the system resets. So with aging, do the barracks get depleted so you would have a lower amplitude? And what about the ability to reset the baseline with aging? Thank you.

Dr. Dhabhar: So thank you for the question. So in terms of the short-term response itself, within about three hours after the cessation of the stressor, everything comes back to normal. So lockdown and baseline. The cell composition may be slightly different and may need about 24 hours to completely come back to normal, but everything else returns to baseline.

In terms of with aging, yes. There may be some evidence for normal aging. So not the successful aging but sort of just the normal response to wear and tear that the barracks are getting depleted and also that they may be getting replenished with the leukocytes that have potential to cause trouble, like Steve said, rather than the ones who may be more able to protect you. So you have two things. Overall cell numbers may come down. The proportions may change so that the more proinflammatory cell types might actually increase relative to the more protective ones. In terms of resetting the baseline, in an aging situation, I don't know.

Dr. Kohanski: And a follow-up. The data behind that come from which organisms?

Dr. Dhabhar: Oh, so thank you for asking that. So basically that barracks to boulevards to battlefields response that I showed you is evolutionarily conserved, at least from reptilian to sort of, well, fish to reptiles to mammals. And you can see it from mouse to person very, very clearly and reproducibly. And that's why some of our preclinical findings in one single step we, as well as others independently, were able to take to the clinic. So nothing in between; lab to clinic.

Dr. Lithgow: I've been asked by NIH to not allow anymore than one follow-up comment. I'm sorry.

Speaker: But he's the boss.

Dr. Lithgow: I know. You made the rule.

Dr. Dhabhar: I'd be happy to talk later.

William Sonntag: : I'm William Sonntag from the Reynolds Oklahoma Center on Aging. And my question is I'm always impressed by the diversity of actions of growth hormone in IGF-1. We heard during the morning talks that these factors are really important for immune function for skeletal and muscle mass. Yet a decrease in both growth hormone and IGF-1 have been shown to increase the lifespan. And so there's two parts, so I do it all at once.

First is, are there opportunities for a window of time when a deficiency or an excess of growth hormone or IGF-1 may be having these effects? That's part one. And part two is, are we vigilant enough about the animal models that we actually use? We all know that a lot of the changes that we make we create animals that are not able to live outside of a two by two box. They are not viable. And so are they good models of aging or not?

Dr. Darlington: So I think you make a good point. The animals that we're using are in a protective environment, as Brian described in the morning, and they wouldn't survive ten minutes out in the wild. However, I think the evolutionary conservation of those pathways would strongly suggest that we will find important benefits in the human population from modulating the downstream targets and perhaps relatively far up in the pathway. I mean, perhaps all the way to the receptors that have partial activity.

And your question about the timing, that's clearly a very interesting one. I think all of us suspect that because these interventions have been sort of lifelong that it may not have application, but we can test that. We can turn things on and off and begin to understand what's our window of opportunity.

Dr. Lithgow: Stuart.

Stuart Kim, PhD: Stuart Kim from Stanford University. I have two questions about loneliness. Do you ever find person-to-person variability in the loneliness genes? I mean, do you ever find a person that doesn't mind being alone?

Dr. Cole: If that's your one question, the answer is yes. So there are what we would call introverts, people that are perfectly happy being alone. And if you put them with a lot of other people, they'd be essentially every bit as stressed or autonomically activated or threatened, whatever you might want to call it, as an extrovert isolated.

Dr. Kim: And so on your heat map they didn't look green and red. They looked unstressed.

Dr. Cole: In the heat map, they weren't there because they tail sampled for people that scored on measures of loneliness, which actually have only a modest input on how many people you actually have contact with but a heavy input on your psychological experience of that. So the measures basically say something like, "I think you can't trust anybody in the world." That's the fingerprint, the deep psychological fingerprint of a lonely person. That's very different from somebody saying, you know, "I'm on my own, but everything's fine."

Dr. Lithgow: You've had your follow up.

Dr. Kim: Oh I get one follow up.

Dr. Lithgow: You had it.

Dr. Kim: I want my follow up.

Dr. Lithgow: You had it. I thought you had it.

Speaker: He's from Stanford.

Dr. Lithgow: Quickly.

Dr. Kim: What happens to loneliness in aging? I mean, do the lonely genes go up during normal aging? And if you're a young guy and you're lonely, is that like switching your lonely genes to the old state? I mean, what happens during aging for loneliness?

Dr. Cole: So at the genomic level, you know, these transcriptomes look very similar. A young lonely person has a transcriptome that looks a little bit more like an older person with more basal proinflammatory signaling, a little bit less of this adaptive immune response signaling, at least through the eyes of a leukocyte. It may be different in the brain. That's a different question.

What we don't know is whether those are incidentally the same thing or whether— There is certainly a very clear and profound trend toward a greater experience of loneliness in the lives of many older people.

So this is such a profound epidemiologic issue that it ranks highly in the National Institute of Aging's sort of behavioral science portfolio, understanding loneliness and its affects on biology. So they're definitely related somehow. Whether they're intrinsically causally connected is a to be determined question.

Dr. Epel: Yeah, and can I just add that in terms of big factors that predict mortality, there have been meta-analyses on social isolation. That's a little different than loneliness, but people who are more socially isolated tend to have earlier mortality and the effect is as large as other factors such as smoking.

Dr. Lithgow: Lots of people want to make comments and ask questions, so let's keep them nice and crisp if we can, thank you. Next.

Constantine Kotsanis: Constantine Kotsanis from the Kotsanis Institute. The question is can stress be inherited by epigenetic factors alone?

Dr. Epel: Yes.

Dr. Lithgow: Crisp.

Dr. Epel: So this area is incredibly complex and there are mind-blowing studies that are in progress both in animals and humans looking at the epigenetics of transmission of depression and stress during pregnancy to placental changes and baby changes. And the telomeric transmission is there as well. Moms who are more anxious during their pregnancy have babies with cord blood that has shorter telomeres.

Dr. Lithgow: And there is evidence from Ambrenes's lab and others that longevity itself can also go through the multiple generations probably to an epigenetic mechanism.

Ian Kremer: Good afternoon, Ian Kremer from the LEAD Coalition. I want to thank this panel and the panel this morning. This has been tremendously illuminating for probably the one nonscientist in the room, but I think it has aged me tremendously.

I have a question related, kind of a two-part question related to differentiation between young onset Alzheimer's and more typical age of onset. So I wonder what the literature, if any, is looking at stress. And to the extent you can comment on this morning's topic of inflammation, where the differentiation may be between typical age of onset and young onset. And then I'm curious about the loneliness aspect for people that did not experience profound loneliness before the onset of either young onset or typical age of onset Alzheimer's but then began to experience severe isolation and loneliness due to the social stigma of the disease as well as the sort of lifestyle limiting factors of the disease. Can you comment on that in terms of how that's affecting the trajectory of the symptomology of the disease?

Dr. Cole: You know, it makes sense as a logical result that they would be connected. I don't think at this point we have much empirical data on whether or not that is actually true, but it certainly seems like a reasonable guess.

Part of sort of the biological phenomenology that makes it seem like a reasonable assumption that that might be the case comes from such actually completely terrifying studies that John Sheridan's group at Ohio State does where they run models of social stress. The mouse pumps out these myeloid lineage cells. They tag them with green fluorescent protein. You look in the brains of these animals a couple weeks afterwards and these GFP positive, once upon a time monocytes — now differentiating macrophages and dendritic cells — have taken up residence in the brain. They come in with an instruction that is basically primed for inflammatory responses. So they're essentially newly implanted time bombs.

They're not going to guarantee trouble, but if they go into an environment where there's already an initiated disease process such as sort of the early stage dynamics in Alzheimer's, they may well amplify

them in much the same way as I showed in the case of that initiated cancer cell where it's going to be a primary tumor unless it gets a lot of monocytes to come in and free those metastases for sort of their dirty deeds and distant colonization.

Peter Nathanielsz, MD, PhD: Peter Nathanielsz, University of Texas, San Antonio. I'm very pleased, literally, probably within the last five minutes, the issue of epigenetics and transgenerational passages crept in because I work in the field of developmental programming and epigenetics. And I was delighted to see early on from cradle to the grave, but I think we have to change that from the womb to the tomb because after all it was T.S. Eliot who said that in my beginning is my end. And it's now so clear from the developmental programming studies in animals that — I'll give four examples — you're born with a smaller muscle mass, you've reset your appetitive peptide thermostat in your RQ at nucleus, you've got different spines on your hippocampal neurons, and perhaps addressing one of the things that's been going through this afternoon, you also completely reset your pituitary adrenal access.

And I suppose my question and my interest in what the panel thinks about this, because I don't think it's been touched on too much, is that this differs in animal studies between male and female offspring. And, in fact, I don't think you get a paper published now in my field unless you've actually studied both.

So I suppose my question is what can we learn from this? What is the significance that developmental programming from the womb to the tomb and my beginning is my end is so different in the male and the female? What can we learn about that for the processes that lead to aging?

Dr. Miller: Well thank you, it's an extremely broad question. And I'll just put the data in front of you. One is that the changes in hypothalamic neurons that can be induced by IGF-1 levels within the first three weeks of life are quite dramatic, but they are the opposite of that which is seen in a situation like a mutation that modulates IGF-1 and growth hormone. You get opposite directions.

Some of the drugs that the ITP has been testing have differential effects in male mice and female mice. And seeing how these drugs interact with the sexually dimorphic hormone levels, with changes in xenobiotic enzymes in the liver, as well as with the sympathetic and parasympathetic nervous system, all of that people are just barely beginning to evaluate in terms of how the antiaging manipulations get read out through manipulations of these CNS and neurohumoral responses. I think it's a very fertile area for investigation, but not much is known yet.

Arlan Richardson, MD: Arlan Richardson from San Antonio. And I think this question would be to Gordon and Rich. You've pointed out very nicely the fact that almost all, maybe all extensions of lifespan are associated with increased resistance to stress. But on the other hand, there are studies where you can get resistance to stress — and I'm not just talking about oxidative stress which we did. But I remember when Tom was first talking about the nematodes, he was saying that while you would see that long-life mutants all had resistance to stress, not all stress resistant models had increased lifespan. And how do you see that apparent dichotomy? Or would you explain that.

Dr. Lithgow: Yeah, the correlation isn't even quite that good, Arlan. There are many mutations. There's over 500 mutations in *C. elegans* that extend lifespan and many of those mutations have not been shown to be stress resistant, at least in young animals. And so that there's a dearth of data there on older animals and middle-aged animals and whether stress resistance comes about. But at least for young animals, the correlation isn't 100% complete.

What I think might be better though is when we drill down into molecular markers of stress resistance or stress response. So, for example, if you look at proteins that become insoluble during normal aging, there's a strong correlation, so far, in our hands of molecules that slow aging tend to always also slow protein aggregation as well.

Dr. Richardson: How would you explain that you can get stress resistance and not lifespan?

Dr. Miller: I think, Arlan, that the oversimplification is the villain here. I would never say that all of the slow forms of aging in mice lead to resistance to all stresses. The evidence is strong that that is much too simple. The challenge is going to be to find out whether there are specific cells that resist into specific classes of stress. People have said, "It's likely to be protein folding or oxidation or modulation or hormonal balance." There are a variety of molecular and cellular hypotheses that now need to be tested. The worm data points us in a lot of interesting directions, but it's certainly not going to map one to one with all of the interesting and more complicated stuff in people like us and the mice.

Dr. Franceschi: Claudio Franceschi. Following the last response, I would like to know if among the difference between mouse strains if there are also differences in coping with emotional stress and if this correlates with the resistance to more classical stressors like mechanical, like say heat stress or reactive oxygen species.

And the second question is I remember the prolongation of lifespan in p66Shc mice, but that they were shown to be resistant to many stressors. But then when they were put in a wild type environment, they were all dead because they were very sensitive to cold.

So I wonder if there are such type of tradeoff between resistance to, one, stressors and being much more weak in response to other type of stressors because what nature did during evolution was to make a compromise in the wild environment between the resistance to different type of stressors. And the animal models that are produced in the lab are monsters in this sense.

Dr. Lithgow: Thank you.

Dr. Epel: I'll brief answer the first part, you get the second. I love the first question asking about stress resistance in, for example, rodent models, psychologically resistant to stress and does that also lead to multiplex stress resistance, cellular stress resistance in the models that we know how to test in a more standardized easy way. I love that question because that's the type of question that will lead, that will bridge the gap here.

So this idea that psychological stress is proaging and we see these physiological examples, but we don't know how that compares to the stressors in these model organisms is an empirical question that we would get so much bang for our buck to address.

So the quick answer is yes. There are stress resistant genotypes. There are at least ten favorite candidate genes that look like they lead people to be maybe less vulnerable to depression under stress, and there's also the idea that these stress plasticity genes lead animals to be more open to the environment period, whether it's aversive or supportive, so they'll thrive more in supportive environments and have more stress vulnerability and disease in more adversity.

Dr. Dhabhar: So, you know, to follow up on Elissa's question, so there are even among mouse strains like differences in stress susceptibility or resistance and also in rat strains. I don't know specifically about how they're within the strains then there are differences to different types of stressors like you asked, cold versus something else; but what's very interesting is that there other vulnerabilities that come in.

So, for example, the Lewis rat is generally stress hyporesponsive to a single stressor, to repeated stressors. So you could say in a way it's stress resistant. It mounts a lower stress response compared to the Sprague-Dawley rat, which is its mother strain and the Fischer rat which is a very closely related strain. The Fischer rat is stress hyper-responsive. So in some ways for any given shot of stress, it represents and mounts a much greater stress response.

Now what's interesting is disease wise the Fischer rat is much more susceptible to tumors and cancer-like diseases and, in fact, it's a model that's used for that. But the Lewis rat, which is more resistant to those kinds of diseases is more susceptible to autoimmune and proinflammatory diseases. And so the tradeoff may come not only across stressors, but also across diseases.

And then even within the same strain if it's a wild type strain, so our tumor model is deliberately made in a wild type strain to keep it as, in a outbred strain, sorry, I mean an outbred strain to keep it sort of closer to the human genetic kind of variance, what you see is within that there's a spectrum of responsitivities, resistance and susceptibility to stressors. And just phenotyping a mouse based on its anxiety characteristics at baseline before you do anything to it and then putting it through the tumor model, that baseline phenotype can let you predict who is going to get more tumors and what kind of immune response is going to underlie its susceptibility and connect it to the stress reactivity. So there is those kinds of models available.

Dr. Lithgow: One minute per question from now on.

Ursula Staudinger, PhD: Hi, my name is Ursula Staudinger. I am from the Columbia Aging Center. And, actually, it follows up quite nicely from just this recent exchange. I was triggered by the presentation by Dr. LeBrasseur on the effects of physical exercise. And I was triggered by comparing it with the work that we have done and we found very significant moderations depending on gene polymorphisms in the reaction to physical exercise. So, again, the COMT gene that we had heard about this morning makes a difference.

And so my more general question really leads on to that that aren't we at a stage in the signs of aging where we have accrued a lot of knowledge and detail at various levels of the causal chain that would now allow us to actually bind this together to a more personalized version of aging. Because if we want to take the next step to powerful interventions, I think we all agree it's not one-size fits all. And if we do not invest in building these very complex models, I'm not sure how we will get there. So maybe some explanation.

Dr. Lithgow: Thank you. Anyone want to comment?

Dr. LeBrasseur: I think you make a great point. And it's interesting to me from the clinical perspective as we talk about individualized medicine, yet we're trying to develop established protocols to treat every condition. So there's got to be a healthy balance in between. In terms of exercise, very few are nonresponders. So we're pretty comfortable with making a blanket _____.

Dr. Staudinger: Yeah, but I mean the gene prototype is just one variable.

Dr. LeBrasseur: Yeah, sure.

Dr. Staudinger: We heard anxiety proneness and then the cumulative effects of biographies. I mean, it's an endless complexity which I think we need to have an eye on.

Dr. Lithgow: Thank you. This side.

Patrizia D'Alessio: Hi, this is Patrizia D'Alessio, University of Paris, Parisuit. I would go back to universal mechanisms I prefer. And the question is to Richard Miller who asked do drugs that enhance lifespan work with stress resistance? And I reminded Linda, back a couple of years ago before getting her Nobel Prize for odorant receptors, writing a paper in *Nature* or, I don't know, *PNS* maybe, in which she proposed 30% lifespan enhancement of *C. elegans* having been treated with mianserin, which is a human antidepressant. At that time it wasn't yet on the market. It has been substituted by another molecule who is called mirtazapine. But the most important thing is that this molecule was myorelaxant like baclofen which is used now to treat alcoholism. So my question, in fact, is would you accept to trade stress resistance against myorelaxation or relaxation in general?

Dr. Miller: I think we don't yet have a very good idea of how to prioritize among suggestions for drugs that need to be tested in mammals for lifespan and health improving capabilities. The NIA has just begun to fund work in worms in which large amounts of drugs, large quantities of drugs will be screened for a variety of cellular properties. We hope at Michigan to do the same thing in worms and flies. And for

cellular stress resistance, our hope as a community is that these cheaper simpler screening systems will do a good job at finding drugs that have a better than average chance at having benefits to health in mice and rats and maybe people. That's my intuition and I'll be happy to find out ten years from now whether that intuition is confirmed by data. But so far we just don't really have a good way of doing it beyond professional guesses.

Richard Morimoto, MD: This is more of a comment for you, Gordon. So when you had that Alice in Wonderland moment — Rick Morimoto, Northwestern — Alice in Wonderland moment of I think probably Budapest, if I remember, and you wandered between a meeting on stress and then heat shock, some of you will remember Nikki Holbrook who is a intramural at NIA. And I'm not sure if this is a stress. Some of you can tell me since I don't work with mice, but she noticed that the naïve mice in the same room as the other mice who were getting their heads lopped off were activating, it turns out, Hsp70 only in the adrenal cortex and in the vascular endothelial. She then teams up with another lab, ours, and it's a full heat shock response. Full activation of heat shock factor.

So if a mouse watching another mouse getting its head lopped off is in emotional stress, then we have the full link. That mouse was activating heat shock chaperones in a very specific tissue probably to remodel the vascular endothelial to allow that massive flux of blood to go through and say, "Get the hell out of here."

Dr. Lithgow: Thank you.

Speaker: That's great.

Dr. Lithgow: Please.

Jacob Siegel: I'm J. Siegel, a private demographic consultant. My comment is addressed to Dr. Miller and it's just an incidental thing that appeared on the screen and one of your comments relating to the fact that you could potentially eliminate both heart disease and cancer.

Now it seems to me given the competing risks between these diseases that this is an impossible dream and that while you may hope to reduce these diseases by slowing aging, there has to be a gradual slowing off of this unless you assume immortality. In fact, as we know, in recent decades finally there has been some significant reduction in cancer, but we notice that simultaneously cardiovascular disease, CV, other, that group of diseases, slowed off that decline.

So I think the comment—

Dr. Lithgow: Thank you.

Dr. Miller: Dr. Siegel, the comment is that it was a hypothetical situation. The point of the slide was not to say that a complete abolition for cancer was a likely thing. It was to point out that the demographic implication of assuming that no one ever died of cancer was fairly small compared to the amount of lifespan extension one gets in laboratory animals by slowing the aging process by diets or now by drugs or genes. It's just a way of trying to point out that interventions in aging are likely to have a very powerful effect on public health and well being because if they were to work in people, they would slow down multiple cause of diseases.

Dr. Siegel: Yes, agree.

Dr. Lithgow: We have two more questions.

George Martin, MD: George Martin, University of Washington. I'd like to get some feedback from the panel about a potential additional variable that might contribute to this interesting heterogeneity among humans in terms of the reaction of stress in various animal models, and that's the microbiome. The

vagus nerve endings in the GI tract they're talking to the central nervous system. And there was a paper, oh what, a year or two ago from Canadian group where they changed the microbiome using a species of bacteria that's quite a cousin of what we eat in our yogurt. And they had an assay for an anxiety and these mice became very laid back, equivalent to what they can give with the valium type drug.

So that has interesting implications like fecal transplants, for example, or eating a lot of yogurt. So that might be one potential, since so much of the microbiome is an important contribution to what's going on in phenotypes. Is any of your colleagues interested in that? Do you know if anything is happening in that area?

Dr. Miller: All of our colleagues are very interested in that but the problem, I think, is analytical at this point just as 15 years ago one was confronted with a list of 5,000 gene changes. And it's taken bioinformatics a long while to start to make sense out of that. Many of the laboratories can now produce lists of bacterial species and evaluate their correlation with dietary and other outputs. Getting meaning out of that, finding correlations that are robust across different environments, different strains of mice, different dietary changes I think it's going to be very important. But I don't think it's something that's currently routine.

Dr. Lithgow: Perhaps the worm may help where you can look at one microbe at a time. Last question, Joe.

Joe Betts-LaCroix: Thank you. Joe Betts-LaCroix from Health Extension in California. So I spent all day yesterday at the AALAS, laboratory animal conference down the road and got a pretty good look. I also have been looking at a number of diagrams in various research facilities to learn more about mammal studies in particular. So this is a question about the relationship between using mammals and stress studies.

I saw a fantastic talk yesterday at AALAS by Joe Garner at Stanford who argued, quite persuasively, that the present mode of studies we have for rodents, they're pretty much all stressed. And, in particular, he pointed to a study — but there were a number of studies he was pointing to — one where animals had added to the normal standard of care that's prevalent across the industry extra actually useful enrichment as they call it in the animal world. And these animals had massively different physiological outcomes. In this particular case, they were looking at a bunch of different cancers and this is not just one specific mouse model that's super vulnerable, but it was a pretty general study. And they had lots of increased resistance to these diseases.

And so what I'm wondering is has anybody done any lifespan studies in rodents comparing the normal standard of care and extremely enriched ones? Not where you just drop in an egg carton or something, but you have actually things that are meaningful to the rodents.

Dr. Miller: I think the idea as to whether antiaging manipulations are robust across changes in environment and diet and psychological situation is very important. My own intuition is that the strongest things will prove to work quite well regardless of that.

Jim Nelson who's here somewhere, I believe, has done a really nice study in which he took mice that are group housed and compared them to mice that were singly housed, which for a mouse is extremely stressful. And he got differences in weight and differences in food consumption, but the caloric restriction regime worked quite well to extend lifespan in these circumstances. Similarly for dwarf mice. You get longevity extension whether they are group housed or singly housed or housed with big mice or small mice.

So the general point that environmental changes could modify the effects of antiaging maneuvers is perfectly valid, but so far, thank goodness, the big, strong ones have proven to be fairly resistant when evaluated from that perspective to any perturbations.

Joe Betts-LaCroix: Okay, that's saying that if you start with group housed mice presently, let's say five per cage, and make them even more stressed by putting them singly housed, that that doesn't make that much of a difference but it still doesn't necessarily address the possibility of making them actually comfortable.

Dr. Miller: I was agreeing with you.

Joe Betts-LaCroix: Okay, thank you.

Dr. Lithgow: Thanks, Joe.

Dr. Epel: A real quick comment that we clearly need more money and funds to study these basic mechanisms in isolation in controlled conditions and model organisms. But we are never going to understand aging if we don't also, in parallel, remember that there's a brain attached, that the brain and body are not separate. So, I mean, you have the example of the microbiome but there's a tremendous number of examples. Inflammation also causes depression of the biodirectionality in that the central regulator in humans is going to control not only the direct stress response that we've been talking about, but behavior.

So in human aging in all of these epidemiological studies, it's really nice to think that we can do caloric restriction, but that would mean we'd have to contend with their behavior and we know that behavior change is one of our biggest challenges to public health and getting people to, for example, not do stress eating of comfort food which causes a tremendous amount of metabolic syndrome that we have.

So, anyway, my point is you can bring stress models to, for example, rodent studies like if you feed a rodent junk food, you see no effect in three weeks on metabolic syndrome. But if you add stress to that, you have a full blown metabolic syndrome and visceral fat. So it's really important to look at interactions when possible even if it comes down to using epinephrine and just biological stressors to help us understand what's happening that make those models more relevant to the human condition.

Dr. Lithgow: Thanks for your questions and your comments.

Dr. Rowland: So I just want to say one of the great pleasures of being a federal employee, NIH employee, is you get to invite really brilliant thinkers and scientists to come and speak and they all agree, even if it means leaving all their costumes at home. And we know from Gretchen's work that the mice are playing while we're here working. So we thank them for that and we thank you for your applause for our wonderful chairs for this particular session, our terrific panelists, and for you for participating in this.

And I would ask you that we're going to take a break now, but as many of the questions have come up onto the next thing is we are going to start very promptly our Epigenetics and Regulatory RNA session very promptly. That will begin at 3:15. So, again, thanks to this outstanding session panel and we'll see you at 3:15.

Break

Session IV: Epigenetics and Regulatory RNA

John B.E. Burch, PhD: Okay, I'm going to go ahead and get started because we do want to stay on time and, hopefully, people in the hallway will hear me and drift in while I'm doing the introductions so that they're in by the time the scientific portion of the session actually begins.

So my name is John Burch and I am from the Center for Scientific Review at NIH. And my co-organizer for this session is Kevin Howcroft, who you met this morning. He's also one of the people who's a main organizer. He's from the National Cancer Institute, and we are both members of the Geroscience Interest Group.

Our co-chairs for this session on Epigenetics and Regulatory RNA are Shelly Berger and Anne Brunet. Dr. Berger is Professor in the Department of Cell and Developmental Biology at the Perelman School of Medicine at the University of Pennsylvania. She will give an introductory overview of the topic for this session.

Dr. Brunet is Associate Professor in the Department of Genetics at Stanford University, and she will be directing the discussion which will follow the last speaker. She also will be giving a summary of the session. So without further ado, Shelley, please.

Introduction

Shelley L. Berger, PhD: So that's me, Shelley Berger. I'm very pleased to be here. Thanks very much for including a section on epigenetics in the meeting. On behalf of Anne Brunet and myself, we want to welcome you to the session, Session IV, and I want to point out — Brian Kennedy brought this up earlier — that aging, of course is endlessly fascinating to humans all through human history. Here Leonardo da Vinci 500 years ago depicting an aging course in humans. Beautiful drawing.

So why do we believe that, the speakers in this session, why do we believe that epigenetics has a basis in age-related disease? Well, much as the other speakers have discussed, in fact all of our slides are pretty much what you've already seen through the course of the day. But we're going to discuss it from the point of view of epigenetics and chromatin biology.

So there's two reasons. So if we look at aging and disease in humans, one is that there's this very profound association of human deterioration, disease with age and this bifurcation at midlife. And the second reason, discussed earlier today, is that the expected lifespan of humans has increased twofold over the last century. So this sort of plasticity strongly suggests that aging and age-related diseases could be due in part to epigenetic alterations. So this is the basis of the questions and discussion that we're going to have in the next couple of hours.

So our point of view— Now, of course, you've already heard a lot of epigenetic issues having to do with epigenetics already as the day's gone on. And there are many, many epigenetic regulators. So what we're particularly interested in are epigenetic regulators at the level of chromatin at the level of the packaging of the genome into this polymer of histones plus DNA.

And so the field as a whole, the chromatin field and the epigenetic field, is very interested in this issue that you see here. Really everything can kind of be reduced to this issue of the interconversion of repressed closed chromatin and open active chromatin that occurs over our genomes.

Now the specifics aspects that we're going to discuss today then are three types of regulatory mechanisms that are epigenetic working at the level of chromatin. One is transcription factor networks, the second is DNA and histone modifications, and the third are noncoding RNA. So you're going to see, over the next five talks, aspects of each of these regulatory mechanisms.

Now one of the views, one that actually my lab favors and others in the field, is that young cells, the parts of the genome that have to be heterochromatic are in that state in young cells, particularly repetitive areas, telomeres, and other areas of the genome. And through the course of aging, the chromatin begins to open. Areas of the chromatin that are not supposed to be open become open.

This then leads to various events that are not good — mutations and also transcription that occurs in an upregulation of transcription. And so just as others have discussed over the course of the day, these events can be modeled in yeast, *Saccharomyces cerevisiae*, budding yeast, through worm, mouse and to humans themselves, of course.

Now why do we think, what are some of the explanations, some of the molecular explanations that suggest that epigenetic changes occur with human aging? So studies in monozygotic twins from, young

twins to old twins, have shown at the level of DNA methylation that young twins, here three-year-old twins, when you look at their DNA methylation, using this sort of depiction, you see that it's all yellow. The two twins have a great concordance in their DNA methylation profiles over these chromosome regions. However, as monozygotic twins age, you can see the green and red features that there's a divergence of DNA methylation signatures across the entire genome.

All right. So this and much more evidence that you're about to see suggest that both genetic and nongenetic factors are involved in aging and, in fact, that the pace of aging can be determined by these nongenetic factors leading to this question that perhaps epigenetic modifications that affect chromatin states mediate the slowing, could mediate the slowing and reversibility of aging. And, indeed, many of the molecules that the speakers you're going to hear today, this afternoon, are focused on are enzymes that regulate these pathways. And enzymes, of course, are wonderful potential druggable targets so that leads to a great fascination with this area.

Now an extreme example of longevity correlating with epigenetics is in the eusocial animals. The naked mole rat was shown earlier and the eusocial insects are other examples of this remarkable plasticity of the epigenome with respect to longevity.

So the females in eusocial insects are either workers or queen and the workers have a shorter lifespan. Queen has a longer lifespan. If you're wondering about males, they have a very tiny lifespan and they don't carry out any of the work. Go figure. So the remarkable thing is that in the eusocial insects — in fact, one of the species that we study in my laboratory, you can interconvert a worker into a queen and this shorter lifespan of the worker is then epigenetically inherited as the worker becomes reproductive and takes on the role of the queen.

All right, so finally, again, a slide that you've seen over and over again, the dietary restriction delays that result in extension of lifespan in many of the model organisms that we've been discussing today. The key point in this session is that some of the key genetic regulators that affect dietary restriction are epigenetic regulators. So you're going to hear about that again this evening.

All right, so our panelists this afternoon are David Sinclair from Harvard. He's going to talk about epigenetic changes during aging and metabolic diseases and are these preventable. Stuart Kim from Stanford is going to talk about an underlying epigenetic clock determining the rate of aging. Li-huei Tsai from MIT will talk about cognitive aging and neurodegeneration. Peter Adams from Beatson came all the way from Scotland for us. He's going to discuss age-dependent chromatin changes could they be at the root of the age dependency of cancer. And Juan Carlos Belmonte from Salk will talk about cellular models for premature aging. Do they provide a basis for a cure?

So with no further delay, then, age-dependent delay, we'll go on to our first speaker, David.

Do epigenetic changes cause aging and disease, and are these changes reversible?

David Sinclair, PhD: Thanks, Shelley. Well thank you, Shelley, for introducing this session. Thanks, Anne, as well for inviting me. Thanks Felipe and everyone else for making this possible. This is one of the most interesting and exciting and broad meetings that I've ever attended. And I think that you'll agree that this is a landmark meeting. I'm very grateful to be part of it, so thanks again.

All right, so here we go. Well what I want to do today in the short time is to, I have two parts to the talk. First part is about new ideas that are emerging in the epigenetic regulation of aging that I think would be interesting to tell you about. The second half is about small molecules that modulate epigenetic systems in animals and I'll end with some promising but preliminary data in humans studies heading towards the ultimate goal that we've heard so much about today using the knowledge about aging and defenses and hormesis to have an impact on society and get us towards that future that Rich Miller held out as a

promise land to us. And I'm hoping that if we go quickly, we won't have to wait more than 100 years to see that. I think that will be too late for every one of us in this room, unfortunately.

All right, so what I want to get into really is the first part which is about an idea that's built on many years of observations, but I think it's finally coming together to give us more of a united theory of how epigenetics and aging comes together. And when Brian Kennedy gave his talk this morning, he pointed out that there are three major areas of mechanisms of aging. And he grouped them into DNA damage, epigenetic regulation, and what he termed antagonistic pleiotropy which included phenomena such as senescent cells and inflammation.

And what I liked about that slide is that it was a perfect set up for what I'm about to tell you which is a new idea that may unite all three of those areas.

Now where this started really for me was a remarkable publication. This came from the Yankner lab, one of my neighbors at Harvard Medical School. They put out a paper that showed that in the aging brain, you can see that as you go through aging — these are ages of people who donated their brains for science — going from left to right you can see that there are fairly reproducible, predictable changes that occur during aging. And this is the brain, but we know from other people's work as well that it's true for other organs.

And so we know for sure that there is a gene expression or there are gene expression changes during aging. The question really is do they have anything to do with aging and, if so, can we do something about it? So the three questions I want to address in this talk are the following: why do these gene expression changes happen in the first place? Do they underlie aspects of aging? Are they fundamental or are they just an associated event? And, if so, are the changes reversible?

And I think that the term reversible is worth emphasizing here because unlike mutations which, arguably, are irreversible, extremely difficult to reverse, what we're talking about here, if epigenetics really takes off in the way that it seems to be, it offers us a wonderful chance to intervene in aging and not just slow down this process but actually rapidly reverse it. And what I hope to tell you about in what's left of the talk is that once you understand aspects of what's going on at the cellular level and the molecular level, you can rapidly reverse aging. It doesn't seem to be that difficult, at least for aspects of aging. You know, we're not going to have old people suddenly look 20 years old. But I think that certain aspects, such as metabolic aging and diabetes, are rapidly reversible, at least in animal models so far.

So this is the idea that I want to put forward. It's based on many people's work and actually originally put forward in part in a theory called the heterochromatin hypothesis of aging. And the idea is that when we're young, when all organisms are young, they have an epigenetic profile and a gene expression profile that is youthful. And this functions very well and the organism is rather optimal.

You can see on the slide here that I've got gene A, B, and C and these could be microRNAs, they could be protein-coding genes. They could even be noncoding repetitive regions that need stabilization.

And what the evidence is pointing to more and more, and you'll hear more about this today, is that what's going on is that we get a reshuffling of epigenetic regulators across the genome as we age. For instance, I'm showing you HDAC1, SIRT1, SIRT6 and neurocomplexes here that are known to redistribute during aging. And, in fact, the first clues to this actually came from yeast. And Brian Kennedy mentioned sirtuins in yeast. Well, when we were working on yeast many years ago and, at the time, the idea was the DNA damage was the major cause of aging. So when Brian and others were looking for genes that regulated aging in yeast cells, we were expecting to find a DNA repair factor.

Instead what popped out was the sirtuin family, or sir proteins, which they showed were involved in epigenetic regulation. And this was really a remarkable breakthrough and we find ourselves today still studying this possibility in mammals.

And what I want to add to that hypothesis that seems to be confirmed in the last few years is that these factors are redistributing in response to DNA double strand breaks. We and others have found that a single DNA break in a chromosome in a yeast or a mammalian cell causes a large reshuffling of chromatin factors, including SIRT1, SIRT6, and Li-huei Tsai has published work on HDAC6, sorry HDAC1 as well.

And so what we think is going on is that, perhaps as we get older, that we are accumulating more and more of these DNA breaks and the cell is overcompensating trying to repair them and that perhaps the reset button to get you back to the original state is defective. It could be that chromatin leave scars, and we'll hear later about these effects. Judy Campisi and many others have now shown that there are scars left on chromosomes after DNA damage.

What we also are very interested in is how can we prevent them from overcompensating? And when I say "overcompensating," you might say, "Well why are you calling it overcompensating?" Well, the idea is that — and this gets to the third part — is antagonistic pleiotropy. What we think is that this reaction is adaptive, it's helpful in a young organism. When you have DNA damage, these factors move from transcription sites here and they go and they alter the chromatin, they help repair, and then they go back to where they came from. And the reason the cell, we think, does this is that these factors are involved in both processes. Of course, it's important to unravel chromatin and change transcription in this area.

But also, largely based on yeast work, what we find is that the genes that are derepressed during this process are typically involved in DNA repair and stress response. So what we think is that this is a short-term transient process in a young animal, young cell, and that the gene expression that comes on is helpful in the short term. But as you get older, this program becomes chronically activated. And so having a liver gene on in your brain, which we see, is probably not a good thing.

And so one of the things we've been working on, which I'll tell you about, is trying to activate and modulate some of these factors to see if we can either delay the process of aging or accelerate it and possibly even reverse the aging process in mice.

So one of the other things I wanted to mention, which is an emerging idea in the field, and this comes from a large amount of work now, mostly from *C. elegans*, is the role of mitochondria in aging and the ability of mitochondria to control the secretion of longevity factors.

What also is interesting in terms of epigenetic regulation is that there's one idea that I think holds some weight, and that is that perhaps as we get older the two genomes that are in our cells, the nuclear and the mitochondrial genomes, don't communicate as well as they should. It's a lot like a newly married couple. When you're young, you communicate really well. And by the end of your relationship, you're not really talking to each other very much, though I'm not speaking from experience. At least not yet.

But, in the cell, this seems to be happening. Actually, what we and others are seeing is that in old muscle in mice, as a good example, the ability of this protein PGC-1 alpha to communicate with the mitochondrial genome here, these circles, really goes down. But the good news is that once you understand what's going on here, you can rapidly reverse that within a matter of days. And so you can take a two-year-old mouse physiology and get it back to a six-month old quite rapidly. And so the idea is that perhaps we could find molecules that could delay or reverse this.

And SIRT1, which I'll talk about just in the last few slides, seems to be involved in this process and we're going to target this with some more molecules and see if we can delay or reverse disease of aging.

Okay, so some people saw my slides and they were asking me what the heck is this. Well, this is actually a slime mould and it produces an enzyme called Ppo1. Ppo1 is a DNA restriction enzyme that cuts very rarely in the mammalian genomes. In a mouse it's predicted to cut about 15 times. So what we've done is we've crossed this slime mould with a mouse and so we've got the ability to induce this cutting, rare cutting in the genome at ostensibly noncoding regions. And ask, "Is this hypothesis that DNA damage

causes this redistribution and the changes during aging?" We could ask, "Is that the trigger?" And the second question is, "Is it a cause of aging?" And if nothing happens, then their hypothesis may not be correct. If something happens, and what we predict is that the mice will get prematurely old, then we have something to study.

And so we did that. We can induce this with a drug. We induce it for either days or weeks. And depending on how long we induce these cuts, we get a stronger and stronger aging phenotype, which this is the first snapshot that was sent to me on an iPhone.

Anne Brunet, PhD: Maybe because we are slightly out of time, we need to wrap up.

Dr. Sinclair: All right, so that's the mouse and what I want to show you is, so this is evidence that perhaps epigenetic changes are actually underlying the aging process in a mammal that we need to characterize this mouse.

So just in the last minute I want to tell you about—

Dr. Burch: Your minute came and went, Dave.

Dr. Sinclair: Oh sorry. So these are molecules that activate SIRT1. We now know that they directly activate. And if you give them to a human disease, we can treat a disease called psoriasis. You can see that this patient, and there have been 40 already that responded well. And so this activator molecule called 2104 works effectively in the mouse. And this is in mouse studies for longevity and the results look promising. Thanks for having me.

Dr. Brunet: All right, so our next speaker is going to be Stuart Kim from Stanford University. Stuart works both in the worms *Caenorhabditis elegans* as well as in human and is interested in transcriptional networks and how they modify gene expression changes during the aging process in both organisms. Stuart.

Is there an underlying epigenetic clock that determines the rate of normal aging?

Dr. Kim: Thank you, Anne and Shelley for giving me this privilege to come and talk today, and the organizers too. This has been fantastic and inspiring.

So I want to talk to you about epigenetic clock that may be working in worms and in people. And so here's the idea. We'd like to know what is causing this. So here's us aging in about 60 years and here's a worm aging in just 2 weeks. And I'll tell you first a story here and then here. And the idea is to figure out what's different between these two states and then to figure out like what causes those differences.

And I came to this field from the field of genomics and systems biology. What I think may be possible is to try to define at the molecular level what is different between young and old. And, of course, right now we're using RNAs and we have defined 1,200 or so RNAs that change. I can tell you that we're also looking at proteins that are different between young and old, turnover rates of every protein between young and old, and not my lab but Gordon's lab and Cynthia Kenyon's lab is looking at all the proteins that are aggregating between young and old.

And now if we can define in the old state what's different from the young state at the molecular level, the next step will be to try to figure out like why does that happen. So what are the upstream regulators that cause genes to start here and these genes to go down and these genes to go up?

So the approach we took was to use the data from the modENCODE Project. My lab was one of the labs that participated in modENCODE Project. We screened about 100 transcription factors for ChIP seq and

then my lab took and looked at all of the 100 transcription factors for the transcription factors that tend to bind to these age-regulated genes because the transcription factor that binds to that could be a regulator.

And then that's how we got to this gene. So this slide is wrong, but L2 starts off high. It's a gata transcription factor. It starts off high. It binds to the age-regulated genes and then it goes low as the worm gets older. And as it goes lower, it's causing these genes to turn off and that's causing a worm to get older.

One thing that is surprising from this result is that this thing is a famous gene, but it's famous because of its developmental roles. This is the master regulator of intestinal function. And so what this was really quite surprising to us because already by middle age the worm has very low levels of this master regulator for intestinal function. This made no sense. And this worm then, before there's really overt signs of damage, it's already doomed because it's intestine has been reprogrammed. It's losing expression of the necessary intestinal genes. It's actually turning on neuronal genes and so in the worm, something very bizarre is happening in that the intestine is becoming reprogrammed fairly early in life.

We could show some of that by taking a young worm and turning off L2 prematurely. And you can see here when we turn off the genes early, this regulator gene early, the response looks like an old worm but even older than an old worm.

Now the opposite is true too. We could take and turn on L2 in the old age and this is like what David just told you about rejuvenating the worm. So we take an old and by turning on the transcription factor, we can reset the genes to a higher state that are younger. So this one looks younger, this one looks older; and because of this we think that this is evidence that we're talking about aging itself.

This worm lives longer. This worm dies faster. And this type of data then makes you think that we're not just making a worm live longer. We're resetting it to the younger state and then it lives longer. And so we're talking about aging itself.

Let me switch topics and tell you that we've done basically the same sort of thing but for kidney. So here is the kidney growing old. These genes are going up, these genes are going down. And we accessed the ENCODE data and looked through 140 transcription factors in ENCODE to find the transcription factor that seems to be regulating this pattern of gene expression.

So here's the way we do that is we're taking and this is an unbiased view. So I'm not actually making a guess and only testing my guess. I'm saying which of the 140 makes the most sense. And there the big winner was a transcription factor called STAT3. STAT3 then starts off at an inactive state and then as the kidney gets older, becomes more and more active. It's binding to the age-regulated genes in the kidney and then it changes the expression of those genes.

We can also show that by turning down STAT3 we can rejuvenate the transcriptome. By turning up STAT3 we can cause a premature aging of the transcriptome. Same as in worms. And we think that has something to do with the kidney going from a young state to an old state here and that we can explain some of the molecular changes between young and old for the kidney.

Now some of the stuff has changed how I think about aging and doing this stuff. So I thought I would tell you about some of the things. One thing that we found is that a lot of the stuff we found is like a reprogramming of tissues and that the young intestine and the old intestine are really fundamentally different. And that the intestinal differentiation genes are off and neural genes are on in the worm. In the kidney, STAT3 is a very powerful transcription factor and so that the old state is not simply the young state plus damage because the whole transcriptome has changed.

And that leads me to think that if we can get our hands on these regulatory molecules, we can change not just one protein at a time. We can affect hundreds of proteins by turning up or turning down the nodes that are causing the age-regulated change. And then because we can switch the transcriptome from the

old state into the young state, I think that's evidence that we have molecular means to try to do rejuvenation. And so it may be possible to, by resetting the regulatory factors in epigenetic aging, to try to reset the epigenome from the old state to the young state.

Thank you very much.

Dr. Brunet: Great. Thank you, Stuart. So our next speaker in this session is going to be Li-huei Tsai from the Massachusetts Institute of Technology. Li-huei is also the head of the Picower Center at MIT and she works on mouse models for Alzheimer's disease moving into humans. And she's very interested in the role of epigenetic regulators, in particular histone deacetylases but also others in this process. Li-huei.

Epigenetics in cognitive aging and neurodegeneration: a slope or a switch?

Li-huei Tsai, PhD: Thank you, Anne. Good afternoon, everybody. So today I would really like to discuss with you I think a fascinating relationship of neuronal activity in the nervous system, the epigenetic landscape, and cognitive function.

So neuronal activity in the nervous system really gives rise to everything we know about ourselves and about the world. And, unfortunately, over the course of normal aging and more dramatically during neurodegenerative disease, neuronal activity is reduced and, therefore, robbing us of our normal cognition, memories, and independence, for instance.

So today I really want to argue for a model where there is this gradual downward slope of neuronal dimming. And I want to argue that this is a driving force for neurodegeneration. So let's just test this idea looking at the existing data about a very devastating disease which is the most common cause for dementia, Alzheimer's disease.

So Alzheimer's disease presents with a very stereotypical pattern of beta amyloid plaque pathology in certain parts of the brain and this part of the brain and these neurocircuits happen to be the circuits that mediate higher order memory and cognition function. So during Alzheimer's disease, this is these particular circuits that actually show gradual reduced neuronal activity. So this was data generated by human fMRI imaging studies, particularly from Randy Buckner. And even more dramatically these are the circuits that eventually display neurogeneration, neuronal loss, and eventually atrophy of the brain.

And a large body of literature in neurobiological studies also show that these beta amyloid peptides, they can reduce glutamate synaptic transmission, reduce synaptic density, reduce synaptic plasticity and impaired cognitive function. So I think when you put all of this together, systems neuroscience and neurobiological studies, I think the picture emerges, at least to me, is that the beta amyloid peptide deposition in certain parts of the brain leads to reduced neuronal activity and this trigger further cascades of events leading to neurodegeneration.

So how about at the molecular level? So what we did was to compile a lot of the published data from gene expression profiling studies in normal brains and in Alzheimer's disease brain. So we compiled this data. You know, altogether there are about close to 200 of normal and Alzheimer's disease subjects. And it turned out that all of these studies are actually quite consistent with each other and show a common trend of differences in gene expression.

So not surprisingly, one sees a huge reduction of genes involved in synaptic transmission, synaptic plasticity, other neuronal functions. And you have heard a lot from this morning, too, very profound inflammatory response, immune response genes and stress response genes, they are associated with aging and also tremendously conspicuously with Alzheimer's disease. And these genes are very conspicuously upregulated.

And I should also point out that this preponderance of increased immune response gene expression is consistent with a lot of the recent genome-wide association studies on Alzheimer's disease where there are quite a large number of genes involving immune response and inflammation, did dysregulation contribute to increased risk of Alzheimer's disease?

So with these differences in gene expression, how about at the epigenetic level? So the large-scale, genome-wide epigenetic study at least so far has not been published in human subjects yet. So here I'm showing you a study carried out in the animal model of Alzheimer's disease. This model really pretty nicely recapitulates Alzheimer's disease pathology and phenotype with profound neuronal loss and a very impaired cognitive function.

So, first of all, you can look at the gene expression profiles. Really, this faithfully recapitulate human gene expression profile that I just showed you so there's reduced expression of synaptic transmission genes and huge increase in inflammatory and stress response genes.

So this is a complicated profile of the epigenomic results carried out in two different stages of the mouse model. In the early stages, this is presymptomatic, there's no neuronal loss or no symptoms; and then there's a later stage with profound neuronal loss and cognitive impairment.

So there are epigenetic or chromatin activation marks and silencing mark performed here. So this is basically chromatin immunoprecipitation followed by deep sequencing. So all I just want to point out is that the epigenetic changes really are very consistent with gene expression changes. In terms of the activation mark, they are reduced in those genes involving synaptic transmission and those marks are increased in the genes involving inflammatory response. And, similarly, the silencing marks show a converse profile.

But there is something here very striking that I want to point out. Okay? So remember in the early stage, this is asymptomatic in a mouse, but one can already see very significant changes in the epigenetic landscape and gene expression also starts to show Alzheimer's like profile. But I think the interpretation here is that even with these changes, the epigenetic landscape still maintains a certain plasticity. Therefore, when one subjects these animals to behavior training, these animals are still capable of responding to the stimulation and induce proper gene expression to support learning and memory function. But at some stage when the epigenetic changes go further and eventually locked into an inactive state, at this point, physiological stimulation can no longer trigger the proper response to support learning and memory.

So in terms of the recent effort in modulating the chromatin landscape to achieve beneficial effect, I think there are quite a few studies now from my laboratory and from many other groups showing that manipulating histone deacetylase, especially inhibition of these enzymes, can be beneficial. And my laboratory and also others show that especially histone deacetylase 2 specifically inhibits the expression of genes involving learning and memory, synaptic plasticity, and so on.

So this treatment has been shown in numerous Alzheimer's model to recover cognitive function. And here I'm just showing you gene expression profile following HDAC inhibitor treatment. And I find this data is very, very compelling. So these are gene expression of control mice without neurodegeneration animals, Alzheimer's disease model without treatment and Alzheimer's disease model with treatment.

So, again, this model shows stereotypical Alzheimer's-like changes in gene expression. But following the treatment, you can see that synaptic functioning genes now their expression is recovered, which is not surprising I think. But what's really surprising are this group of genes. Their expression are tremendously increased in the Alzheimer's model; but following HDAC inhibitor treatment, they now show a much reduced expression pattern such that the treated mice show a pattern much more similar to the control mice then the Alzheimer's mice. So this suggests that even though the epigenetic landscape is locked in an active state modulating the chromatin landscape, still has the capacity to reverse the inactive state of the chromatin.

So, in conclusion, I just want to explain, again that I would like to argue for an activity-driven model of Alzheimer's disease pathology. I would like to propose that amyloid deposition in certain brain regions lead to reduced neuronal activity, and this induces cascades of events involving epigenetic dysregulation, transcriptional dysregulation leading to cognitive decline. And I also show you some promising evidence that even when the chromatin is locked in the inactive state, modulating chromatin modifying enzymes can still maybe have the potential to revert the pathology.

So, finally, model and perspective reduced neuronal activity can lead to decreased synaptic plasticity genes and increased neuroinflammation and stress response genes. And we can discuss later, but I sort of feel that these two classes of genes almost have this reciprocal kind of relationship. And I also show you that once the epigenetic landscape is locked in the inactive state, physiological stimulations are unlikely to reignite neuronal function necessary for cognition. But pharmacological modulators of chromatin-modifying enzymes can restore an active chromatin landscape and recover cognitive function.

And I think you for your attention.

Dr. Brunet: All right, thank you Li-huei. Our next speaker is going to be Peter Adams from the Beatson Institute and the University of Glasgow. And Peter is proposing a new model that cancer is an epigenetic disease and not just a disease of genetic modification. So, Peter, thank you.

Why does the incidence of cancer increase with age?

Peter D. Adams, PhD: Thank you very much for the invitation to speak and also for organizing this fantastic meeting.

So I'm going to talk about cancer and aging; and, specifically, I'm going to ask the question, why does cancer increase with age? So, as we all know, the incidence of many cancers increases with age and, in fact, for many or most cancers age is the biggest single risk factor for cancer. We've also heard today that the link between aging and cancer is further underscored by the demonstration of various manipulations, whether dietary, small molecule, or genetic. Those interventions which extend lifespan will often suppress cancer. So, as I said, the critical question is, why does cancer increase with age?

And I think it's true to say that within the cancer field itself, there is a general assumption that cancer increases with age because growth of a cancer is a multistep process that depends upon multiple genetic and epigenetic alterations. And so there's a long history for this going back to the 1950s from epidemiological modeling data from Richard Dahl and Carl Nordling, Knudson to hit hypothesis, demonstration of oncogene cooperation by Bob Weinberg in rodent and then human cells, a genetic paradigm in colon cancer established by Bert Vogelstein and coworkers depicted here, and, obviously much more recently, whole genome and epigenome sequencing efforts have uncovered hundreds or thousands of genetic and epigenetic alterations in cancer.

As I say, this model has been extended to include epigenetics because it's now in that, for example, epigenetic silencing of tumor suppressors such as APC can drive inactivation of those tumor suppressors instead of their genetic mutation. But I think what we need to remember is that just because cancer is a multistep process doesn't mean that it takes 50, 60, or 70 years for those events to accumulate and for the cancer to manifest itself. That doesn't automatically follow.

And so, in actual fact, I think if we look closer at that idea, there's actually quite good evidence that it's more complicated than that. So, for example, there are cancers which have a large number of genetic alterations but their peak incidence is much earlier. So, for example, osteosarcoma has a highly complex genome and yet its peak incidence is in young adults and adolescents.

People such as Jan Vijg, who's here in the audience, have actually measured mutation rates within development and growth of young organisms and then into aging. And what these studies tend to show

is that the rate of accumulation of mutations is high during development and early growth, as you might expect, because that's when there's most cell proliferation going on. But then if you actually look in adults, and this is mouse aging here obviously. In adults, the rate of accumulation of mutations is variable between tissues but actually quite often relatively slow so that the gray line here — and this is a figure from Jan — shows the survival of these mice which approximates to the inverse of cancer in these mice because most of these mice die of cancer.

So what you can see is that the cancer incidence increases exponentially in late life, but the accumulation of mutations is relatively slow throughout life. So there's not a clear relationship between the accumulation of mutations and the onset of cancer.

And, finally, going back to the Vogelstein model that I mentioned earlier, the so-called Vogelgram, turns out that it takes about ten years to go through that entire sequence from an adenoma to a carcinoma. So that model doesn't tell us why the average age of diagnosis of a colon cancer is 72 and why we don't even screen for colon cancer before the age of 50. So we don't know what's going on in that first 50 years.

So I think putting all this together, I think we should think about a revised model. And this isn't my model. This is very much a synthesis of other ideas, which are already out there but I think need to be thought about more within the field, and that is the idea that even in young, normal tissues, we already have a certain number of mutations, oncogenic mutations. But those oncogenic mutations are actually quite well tolerated by the tissue.

As we age, additional mutations accumulate and the development of the cancer is driven by the interaction of these mutations with other changes to aspects of molecular cell and tissue biology. But the key thing about these changes, that these are essentially progressive age-associated changes to aspects of cell and tissue biology which are essentially quite plastic. So that could refer to epigenetics, metabolism, the immune system, inflammation, stem cell niches; pretty much anything you can think of apart from DNA sequence. And so epigenetics is a very good example of this because we know that in young, normal tissues, most CPG islands are in an unmethylated state.

In a cancer, many CPG islands are methylated and it's thought that that methylation contributes to silencing of tumor suppressor genes and progression of cancer. But we know from the work of Theas Hillstay and Jean-Pierre Issa that this transition from unmethylated to methylated can actually be progressive through age. So if you look in histologically normal tissues, you can see these intermediate states of methylation suggesting, as I say, that this is a progressive process. So essentially a shift in a dynamic equilibrium of these processes.

So why is this important? First of all, what we're finding from targeted therapies in cancer, and most of these targeted therapies are directed towards the dominant genetic alterations within those cancers. So, for example, Vemurafenib in melanoma is directed toward the activated BRAF oncogene. Most of those targeted therapies in advanced cancers actually have a relatively modest benefit for the patients.

So I, and I think many others, believe that really to beat cancer what we're going to have to do is to do risk assessment, early detection, and chemoprevention in young to middle-aged adults. And at the moment, we don't know how to do this.

So in terms of what I would consider to be a way forward, I think we need to continue mapping age-associated changes in these diverse aspects of molecular, cellular, and tissue structures and organization. Those changes need to be modeled. Those age-associated changes need to be modeled onto *in vitro* and *in vivo* models of cell transformation and tumor genesis. Obviously, we need inhibitors to these changes. And those inhibitors need to be tested in mouse models that are designed to recapitulate the age dependence of cancer.

And we've heard about mouse models already today and the problems with some of them. And I think in the cancer field, this is a particular problem. So many of the mouse models commonly used in the cancer field, they're actually doing now a pretty good job of recapitulating the genetics of human cancer, but I would argue actually a very poor job of recapitulating the age dependence of human cancer. And I think there's various reasons for that that we can discuss. And that's not simply because a mouse only lives two or three years and a human, obviously, lives much longer than that. So a more fundamental problem.

And so with that, I'm done.

Dr. Brunet: Thank you, Peter. So our next and last speaker is going to be Juan Carlos Izpisua Belmonte from the Salk Institute. And Juan Carlos is going to talk to us about using iPS and reprogramming to model aging and age-related disease and ways that epigenetic changes can be used in that context. Thank you, Juan Carols.

Can stem cell models provide new insights on the mechanisms of human aging?

Juan Carlos Izpisua Belmonte, PhD: First, I would like to thank the organizers and Ann and Shelley for their kind invitation. It's a privilege and an honor to be here.

I was asked to discuss how cell reprogramming could give insights into aging and with the particular focus of the epigenetic session of this afternoon. The reprogramming of a cell that is adding these four factors of any somatic cell and obtaining a pluripotent cell is, in fact, an epigenetic rejuvenation experiment. You see global demethylation, opening of the chromatin, and regulation of RNA and others.

But what this experiment allow us is from these pluripotent cells that we have generated to differentiate to any of the cell types that we have been discussing today here that could be the target for aging and then re-establish these tissue-specific epigenetic marks, the aging marks. And any of the first basic experiments will go as follows.

So what you would do is to identify a particular gene that is involved in any of the different aging factors and create two different cells — one in the absence of that gene and the other one exactly the same one, a perfectly isogenic line where you have corrected these mutated genes. And from there, from these two pluripotent cells, then differentiate then to different cell types.

Two examples of this experimental setup is, for instance, this one here. The Hutchinson-Gilford Progeroid Syndrome. This is a mutation on lamin A and one of the characteristics of this mutation, as you can see in the middle panel, is the alteration in the nuclear envelope. This alteration in the nuclear envelope will have really dramatic consequences for the aging of these kids.

Once the gene is corrected, then we have perfect situation where we can study during differentiation how these phenotypes develop. And these also, here is another example, help to uncover novel hallmarks of disease related to aging. For instance, Parkinson's. Parkinson's is a disease that certainly correlates with aging and perhaps the gene that is more often affected in Parkinson's is these LRK2 genes, but it happens as well to correlate with alterations in the nuclear envelope. And these phenotype that was not previously known, that was obtained through this technology, certainly reflect what is happening *in vivo*.

Here you can see to the right, here to this side you can see a brain from a patient affected with Parkinson's. And you can see how the alteration in the nuclear morphology is clearly displayed compared to the control one. So what these models give us is not just novel features and hallmarks of the disease, but after looking at these and other models like, for instance, the Werner Syndrome model, the conclusion that we can extrapolate from all these progeria model and some disease-aging associated models is that they present epigenomic changes that are characteristic of a global open chromosomal structure, what Shelley told you at the beginning of the session, characteristic of the aging process.

Now the idea is that this technology could help us to understand better the disease, perhaps to even introduce and challenge the cells where they differentiate an age with chemical compounds and eventually they can help us in cell therapy and rejuvenation.

Now it is clear that the genetic factors, and I showed you a couple of examples, the role of these factors can be addressed in this model. But what about the role of epigenetics? And for that there is one tool that we still need to develop and that is the tool of the epigenome editing because this will allow us to really make shifting of these whole genome expression program and perhaps better understand physiological aging.

One of the major problems that the field has is the differentiation of particular cell types and why that is a problem. This is a problem because we do not have any protocol in the field that leads to a postmitotic cell. And this is very important, as you can imagine, in the idea of aging. We can really not try to attack a major problem. We can go after replicative aging, but we cannot attack in a study chronological aging until we don't have protocols that lead to postmitotic cells.

Another problem is that cells are not organized structure, they are not organs. And certainly the conclusions that we obtain from cells cannot be immediately extrapolated. And the future directions, the near-term future directions, an open question that I think that this field faces in order to help and give insights into the field of aging, I have summarized them in three questions.

One of them to try to know whether the epigenetic is cause or consequence of the aging process perhaps can be helped by the derivation of iPS from old and young individuals. And after analyzing of these epigenomic recapitulations perform epigenome editing on these cells.

The other one to develop protocols for differentiation of postmitotic population that will give us tools to study cellular division or time for replicative versus chronological aging.

And the third one is a question that cells probably are not enough and to study whether organ models can be used to model physiological aging. And in the last few months we are seeing how different laboratories can develop 3D protocols for the generation of many organs *in vitro*: brains, liver, and we recently are developing a protocol for kidney.

And this is all what I wanted to say. Thank you.

Wrap-up

Dr. Brunet: So could everyone from this session come up for the panel. And, all right, as our panelists are taking their place, I will do a brief summary of this session.

So I guess the two key concepts that are brought about by this epigenetic and aging sessions, one is the possibility, the concept that epigenetic changes are long-range, long-lasting type of changes. And the other key concept is that epigenetic changes have this amazing capacity of being reversed or reversible. And this brings the notion of the rejuvenation or the reversal of an already old or an already diseased organisms.

So I think really, in summary, it's clear now that there are epigenetic changes changing to the genome during the aging processes. It is clear from the talks that one can manipulate the enzymes or the transcription factors that do impose those changes on the genome accessibility and the gene expression, and that that those changes can have some impact on the reversal of some hallmarks of aging in some disease.

It is also interesting that cancer, one of those disease, could be a disease of epigenetic. And I think there are two revolutions in the field that touch on epigenetics that are very interesting for the future. One is the capacity to model some aspects of aging and aging-related disease with cellular models; and we've that.

And the other one that you've seen in many of the slides with those large-scale data is really the revolution in genome technologies, whether it's RNA seq, microarrays, chromatin immunoprecipitation. And this really, for the first time ever, gives us the capacity to look at changes in the genome at really a very fine resolution which finally empowers us to understand and also manipulate more specifically those changes. So that's for the summary.

In terms of challenge, I will, of course, leave that to the questions; but I will just pose a few challenges. In terms of first drugs that can manipulate the epigenetics, are they going to be safe, are they going to be effective against diseases? It seems that at least some of them will be so that's very exciting because cancer and the neurodegenerative or degenerative diseases are almost antagonistic of one another. One could wonder whether some drugs that are designed for one disease, let's say cancer, might have a degenerative effect and vice versa. So one needs to think about that in the challenges. So that's for the disease aspect.

And then for the prevention aspect, it's very interesting to see all these other panels because it's interesting to think about epigenetic as maybe an underlying theme in many disease. And if it is, it could be an underlying theme that could be prevented perhaps by those of the interventions that have been mentioned, natural interventions that have been mentioned to be so good with health such as dietary restriction, exercise, lowering the stress levels. So could they have an impact in resetting epigenetic changes and long-lasting effect from early development to later and even being passed on upon generation and transgenerational epigenetic aspects. So that's for the challenge. And now I will open the session to the panel for questions.

Discussion

Dona Love, PhD: Hi, Dona Love from NIDDK. And this question is for Stuart Kim. I was curious about your microarray studies. Was that done in the whole animal or have you done tissue-specific analysis? Do you find any tissue driving the aging process more than others? And then I wanted to give you a chance to expand on the old does not equal young plus stress.

Dr. Kim: So, first, with the work I showed was a human kidney and we could do the— In that paper, we did the cortex and the medulla. We've also done a fair number of mouse studies. So we looked at, with Kevin Becker, we looked at 17 different tissues in the mouse. We did the game of like which tissue was aging the fastest. We did other experiments. We tried to figure out which tissues were aging in synchrony. And so amongst the different mice, we could ask is the mouse with the oldest heart also the same mouse with the oldest brain kind of thing.

Dr. Love: I'm guessing in *C. elegans*. That's what I was curious if that's possible.

Dr. Kim: In *C. elegans*, you know, we have technologies to do tissue-specific expression in *C. elegans*. One way is we can express an RNA binding protein that grabs the RNA but only in the tissue that is expressed and immunoprecipitate sequence that RNA. But in the work I showed there, you can just do an informatics study on that and just say, "Of the 1,200 different genes, what tissue do they come from?" And if you do that, you find that most of them are in the intestine. So that's the big winner in what's changing between young and old worm.

Another one that changes is the skin. And something that doesn't change, like not one gene do we find different, are neuronal genes. So we would love to try to get an angle into neuronal aging, but I think worms are pretty well preserved for neuronal aging. I think Monica Driscoll's lab has made some good inroads into possibly studying neuronal aging in *C. elegans*; but if you just ask what's changing molecularly, there's not much changing.

Dr. Kotsanis: Constantine Kotsanis from the Kotsanis Institute. All day today I heard wonderful presentations on biochemistry and very little was touched about the physics. And the question is how important is voltage as an epigenetic factor?

Speaker: How important is?

Dr. Kotsanis: Voltage and electromagnetism.

Dr. Berger: Are you talking about changes in charge inside?

Dr. Kotsanis: Yes, yes, voltage. Like the cells have minus 70.

Dr. Berger: Right. So these modifications, some of the modifications we're talking about such as acetylation actually changes the charge in the DNA chromatin polymer. So there's definitely changes in charge that lead to changes in structure in the chromatin. So I think there are changes.

Dr. Kotsanis: Does the voltage drop when you have the changes?

Dr. Berger: Voltage drop.

Dr. Brunet: You mean in the neuronal cells, for example?

Dr. Kotsanis: Everywhere. Everywhere in the body. Neuro cells especially.

Dr. Brunet: Do you know, Li-huei?

Dr. Tsai: So that's the whole point. If we want to talk about like the excitability of nerve cells during aging and especially in Alzheimer's disease, it really is very tremendously reduced. And even when you use electrode or all kinds of methods to try to excite the cells, the cells no longer respond properly. So I think that is a primary problem of cognitive aging and cognitive impairment.

Dr. Kotsanis: Yeah. We know when the protein is active is a higher voltage and when it goes back to normal or to a quiet stage, it drops voltage to congregate. So if you have low voltage to begin with, you will lower then to a lower voltage state which will also promote epigenetic disease, wouldn't it?

Dr. Brunet: Yeah, so that's a possible interesting point. So I'm going to take another question then.

Josh Mitteldorf, PhD: I'm Josh Mitteldorf. I do evolutionary biology. I'm from Philadelphia. I have a conceptual idea I'd like to introduce. A theme that I hear in this session that's so exciting is that not only are there epigenetic changes that are clearly differentiating young from old, but there are consistent patterns that everybody has the same genes turned on and turned off as they get older. And this is really surprising from an evolutionary perspective. Why would evolution put up with these gene changes, gene expression changes that are self-destructive? We know that gene expression is tightly under evolutionary control, that evolution cares a lot about what genes are expressed when and where.

So I want to introduce a concept that people who aren't in the field may not be familiar with, that there's a revolution going on in the evolutionary biology field now from the selfish gene idea which has predominated the last 50 or 60 years to a more communal idea that often what's good for the community can evolve even when it's bad for the individual.

And my suggestion is perhaps what we see in gene expression changes is a result of evolution at the group level. Aging is bad for the individual. Destroys individual fitness but aging is necessary at the group level to preserve, for example, population homeostasis, aging levels out the death rate. Maybe this is the deep meaning behind the epigenetic changes that we see.

Dr. Brunet: Who wants to take that?

Dr. Tsai: I can try first. I don't know about aging at the group level, but you mentioned earlier about some cellular mechanism created to just destruct the cells. I would like to argue differently.

So remember this morning Brian Kennedy's talk. You know, it's really because of the medical advances and people live longer and longer. So, in ancient times, people really only had a very limited lifespan. So when I talk about reduced neuronal activity, and I actually believe that this process operates say during nervous system development. So during development the system tends to produce exuberant processes and synapses and so on. And if you have too much of good things, that actually can be bad for you as well.

So there's this mechanism to eliminate certain unwanted synapses or neuronal processes, dendrites, and so on if the activity of those population is lower than the system especially, actually, if you look at Ben Behr and Beth Stevens publication on *C. elegans* that when certain synapses show lower activity, that the immune system microglia actually gets activated and they will preferentially attack those synapses and then eliminate those synapses. And that's a very important normal process for proper brain development.

And my hypothesis is that this system is more adapted during aging and during neurodegenerative disease that when there is a systemic reduction of excitability due to beta amyloid accumulation or other stress, oxidative stress and so on, this same system gets activated again and that becomes detrimental. So I will stop here and see if other people—

Dr. Brunet: So maybe one answer from David and then we move on to our next question.

Dr. Sinclair: So I'm a disciple of the old school. So Williams and others have gone into this in depth. And as far as I know, there isn't a big revolution in terms of group selection for longevity. I think that would be a discussion over some beers later. But in general, in our field, at least within our circles, there is not a belief yet that, certainly for humans or for any larger life forms, that there's group selection within individuals.

That said, you raise an important point that within an individual let's say, cells within our body they are acting communally because they need to all survive together. And perhaps some of the changes that we see here are adaptive and beneficial to the organism, but not so much for themselves, the cells. And so the senescence program that we've heard about is a good example of that. The cells will arrest themselves to benefit the rest of the organisms, but, again, there are always tradeoffs to that as well getting to antagonistic pleiotropy.

Dr. Berger: I could follow up with that. In the ant societies it's exactly like this only on the organismal level that individuals have very short lifespan. Males lives three months, workers live two years, and queens live ten years. So there's this huge disparity. So, again, for the good of the colony, the individuals carrying out different tasks live different lifespans. So I agree with David's point of view.

Dr. Brunet: Thank you.

Dr. Mitteldorf: That sounds to me the opposite of what David just said, but I agree with it.

Dr. Berger: No, no, the individuals in the— You have to think of the colony the same as one soma. And in that case, each individual cast is sacrificing its lifespan for the good of the soma.

Dr. Brunet: Question on the right.

Chunyu Zhang: What an exciting session. My name is Chunyu Zhang from The Johns Hopkins. I have a question for David and Li-huei. I know the time limitation so you didn't explain a little bit about the HDAC1. My question is did you see aging depend on increase or decrease HDAC1? How HDAC1 did location, I mean, did distribution between young and old? These are the ones that, yes, this is for David.

Dr. Sinclair: The HDAC1 work I think is better for Li-huei to answer. That's her work specifically. I would say let Li-huei talk about that.

Dr. Tsai: Okay, so there are actually quite a few papers published about HDAC1 distribution. So it's a histone deacetylase so normally it functions in the nucleus. It has a number of nuclear substrates. But during aging and during toxicity situation, HDAC1 seems to redistribute to the cytoplasms. Okay? So this seems to represent HDAC1 loss of function scenario. So, in other words, it seems that HDAC1 activity is downregulated during aging.

Dr. Zhang: Uh huh. So because HDAC inhibitor is not very specific, how do you address specificity related to HDAC?

Dr. Tsai: Yeah, so it's a very, very good question. Right now all the available small molecule HDAC inhibitors cannot differentiate HDAC1 from HDAC2. So, clearly, you know, more chemistry needs to be done to achieve more selective inhibitors.

Dr. Zhang: Okay, thank you.

Dr. Brunet: Question.

Speaker: My question is mostly for Li-huei. In your mouse study, it seemed like the transcriptional change is gradual, that in the end you end up with upregulation of inflammatory genes and downregulation of neurogenesis type of genes.

My question is that, you know, if you really provoke its amyloids, then that seemed to be a bit late. I wonder if the field considered the possibility that it's actually the glial cells somehow has gone haywire and has become inflammatory and then destroying the neurons. So one question then following that is when you analyze gene expression, did you separate glia and neuron? And what does it look like if you separate that?

Dr. Tsai: Great questions. So right now I think for all the epigenomic/epigenetic studies in the field, the most important task for everybody is to do to cell type specific analysis. This is not easy to do it in the brain. I mean, in mouse, probably one can use a genetic marker. For instance, expressing GFP and the result have specific promoter and then isolate those cells. That's probably still possible. But in postmortem human brain, it really represents a very tall order to isolate specific cell type.

What I think one way that possibly can get around this dilemma is what Dr. Belmonte present. They actually used the induced pluripotent stem cell model. There you can try to induce a cell to a pure neuronal population versus a pure glial cell population and do your gene analysis separately.

Looking at the profiling, right now I would bet my money that most of the downregulated genes represent neuronal genes. Most of the upregulated genes represent glial genes, such as microglial or astrocytes but, clearly, that needs to be validated with better models.

Speaker: So do you or the field believe that one driver of Alzheimer's is actually glia more so than the neurons?

Dr. Tsai: That depends on what's your definition of driver. If you talk about the initial event.

Speaker: Well, yeah.

Dr. Tsai: There has to be a trigger.

Speaker: The trigger is probably inflammation caused by glial genes. Something intrinsic happening to the glial cells and then that kind of sets off the fire.

Dr. Franceschi: Okay, I have two questions. We did in our lab recently two genome-wide studies on DNA methylation in centenarians and their offspring and in Down Syndrome as a model of accelerated aging,

their mother, and their unaffected sibs. And, apparently, the epigenetic changes cluster in families. So the question is are the epigenetic changes which occurs during aging are genetically controlled? And the second question is in Down Syndrome, for example, of course, the chromosome 21 is deeply affected but there are many other chromosomes which are also affected; not all at the same way. So we wonder if is the global architecture of the chromatin which matters to understand what is going on with these epigenetic changes with age.

Dr. Brunet: Maybe like, Peter, you want to comment on that and the cancer aspect?

Dr. Adams: Yeah. Well, I can at least comment on the first part, the extent to which the epigenetic changes are genetically determined. And I guess, therefore, how they might differ between individuals depending upon the genetic makeup of the individual, SNPS and what have you. I think it's a very good question. I'm not sure we really know the answer at this point. My guess is that there is a kind of a genetic determination, to some extent. But I think one of the things that we know about chromatin is that it's inherently a quite plastic, dynamic, stochastic structure. So, for example, even going back to the phenomenon of position effect varigation in flies, we know that it has an element of stochasticity about it.

So I think, you know, the genetic perhaps predetermination versus the inherently stochastic behavior of the chromatin are going to play off against each other, as well as, I guess, environmental influences coming in as well. And all that is going to feed into the endpoint. I don't think we know the relative contributions of those three factors at this point. I think that's a major question.

Dr. Brunet: If I can just add on that. Like there are recent papers from just in the last issue of *Science*, actually, where people have looked at - Mike Snyder, Jonathan Pritchard, they've looked at SNPS and how they influence chromatin changes and, indeed, there is a link between QTL, so quantitative trait loci that have been identified on the genome and changes to the chromatin. Those are correlative, but still they point to exactly what you are talking about. So that's pretty exciting, in fact.

Dr. Franceschi: And the fact that chromatin is affected, the different chromosomes are differently affected?

Dr. Brunet: For the Down? Well I would imagine, sir, that—

Dr. Franceschi: Well even in centenarians and in their offspring.

Dr. Brunet: In general? Yeah. It would be interesting, actually, to overlap the data that they got with some of your data in centenarians because it may be that, indeed, they correspond to the same loci. It might be very exciting, in fact.

George.

Dr. Niederehe(?): So I was going to ask Li-huei a question, and I'll get to that in a minute; but the discussion is raising another question I wanted to clarify starting with Josh's discussion. So if it's determinative adaptive, so it's like determinative sequential gene action in development, and you go onto into an adult life, certainly, the changes that you find in neoplasms, right, Peter, where you're silencing a tumor suppressor, it must be stochastic basically. And then you have lots of evidence of epigenetic drift.

Jan Vijg has given us good information about drift in post replicative cells. But to clarify, to make sure that what Josh was saying, everybody gets the same genes on, the same genes off, my notion is that there's tremendous varigation and it's a lot of stochasticity. So fill us in on that and then I'll ask my quick question about the cognitive story.

Dr. Brunet: Stuart.

Dr. Kim: I mean, the data is what it is so, you know, not making any sense. If you just look at the tissues between young and old, you do see reproducible changes in gene expression. So the issue is not could it exist, but what are reasonable mechanisms that you can use to understand how it exists?

You know, I've talked with— One of the mechanisms that's consistent with the evolutionary theory is that all of this is beyond the force of natural selection. We are just watching events happen beyond the force of natural that happen to be reproducible. It seems to me like cancer is not an evolved program. Cancer has transcription factors. Myc is a transcription factor. Results in very reproducible changes. P53 results in very reproducible changes in cancer but that's not how they evolve. They evolve for their positive roles during development and everything we're talking about could— Everything we talked about has a positive role sometime during young adulthood and we're just watching what happens.

And Brian showed, and I think all of us agree, that when we're talking about these 80 year olds and these old worms, they not, obviously, happening in nature just like cancer isn't an evolved property. We could still study it as something that happens very reproducibly. Just like the Weinberg pathway and the Vogelstein pathway is really quite reproducible, it's not stochastic; cancer isn't a bunch of stochastic things that just happens willy-nilly because it didn't evolve. P53 is a highly evolved mechanism that happens and it happens to just be one of the ways that you get to get cancer, but it is reproducible in cancer. So it's possible for me to just believe that one of the ideas is that when aging, you see reproducible things happen. But not because it evolved for old age.

Dr. Brunet: And, David, you want to add a brief like something, follow-up?

Dr. Sinclair: Yeah. Just so Jan doesn't run up on stage and scream. To speak up for his work, he's been able to show at the single cell level that there is great variability within a tissue between individual cells. And where I think the middle ground will turn out to be is that, and we agree with Stuart as well that if you look at larger amounts of cells, it evens out and you can see definite— What looks like a program, though probably is not, but in my talk, and I have another ten slides if anyone's interested. Maybe another time. But what I was trying to get across was the idea that random events, such as randomly placed DNA breaks, can give rise to what looks like a program or reproducible effect because it's a cell's response to that random damage. So I hope that helps.

Dr. Brunet: So you have another question maybe, George, or that's it?

Dr. Niederehe: Just really quickly, I also wanted to ask about the histology of your mice with AD model. Do you still see gliosis? Do you still see microglia that are still activated? Is there shift and, also, can you measure synaptic density?

Dr. Li-huei: Yes, tremendous.

Dr. Niederehe: Does the synaptic density come back?

Dr. Li-huei: Hugely decrease.

Dr. Niederehe: It does decrease?

Dr. Li-huei: In the Alzheimer's models.

Dr. Niederehe: Oh yeah, but I mean with your HDAC inhibitor.

Dr. Li-huei: Oh.

Dr. Niederehe: Is it come back?

Dr. Li-huei: Yes.

Dr. Niederehe: You can actually quantitate?

Dr. Li-huei: Very dramatically. Very efficiently.

Dr. Niederehe: That's cool. Yeah.

Dr. Li-huei: Yes.

Dr. Niederehe: And quickly tell me your cognitive assays.

Dr. Li-huei: Really, mainly in mouse the easiest to do is hippocampus dependent cognitive function so there are quite a few tests one can do — spatial memory assays, associative memory, object recognition, object location. So there are quite a few different kinds.

Dr. Niederehe: Quite a few, okay.

Dr. Li-huei: Yeah.

Dr. Niederehe: Thank you.

Dr. Brunet: All right, one question here.

Christian Sell, PhD: Hi, Chris Sell from Philadelphia. So my question is triggered by Peter's talk and his analysis of the exponential rise in cancers with age. And it made me wonder about a threshold effect within the chromatin. Does chromatin epigenetic remodeling have a threshold effect such that large regions of the genome are remodeled rapidly, such as during the iPS dedifferentiation process? And, if so, does that have implication for aging? So you're getting to a certain point suddenly you've got that trigger that's creating a propagation wave of changes that may have implications for the late life diseases.

Dr. Adams: Yeah, that's a really interesting point which I hadn't thought about. But I think just thinking about the biochemistry of chromatin, it seems entirely feasible because we know the chromatin structures, heterochromatin in particular, is maintained through positive feedback. So it's a dynamic structure and it's maintained through a system of positive feedbacks. So I can imagine, therefore, that if it starts to collapse then eventually it's going to get to a point where the positive feedbacks also collapse. And so then you could well cross a threshold effect when, yes, all hell is let loose. And that might be contributing to the rapid rise with age when perhaps that intracellular threshold of chromatin structure, or any other structure in the cell, is crossed. I mean, we could be talking about, you know, structures within metabolic networks here as well.

Dr. Sell: Right.

Dr. Brunet: Thank you. Gordon.

Dr. Lithgow: Gordon Lithgow, Buck Institute. So, Peter, I just wanted to hear more about your concerns with mouse models and the age-related component of cancer. Is it an epigenetics issue and does it also threaten other diseases in terms of their modeling?

Dr. Adams: I haven't thought so much about other diseases, so I'll maybe let others comment. But my concerns about the mouse models that are commonly used in cancer, there's several. First of all, we know that, with respect to cancer, mouse cells and human cells are fundamentally quite different, which is why when Bob Weinberg transformed mouse cells I think in 1983, it took him another 15 years to do the same experiment with human cells. It's just fundamentally more difficult to do it. And there's various reasons for that which we could go into.

But there's other problems specifically with the way the models are set up as well. So, for example, you know, one of the basic principles of human cancer biology is that it's a clonal disease. It comes from a mutation in a single cell which then clonally evolves. The vast majority of the mouse models of cancer out there, okay, whether it's a mouse model of colon cancer or pancreatic cancer, they have a tissue-specific cre which activates an oncogene in every cell in the tissue.

So you've immediately lost what is perhaps the biggest determinant of the age dependence which is how that initial oncogenic event within one cell in a tissue is actually handled by the tissue because what you've done is you've introduced a mutation in every cell in the tissue all at once, which is a catastrophic thing which I think very rarely, if ever, probably happens in a human tissue.

The other thing is just the way the, you know, the mindset of the investigators. I mean, the investigators want results. And this is arguably — and maybe this is driven by the drug industry — people want results as quick as possible. They want to put the drugs through the mouse as quickly as possible so they can get it into the clinic as quickly as possible. So the emphasis is on fast models, models that give you results quickly.

Some of the mouse models of cancer which are out there where, you know, they'll develop cancer within weeks or months. And that's generally considered to be a good thing within the field because you get your data quicker. People will speed their models up by, for example, giving DSS to colon cancer models or inflammatory agents to pancreatic cancer models. So there's not really a mindset within the cancer field, I don't think, of actually trying to recapitulate that age dependence.

Joe Betts-LaCroix: Joe Betts-LaCroix from Health Extension in California. So aging and epigenetics, probably the most exciting thing I saw so far this year on that was Greg Hanneman's paper from UCSD in Kang Zhang's lab down there where he found the best biomarker for aging I've ever seen. And it has about like a correlation R of .96 between age of human beings and something you can measure in their blood. So I don't think we've ever seen anything that good and that's looking at methylation.

So some interesting things he saw in there also were that seemingly men age faster than women. So if you use his same, the way of creating the features for his component analysis, then on the same coordinates you'll see a different slope for women and for men, which I thought was pretty interesting. And I wonder if you guys have seen anything like that. Also different tissues.

Speaker: I completely agree with you that that's an incredible result. I mean, it's the DNA methylation state, in case you guys don't know. It's the DNA methylation state in the lymphocytes from people and there's Trey Ideker and others developed this algorithm that can predict the age of the person without seeing it with this correlation of .96. I talked to Trey Ideker last week and he agrees this is magic, but he didn't say he knew why it worked. But that kind of an incredible link says something important is going on. but I think it's an amazing result that I think should be followed up.

Speaker: Well we published a paper in aging cell showing that there is an increased methylation of the L of L2 genes and other couple of genes, and the correlation with age in 500 people is .99. So this is published.

Speaker: So what's the mechanism? That's awesome.

Speaker: Next paper.

Speaker: Next paper.

Dr. Brunet: All right. So this closes the session. So thank you very much for your time and attention.

Speaker: So as everyone is heading out, I want to remind everyone that we'll reconvene here tomorrow at 8 AM sharp for the session on metabolism. On behalf of the NIH program staff involved in this

meeting, I really want to thank all of the speakers who came here today. You gave us a lot to think about. One more round of applause as you're heading out. Thank you very much.

END OF DAY 1

DAY 2: THURSDAY, OCTOBER 31

Session V: Metabolism

Aaron C. Pawlyk, PhD.: All right. Good morning, everybody. Welcome to the second day of the summit. This is the first session. It's the session on metabolism. My name's Aaron Pawlyk. I'm at the National Institute of Diabetes and Digestive and Kidney Diseases. My coorganizers for this session are Xiaoling Li from the National Institute of Environmental Health Sciences, and Ron Kohanski, who you all know from the NIA and we're all members of the Geroscience Interest Group, which has been instrumental in putting this scientific program for this together.

The external cochairs for the metabolism session are Christopher Newgard and Jeffrey Pessin. Dr. Newgard is professor in the Departments of Pharmacology and Cancer Biology and of Medicine and director of the Sara Stedman Nutrition and Metabolism Center at Duke University. He will give an introductory overview of the topic for this session as well as be introducing the speakers throughout the session.

Dr. Pessin is professor in the Departments of Medicine and Molecular Pharmacology and Director of the Diabetes Research Center at the Albert Einstein College of Medicine, and he'll be handling the discussion after the panelists give their presentation, as well as giving a summary of the session.

So without further ado, Chris will take over.

Introduction

Chris Newgard, PhD.: Thanks very much, Aaron, and it's a pleasure to be here today. This has been a very interesting symposium so far. I think we can all agree to that. Maren Laughlin, Senior Program Manager for NIDDK saw me yesterday and asked, how many of these NIH workshops have you been to over the years, Chris? And, frankly, I couldn't answer. I know it's been a couple, at least, a number of them. But I have to say, this one is unique. There is a diversity of presentations, a breadth of science, an engagement, in terms of the questions that occur after the sessions that has been really remarkable. And I think it's partly because aging remains a topic which is very difficult to get our arms around about really defining the most concise cause and effect experiments and picking out what are the causal drivers of aging phenotypes.

It seems that for almost every target and every idea that we have about drivers, there is both pro and con information. And I wish I could tell you that now that we're going to do a little unit on metabolism that it was going to change and everything's going to be crystal clear but, in fact, we are bedeviled by some of the same issues here, in speaking of metabolism in terms of cause and effect. And to make it even more complex, our panelists are not necessarily card carrying, aging researchers. Some like, Bill Evans, that is known to many of you, certainly has worked in the aging field for quite a number of years, but others such as myself, Jeff Pessin, Morrie Birnbaum, I think we would characterize ourselves as metabolic disease researchers but not necessarily focused on aging.

So I hope, though, that bringing people together of different stripes gives us a diversity of opinion, a freshness of approach that will somehow be useful to the gathered group here.

So I'm going to just very briefly make a few remarks. This is an attempt to summarize molecular pathways of aging and this was a really painful experience in writing a little review article for *JCI* with Ned Sharpless. As a nonaging researcher to go into the literature of the aging field and attempt to come up with one diagram that summarizes the molecular pathways of aging, was a tortuous experience, I have to say. And with regard to this morning's topic, already there's something presumptuous and provocative about this slide, in that it puts the metabolic events very proximal in the scheme. So if we think of over nutrition, the pandemic of world obesity, anabolic hormone signaling has been talked about here. Insulin and IGF-1 as drivers of aging phenotypes. Chronic mTOR inactivation, decreased sirtuins, reactive

oxygen species, mitochondrial dysfunction, sometimes fueled by a slow rate of mitochondrial DNA mutation.

Presumptively, we have put in this particular diagram, these metabolic events as proximal in the scheme, leading to the more sort of, core cell biological changes that we've been discussing at this meeting such as senescence, the SASP response, increased cytokine production, impaired stem cell renewal, and these cell biological events then leading to aging phenotypes that have also been under discussion here: reduced immune response, chronic inflammation, frailty, impaired capacity to respond to regeneration therapies. But right off the bat, perhaps I'm introducing something that we can debate and consider it to be controversial, is metabolism early and causal or is it more a reflection of the process of aging? Does metabolism change as a consequence of aging? Does metabolism change early and drive aging is one of the fundamental questions that we can address for which, I don't think we have an absolute clear answer but maybe some of the ways to attack it will come out in this session.

So another way of thinking about this is to say, what do we think we know about aging and metabolism? And these are some sign posts, some things that you think you can hang your hat on from the literature. Agents that suppress anabolic, metabolic processes. Rapamycin has been talked a lot about here, an inhibitor of mTOR, increase lifespan. But interestingly, other agents that stimulate oxidative fuel metabolism, for example, metformin, which we've now learned is an inhibitor of the mitochondrial electron transport chain acting at site one of the electron transport chain, resulting in a rise in AMP/ATP ratio and activation of the AMP responsive, five prime AMP kinase leading to activation of fatty acid oxidation, metformin has also been ascribed lifespan promoting activities.

Another metabolically relevant intervention that we've discussed here is that caloric restriction increases lifespan. There's a lot of literature and a lot of debate and controversy around the idea that agents that limit mitochondrial stress and production of reactive oxygen species, antioxidants, will enhance lifespan, and we've also been discussing sirtuins. We focused a lot on SIRT1 here, the mitochondrial sirtuin, and SIRT3, may also be involved in aging biology. And both of these via their interaction with NAD metabolism which is a very interesting area.

So some bullets of things we think we know but, when we look at this a little bit harder, fundamental issues arise. Does rapamycin slow appearance of aging phenotypes or does it increase lifespan independent of those phenotypes? And, as you all know, there's literature out now that says that these are potentially separable.

If reactive oxygen species generation is toxic, why does an agent that increases metabolic rate, metformin, and presumably RAS generation, have positive effects on longevity. And, conversely, why does caloric restriction and intervention that slows energy expenditure, also enhance longevity? One of the interesting things, just from a fuel selection point of view, is that a common feature of these two maneuvers, metformin and caloric restriction, is that they both favor a shift toward fatty acid oxidation and away from oxidation of glucose and other fuels. Is there something relevant to the preference for fatty acid oxidation that actually is a link to longevity? And how does NAD regulate aging and are sirtuins really involved?

So these are some of the sort of bedeviling questions of the metabolism in aging field and three other topics that are going to be covered by our speakers. How are obesity-related and aging-related metabolic dysfunction syndromes similar but also distinct? Do metabolic futile cycles have a role in regulation of the aging process? That will be covered by Debbie Muoio. And what's the role of central regulation of metabolism including control of circadian rhythm in aging biology?

So I haven't helped you at all to get clarity. I've just thrown up a bunch of things to confuse you and I'm going to rely now on our speaker panel and hopefully our discussion session to try to provide that clarity. So we have a wonderful group of speakers that will be covering some of the topic areas that I just described and we're going to start with Morrie Birnbaum who, of course, over many years has been a leader in molecular signaling pathways that control metabolism. Morrie.

How do signaling pathways that regulate growth and metabolism contribute to aging?

Morris J. Birnbaum, MD, PhD: Thank you very much, Chris, and Jeff and thanks for the opportunity to talk to you but even more than that, the opportunity to listen to the interesting and provocative presentations that went on yesterday and I'm sure will happen today.

So my task, I was tasked with talking to you about signaling pathways involved in anabolic metabolism and how they relate to aging. And obviously, that's a challenge which is much too complicated and much too extreme to cover in ten minutes, even if I were able to show reams of data.

So instead, what I'm going to do is really talk a little bit about this relationship between anabolic metabolism, between more conventional metabolic regulation as it relates to diabetes, that is in thinking about glucose metabolism, and really pose some questions in context to think about it and then if there are more specific issues to cover we could do that in the panel discussion when I have the option of deferring to colleagues.

So, yesterday, a number of speakers showed slides of various experiments reaffirming the long held observation that caloric restriction extends lifespan. Once one accepts that, it's a logical extension to believe that caloric restriction is working by suppressing an anabolic response. And the key to anabolic responses, or at least what's now believed to be the central integrating node for the stimulus for anabolism, is the TOR complex, which is what I'm going to be talking about today.

Now much of the support for the critical role of this complex and anabolic metabolism in the aging process comes from parallel work in three different classes of organisms which are shown in this slide. So I just wanted to begin by emphasizing the differences in the way the signaling pathways link up in these three organisms. And, of course, yeast is unique in the sense that it's really primarily a cell autonomous organism which is relying on nutritional signals to regulate the growth response. Flies and worms are more complicated in the sense that they also have this nonautonomous hormonal input, in this case, regulated virtual exclusively by the insulin in IGF-1 signaling pathway, but it's also worth remembering in these genetically tractable invertebrates that there is a single system that regulates this pathway.

Now there are multiple ligands, five or so in flies, a lot more in worms, but there's a single receptor that conveys the signal both for metabolism and growth, and clearly in these organisms the growth response is dominant, much dominant to metabolism even though there is a metabolic pathway.

Now this very sketchy diagram is not to imply that this is the only thing that insulin does. We know it regulates another pathway, most importantly, the FOXO antistress pathway, but this is really to emphasize that there's a signal receptor that transmits this. And then the organisms we care most about, mammals, this really emphasizes that this system is duplicated into two parallel pathways, one which is primarily, though not exclusively devoted to the regulation of metabolism, that is insulin, and the other one, the IGF signal which is regulated primarily, though not exclusively, to the regulation of growth.

And this complicates things enormously because these two pathways talk to each other, they influence each other, and when considering the regulation of one of those pathways, that is the anabolic pathway, either in the etiology of aging or even more profoundly, as a therapeutic target, it is impossible not to consider the implications for the parallel metabolic pathway and that's one of the things I'm going to talk about today.

Now the fact that blocking this TOR pathway and suppressing the canonical growth response extends lifespan in these three organisms, has been given rightly as one of the most compelling pieces of evidence that it is TOR itself which is regulating the aging process and not some secondary consequence of altering that signaling node. And that obviously, is a very compelling argument but I want to emphasize that it still remains an argument. Just because altering one particular signaling molecule has parallel

effects in two different classes of organisms, it does not in itself prove it's accomplishing that by the same mechanism.

Now humans are even more complicated and that's because there is this element of choice. Now obviously a mouse can choose how much it can eat in a cage but it can't choose substrate selectivity the way people can. And I don't know if this is the reason but it's important to realize that in trying to apply what we've learned from mouse energy metabolism to humans, it's been remarkably unsuccessful. That is to say it is very easy at this point to influence to prevent obesity genetically in a mouse. It's easy to prevent to uncouple the link between obesity and insulin resistance in diabetes in a mouse but so far these observations have not been transferable to humans. There are many reasons why that might be the case but one of them, of course, is the fact that humans have much more control over their environment, their diet, their exercise than mice do.

So what are we talking about? We're talking about this molecule here which is TOR. This is a complicated slide, obviously, with a lot of different components. I'm not going to talk about it except to say that there are many things that influence it and for the rest of the talk I'm going to break them all down into energy stress which suppresses it, and anabolic inputs, either from nutritional cues or from hormones which stimulate it. This also emphasizes that TOR has a lot of outputs and even though this review was published in this year, 2013, this list is already incomplete. So the other question, of course, is, which of these downstream pathways are responsible for the aging effects of TOR and the anti-aging blocking it. I'm not going to treat that at all now but again, we can talk about that later in the discussion if people are interested.

So, of course, the TOR pathway is complicated by the fact that there are two different TOR signaling complexes, one, mTORC1 which goes to growth, the other mTORC2 which signals to metabolism and even though I haven't put it up here, it regulates the pathway which suppresses FOXO. So in looking at the various studies that have been done looking at this pathway, we have to realize that some influence both or one. When using hypomorphic approaches influencing this pathway we decrease both the TORC1 and the TORC2 response and that clearly extends lifespan genetically.

Now the one thing I should emphasize is that even though mTORC2 regulates and is necessary for this pathway, there's a lot of spare signaling in this pathway so that when you turn down the mTORC2 arm you can do that quite a bit without seeing functional consequences on insulin signaling to metabolic regulation, at least under steady state conditions. And that's probably why dropping TOR to both has a profound effect on growth but has less of an effect on metabolism.

The other strategy, of course, is to selectively decrease TORC1 either genetically or therapeutically. I'll remind you of one experiment, in a way that doesn't affect TORC2. One way that has been done genetically is to knock out a major target of TORC1 and that, of course, is S6 kinase. That experiment has been done and it's been shown to extend lifespan. But the interpretation of that experiment teaches a very important lesson in terms of interpreting metabolic phenotypes and that's shown up here, of course. Here's the S6 kinase knockout which extends lifespan but this is the key figure in that paper and that is when a whole body germ line knockout S6 kinase is studied, adiposity is decreased.

Now when you knockout S6 kinase, a product of TORC1 signaling, one would like to believe that you're mimicking caloric restriction. That is the way the pathway goes, caloric restriction to TORC, S6 kinase, to lifespan extension. But I would argue that in this model, that pathway is reversed. That what's happening is the effective loss of S6 kinase selectively in the brain, is causing a change in energy metabolism so the way the pathway goes is decreased signaling in the brain, decreased caloric intake, an increased lifespan. That is to say, that this is working upstream of caloric restriction and really going through a classical response because of the cell autonomy of the response.

There's a more complicated pathway with rapamycin. Rapamycin, of course, through a signaling route which I won't go through here, not only blocks TORC1 signaling, also blocks TORC2 signaling. And that has been really nicely shown in a recent study from Joe Baur, a colleague of mine at the University of Pennsylvania in collaboration with David Sabatini who showed that when you knockout or when you treat

an animal with rapamycin, yes, you do extend lifespan but you also make that animal insulin resistant, which creates that very interesting problem, how is it that you can have an animal which is increased insulin resistant but also extends lifespan we know that insulin resistance extends.

And there are two possible explanations for that. One is that rapamycin actually causes insulin resistance in a way that's fundamentally different than obesity, that's certainly possible. The other is, and the one that we really worry about is that the mouse is not a sensitive organism for looking at the adverse consequences of extended lifespan. And I think there's really data to support that. Diabetes in mice doesn't cause profound cardiovascular disease. In humans, two-thirds of morbidity mortality and expense is cardiovascular disease, and secondarily diabetes and obesity in mice don't cause severe liver disease. So I think we have to be really cautious that interpreting that the adverse side of rapamycin signaling in mice might not be having the consequences that it might well have if given to human beings.

Thank you.

Dr. Newgard: Thank you, Morrie. Our next speaker is Bill Evans from GlaxoSmithKline and he's going to speak to us of implications of age associated increased nutrient requirements and decreased energy needs. Bill.

Implications of age-associated increased nutrient requirements and decreased energy needs.

William J. Evans, PhD: Thank you very much. It is indeed a pleasure and an honor. Thanks for the organizers and thanks for the previous speaker who sets this up, a beautiful talk on the implications of what we call anabolic resistance.

And I'll just start off by saying, some years ago, we were conducting a study to look at the effects of dietary protein and exercise on changes in muscle mass and one of the things we observed, when we put healthy older people on the recommended dietary allowance for protein, is that they were all in negative nitrogen balance. Now, if the RDA for protein is adequate we shouldn't be seeing that. And we speculated at the time that there was inadequate amount of data to say anything about the dietary protein needs of older people except to say that this was an interesting result.

If you go back and look at the data on how the RDA was arrived at, fundamentally it is in young MIT and Berkeley students, 90% of whom were male, almost no older people. So that I think is an early indication that, in fact, there is some anabolic resistance to the effects of dietary protein. So we decided to test the hypothesis then if the recommended dietary allowance is inadequate to meet the needs of older people, what happens if you give that amount to them over a long period of time? And what I can tell you is that if we give healthy older people the RDA for protein, they move into positive nitrogen balance over that three month period but they do it at the expense of muscle. And so we have a strong indication that the dietary protein recommendation is inadequate. Now, that's important information because at the same time, aging is associated with a loss of energy requirements. And that almost certainly is due to the result of a decrease in the amount of muscle.

Data from the Baltimore longitudinal study on aging show that fundamentally all of the reduction in metabolic rate is simply a consequence of reduction in muscle mass. I'll show you this slide, you saw it yesterday from Linda. This is here kind of operational view of frailty, but I want to point out this one right here. We think that chronic under nutrition of protein, in particular, but a number of other nutrients, is fundamental to the loss of homeostasis and certainly the loss of skeletal muscle. Now, at the same time, if dietary protein intake or requirements are going up and energy requirements are going down, that sets up an extraordinarily difficult situation in trying to come up with strategies to feed older people an adequate diet.

And so one of the consequences of this decrease in energy need is an increase in body fatness. And that is also one of the most frequently measured consequences of aging. It's interesting, if you look at the

statistics, we know that there is this obesity epidemic in the United States. Among women 20 to 39, the obesity percentage is 32%, among women 40 to 59 it's 36% and among women over 60 it's 43% in the US. So 43% of all women in the US are obese, and we do know, and studies that were done at Wash U, 96% of obese elderly are frail by Linda's criteria. Obviously, obesity isn't associated with shrinkage or loss but it is associated with poor function. In fact, body fatness is one of the most powerful predictors of late life disability.

These are data from the Health ABC study and I just want to show you this is very interesting information. These are in postmenopausal women. In young people, when young people gain weight, about a third of the weight gain is lean mass. But look at this, as older women increase their BMI, a BMI about over 26 there is no further increase in lean body mass. So 100% of the weight gain is fat. And when they attempt to lose weight via energy restriction, about 50% of the weight loss is lean. In fact, we know that weight loss among postmenopausal women is associated with a two-fold increase in risk for hip fracture, irrespective of when they start losing weight or intent to lose weight.

Now we've talked about the effects of exercise and the last thing I wanted to show you are some data that we've generated showing the opposite effect. In fact, a situation that elderly people are far more likely to encounter, and that's bed rest. So we know that changes associated with aging included decreased fatty acid oxidation, decreased muscle mass, and all of these things that we've been talking about, but when you put someone to bed many of these things are accelerated and accentuated. There is a real decrease in the rate of protein synthesis, an increase in the sensitivity to cortisol, which further downregulates rate of protein synthesis. Insulin resistance which further downregulates protein synthesis. A decrease in fatty acid oxidation which may cause an increase in intramyocellular triglyceride content which causes insulin resistance which further downregulates muscle protein synthesis, ultimately resulting in rather profound losses of muscle leading to decreased strength and frailty which kind of has this cyclical effect.

I'll show you, when we put healthy old people to bed for ten days this is what we see in terms of nitrogen balance. Now the first thing I'll show you is this is the pre-bed rest period, so we feed these elderly people the RDA for protein and adequate amounts of energy. The fact that before they start their bed rest period, they're in negative nitrogen balance again indicates that the RDA for protein is inadequate and almost certainly as a result of this anabolic resistance, whether it's a failure to activate mTOR or insulin sensitivity which fails to increase blood flow, but what you can see is that inactivity results in these subjects moving into profoundly negative nitrogen balance.

The consequence of that is interesting. If we put young people to bed for 28 days they lose about 400 grams of muscle from their legs. When we put elderly people to bed for 10 days they lose 1.2 kilograms of muscle. It's an astonishing change in a very, very short period of time. And what we just published is that there is a really striking increase in hepatic insulin resistance which we didn't really recognize starting out, also an increase in peripheral insulin resistance, but this hepatic insulin resistance may be driving a continued increase in glucose production that may be driving down fatty acid oxidation in muscle which may also cause an elevation in triglyceride levels. But this loss of muscle mass is really quite striking.

And in older people it's associated with a 40% reduction in muscle protein synthesis. And this is one area where rodents and humans may differ because inactivity in rodents is associated with an increase in protein degradation. We don't see any evidence of that. We see pretty much all of the change is in rate of synthesis.

So, in conclusion, it's interesting that the emerging Baby Boom generation enters old age with, what we call, sarcopenic obesity. Obesity associated with low amounts of muscle mass. I think it's going to be an extraordinary challenge to everybody here on how to deal with that because traditional energy restriction to lose body weight is not the appropriate strategy for older people. So we need to think about the best and safest way to maintain muscle mass and bone, at the same time decrease body fatness. And I think that this anabolic resistance to protein is amplified by inactivity and decreased energy intake.

Thank you.

Dr. Newgard: Thank you, Bill, for those very provocative and very interesting comments. Now it's my pleasure to introduce my friend and colleague, from the Stedman Nutrition and Metabolism and the Duke Institute of Molecular Physiology, Debbie Muoio. She's taking on bravely, a really difficult topic, futile cycles as targets of metabolic control in aging and disease, and I'm glad she's doing it and not me.

Fueling healthspan through futile metabolic cycles

Deborah M. Muoio, PhD: Okay. First of all, I'd like to thank Chris and Jeff and all the organizers for giving me the opportunity to be here and, also, a special thank you for assigning me a challenging topic. I was told to cover metabolic futile cycles and keep it simple. So I'm going to try to do so. Also, I want to thank Morrie and Ron, actually, for providing very nice context for my talk.

So the concept that I'm going to cover today is really the potential approach to extending healthspan by targeting futile metabolic cycles. And the simplest definition of a futile cycle is one that harnesses the potential to waste energy. So I'm going to start with the question of why this might be beneficial, particularly in the context of aging. And the rationale for the idea stems from what I refer to as the energy balance model of aging which suggests that chronic positive energy balance results in excessive storage of energy or excessive potential energy and that this energy fuels damage to cellular constituents such as DNA and proteins and lipids through a broad range of mechanisms that we've been discussing at this meeting. So the idea then is that excess energy fuels damage and thereby accelerates the aging process.

By contrast, if energy intake can be matched more precisely to meet only what's required for optimal cellular function, then energy storage and damage are minimized and aging is delayed.

So what's the evidence for this model? Well, the evidence stems from three very basic observations. And the first is that obesity lowers healthy life expectancy. So obviously obesity is a state of excessive energy storage. And the second observation is that habitual physical activity which increases fuel burning and minimizes fuel storage is associated with enhanced life expectancy. And then third, an issue that's been a hot topic of discussion here, is that in studies applying models ranging from worms to nonhuman primates, caloric restriction extends lifespan and lowers disease incidence.

So I think one conclusion that we might come to based on these observations, is that energy balance matters and perhaps the simplest prescription for a long and healthy life is to eat less and to move more. The problem with that is, as many of us are well aware, that lifestyle modification is much easier said than done. So that brings us to the next question of whether or not we can develop alternative strategies to minimize energy surplus. So that's where we come to the possibility of tweaking these futile metabolic cycles.

A more elaborate definition of a futile cycle is a set of opposing nonequilibrium reactions catalyzed by different enzymes that act simultaneously. So I'm going to highlight two important elements of these cycles. The first is the forward reaction shown in green and the reverse reaction in blue, must be catalyzed by different enzymes. This is important because it allows the reactions to occur simultaneously and then also presents the opportunity to regulate flux at at least two different independent sites.

In this cartoon the forward reaction is catalyzed by A, the reverse reaction by B, and the net flux is the rate of the forward reaction minus the rate of the reverse reaction.

Another critical element to these cycles is at least one of these reactions must depend on ATP hydrolysis. So that these cycles can consume ATP and generate heat without a net change in the cellular ration of substrate to product.

So another question that is still up for debate is, why do these cycles even exist? I think the conventional thinking is that evolution favored processes that are more energy thrifty. So it might seem counterintuitive that the evolution that actually preserve a cycle that essentially wastes energy. And the theoretical

answer for that over the years, originally proposed by Eric Newsholme, is that these cycles introduce or impart sensitivity. So a cell or a system that operates at a high cycling rate will be more responsive to regulatory cues that target one or both of these arms of the pathway. So then the advantage of the cycle is enhanced sensitivity and metabolic flexibility and the cost is ATP consumption.

So the next question that I'll address is, why would sensitivity be important? And one reason is because in many cases the metabolic intermediates generated by these cycles can be used as substrates for energy provision under circumstances when energy demand suddenly increases. So the analogy that's often drawn is one of an idling engine. So an engine that is idling at a high RPM is well poised to accelerate from 0 to 60 in a very short notice. And so you can think the same might be true of a resting skeletal muscle in an animal that is anticipating the need to move quickly. So then the anticipation might increase the cycling rate and the right regulatory cue that then inhibits the reverse reaction would cause a very dramatic increase in substrate B that can provide energy and ATP production, thereby providing for a very robust fight or flight response.

So in addition to providing energy some of these intermediates of these cycles can also be used in a signaling capacity or can be used for biosynthesis of macromolecules such as DNA or protein and lipids. So then another advantage of these cycles is that they might enhance signaling capacity or provide a constant stream of substrates for metabolic biosynthesis, which could be important under circumstances such as growth and repair.

So this brings me to the triglyceride/fatty acid cycle, which I'll explain is just one example of a substrate cycle that I think could be particularly relevant in the context of age-related obesity. So the triglyceride molecule is the major storage form of energy in most cells. It's comprised of a glycerol backbone, esterified to three fatty acids, and in most, if not all cells, these energy reservoirs are undergoing constant remodeling. And so the process of lipolysis will convert the triglyceride molecule to fatty acids in glycerol. And if these products are not needed either for energy production or some other purpose, then they will be quickly re-esterified through reactions that would require ATP.

And then another interesting aspect of this particular cycle is that the glycerol that's generated through lipolysis can also engage in a second cycle that has the potential to produce NAD. And NAD is a required cofactor for the sirtuins and thereby is widely considered a potential longevity signal.

So then, if we pull all this information together, we might suggest that we could mimic caloric restriction by activating these cycles which, in theory, would consume ATP, reduce energy storage, and potentially generate a longevity signal.

So also interesting is that maneuvers that are considered to be more anti-aging such as caloric restriction and exercise, actually activate this cycle and some of the signals that Morrie just talked about, so insulin signaling and mTOR signaling, are known to inhibit this cycle. So again, that kind of brings us to the provocative idea that we might be able to mimic caloric restriction by activating the cycle.

So the triglyceride fatty acid cycle is just one of many potential thermogenic cycles. I've listed several here. These include protein and glycogen turnover.

And then I'll just end by raising some of the important knowledge gaps in this field. I think first and foremost, we need to understand where and when these cycles are activated in vivo, and are they affected by aging? Again, whether or not we can mimic caloric restriction by increasing substrate cycling, whether they're involved in metabolic fine tuning and generating longevity signals, and are they modifiable?

Thank you very much.

Dr. Newgard: Thanks very much, Deb, and we'll move on to our penultimate talk, which will be given by Dongsheng Cai from Albert Einstein. Dongsheng has done some really nice work on the central

regulation of inflammation and he's going to take it a step broader here with a talk about hypothalamic neurodegeneration, a common link between metabolic disease and aging. And I'm going to follow you and handoff a pointer. Here you go. Thank you very much.

Hypothalamic neurodegeneration: a common link between metabolic disease and aging.

Dongsheng Cai, MD, PhD: Thank you. Thank Chris and Jeff for the kind invitation, also thank the organizers for putting together this terrific program and it's very exciting and stimulating.

So I think I have heard quite a few interesting topics from Morris, from Debbie, from Bill about the different type of metabolic signal and changes in relevance to aging. And you also heard of some interesting concepts about inflammation yesterday. And there was a whole section about that and even followed by the stress related to inflammation. I also noticed some speakers mentioned about the brain dysfunctions, functions, and although it hasn't been described whether brain dysfunctions could be, have some causative role in the aging development or aging biology.

So what I'm trying to do is put all these few points together considering perhaps the connections of those major points, may have particularly important role in, for our understanding of aging biology and aging related diseases.

And suggesting potential pathway that neuro information, particularly hypothalamic information may have some, form a common basis between the metabolic disease and aging and partially through neurodegeneration.

So to dissect this kind of a comprehensive network, I think there are two levels we can approach. So one is on the left hand in the context of caloric over nutrition. We know this provides important background for the development of metabolic syndrome which is a collective term of a number of interconnected diseases or disorders, such as obesity, overweight, hyperlipidemia, glucose intolerance, insulin resistance, prediabetes even hypertension. So all those are parts of the metabolic syndrome, and even aging has recently been added part of the metabolic syndrome. So we know that uncontrolled conditions of those metabolic syndromes can lead to eventually, the development of Type 2 diabetes, cardiovascular disease, and neurodegenerative disease, and etc., and all these problems can be further promoted under aging conditions.

So over the last some years, six or seven years, our lab as well as some other labs, have obtained quite significant evidence suggesting that the neuro information, especially the hypothalamic information may represent a link between this metabolic environment or induced metabolic challenges in this metabolic consequences.

This is one arm, and meanwhile, on the right hand, so this neuro information or hypothalamic information system can be also employed or utilized under the condition of aging increase postdevelopmentally and seems like has an overall in the aging development per se, including aging related diseases.

And we know in reality, so these left and right, actually quite often simultaneously take place. But at the laboratory level we can try to dissect then, for example, can use animal models with over nutrition at a young age to understand this or we can study the old animals on a normal nutrition to start this, and then they're put together to get more overall view.

So I think, so this type of information probably is a little bit different, although share a lot of similar changes compared to the classic information and one similarity is this is very important master control for classical information NK-Kappa B, nuclear transcription factor and upstream kinase IKK pathway. So I just give you a little bit of the background of this pathway. So we have significantly appreciated that, the activation of this pathway, in the classical immunity information reaction. For example, in response to pathogens and the environmental invasions, these lead to a robust quick, or chronically, activation of this

pathway in the high magnitude, and quite often associated with morphologic changes. And this pathway can now, hundreds of genes that I involved for the information immunity and the genesis apoptosis to pseudomonas, etc.

But this pathway, typically known in the immune systems, immune cells, however, in the context of aging or metabolic syndrome, so this system can be ectopically and atypically activated in nonimmune system, nonimmune cells, especially in the CNS, very sensitive in the CNS. If you have, for example, a few days of high fat _____ or at the very early stage of aging, so this pathway can be turned on in the hypothalamus and we have to particularly appreciate the activation of this system involves a number of intracellular organelle stresses or dysfunctions. For example, in the form of ER stress, mitochondrial changes, autophagic defect, lysosome changes, also nuclear abnormalities such as DNA damages in a histone dysfunctions.

So all this organelle changes, they have their own ways to crosstalk to this intracellular inflammatory machinery to induce the activation, I think perhaps initially, is to try to adapt, try to resolve some of the bad events but this is, when this is not completely resolved, it's always on and has been known to be problematic.

And in a context of metabolic disease, metabolic syndrome, I think some years ago, six, seven years ago, I gave a very briefly term, the metabolic inflammation, and, actually, many of this is aspects of also shield on the aging condition. I think we still don't have enough understanding of what the detailed characteristics of this type of atypical inflammation under aging or metabolic disease condition.

But at this we have begin to appreciate the consequences or the impact of this type of inflammation in a number of metabolic conditions including the regulatory site in the CNS in the hypothalamus, for example, I list a few here. When this inflammation is induced in the context of over nutrition or age increase, it can affect different types of neurocell types including neurons so the change in the neuronal signaling, a change in neuronal control of whole body's physiology and can affect the glial cells and the glial functions, therefore, affect the glial neuronal crosstalk and interactions and can also affect vasculature, may not only contribute to stroke, maybe or as well as the brain dysfunctions because of changes to blood supply of the brain.

And most recently have appreciate another level at the neuro stem cell level. And I think these are going to talk about neuro stem cells, we have a session this afternoon on this so I'm not trying to talk too much about this subject but just briefly, I think over the last decade the literature has been convinced that there's several types of neuro stem cells in the brain in adult animals, at least in rodents.

So two important regions have been identified. One is the dentate gyrus in the hippocampus. Another is subventricular zone of the _____ ventricle in the brain. So these two regions have a high level of neuro stem cells. In the last few years we have seen that the hypothalamus is perhaps the third important brain region that can have adult neuro stem cells, and some additional experiments, for example, ablation of the stem cells have been done, suggesting that those cell populations, this cell population does have physiological relevance. And I just put this in, I guess, this _____ is the real experiment, can give a little bit illustration.

So this is from the hypothalamus, neuro stem cells isolated from adult mice in a culture in which in a form of ne___ and differentiate into neuro cells including neurons in red and astrocyte in green. And you can appreciate that the ratio and how nicely they can differentiate in the neurons and astrocyte.

And we're struck by the observation that on the chronic prolonged over nutrition this is significantly compromised, not only depletion of neuro stem cells in the hypothalamus but also they have a poor differentiation, and you can see from here.

They barely differentiate into neurons, although they can differentiate into astrocyte in quite a limited manner. And altogether I think I would suggest that this model here, in a normal physiology the

hypothalamus stem cells can contribute to a fraction of neuro cells, including neurons, perhaps to replace certain number of dysfunctional neurons. And this population, this turnover probably is small in the short time window and is not easily detected just within the short time window but it could be very significant over time. And the disease condition I mentioned, there's a degeneration and there's also reduced neurogenetic function of those cells.

So altogether I think just add this in the paradigm, we think that chronic overnutrition activates the neuro inflammation and lead to a number of events including, impaired neurogenesis and promoting neuro degeneration and partially contribute to the development of metabolic syndrome as well as aging related disease, perhaps a common basis. I list a few points for you to consider as potential discussion.

That's it.

Dr. Newgard: Thank you, Dongsheng, and now the final talk of this session will be given by Roman Kondratov from Cleveland State. He works on molecular clocks and circadian rhythm and will particularly speak about circadian desynchronization as a key player in metabolic disease of aging. Roman.

Circadian desynchronization: chronic metabolic diseases of aging

Roman Kondratov, PhD: First, thank you organizers for the invitation and this opportunity to speak here. Before I will address the question I've been asked, and it's very long question and probably actually it's multiple questions inside of this question, I will start from this slide just to tell you that circadian clock is little bit more than cause of jet lag.

So for ages we know that circadian clock dysfunction is associated with sleep disorders and psychiatric disorders. What we know now from epidemiological studies is that circadian misalignment in humans will increase risk of cardiovascular diseases and increase risk of metabolic syndromes, obesity, diabetes, and other pathologies.

What is circadian clock? We know rhythms and sleep wake cycle, rhythms _____ and behavior, and this is a function of central clock which is situated in hypothalamus, in the brain. But what is probably less known that circadian clocks operate in every cell, in every tissue. And this circadian clocks and they're called peripheral clocks from circadian point of view.

So these clocks generate rhythms in gene expression. So about 10% of genes in every tissue show circadian rhythm in gene expression. And this rhythm and gene expressions somehow transform into circadian rhythm and physiology in metabolism.

First question I think needs to be answered, what is the effect of aging on the circadian clock? And answer will be not a simple one because we're dealing with central clock, and effect on the central clock will affect sleep, will affect response to light, will affect feeding behavior. And all this could contribute to development of different metabolic diseases and other diseases, because for example, it is very well known that sleep disruption is associated with metabolic diseases and probably contributes to this.

What is much less appreciated from my point of view, is affect of aging on peripheral clocks, but activity of peripheral clocks, I think, is extremely important for control of metabolism and for control of physiology. What we know about changes in the peripheral clock, that amplitude of circadian rhythms are reduced with age and when we have reduction in amplitude it can be achieved in two ways. So one way is a clock in all tissues or in cells will stop and we will see as a flittering of amplitude. So another possibility is desynchronization of individual clocks and the tissue. So if every cell will have its own phase of isolation, when we measure rhythms as a gene expression and as a micro parameter, we will also see an amplitude of rhythm will decrease.

If clock is stopped with age, or let's say upon disease, the question is, at what timepoint clock was stopped because this is an example of two types of circadian mutants so it's deficient, so one circadian

clock gene and deficiency of another circadian clock gene. So both of these genes expression of activity isolate under normal circumstances. But upon mutation isolation will disappear but you will see that isolation, or let's say clock will be stopped at exactly opposite times. And the effect, metabolic effect and physiological effect in these two mutants will be different.

So why it is important to understand is stoppage or is it desynchronization. To continue parallel with driving a car, if you have a clock which is stopped to the car with transmission where so your gear is locked but first or last gear. And if you are dealing with desynchronization you are dealing with car where transmission is actually kind of switched in a random way constantly. So, therefore, in order to fix the problem you need to know what's the problem.

The second question is, how this circadian misalignment, how this circadian dysfunction could affect aging and, specifically, chronic diseases of aging. Again, answer is not simple and my feeling is that it will be tissue and cell type specific because circadian clock controls different set of gene and different tissues.

And again, aging would affect central clock and peripheral clock and many things will be changed. But even when talking about metabolism and effect of clock disruption on metabolism. I think there are several pathways which could be universal and need to be considered. So one thing is control of cellular redox state and oxidative stress response and there is a big body of data on this subject. Another important pathway is SIRT pathway. So it was demonstrated that circadian clock and SIRT1 signaling pathway are interlinked and it was demonstrated by laboratories of Pablo Sosoncorsi, Leo Guarente and Joe Bass, and I think this is important.

My lab has interest in circadian clock dependent regulation of TOR signaling pathway. I don't think I have to introduce this anymore in this meeting. What we found that TOR signaling pathway under normal physiological condition is controlled by the circadian clock and this control is actually feeding independent which is important and this control is disrupted in circadian mutants. And I think that's what could contribute to metabolic disorders of aging.

So final slide, why it is important to target circadian clock as therapeutic target. So one advantage is that when you target the clock you actually target multiple physiological systems. The second advantage and probably not advantage from a morphological company point of view, but you can target clock through behavioral or dietary intervention. So you don't have to use drugs and there are a few examples of this. So restoration of circadian rhythms in behavior delay development of pathology in animal model of Huntington's disease.

And as example of diet behavioral intervention would be a time restricted feeding which was recently demonstrated by Sachin Panda that time restricted feeding prevents development of obesity and metabolic syndrome in mice on high fat diet. I'm done.

Discussion and Wrap-up

Jeffrey Pessin, PhD: I would like to invite the panelists to please come up to the stage and while they're doing that, I'll make some comments.

I think from this discussion, it's very broad and a variety of areas in metabolism, but I think as everybody can see, metabolism affects many parts of aging, and aging related disease processes. And I think that is the primary take-home message. And as Chris said at the beginning, cause and effect. We have no idea. This is an interrelated complex pathway and all of the systems, biology that goes on, whether it's DNA methylation, histone modification, protein acylation, all these things require metabolic substrates, metabolic products, and any dysregulation of one affects the other one.

So, for example, you think about acylation of proteins acetate. Acetate doesn't come on to a protein as acetate. It comes on through acetyl-CoA which is made through metabolism. You heard about NAD, NAD cycles.

And the other point I'd like to make is the cycling parts of metabolism. This is a very fundamental aspect of metabolism which is not really well appreciated and I think Debbie did a very good job of describing how the futile cycles can be poised to change various functions. And you can think of circadian rhythms which we also know are very important in aging process as Roman described, that that also is sort of a cyclic pathway. It's kind of like a futile cycle where things are cycling around. So with those comments I will let the panel now answer questions.

Robert Jilka, PhD: I'm Bob Jilka from the American Society for Bone and Mineral Research. I have a comment, mainly that a lot of the speakers who have dealt with age-related bone loss and related to sarcopenia, have portrayed it as sarcopenia being the cause of the bone loss. But, in fact, there's a lot of confusion about that in the literature now and there's quite a bit of evidence that bone loss takes place quite independently of sarcopenia, especially in the mouse. I admit that the data in the humans is not well-established.

And in my own lab we have shown that the, and the field, in general, has accepted the idea that bone loss with aging is due to a deficit in osteoblasts that seems to occur quite independently of changes in muscle function.

So I would invite perhaps Dr. Evans or others on the panel to comment on that.

Dr. Evans: Hopefully I didn't suggest that sarcopenia is driving loss of bone. What is clear in the longitudinal observational studies is that weight loss results in bone loss and the greater the amount of weight loss, the greater the amount of bone loss. That's for sure.

We also know that there is actually a pretty good relationship, Bestoff and Hughes has shown that dietary protein intake is associated with bone health as well. So I have no doubt- There has always been a strong relationship between bone mass and muscle mass, what drives the other is what's less well understood. I appreciate that, Bob.

Dr. Jilka: Thank you.

Richard Miller, MD, PhD: I'm Richard Miller from the University of Michigan. Dr. Cai's studies have indicated that the gonadotropin releasing hormones play a substantial role in regulation of hypothalamic inflammation in some of the mouse models. So I'd be eager to hear your ideas but anyone else's ideas as to whether this may or may not be a major driving force behind the sexual dimorphism in responses of male and female mice to either diets or drugs, some of which have very specific effects on one sex or the other. To what extent do these anti aging interventions interact with male specific or female specific pathways? And is the hypothalamus the place to look for the answer?

Dr. Cai: Thank you, Dr. Miller, for this very nice comment. Yes, GNRH is at least one of some molecules downstream of hypothalamic inflammation. I do believe there are others but we have studied GNRH as we recently reported and I think this could be an interesting point to link the connection between the production physiology and the biology and aging. We know there's always a relationship between these and the agent and there's many models showing that suppression or ablation of ___ control molecules can affect the aging, the lifespan in a positive, or in some cases, in the negative manner. So it's very possible that GNRH level may explain some of the dimorphism between male the female in terms of her lifespan expectancy. So there's even some examples in society. For example, in males in the history in the Royal family there's some cascation, cases that there's been shown have increased, there's high incidence of ____ from that population.

And in animals it's been also seen the situation. And maybe in terms of male and a female the post m____ change of sexual hormones and an influence that the GNRH may contribute to the different profile of life for aging speed in males and females.

Dr. Newgard: I want to push on that just a little bit. One of the things that I said in my remarks, that some of us are coming from outside every day work in the aging field and I was struck by the acarbose observation. The idea that a blocker of intestinal carbohydrate and glucose absorption leads to a sex specific effect on aging. So has anybody looked at in the acarbose studies, as to whether there are changes in hormones that we would view as sex specific or sex driving? And if so, maybe that begins to suss out a connection. I'm not aware of an agent that blocks glucose entry having any particular effect on sex hormones, but that's maybe because I was just not aware of it.

Constantine Kotsanis, MD: Constantine Kotsanis from the Kotsanis Institute. The question is on Stage IV cancer and diet. There is a tug of war between different academies on carbohydrate metabolism and intake and ____ metabolism and intake. And some of them lately recommend ketogenic diet as the only way to fight cancer dietarily.

Now we know this promotes sarcopenia so what's the balance, what kind of diet would you recommend to balance cancer and sarcopenia at the same time?

Speaker: The only thing that I would say is that in cancer, what we need to do is just increase food intake, period. And we know that cancer is associated with a profound cachexia which is associated with accelerated protein degradation and we know that it's unresponsive to nutrition. One of the hallmarks of cachexia is that nutritional strategies have no effect on muscle loss. So whether you have to combine it with an anabolic agent or some other intervention like physical activity or exercise, but there is very little evidence that nutrition per se affects muscle loss. We know that from the sepsis data. You can provide TPN to septic patients and you can maintain their weight absolutely but they will continue to lose muscle mass.

Dr. Kotsanis: How do you correct anorexia?

Speaker: Pardon me?

Dr. Kotsanis: These people have severe anorexia.

Speaker: I don't know.

Peter Nathanielsz, MD, PhD: Peter Nathanielsz, San Antonio. I really enjoyed the two talks on the hypothalamus. If you look at the regulatory thermostats and clocks in the hypothalamus, there's sort of a ying yang system. If I could focus on the appetitive control because we've talked obesity in aging and if you enter life, as somebody suggested yesterday, that he did, with an increased appetitive drive, more orexigenic protein drive in your arcuate nucleus. And if we're looking at things like effects of inflammation, sirtuins, etc., then would the panel enlighten me, am I right that in the orexigenic system one of them would have to go up and in the anorexogenic system, they'd have to go down. So perhaps we need to look more carefully at the specificity within these systems because if inflammation is affecting them both the same, then theoretically it wouldn't change the drive.

Dr. Pessin: Dongsheng, you want to try to take that one?

Dr. Cai: Yeah, I think this is an interesting point. So there are a variety of different types of cells in the hypothalamus and maybe some cells outside of hypothalamus are also critical. And the inflammation has an impact on them – I guess that the upstream changes probably is shared but the downstream just depends on the context, depend on the specific signaling, cascade and the functions. You could have the different even opposite physiological impact depending on what kind of a neuron like POMC neurons, for example, versus the AgRP neurons, so one suppress food intake, one promote food intake.

But when the functions, the regulatory functions of those neurons are impaired, they respond less to upstream signal, for example, lactin or usadin, they can have in the same direction, they both contribute to the hypophagic and a metabolic syndrome like phenotype.

So I guess it's quite dynamic and I'd have to consider the context of specific cell types in upstream, downstream events.

Dr. Pessin: Roman, did you want to say something also?

Dr. Kondratov: It's hard to comment but again, as I had mentioned-

Speaker: I can't quite hear you.

Dr. Kondratov: What I am trying to say that I definitely agree with you. We have to take into consideration specific cell types and probably response will be different in different cell types, that from the circadian point of view. The question would be what time of the day your _____ is. And the response would be different during different time of the day. And set of genes which are regulated in a different part of the brain would be different, too. So I cannot answer this question in one way or another before we will have all this.

Dr. Nathanielsz: I just wondered if there was any evidence, that's all.

Dr. Pessin: I can make a comment. So the NF-kappa-B signaling pathway can be different in different cell types and it's not published yet, but we have data that NF-kappa-B signaling adipocytes gives you a different output than you would get when you do it in an immune cell, for example. So you get different cytokine production and different responses.

So some of them are similar and some of them are different. So your question's a very good valid one, and that is that you might have very different signaling outputs from the different cell types and, of course, with feeding AGRP neuron suppressed POMC neurons and so you could have a decrease in inflammatory signaling in that case, but perhaps you have an increase and you get a different output. And those are the things I think you're absolutely right, are very important to sort out.

Benjamin Best: Ben Best from the Life Extension Foundation. Dr. Newgard has written that branch chain amino acids can increase insulin resistance and didn't discuss that at this presentation. And Dr. Evans has said that energy restriction increases the need for protein but spoke only of energy restriction rather than something like a carbohydrate restriction. So I'm wondering if we couldn't get more specific dietary recommendations to address these problems.

Dr. Newgard: Well, first of all, I'm right. No, no, no. What we have observed is a very strong association of an increase in branch chain amino acids and related metabolites in multiple human disease settings. And then, as we brought that observation back to the laboratory, we can promote insulin resistance but only in the context of a high energy diet. We can also promote behavioral changes with branch chain amino acids in the context of high energy diets. So I think branch chain amino acids really begin to exert negative effects in middle age, not necessarily in the older and sedentary subjects that Bill was speaking about earlier, and in the context of high energy diets. They become a bad actor when the other macro nutrients are abundant. That's when they can begin to exert effects on metabolic homeostasis in our experience.

Conversely, if we restrict branch chain amino acids in obese, for example, obese Zucker animals, we can resolve a significant amount of the insulin resistance and that's the data that we have to this point. Bill.

Dr. Evans: To the extent, I didn't really discuss it because I didn't have time. In our bed rest studies, if we supplement our healthy older people with just a solution of essential amino acids, 15 grams in the morning and 15 grams in the evening, it almost totally obliterates the loss of muscle. So while there is

this anabolic resistance in older people, I think that you can overcome it with sufficient quantities of essential amino acids which also, obviously, include the branch chains.

So I think one of the challenges that we have is to provide adequate protein nutrition. We know that the higher the percent of essential amino acids there are in the food, the greater the stimulation of protein synthesis is in older people. And so, high quality, low fat protein is my recommendation.

Nir Barzilai: Nir Barzilai from Einstein. So in honor of Sierra and Kohanski that brought us together, NIDDK, NIA, others, I want to do a provocation that it has to do with geroscience, and it's to you guys who are funded by the NIDDK. From the first day of me coming from NIDDK, switching from NIDDK to NIA, the concept in the NIDDK was that insulin resistance is central to diseases. And in aging insulin resistance is central to longevity. They were totally different views. And it was insulin resistant in NIDDK because we humans have poor beta cells and we develop diabetes, okay. And that was the problem.

While using models every time you cause insulin resistance you have basically longevity. Luigi Ferrucci and I have published in the *Journal of Gerontology*, all the studies that have looked at endpoint of lifespan. In animals, they were either insulin resistant or insulin sensitive and there's no relationship to longevity; maybe pointing to Newgard's point that it's upstream somewhat also. But there are lots of models that are insulin resistant like the Morris White IRS, that live longer, or Maureen Charron GLUT4 overexpression that are very insulin sensitive and live a little bit shorter.

Why is it so important for us to discuss those things because, and it has to do with humans healthspan, because when we are deciding to treat people for insulin resistance, knowing that insulin resistance is a stress response also, why are we so surprised that we have outcomes that are not so good? Why are we surprised that TZD does cardiovascular disease? We just removed the stress response. So the holistic approach that comes from aging and the practical approaches that come to NIDDK are really exemplified by what you said, Morrie, and said at the end, I don't understand the insulin resistance.

But I think there are things we have to consider about the insulin resistance, are very relevant to the institutions.

Dr. Pessin: Go ahead Morrie.

Dr. Birnbaum: They're looking at me, which doesn't surprise me. I think we have to start, before I even, I was going to say answer the question, I'm not going to answer the question, I'm just going to talk for a while.

But I think before I even do that, I think we have a real problem here and that is the definition of insulin resistance. And the problem is that that phrase has ceased to mean anything that everybody can agree on. Okay. So when you say, when you refer to insulin resistance in the population of obese individuals that's increasing in prevalence today, I'm not sure what that means.

If you take the classical, strict definition of insulin resistance which somebody far into this field would think about, it's an impairment in insulin signaling and an impairment of insulin signaling which includes impairment in the actual signaling intermediates as well as the biological actions of insulin. That is best replicated by somebody with a mutation in the insulin receptor or in many ways, a Type 1 diabetic because they don't have insulin signaling. The thing that I think all of us know is that an obese patient with insulin resistance does not phenocopy a loss of insulin signaling. Now there are many reasons you could argue about that, you can argue that's the case. One is because of this concept of selective insulin resistance that some pathways are resistant, others aren't.

The other, of course, is that our fundamental concept of insulin resistance in its etiology of Type 2 diabetes is wrong. Again, there's not time to discuss all the reasons why that might not be the case, but I think the important message to get across is that when we refer to quote, "insulin resistance in Type 2

diabetic patients in obese individuals," that is not an exclusive selective but selective to the insulin signaling pathway but generalized in terms of all tissues, did decrement in insulin signaling.

Now when you talk about manipulating insulin signaling to extend lifetime, that's what you're talking about. You're talking about knocking down AKT and affecting AKT signaling as well as affecting all outputs in all tissues. So I think, unfortunately, the term resistance is applied both to genetic uniform reductions in insulin signaling and to this very strange syndrome that obese people get but it's the same term being applied to two very, very different situations.

Dr. Cai: I think that in my mind that Morris is right, the definition of insulin resistance probably is not that kind of clear cut. So there might be a physiological insulin resistance versus a pathological insulin resistance. So I don't think that the insulin resistance at the pathological level like diabetes, is beneficial for aging retardation or longevity. So and, actually, in normal physiology, we do need a certain level of insulin resistance under certain circumstances, like starvation or stress condition. Particularly, even some tissues, for example, brain, a little bit level of insulin resistance may help the brain to cope with the stress response maybe, just to be consistent with the discussion yesterday afternoon about stress resistance, maybe it's consistent.

So we may have same language between two institutions I guess.

Dr. Birnbaum: Let me make one additional point, just to say something a little bit different than Dr. Cai. There are many people who plausibly believe that there is no difference between physiologically and pathological insulin resistance. That, in fact, we call pathological insulin resistance is an appropriate response to overeating and overnutrition and, in fact, if people didn't have that resistance response, they'd be in worse shape than they would be with the resistance response. So I think that's an important potential concept to have out there.

Rod Levine: Rod Levine from NIH. I have a question for Debbie Muoio. You pointed out the effect of a futile cycle is really to create an ATPase to convert the energy of ATP into heat and that one way to do that is to have two enzymes, one that catalyzes a forward reaction, and one the other. But wouldn't it be sufficient to simply target any forward reaction that uses ATP? For example, in the glutamate, glutamine pair that was on you list, if you had a drug that interfered with the full catalytic cycle of glutamine synthetase you'd just be hydrolyzing ATP.

Dr. Muoio: So I think that one possible answer to that question is that if you lack the other arm of the cycle then, theoretically, what you're going to be missing is the metabolic control aspect of the cycle and not just the energy wasting aspect of the cycle. So there's a lot of debate about why these cycles exist and just conceptually it doesn't make so much physiological sense that there would be some system to simply waste that energy. And the architecture, the network would have to be designed so that process can be turned off when it's physiologically appropriate. So I think just expanding the definition and the utility of those cycles to mean important in regulating signaling and regulating metabolic precursors, that it's important to have that flux control at various sites other than just the one enzyme.

Dr. Levine: I agree, but if you wanted to intervene you'd have the other strategy.

Dr. Muoio: But I think, simply for purposes of energy wasting, that could be beneficial, but then as part of the discussion that we've been having here is that there's going to be some detriment to uncontrolled energy wasting. So I don't know that turning on a pathway that would waste energy uncontrolled would be favorable. And one example of that is metabolic uncouplers, so turning on the process of uncoupling without having some regulatory arm is probably going to be detrimental.

Dr. Pessin: Okay.

Nicolas Musi, MD: Nick Musi from the Barshop Institute in San Antonio. So similar to Nir, I'm an endocrinologist interested in aging, so when Arlan Richardson or Randy Strong, four or five years ago,

asked us whether we would study the effects of rapamycin in human subjects, we thought it was a crazy idea, was ____crilogy because rapamycin was thought to induce insulin resistance and promote glucose intolerance.

But as we've been learning more about the effects of rapamycin, I think it's warranted to study it in humans, and also in healthy subjects for several reasons. The effects on glucose metabolism, I think they're still unclear. There is evidence from Dave Harrison that if you study a heterogeneous mouse insulin resistance is not observed. There's some people in San Antonio that have done pilot studies in rapamycin in healthy subjects. We haven't seen hyperglycemia, at least with fasting glucose.

The other issue has to do with cardiovascular disease. There's several groups demonstrating that rapamycin has a strong effect to prevent atherosclerosis and APO E in knockout mice and that's why rapa logs are used and coronary stents.

And lastly, as you pointed out, it may be that the effect of rapamycin to extend longevity is related to a potential effect on the insulin action.

Dr. Birnbaum: Right. I agree with everything you said. I think the difficult situation we're in now is that there is a lot of compelling reasons why you would want to try rapamycin in people and there are some, admittedly theoretical, but nonetheless equally compelling reasons why you would worry about it.

One of the issues, of course, with rapamycin and insulin resistance is it's indirect. We didn't say it explicitly but of course, the thought is based on David Sabatini's work is that when you have rapamycin you sequester TOR into this inactive TORC1 complex and eventually turndown TORC2 signaling and since TORC2 is important for the activation of AKT, which is an integral intermediate, classical, canonical metabolic signaling pathway, you're going to get insulin resistance.

Again, let me just say, two alternative explanations why, or at least one possibility why that's not an issue, and that is the assumption in the community, that is the prevalent belief is that Type 2 diabetes in insulin resistance is insulin resistance is etiologically a decrease in activation of AKT. That is the most prevalent belief.

But there are other plausible sequences of events that would ultimately lead to the decrease in AKT activation as a consequence of the disease and not a cause of disease. If that's the case, then rapamycin decreasing activation of AKT is not going to be bad at all. It's going to phenocopy at the final step, but it's not going to be bad.

So in order to resolve those, yes, we have to understand a lot about rapamycin but we also have to really be much clearer on the sequence of events that really leads to quote "insulin resistance in Type 2 diabetes."

I think you can summarize it by saying, there's 100% certainty that rapamycin will extend your lifespan if you don't die first.

Christine Liu: Hi, my name is Chris Liu and I'm from Boston University and my question is for Dr. Evans and it's regarding interventions to prevent the nitrogen loss that you see with bed rest. And so I'm just curious to know if you, you mentioned that you looked at amino acid supplementation as an intervention, but if you've looked at physical activity as an intervention and, in particular, I'm thinking about Cynthia Brown's work at University of Alabama, where she did an intervention to increase mobility in hospitalized elders.

Dr. Evans: Yes. So one of the interventions we did is we got each of the older people out of bed for 15 minutes per day and they walked on a treadmill at a specific percentage of their maximal aerobic capacity. And it had absolutely no effect on the loss of muscle. So 15 minutes a day of walking was inadequate to prevent the loss.

Now, it may well be that the studies funded by NASA show that one or two bouts of resistance exercise exert a far more powerful effect than just getting up and moving around. So I think that that's probably the best and most appropriate way to exercise the patient.

There were a number of other effects. One of the things that happened, again these are healthy people, they weren't sick. So this is the best case scenario. They became really profoundly hypotensive when they tried to ambulate and move around. So I think that there are a lot of changes that occur both in the cardiovascular system as well as in muscle protein metabolism. I do think that the insulin resistance that we see also impairs the amino acid stimulated blood flow to muscle and that may be one of the sources of the so-called anabolic resistance.

Dr. Liu: Thank you.

Dr. Pessin: We have ten more minutes left in the session so try to keep the questions and the answers pretty brief.

Dean Jones: Dean Jones from Emory University. I just wanted to broaden the scope of the discussion a little bit. It's now possible with metabolic profiling to measure 20-, or 30,000 chemicals in a living organism. And we've done that in association with aging in flies, in marmosets, and in humans. And what's quite remarkable is that there are hundreds of chemicals that change significantly in association with age. Now we don't have any information with regard to association with longevity but you can do a metabolone wide association study on this.

But the question really comes from that and that is that, has anybody or do you know of experiments where people have taken the calorically restricted models and asked the metabolic questions and the interventional questions that you're discussing? Because it would seem that if you maximally extend longevity with the caloric restriction, you're going to have much better opportunity to really identify the other contributing mechanisms.

Dr. Newgard: I'm not aware, as one of the centers that does metabolic profiling, we've certainly looked at multiple models of caloric restriction and looked at metabolomic outcomes in such but not in the context of the lifespan extension timepoint. We can tell you what happens metabolically with eight weeks, for example, of caloric restriction in a DIO high fat diet fed animal. And the changes, of course, are profound as you switch to a very much more catabolic condition.

But how that plays in terms of then the subsequent temporal pattern of metabolic change in an animal that experiences lifespan extension versus one that does not, that is maintained on an ad lib fed regimen, I'm not aware of data sets of that nature. If anyone is, please speak up. But it's an interesting- Like so many things in this field, even thinking about that experiment hurts my head. But yeah, go ahead.

Speaker: There is a publication from the Wisconsin nonhuman primate calorie restriction study. Resi is the first author from 2009, I think it's, *Experimental Gerontology*. So they did NMR profiling at three timepoints through aging as part of the longitudinal study and the trajectory of change with age was different for the control animals than it was for the CR animals.

So there were significant differences at each of the timepoints but that dynamic of change through aging was different for both groups as well. But I think that's the only one for the nonhuman primates that I'm aware of published.

Dr. Newgard: Okay.

Dr. Pessin: I think we better go on.

George Martin: George Martin, University of Washington. First of all I want to congratulate, I'm not into metabolomics but you did a great job and I learned a lot. My question is something else I don't know

much about. The hippocampus, if we can go back to that, Dr. Cai and Roman. And I was wondering what's the proximal stimulus for infiltration or activation of the microglia and the inflammation? Could it be Judy Campisi's SASP, with astroglial cells which are known to undergo replicative senescence? Or, is it something to do with proteostasis, protein aggregates that triggers it? How do you think about that?

Dr. Cai: This is an excellent question and I think at this moment we don't have a clear answer. I guess we need quite some years to develop a picture. But I guess, conceptually, I think it just depends on what kind of stages you're talking about. Is it initial stage or during the development of aging?

If you're talking about during the course of aging development there's certainly that crosstalk between microglia, astrocyte and neurons, and they influence each other. Clearly, they're paracrine pathways mediated by cytokines and maybe more than cytokines. And if you're talking about initial change for let's say inflammatory signaling. So I think intrinsic homeostatic changes intracellularly may, as I said in the talk, may have a good contribution but we still don't know extra cellular stimuli what kind of important factors could be most important in that process. But that's I think, is a significant area for the future.

Dr. Martin: And very quickly about brown fat. We didn't hear anything about brown fat, Deborah, in this story. It says, uncoupled, and generates a lot of heat, and we're losing it as we get old, right, losing a lot of brown fat.

Dr. Muoio: I'm not aware of any study showing that. Is that true?

Dr. Birnbaum: I think the activity goes down, whether it's less brown fat or less activity.

Dr. Muoio: But due to a change in sympathetic nerve activity. But there's certainly, I think, a lot of evidence that modifying the activity of brown fat can be beneficial for the health outcomes that we study in the context of obesity and diabetes. I think it's an intriguing possibility that you might be able to target some of these cycles in the brown fat to then generate signals that would be favorable for lifespan.

Dr. Evans: There is some data that in animals that have more brown fat that they do live longer but whether or not they're healthy aging or just protected against disease it's still not known.

Jim Nelson: Jim Nelson, Barshop Institute, San Antonio. A comment and a question. With respect to this paradox, if you will, between insulin resistance between mammals and invertebrates, I just wanted to bring to attention a fascinating paper that was published in *JBC* about seven or eight years ago by a Japanese group who replaced the murine insulin receptor with a mutated insulin receptor by knocking in one that had a mutation at a site that was common to the DAF2 mutants that live long and *C. elegans*. And what they found out was that this replacement increased, quote/unquote "classic mammalian insulin resistance" as defined by glycemic responses, but it also had a remarkable effect on increasing resistance to oxidative stressors in vitro and in vivo. And then they went on and for five years later published the lifespan data and showed that despite continued insulin resistance there was no difference in lifespan so it was a very solid study.

My question is with respect to Bill Evans' very fascinating and thank you for bringing that to consciousness, this discussion of anabolic resistance. Back 40 years ago when NIA was first formed there was, the discovery had recently been made of the steroid and peptide hormone receptors in signaling pathways and there was a big push then to look at the effect of aging on those pathways. This has kind of fallen by the wayside and I was just wondering, with respect to these elderly individuals, what we know about anabolic hormone receptors signaling pathways. Is there resistance in those pathways?

Dr. Evans: The limited amount of data that has been published would indicate that there is continued to be some resistance to a straight anabolic therapy although it's not clear where the resistance lies. There are data that show that if you stimulate artificially blood flow, that is the muscle sympathetic nerve activity is reduced with insulin resistance and diabetes. I think that translates to a decreased production of NOS and a decreased vasodilatation associated with feeding. I think that's where a large part of this insulin

resistance lies. And if you change blood flow along with the anabolic therapies you see almost no anabolic resistance at all to either amino acid feeding or to the anabolic effects of testosterone, for example.

Dr. Pessin: All right, we only have two minutes left so please, very brief.

Michael Anson, PhD: Mike Anson, CCBC. I think this question is probably best for Bill Evans. In most macro nutrient studies there is a time of adaptation to the change in protein level or the change in carbohydrate level, and in real life with nonexperimental, when you're hospitalized, a lot of times it happens unexpectedly. Do we know anything about whether we lose our ability to adapt to sudden changes in macro nutrient? Does the time course of adaptation change with age? Do we know that?

Dr. Evans: I'm not aware of any, but one of the things that clearly happens in hospital setting is that older patients become anorectic or even worse, as a colleague of mine showed that in most hospital settings older people are given 50% or less of what their true energy requirement is. That's what they're given, not what they eat. And those patients do extraordinarily poorly and the number one reason for that is doctors forget to take NPO off their orders. And that's 30% of elderly people in a VA system. So I think we have a lot of work to do.

Speaker: I think this is a question about futile cycles. But that's because I don't know anything about futile cycles. Methionine restriction extends rat lifespan by 40% even though they eat like crazy. They're not caloric restriction, it's just that they can't get that next methionine residue. How does that work? How does that extend their lifespan?

Dr. Muoio: I'm going to defer that to Morrie.

Dr. Newgard: No, let me jump in on that one because we've actually been studying an inhibitor of an enzyme called METAP2, which is the enzyme that locks methionines off of the end terminus of proteins. And remarkably, if you inhibit that enzyme you drop cellular methionine levels by 40%. So there's a very important role for the removal of that methionine in terms of the homeostasis of the methionine pools. That intervention, a block of the METAP2 inhibitor causes a dramatic weight loss, a reduced caloric intake. So I do think that more study of this methionine mechanism in terms of longevity and also feeding behavior is very well-warranted. There's literature out there, like other aspects of the nutritional literature, it can be a bit occult at times, and I think it's something that we should have a fresh look at.

Dr. Muoio: I'll make one real quick comment. It's just that in preparing for the conversation I had here, is that the research on these futile cycles really goes back to the mid '50s and '60s and it was very active at that time and fascinating ideas. I think that a lot of these cycles that we think about, protein breakdown and resynthesis, for example, in the context of the methionine diets, very well could be activated. The gap has been is that that field has been relatively silent other than the uncoupling proteins for the last couple of decades. And I think now we have the tools to really start asking those questions in a much more robust and deeper way.

Dr. Pessin: Last question.

Rochelle Buffenstein: Rochelle Buffenstein from Barshop Institute. This is a question on circadian rhythms to Roman, I'm sure there must have been some studies that have been done on humans and animals that have been kept devoid of ____ givers or in the dark. Is there a change in the peripheral and central circadian clock genes and does this impact on metabolic syndrome and insulin resistance kind of features?

Dr. Kondratov: To my best knowledge nobody did this experiment. I mean, people did experiments keeping animals under constant condition but nobody checked for expression or anything like this. So it needs to be done.

Rochelle Buffenstein, PhD: Are there any diseases that have been linked to being kept permanently in a disrupted free running rhythm?

Dr. Kondratov: What is known very well is that if you take disease, let's say obesity or metabolic syndrome, at least in animal models, in all these conditions, circadian in gene expression and production of some secretory molecules, they are disrupted. So that's what is known. For disease state result in disruption of circadian rhythm and gene expression. To what extent this disruption and gene expression contributes to the development of disease is a question.

Did I answer your question or not?

Dr. Buffenstein: We'll talk about it later. Thank you.

Dr. Kondratov: Yes.

Dr. Pessin: I'd like to thank all the panelists and the audience and I'm sure they'll be ready to talk later during the break. Aaron do you have an announcement?

Dr. Pawlyk: Thank you. We'll take a break. Be back at 10:15. We'll start with the macromolecular damage session promptly at 10:15.

Session VI: Macromolecular Damage

Ron Johnson: Okay, everyone. If you could take your seats, we're ready to start the next session which will be on macromolecular damage. My name is Ron Johnson. I'm a Program Director in the National Cancer Institute. For today's session our cochairs on the session for macromolecular damage is Arlan Richardson and Eric Schadt. Dr. Richardson is Professor in the Department of Cellular Instructural Biology at the University of Texas, Health Science Center in San Antonio and senior research career scientist in the Barshop Institute. He will give an introductory overview of this topic for this session.

Dr. Schadt is Professor and Chair of the Department of Genetics and Genomic Sciences and Director of the Institute for Genomics and Multiscale Biology at the Mount Sinai School of Medicine in New York City. He will be directing the discussion which will follow the last speaker and give a summary of this session. Dr. Richardson.

Introduction

Arlan Richardson, PhD: Thank you very much, Ron. Before we start I want to really give Ron credit because he's done all of the legwork behind the organization and keeping us on course. He would always send out these nasty emails and I would then play the good cop who says, well, you know, maybe you can get by with another day or two. So thank you very much, Ron.

I'd like to start out the session- I wanted to kind of capture the importance of DNA damage and aging and I appreciate Felipe Sierra giving me this picture of his car which would kind of demonstrate the potential ravages of damage in aging. It would have been nice if you wouldn't have thrown away the hub caps on you car, there, Felipe. And we wonder why taking the senators and congressmen around in this kind of a car, we aren't getting more money in aging.

But seriously, the concept that damage was important in aging is a relatively old concept. It started with Bjorks in '42. He was a chemist with Kodak and he came up with the concept that protein crosslinks might be important mechanism underlying aging. And then, of course, in '56 Denham Harman came up with the free radical theory of aging which is now called the reactive oxygen species theory of aging, or oxidative stress theory of aging. And Denham came up with the concept that free radicals were causing damage and this damage would give rise to aging.

Subsequently, Leo Szilard, who is the father of the atomic bomb, a physicist, came up with the concept that DNA damage was a key in aging and, subsequently, Leslie Orgel, came up with the error catastrophe concept which damage or mistakes in those proteins and molecules involved in gene expression would cause more damage when they were in transcription and translation, and that these would build up and you'd have this cascade of this error catastrophe.

Now the question is, at that time was, does damage increase with age? And there was a great debate for a substantial amount of time but in the late '70s and '80s it was shown that yes, indeed, damage did occur. These are two of the leaders that I remember speaking at almost every aging conference that I went to back in the '80s and Bruce Ames was focusing on DNA damage and the slide below shows data from our studies where we were looking at eight oxy deoxyguanosine going up with age. Earl Stadtman's group not only showed that oxidative damage, in this case carbonyl groups on proteins, increased with age. But Earl's group was the group that essentially discovered that proteins actually did become damaged or oxidatively modified. And it's interesting, Rod was telling me that Earl essentially was known up until his 60s, as the person in studying regulation of proteins and it was in his 65, his later years, that he really got into aging and this became one of the key factors we remember him for.

Okay, so damage increases with age. What is the evidence that damage plays a role in aging? And that's kind of the theme that we've heard all along here. In other words, does it cause aging or does the damage arise from aging? And the evidence for DNA damage is several-fold. It's first DNA damage, particularly oxidative damage, is reduced by dietary restriction which increases lifespan and is believed to slow down aging.

The other interesting thing is that deficiencies in DNA repair, and this was observed in humans, would exhibit accelerated aging phenotypes, and this is the study of the Werner Syndrome patient, but there are other damage. And this has also been found in mouse models where they've knocked out various DNA repair genes. In addition, mice have been made that make mutations in the mitochondrial DNA and these also show accelerated aging, indicating that damage in mitochondrial DNA is important.

Now, however, even though there is this evidence supporting this concept, there's a few problems. First, the levels of mutations or deletions are damaged that you see in these models of accelerated aging. The amount of damage that you see here is very high compared with the damage that you normally see. The other fact is that, although we see this difference between damage and accelerated aging, it would be really, I think, important, to show the reverse and this has not been done. Where if you would reduce damage in DNA would this lead to increased lifespan?

Now with respect to protein we see that protein, oxidative damage to protein is increased in many age-related diseases including subjects with progeria. Dietary restriction, again reduces oxidative damage to protein. However, again, there are no studies that have shown where you've been able to reduce oxidative damage to DNA and lead to increased lifespan, and the studies from Shelly Buffenstein's lab with naked mole rats show, in contrast what you would predict because these are longer lived, they actually have more damaged protein as well as DNA damage.

So in general, when we think of protein damage we think that it could be having a physiological effect; initially it was argued that this was due to decreased enzyme activity or biological activity of the protein. Now the argument is that this damage results in oligomerization and aggregation, and this is something that the cell wants to get rid of is potentially toxic. So we talk about proteotoxicity.

And the next session will be focusing on that. However, it's also, as Dean Jones will talk about, is the damage to those proteins that are in, what he would term, the redox proteome. In other words where you have a free file group that's important in biological reactions, this could have a multitude of effects, not only in transcription factors but in a variety of other places so that you would have this global effect.

So what we're going to do today on our session is that the speakers in the first two presentations are going to focus on the importance of DNA damage in aging. The next two speakers are going to be focusing on protein, and then Mike Yaffe at the end is going to be looking at damage with respect to

systematic approach because one of the problems in this field or limitations in the data in this field, is we've usually looked at either DNA damage, or protein damage, or maybe damage to lipids, and even when we're looking at DNA damage or protein damage, we're looking at one or two types of damage. No one has really looked at what would be the concentrations of a little bit of damage everywhere? And so I think this will be important in the future. So with that, we'll go to our first speaker.

This is Jan Vijg. He's a Professor and Chairman of the Department of Genetics. I knew Jan, I'm so old that I remember Jan when he was just a junior faculty member. He was just a short little guy then and had really long hair. So, Jan, for those of you who don't know it, Jan really started out, he developed the first, it wasn't called big blue mouse, but the first mouse to look at mutations to study the potential, to really ask the question, did DNA mutations increase with age? And this is an area he's been a leader in subsequently. Okay, Jan.

Do DNA damage or mutations ever reach high enough levels to cause functional decline?

Jan Vijg, PhD: Well thanks, Arlan, for this nice introduction. I just want to say, actually seriously, thank you, Arlan, I really learned a hell of a lot from you in the time I was in San Antonio.

So I can tell you already, I'm not going to be able to answer that question, actually, perhaps partly. Why DNA, why damage in DNA? Well Arlan already you mentioned that, I think it's really the only cause of aging for which we have shown that when you are going to look to the cellular defense systems that the repair and neutralize in any other way, DNA damage, when you have inheritable mutations there you show premature aging. That's true for humans and for mice. I'm not aware of any other multi T systems that shows it.

But the other reason, of course, is that some of the damage in DNA, some of the alterations in DNA are actually irreversible. And I think that's really also not true for most other biomolecules. Now when we really became aware of how important DNA damage was, that goes back to work in the 1970s by Thomas Lindow who demonstrated that DNA is actually a very unstable molecule under physiological conditions. There are probably thousands to tens of thousands of DNA lesions in each cell, each day and, of course, the first question that really came up here was, if that is the case then can DNA damage itself and DNA damage are really physical alterations in the DNA molecule like breaks or cross links or depurination, depermutations, not to confuse them with mutations which I'm going to talk about in a minute.

So the idea was perhaps, that DNA damaged itself at some point, reached levels at old age, high enough to have immediate adverse effects. For example, on transcription. I can answer that question. The answer to that question is almost certainly, no. It's very, very unlikely that that's going to be true. First of all, several laboratories, including my own, have demonstrated that, actually, the steady state damage level is very, very low, even at old age.

So now the reason that it is low and actually stays low, virtually undetectable, in fact, is of course it has to do with the complex of genome maintenance systems that you can roughly divide in two arms. That's, of course, actual repair of damage, so excision of damage and replacement of the damaged piece of DNA, rekneeling of breaks, double strand breaks and so on.

But the other form actually is a response to damage and I think David Sinclair already mentioned that because DNA damaged itself, is of course, random. But by signaling to systems that turn that even rare damage like double-strand breaks, turned that into phenotypic endpoints like, for example, apoptosis in cell senescence, you have a very nice example that you don't really need programmed aging to get very consistent effects of DNA damage that are very similar from individual to individual and even across species.

So, as you have already heard, loss of cells, atrophy, and also, of course, accumulation of senescent cells is probably a major phenotypic endpoints of aging and those could be the result of DNA damage.

Now, on the other end, repair of damage, in fact, can also lead to chromatin alterations. David also mentioned that briefly and that's something for the next speaker.

What I will be specifically talking about is errors that are being made during the actual repair of damage. Damage repair is good for the survival of the cell but there are inevitable errors and those errors we call mutations. So mutations, not DNA damage. That's a really important point because DNA damage is reversible, you can repair it. Whereas, mutations cannot be repaired. Once they're there - they're there. You lose the information in the DNA template. So the question here is really only how many mutations are there and can they exert a phenotypic effect?

Same is actually true for random changes in the epigenome. Not to be confused with program changes in the epigenome, and then, of course, occur all the time. But I'm really talking here about say a pattern of DNA methylation or histone modifications that need to be restored after repairing damage.

Now you can put it in a model as I have tried to do here. This is a young healthy tissue with the color indicates increased numbers of mutations and possible functional decline. That's already the case actually, in a young tissue. But after aging, you see accumulation of senescent cells, irresponsive DNA damage. You see actually atrophy, cells that have been removed by apoptosis and sometimes that's a very unfortunate combination of mutations that give rise to a tumor. So in principle this could explain a fair part of the aging process.

Now what I will do now is to spend a little bit of time on the possibility that actual mutations will accumulate in individual cells to levels that can exert a phenotypic effect. Now this is the situation with respect to mutations. Those errors in repairing DNA damage already occur from the very beginning. So you'll see that DNA mutations begin to accumulate during development and, of course, the aging process. You can see that here. Each color dot is a different mutation, it's just a completely random process. But occasionally there's a mutation that provides a selective advantage and that is the one that grows out to a tumor. You can take that tumor from the tissue and sequence a complete genome. It has been done. And what you will see then is that patients that were older from which you took the tumor and you sequenced, contains many more mutations than younger patients. And this, of course, is because most of the mutations that you find in the tumor actually were accumulated in the normal cells before the tumor even took off.

Occasionally that's also what you can call drift. There's a particular mutation that's actually showing up in a fair fraction of the tissue or cell population, let's say, 15%, 20%. Those can be detected directly by assay and it is being done. There are a number of papers on that. But the vast bulk of mutations you cannot detect. So that's really a major problem for us to answer this question that I mentioned to you before because if you look at this random mutation some people would argue, well that can never have a functional consequence because the frequency's too low.

But that's actually quite incorrect because, of course, function is not provided by genes. Function is provided by genetic pathways and networks and they consist of multiple genes in the pathway, many other genes that encode proteins that are going to affect those genes. And then, of course, assist in transfer noncoding regulatory sequences. So the total amount of sequence code that can be affected by random mutation is fairly large. So wherever a mutation lands in this system, it will of course, have an adverse effect on the function of that network.

So here is another example of how completely random mutations can actually have a phenotypical sequence that, again, is very similar from individual to individual and even across species.

Now of course, what you would like to do and it's the last slide that I'm going to show, is can be used, this great series of advances in sequencing to actually look at somatic mutations and the answer initially is no or very difficult because again, remember this whole tissue, where mutations are random, this is one of those mutations, one single cell. You grind the tissue together, you start to sequence it and this is typical what you get with next generation sequencing, many, many reads. And here you see the mutation. Well you know that this is critical mutation. This is what I told you just moments ago.

How you could distinguish between this mutation and one read, all the other reads were clean. And here you see two others which are really sequencing errors. You cannot distinguish. So it's really not possible to get information about somatic mutations. But you can take individual cells, do a whole gene amplification and now you will see that the same mutation actually shows up in about 50% of the reads.

Why 50%? Well because it's only one allele, and the chance that a random mutation will hit both alleles at the same time is very small. So when you do single cell sequencing you can get a handle on finding out how many mutations there are in a somatic tissue. And this is just one example where we sequenced a single nuclei from the Sofala flight missile. This is a _____ of course and you can't really isolate single cells. We isolated a single nuclei from the flight missile from young flies and old flies. And this is six different nuclei and here eight. And you can clearly see that you see a particular dramatic increase in the mutation frequency. This is mutation SNV single nucleotide variant per mega base, so it's about .25 per mega base in these flight missile nuclei and it's much higher in the flight missile nuclei from the old animals, and you also see, of course, that a variation is much higher. We recently got data from mouse hepatocytes and they show essentially the same thing.

So finally, looking at the conclusion. You can see there are three here. Even if you have very low levels of DNA damage like double-strand breaks, that can drive apoptosis on senescent cells. So here you got yourself a clear possible function from DNA damage.

Of course, random mutations, even if they are occurring individually at low frequency, can definitely affect gene functional networks and finally, when you do single cell genomics you can actually get access now, finally to this landscape of somatic mutations in old age.

Two last conclusions that are not on here. I think it is very important to monitor when you are developing interventions in aging that you're not only focusing on cell survival because cells may survive but they will contain more and more mutations. That's not good. So it's important to have a measure for the occurrence of random somatic mutations. Same will be true, by the way, for stem cells to maintain stem cells in culture.

Finally, and perhaps something for the discussion, you can ask yourself the question, if those mutations will definitely occur, are inevitable, and also irreversible, is it actually possible to do something about aging in the first place. Will we ever reach a state if we can in one way or the other, sort of refer that. I'm not so sure about it. Thank you.

Dr. Richardson: Thank you very much, Jan. The next speaker is William Bonner. He's from the Laboratory of Molecular Pharmacology at NCI and Bill was involved in identifying several histone variants and showing their association with double-strand breaks. So, Bill.

Are chromatin changes a result of aging, a cause of aging, or both?

William M. Bonner, PhD: Okay. Thank you very much. So I'm going to talk about chromatin and so in fact, I'm going to change the title a little bit to nuclear changes. And the reason is, is because of what I've written up here, the chromatin, of course, we have the DNA and the histones, and nonhistone proteins and RNA and lipids, and it resides in the nucleus and it also takes up the whole nucleus, virtually.

And, of course, its job is to control gene expression by the timing and the level. Now, observations that have been made in the literature is that aging leads to telomere shortening, heterochrominization with these senescence associate heterochromatic foci, which contain gamma H2X. Then there's other things, loss or changes in 5 Methyl C, poly ADP ribosylation and some other markers.

So, first of all, we have telomeres and we know that telomeres get shorter with aging because of the preliminaries can't make it all the way to the end of the DNA, and this was, I guess, Hayflick's theory of aging, but there are multiple lines of evidence that do not support it. I mean, for one thing, mice have

very long telomeres, even when very old and when they're dead. So we can see now, what about the senescence associated, heterochromatic foci?

Well, here's a paper that says they are dispensable, at least in certain cases. Here we have, when we talk about epigenetic modifications, here is sort of the catalog of many of these epigenetic modifications. As you can see on the four core histones, primarily on the amino terminal ends, as you can see the histones are packed into these nucleosomes and so you have various acetylations, methylations. We're all sort of familiar with this.

This is the one that has been remarked in the literature as being enriched during aging. And also we have 5 Methyl C changes. This is a paper that I came across, this just came out last week, which I think is quite interesting. It talks about a DNA methylation clock. Steve Horvath has found 353 methylation sites which, when you graft them out, track age, very, very closely so that you can, actually, and in virtually every tissue of the body, so that you can calculate the age of a tissue by looking at these methyl groups.

So you can see from birth on there's a very rapid increase in these parameters and then a more gradual straight clock. Some interesting observations from this is, well this happens in quiescent cells and neurons as well. So what about progeria, the premature aging disease, Huntington's? Well according to this, you do not get, these things do not fall on this line. So maybe there's some question about progeria being a model or maybe there's some question about the methylation clock. But anyway here's sort of a little area of disagreement.

What is interesting is that when you induce pluripotent stem cells you do reset the clock, which I think is probably sort of encouraging for the people in that field.

So now let's talk a little bit about what's in the nucleus besides the chromatin. Well, there's a whole bunch of little factories that hang on in between the chromatin. For example here, this is the nuclear speckles where you have the splice-sites. In fact, a faulty cryptic splice site is what turns lamin into progerian and causes the Huntington's disease. And here is the lamin, this little sort of wiggly line along the outside of the membrane.

Another thing that sort of came up earlier in the conference is nuclear stress bodies, obviously, a decent name, that work to counteract stress. And you can have both mild heat stress which is thought to be mainly correcting nascent proteins, and then you can have severe heat stress. And I thought it interesting, the temperatures here and heat stroke in humans happens at about 105 degrees so certainly we could be undergoing mild heat stress. And whether this is in any way equivalent to sautéing nematodes, I don't know.

I thought when that talk was given, if you looked at people who spent hours and hours in steam baths all the time, do they live longer? I don't know.

The transcription factor is, okay, here we have senescence associated heterochromatic foci and some other things. PML bodies are thought to have to do with telomere maintenance, so you have those. So we have a whole bunch of other things in the nucleus. So we have a very complex thing.

Now here's a list of the progeria-like diseases and the defects. As you can see here, Werners, Bloom, Rothmund-Thompson, these are all hela cases. Here we have, and the other ones, with one exception, these are all nucleotide excision repair proteins.

So we have Cochranes' xeroderma and tri-whatever it is.

Now here we have telomere associated, dyskeratosis congenita, which has to do with defects of the telomere. And then we have Hutchinson's-Gilford progeria with the lamina protein, two forms, one in

which the lamina is actually faulty and the other in which the protease which chops the prelamin into lamin is missing. And so it's interesting that these are all single-strand damage.

Double-strand damage repair pathways, of course, ATM mutants and things like that are serious but they don't seem to show any effects of accelerated aging. And I was thinking, it was alluded to before, when we're talking about single-strand damage we're talking about perhaps 50,000, 100,000 events per day per cell. So these pathways are always on somewhere looking around doing something.

On the other hand, double-strand breaks, we're talking maybe ten per day and that may be more in cycling cells, so the demand for that may just be a lot less and so it may have enough time to take of stuff even under highly suboptimal situations.

And just to finish up I thought it would be interesting to present this as other options for employment. As you can see, there's already something around here to take care of progerin problems, by this cosmetic company, and you can also get a certificate of telomere science if you would like.

Thank you very much.

Dr. Richardson: Our next speaker is Rod Levine. I've know Rod for a long time. Rod is the Chief of the Laboratory of Biochemistry at the National Heart and Lung and Blood Institute. As I said, I've known Rod since he was a real tall person and he had no beard. And now he shrunk and he's got a beard. But seriously, Rod was part of Earl Stadtman's group and was really involved in the discovery of protein oxidation and he's made major contributions in this field and so he's going to talk about the role of protein oxidation and aging.

Can oxidized proteins be repaired?

Rod Levine, MD, PhD: Good morning. Thanks, Arlan, and happy Halloween. You know, there's a lot of official celebration of Halloween here at NIH. It's not just the candy that was put out at the registration desk, at lunch, check it out. Go out the cafeteria doors. Walk over to the sidewalk and look across the green area to the trees and you'll see something scary. There is a coyote moving there. Some of you may already have spotted this. It apparently wasn't put out just to scare us, it actually was put out because we have an invasion of Canada geese which make a God awful mess and they love this area. And so the grounds people actually put these things out and they look pretty realistic and they move, and according to their poorly controlled study, it actually works.

This is supposed to be a TED like talk so there's a simple question, can oxidized proteins be repaired? And I'll raise just a couple of simple points.

We know that sulphur-containing amino acids, cystine and methionine, when oxidized, can be repaired. But what about beyond that? The point I'd like to make first is, we don't actually know what's repairable because most of us haven't actually had the strength to tackle it. And I want to suggest that we really might benefit by determining which of the covalent and noncovalent modifications really can be reversible, that is that can be repaired.

And there's a key caveat that I'd like to offer you despite the fact that my undergraduate degree is in chemistry, evolution isn't run by organic chemists but a lot of our thinking about what's repairable has been dominated by that. And my colleagues who are chemists are much more certain about what's possible and what isn't possible than most of us in biological research.

So here's an example. Suppose you want to oxidize acetate to carbon dioxide. Talk to a chemist. It's hard. You've got to use very high temperatures and elevated pressures to do it. It turns out though, the cell, no problem. Activate the acetate with Co-A, pop it into the TCA cycle and you're good to go and it even works at room temperature. And this is meant to be more than just glib, it really is a contrast to the way of thinking.

So here's what I think is a relevant example, repairing overoxidized cystine in proteins that have an essential cystine thiol at the active site. In such enzymes conversion of the thiol to sulfinic acid is critical for the catalytic cycle. Peroxiredoxins, reductases, some dehydrogenases and others. But this is a fragile modification in that the sulfinic acid is very susceptible to further oxidation. It's pretty easy to go on to sulfinic acid, two oxygens added. And from the chemist's point of view, I learned this, many of you did, that's simply irreversible and we've known that for years, simple.

But it's wrong. Sugu Ri and his colleagues during the course of studying antioxidant defenses mediated by peroxiredoxins, showed that extracts from hela cells and then later a number of mammalian cell lines could actually reverse it. And they published this observation in this first paper. They didn't know how it was being mediated but it was quite an impressive discovery that it could happen because it wasn't supposed to be possible.

So how is it repaired? And Sugu Ri and his colleagues didn't succeed in isolating the activity, identifying it and telling us what it was. And it's because Sugu, who is an outstanding biochemist, had a handicap. His PhD was in chemistry and he made some assumptions. But Michel Toledano and his colleagues did find it, they were thinking outside the box, and they named it sulfiredoxin. What went on here? Well, it would have been ridiculous if you were thinking about this logically, to consider that ATP might have been required for this activity. And Sugu Ri and his folks never thought about that. They threw all sorts of reducing agents in to try to get this to go. But Toledano said, "I haven't got any idea what's going on, I'm a geneticist, a molecular biologist, we'll throw everything in." And ATP was the key. So I repeat, for the last time, evolution isn't run by organic chemists. Maybe we ought to have a more open mind.

How far can repair go? There are a lot of modifications that I would have liked to talk about but the task master, Felipe, got his whip out and told me that I had to cut at least one slide and the list of modifications went.

I'll mention nitration, particularly of tyrosine, since that's rather an important modification that can be mediated in inflammatory changes initially through modification of nitric oxide. But let me focus on one thing that Arlan did introduce, and many of you are familiar with, and that's the accumulation of carbonylated proteins which can come from a variety of sources, but let me just mention this one.

And that is, a common oxidative modification of proteins that have metal binding sites, so structural proteins and enzymes is the oxidation of arginine. And the guanidine group with its three nitrogens is just blown to pieces by an oxidation that occurs at the metal binding site and affects the arginines. And you end up with the carbonyl group that's just the aldehyde analog of glutamic acid, gamma glutamyl semialdehyde. I hope that, I've not done this, I might have, but it's widely stated that this is an irreversible reaction and the only way you're going to deal with this is to degrade the protein. Is that true?

So let me show you my "oh hell" slide, that's because when I show you the next part, you're going to be thinking, "Oh hell, is he going to go through all that?" Well, I'm not. I want to zoom in here on the arginine metabolism and show you something that microorganisms and a number of lower organisms do with no problem, some higher ones. They make arginine. You know how they do it? It's a simple two-step process starting with gamma glutamyl semialdehyde. Ornithine transaminase converts it to ornithine, goes onto citrulline and you're popping this into the urea cycle and what do you end up with? Arginine.

All you have to do is follow this. It works on small molecules. There's no reason why it couldn't work on proteins if access were available and the enzymes existed. Nobody's looked. So I had two other slides that the whip made me take out, and it's simply to say the same thing happens with lysine oxidation, two-steps, yeast are really good at it. You've got your oxidized residue and you're back to lysine.

So my conclusion is simple. There's an opportunity here to understand protein repair and that might be important, obviously, in certain aspects of the mechanism of aging or in our ability to intervene in the rates of aging. And I close with a reminder from Albert Einstein, and thank you.

Dr. Richardson: Very good. Excellent. Okay, our next speaker is Dean Jones from Emory. He's Professor of Medicine and Director of the Clinical Biomarkers Laboratory. And Dean is going to be talking with respect, he's been involved in looking at the redox system in organisms and in the last, five, ten years, it's become more and more important that this might play an important role, particularly in any oxidative stress mediated problems. Dean.

What therapeutic strategies prevent or remedy changes in redox potential?

Dean P. Jones, MD: Thank you. And I just have to follow-up with the compliments to Felipe for directing us so clearly. And I have to add one point, and that is that many years ago, decades ago, actually, in a conversation with my father, it came up, what is the meaning of taking scientific data away from a scientist which Felipe did for all of us. And what my father said, is that if you take the scientific data away you have a preacher. So I'm going to be a preacher.

So the question that I have been asked to address is really what we do about the reversible redox change that occurs with aging. And so I've broken this into really three components. One, I want to address just briefly, why this occurs and then does it matter and then talk about potentially what we might be able to do about it. But for those of you who are not familiar with the concept, a bit over a decade ago we found that there is a slow change in oxidation and redox potential that's associated with age. Specifically, beyond about 50 years old we become one millivolt more oxidized per year.

So the questions of why does this occur and does it matter, we have expanded the research a bit. We just had a paper come out on the common marmoset model of aging and found that also in marmosets, just as in mice, and also previously found in rhesus, that they similarly change in the redox potential with age. And so the question, why does this occur, I think to address that, what I would like to go to is the concept of the exposome, as Christopher Wilde defined it, now several years ago. This concept that we have a cumulative life-long exposure to many different dietary, environmental, infectious, exposures, as well as the various contributions to the microbiome therapeutic drugs, lifestyle, behaviors, and so forth.

And so all of these, the cumulative nature of these, is that they impact the organism and they impact the organism principally along this axis between the proteome and the metabolome. Now, of course, some things dribble through and do cause direct damage to the DNA but I think our major interface is right here.

Now what we found over the past ten years or so, in studying this, is that the redox proteome, that is the components within the protein that can undergo changes in response to oxidation, that it's really following up on what Rod Levine said, in terms of believing the chemists, here you have to be really careful about believing the biochemists because the attitude in biochemistry is effectively that the cystines should all be reduced and if they're not all reduced then we simply have an artifact from our biochemical analysis.

Well what we found now is using mass spectrometry based redox proteomic methods is, in fact, that is really completely wrong. That in fact, the redox proteome exists as a spectrum of oxidation states of different proteins and that this, the concept is that this redox proteome is really our primary interface in interacting with the diet and environment and indeed, at a molecular level, it's a follow-up to what Linda Fried talked about yesterday. This is a primary adaptive structure. This is a primary way. It has evolved since the evolution of oxygen atmosphere and it's, obviously, it predated that but in terms of the character that's in us and in mammalian systems, at least, it is this adaptive structure that the redox proteome provides that I think is really the key in terms of the environmental aspects, the diet, environment, and all these other interactions that we have, the stressors that we have.

Now there are 214,000 cystines encoded in the mammalian genome. Those are, obviously, not all of them have been characterized functionally but as we're developing methods, about 4000 of them now, have been measured in a redox sense, almost all of those are at least partially oxidized as they exist in biologic systems.

Now what's very important and links things back to the session this morning, is that the redox proteome is dynamically controlled by the redox metabolome. So the redox proteome, as Arlan indicated, really impacts all aspects of the function of cells. But what's very important in terms of closing the loop, is that the gene directed activities are controlling the metabolome and the metabolome also is interacting with the diet and environment. And so this includes, most notably, the NAD, NADH system, the NADP, NADPH system, we've talked about a bit. It also includes glutathione, glutathione disulfide, cystine, cystine, lipoic acid that we haven't heard anything about here but also coenzyme A. There are many systems that are actually involved in the redox metabolome, and these are, in fact, directly interacting with the redox proteome.

So the oxidation of the redox potential then reflects a loss of adaptability to this external environment. The question is, does it matter? And we've also discussed the problems of correlation analysis And what we know from our studies, I'm in a clinical department, I'm a biochemist, but I'm in a clinical department and so I've been involved in many different studies of this thiol redox system. And what we find is that in obese people, they're more oxidized. If we look in diabetics, they're more oxidized. If we look in people who have age-related macular degeneration, they're more oxidized. We can go down the list, atrial fibrillation, other aspects of cardiovascular disease, idiopathic pulmonary fibrosis. So there's this long list of conditions associated with oxidation of these thiol systems

But that does not establish cause effect. And so the question is, what's the evidence in terms of a causal mechanism? And so, one of the things that has been done is, we and others have looked at changing the redox potential in system and ask the question, what does that do? And two of the very important things that it does is it stimulates proinflammatory signaling and it stimulates profibrotic signaling. So I don't have to go into any details on that but I think the key point is, what can we do about this.

One of the aspects that follows from the last session, I think that this needs to be paid attention to, that American intake, the median American intake of methionine is 2.5 times the recommended dietary allowance. And what my colleagues and I have shown is that if one takes in excessive methionine and cystine what happens is that we increase this oxidized formed cystine and it's very poorly cleared. It's unclear as far as whether or not we should decrease total protein intake. I think it is relatively straightforward that we should at least consider the possibility that we have a pretty wide margin of intake, on excess intake on methionine and it would be worth looking into the possibility that this would be beneficial.

A second is the dietary zinc. We had an ancillary study to AREDS 1 in which we examined the effect of the zinc supplementation on the cystine level, the oxidized form, and what we found indeed, this was the first evidence that the redox actually changes longitudinally within individuals and what that showed was that zinc decreased or prevented the increase with age, and what colleagues have more recently shown is that that zinc mechanism occurs through activation of Nrf2.

I don't have time to talk about selenium, but I think if we had methods to measure how much selenium we should be eating that this is a good target. Because we are now out of time I do want to jump back as a preacher just for one second.

And this preacher is right here. I think that if we have missed the boat as far as longevity research, it's right here. And that is that we need to pay attention to this concept of the exposome and we need to move forward just as the genetics and genomics community sequenced the human genome, we need to develop strategies to sequence the human exposures. We need to sequence the exposome because I think this is going to give us a tremendous advantage in understanding age-related diseases and prolonging health.

Thank you.

Dr. Richardson: Thank you very much, Dean. The last speaker in our session, I can say that I didn't know him when he was little, so this is my first time to meet Dr. Yaffe, and I'm very interested because he's going to - I should point out, he's Co-Chair of Biology and Bioengineering at MIT - and he's going to

be bringing a systems approach to looking at this. I think that, particularly on the last speaker, you can get a feeling, as well as from Jan's speech, is that we have lots of different types of damage and how do we look at all of the end product of all of these together. Michael.

How does signaling in the surrounding microenvironment affect the response of cells to macromolecular damage?

Michael B. Yaffe, MD, PhD: Let me start by thanking Arlan and Ron for the opportunity to speak. What I was charged with telling you about was how signaling in the surrounding micro environment affects the response of cells to macromolecular damage from a systems biology point of view.

Now I was really intrigued by Arlan's analogy of transportation and the car. And so I'm showing you here, a diagram, it looks a bit like Rod's arginine metabolism diagram. It's a systems level diagram, obviously, but what I want to point out here is that this systems biology diagram is the railway lines in Tokyo in keeping with that transportation model. So you can find complexity in everything. You don't have to look just at biology for it.

Now the first point I want to stress to you is that there's a lot of crosstalk between the innate and adaptive immune cells and the micro environment in which damaged cells find themselves and the ultimate outcome of macromolecular damage. So in the case of inflammation where we know that neutrophils and T lymphocytes and macrophages contribute to some of the radicals that cause DNA or protein damage, these cells also play an important role in determining what happens to those epithelial cells when they're damaged.

Probably the best example with which we understand this is in the setting of cancer. And what I'm showing you here is a section of epithelium that's undergone damage. And when cells are damaged they really have a limited repertoire of responses. The cells can repair the damage, either fully or only partially and maintain that epithelium, or alternatively, if you've denuded an epithelium like this you get some type of a repair process and a couple things can happen.

First, we know that cells like neutrophils and monocytes and macrophages move into that area. I want to stress what Russ Tracy alluded to. There's a very important role here for platelets and for fibrin and thrombin. And this, in turn, can do two things, both of which result in a loss of function, one of which results in loss of function. You can lead to substitution of this epithelium by a healing response. You can end up with fibrotic degeneration in this area and that limits function or, alternatively, you can stimulate the division of some type of precursor cell, a stem cell, if you will, that can repopulate this epithelium.

Now the problem is, that in chronic inflammation where this precursor cell itself undergoes continual cycles of damage and repair, damage and repair, it can turn into a malignant clone and I would argue that its signals that emerge from the micro environment that can stimulate and maintain that malignant clone until it turns out to become a full blown tumor. And in particular, I want to stress that there's a very important role here for the endothelium, I'll say more about that in a moment.

So it's really crosstalk between what's happening to macro molecularly damaged cells and though intrinsic pathways and extrinsic pathways through the micro environment, through macrophages, and neutrophils, and other cell types that determines what type of a response we see. Furthermore, it's these extrinsic pathways, it's these extrinsic cells and some of the cytokines and growth factors that they release that modulate what it is that the damaged cell itself does.

Now we have some understanding of some of the cytokines and growth factors that play a role in this. You've heard a lot about this over the last two days, proteins like IL-6 in particular, and IL-1, IL-8, and TNF-alpha but in addition growth factors like IGF-1 that Morrie alluded to earlier and VEGF and PDGF, all play an important role. Now all of this starts, of course, we think at least in part, by ROS generation in response to stress but tumor cells themselves, when they undergo that change in metabolism that we're

all aware of, also generate ROS and this leads to a cycle where tumor cells modify and modulate their micro environment at the same time that the micro environment modulates the tumor cells.

But there's one cell that we haven't heard that much about that I think plays a particularly important role in tumor genesis and probably aging in general, and that's the endothelial cell because we know that some of the things that tumor cells secrete stimulate endothelial cells and endothelial cells, in turn, then secrete cytokines and chemokines that control what happens to those damaged cells, whether they proliferate, whether they migrate, whether they then, in turn, induce further angiogenesis.

And I would remind you that in the case of lymphomas, for example. When you treat lymphomas with chemotherapy, a subset of those lymphoma cells become resistant and lead to ultimate recurrence of the disease and those resistant cells exist in the thymus. And the reason that they're resistant isn't because we haven't damaged their DNA, it's because IL-6 produced in the thymus makes a chemo resistant niche where those macro molecularly damaged lymphocytes can be maintained.

Interestingly, the cell type in the thymus that's responsible for the IL-6 production is not an immune cell. It's in fact, the endothelial cells in the thymus. And so I think, as we begin to look at signaling mechanisms that are involved in what happens to macro molecularly damaged cells, we need to keep these endothelial cells in mind.

It means that we have to start thinking about signaling pathways that are involved in these growth factor and cytokine responses. Now from a systems point of view, I'm going to give you sort of the TED talk on systems biology. What you get out of it depends on the level of granularity at which you want to look.

Now we heard earlier, from Linda, that the types of things that we can look at epidemiologically in patients, are things like nutrition and infectious status, and what happened to the patient, and whole body dynamics. But in fact, where systems biology is best suited, at the moment at least, is down here at the level of individual cells, where we look at certain cues that the cells see, certain responses that they have, and then we assay the things that we know how to assay, mainly signal transduction networks, in order to build a model where we try to correlate the cue that the cell saw to the response that this cell provide based on the signals that it saw.

And I want to give you a quick example of two ways in which one can do this. So what I'm showing you here is crosstalk between, for instance, modifiers of metabolism like insulin and cytokines like TNF that ultimately result in cell death. And if we want to understand how it is that signaling pathways tell us whether a cell in response to insulin, in TNF, die, survives, or replicates. We have to start to look at pathways that control each of these responses and how these pathways talk to one another. So we can break this down into pathways that we think insulin drives, survival pathways like ERK and mTOR and AKT. Stress pathways that we think TNF-alpha drives like NF kappa b and JNK and P38. And then outcomes like DNA damage responses in caspase activation. And we have to come up with assays that allow us to measure what's happening dynamically in this entire network.

And there are a subset of proteins, many of which you see marked with the letter P for which we have medium or high throughput assays that allow us to do this. And the way we do these assays is either by doing flow cytometry or bulk measurements on lysates of cells using reverse phase protein arrays or Western blots or fluorescent based bead assays or in vitro kinase assays. And from this we can gather a fair amount of data.

Just to show you one example of how the DNA damaged pathway crosstalks with the growth factor receptor pathway. We can look at everything you see here shown in white and this is the type of data that emerges from these systems biology studies.

Now I want to stress, if you're going to do systems biology, you can't do it with one cell type and one stimulus and one response. You either have to take the same response, the same stimulus in a bunch of different cell types, or even better, one cell type and a lot of different stimuli or the best ever, take multiple

cell types and multiple stimuli. And then measure densely, you have to measure a bunch of things. You have to sample as many pathways as you can and you want to sample them, two aspects of the sampling.

First, you want the sampling to be dynamic. So you want to measure the responses in some sort of way that's dynamic. You can't just pick one measurement. So you want to measure, in this case we did 12 different measurements. And then the measurements have to be rigorously quantitative because you want to use math to explain the results. And this means that you have to do some kind of a technique where what comes out of it are numbers that are meaningful. You can do this with Western blots, you can do it with reverse phase arrays, but you have to get measurements that tell you something about what's happening. And then you also have to measure, in a quantitative way, the responses. You have to be able to measure, for example, what happens to the cells in response to macro molecular damage. Do they die by apoptosis? Do they proliferate or not? Do they get stuck in the cell cycle? Do they undergo autophagy? And again, you have to do this in response to multiple stimuli and multiple cell types to have the ideal data set.

And then you have to use mathematics to somehow connect those signals to the responses. Now most people when they think about systems biology, immediately think about differential equation based models. But what I want to stress is that's just one extreme of a broad range of models that you can use. And, in fact, you can often get more insight about the larger series of networks that are involved in controlling the response if you use a regression model. If you use regression models, you require a lot less prior knowledge. They're easier models to calibrate and you can use the broad sampling of many pathways.

The downside of this, of course, is you don't get as much mechanistic insight. You get correlations with this rather than causations. But I think that the results that come out of it are actually quite important. The idea with all of these models is you somehow want to relate the responses to the signals and the key thing is whether this function that you derive is a mechanistic one as comes from differential equations or if it's just a correlate of one that comes from these regression models.

Now there are a bunch of signals that are probably important in aging and in macro molecular damage that we don't measure. If you believe that it's actually pathological repair, as I do, that controls the ultimate outcome, then you have to think about the pathways we can measure which I've shown you, and then what's emerging is really important pathways that we don't do a good job of measuring. We don't do a good job at the moment of measuring activation of the notch pathway or of the beta catenin Wnt pathway. And in these cases it's largely because there aren't post translational modifications that are easy for us to set up to measure. We'd have to look at individual cells and things like protein translocation here.

And so, to sum up, I want to leave you with what I think are the key challenges and the unmet needs. What are the cell types that are most important in controlling the response of cells to macromolecular damage? Is it the damaged cells themselves or is it specific cells in the micro environment that we should measure? What signaling pathways are the most important ones to measure? I think this is a case where it's a bit like the drunk under the lamppost. We have great tools for measuring some pathways and poor tools for measuring others.

What should we measure as a response? Should we be measuring phenotypes like senescence or apoptosis? Should we be measuring actually something that's easier to measure quantitatively like IL-6 or TNF? Or should we be focusing on some of the cell markers like Bill Bonner alluded to, modifications of histones, for example, or some levels of oxidized proteins or lipids or thiols. How do we measure signaling events dynamically in vivo? It's easy to do this in cells in culture but there's no way that I know of to measure multiple pathways in cells within a tissue at the moment.

How important is this pathological repair process in signaling and pathological repair models? How granular should our mathematical and mechanistic models be? Linda talked about a model of frailty where the granularity is at the whole person level and I've shown you examples where the granularity is

all the way at the level of individual molecules in a pathway. How can we use fixed patient samples, the type of thing that we can collect in order to get at this data. And, finally, how can we make the appropriate kind of mouse models that allow us to test specific predictions of signaling in vivo?

Thank you very much.

Dr. Richardson: Okay. I'll turn this over to Eric. I want to thank the speakers for keeping on time. Eric.

Discussion and Wrap-up

Dr. Schadt: Thanks, Arlan. Yeah, so the speakers will come to the podium. No shortage of things to be thinking about given those great mini lectures. So the people who want to ask questions can just come up to the microphones. And I particularly liked the last part of Michael's talk on how to, all the different questions, and it really is going to come down to both collecting and aggregating all of this information over multiple different systems to integrate those data and take a more data driven view perhaps of aging.

So we're ready to engage.

Speaker: I think is a point of clarification for Rod, a question. So the oxidation of the arginine and lysine, you suggested that that only happens to metalloproteins. So is that absolutely the case the oxidation of arginine and lysine in the way you described only happens to metalloproteins?

Dr. Levine: No, not at all. It certainly happens without metalloproteins, particularly with the many lysines that are on the surface of proteins. What I didn't make clear is the functional effect, was what I was emphasizing. So if it's a metalloprotein with an arginine that's forming part of the metal binding side, or right nearby, then it becomes dysfunctional or nonfunctional. Whereas if it's surface exposed, it's unclear how much difference it may make. The fact is that oxidizing that lysine on the surface might even be an antioxidant protection mechanism.

Speaker: I see. Thank you.

Richard A. Miller, MD, PhD: I'm Rich Miller from the University of Michigan. I have the same question for Jan and also for Rod. I'd like to ask Jan if there's any evidence that the lifespan of a normal healthy animal, say a mammal, could be improved by fixing DNA mutations or slowing their accumulation.

And for Rod, I'd like to know, is there any evidence that the lifespan of a healthy normal mammal, not a mutant, not some sick little thing, can be improved by preventing oxidation of proteins or repairing them?

Dr. Vijg: First of all, of course, there have been many thoughts about trying to improve DNA repair, but that turns out to be extremely difficult. If you really overexpress, for example, particular components of basic DNA repair the effects are merely toxic. I don't think there's any evidence for that at all. However, what, for example, our own work shows is that a mutation accumulation, age-related mutation accumulation, definitely slows down, for example, in dwarf mice. It's much slower in dwarf mice than in control mice.

The same we see with drosophila in chico flies the mutation loads are clearly - about 50% less actually at the same age level.

Dr. Miller: That's the answer to a different question. The question I asked is whether you can accomplish the goal of lifespan extension specifically by improving DNA hygiene.

Dr. Vijg: As I said, immediately, nobody has really tried to improve DNA repair but in a sense you slow down, that's at least the idea. When by lowering IGF signaling, is in fact, improving you cellular defense system stress responses, and geno toxic stress is stress also, then that is, I think, evidence that there is at least correlation between the level of IGF signaling and there is a correlation, inverse correlation.

Dr. Levine: My answer to your question is, no, there isn't any evidence at this point. It's primarily because people haven't really looked and that's your point, haven't looked in the healthy normal animal, although stay tuned for what the proteastasis people have to say about that.

Dr. Martin: So my question is mostly for Jan also, a kind of follow-up for Rich's question.

Dr. Schadt: So before you go on I think we had one other comment.

Speaker: Yeah, I just wanted to add one comment to the last answer and that is, for example, double-strand break repair, nonhomologous enjoining is inherently mutagenic. Rarely will you get, I don't know about rarely, but and this is the only type of double-strand break repair you have, for example, in G1 cells. So the question about can you completely prevent a genome from changing its base sequence during its lifetime I have a feeling is, no because there are inherent processes which lead to that.

Dr. Martin: George Martin, University of Washington. A follow-up to Jan mostly. So in relation to Rich Miller's question, bring us up to date on what comparative geroscience tells us that what about the intrinsic rate of somatic mutation as a function of lifespan when you compare species and fidelity of DNA replication, for example. I had one other follow-up question about somatic mutations.

Dr. Vijg: The answer is that we simply don't know because we never had methods to really analyze DNA, somatic mutation rate in different species. It was simply not possible. There are like reporter genes, as you know, so we compared, for example, drosophila with mouse but not for all species. There are reporter genes in mouse, the mutation frequency is much lower than in the fly, somatic mutation frequency.

Germ line mutation is a whole different matter. Germ line mutations are generally lower than somatic mutations and they seem to function in a sense. For example, yeast germ line mutations, that's the same as somatic mutations, is much lower than, for example, in humans. You actually see a very clear relationship than in more complex organisms is generally higher mutation rate. So that's the answer to that question I think.

Speaker: I'm glad you brought up the somatic mosaicism story. Actually when I read Leo Zilard's famous PNS paper it looks to me it's more what he's talking about is the inactivation of an entire chromosomal arm. It's like a line hypothesis. And now Andy Chess' story, that's what I want to hear some follow-up on. Where he finds evidence that there's somatic mosaicism for autosomal genes, lots of them, including the beta amyloid precursor protein. So little patches of tissues, he says. You're operating either monoallelically, either on the paternal or the maternal allele. Sometime it's diploid sometime it's hemizygous. So what's the follow up on that?

Dr. Vijg: This is, of course, interesting. There is now a lot of very recent evidence for somatic mosaicism, genotypic mosaicism. And this, of course, because there's enormous amounts of sequencing and all sorts of chromosome analysis. So you can really see now that the subpopulations of cells they are showing up. Sometimes where you have a clonally developed mutation you can actually detect it. Of course, the very low ones, it's really the tip of the iceberg that it's now being discovered slowly. But post psychotic mutations have now been implicated in for example, autism and schizophrenia. So the role of somatic mutations in actually disease pathogenesis is becoming clearer and clearer.

But again, the only way to really get a good level of information about it is to take a single cell approach and simply begin to sequence all genomes of many single cells.

Speaker: And I think on that point there's a nice paper that just came out in *Scienc*e today on the somatic mosaicism in neurons so these copy number variation alterations they're averaging from 2 mega basis to 70 mega basis, half of the cells that they sequenced individually, harbored at least one of these rather large copy number variation mutations. So maybe it is actually a fairly natural thing that the genomes are being shuffled around.

Dr. Vijg: Thank you.

Dr. Laughlin: Hi. Maren Laughlin from NIDDK and my question is for Dr. Jones, stimulated by this nice slide behind you. Are there interactions between physical activity and the redox proteome? You talked about diet but do you know of anything?

Dr. Jones: Certainly there's a lot of literature on that. It follows in two phases. Immediately after exercise there is an oxidation response, an oxidative response and then a prolonged response from that is a more reduced state. As far as following that over, in terms of a long term in individuals, I don't know of studies that have done that. Certainly, the studies in younger individuals and more in terms of conditioning and endurance athletes, it's very clear that the exercise does have an impact on the redox state.

Penny Dacks, PhD: Penny Dacks, Alzheimer's Drug Discovery Foundation. I think there's a lot of use in the general public already in terms of assaying their telomere length, right, to get an idea of their age. And this, I think, was clear from the talk today, that's probably not necessarily the best marker that anybody could be using but it's already out there and it's being used. So I'm just very curious on all the different types of macro molecular damage that were discussed in this panel. What might be feasible for testing in humans as a biomarker? And both from the perspective of people who are looking for that and buying that and maybe the dangers. But I mean, maybe on a more practical perspective, epidemiology, what might be useful? There's a lot of data that telomere length might be associated with cardiovascular disease. And so, with these various approaches what's practical for testing in humans?

Dr. Levine: I'll start just by making the point that we need to distinguish those biomarkers that are useful in a screening of population or a group to give us a clue about mechanism, etc., and those that are actually useful for a specific individual. In the case of protein oxidation there's a remarkable number of diseases that have a strong correlation with plasma oxidized protein level and often this can be observed before the clinical onset of the disease. But I don't know of a single one of those whose variability is low enough to make it useful for individual use.

Dr. Evans: I was just going to mention, there have been some studies, for example, for gamma H2X this might be inflammation rather than aging specifically, but in Italy they screened a bunch of children and found gamma H2X levels increased with the BMI of the individual. And so there are some things that you can measure.

Dr. Vijg: I think it's really not wise to focus on one particular aspect like telomere erosion is really only one of many forms of genome instability. So I always was a little bit wondering why. Of course, I can sort of guess why there is so much interest in that, but nevertheless you should really look at genome instability in a more broader sense. So to also look at other markers, for example, even cytogenetics assays to do that RICGAs, and as I hopefully tried to show in the future, sequencing, actually, to try to capture all these variants in one stroke.

Speaker: It's probably going to turn out to be useful to measure not just markers of damage in a particular cell. And I agree with Bill, I thing gamma H2X is probably a very useful marker as a place to start. But also to measure things like circulating cytokines or circulating coagulopathy profiles and see if there isn't some way to put all that data together to stratify risk. I think that if we just focus on damage and we ignore the repair component and we ignore what it is that controls whether that damage results in functional loss or not, then we're only looking at one part of a bigger equation.

Dr. Bonner: I don't know about humans but certainly in mice, we've used the assay of isoprostanes measuring and that's not too bad because it kind of does give you the tissue, it leaks out into the blood. But I agree with Rod. I think that there would be so much variation, it's complex. And I was going to ask Dean, because I know Dean has done some things where he's looked at the glutathione oxidized reduced ratio. And it would seem to me that would be an interesting thing. And I know you've done that in humans and have data.

Dr. Jones: We've looked at glutathione redox potential cystine and redox potential variety of other biomarkers and I think the emerging view is that the concept of a galceon type of distribution or a distribution that is monotonic is really probably an erroneous way to think about things as we go from a population to an individual based health assessment. We're probably going to, as we get large enough systems in a systems biology sense, we're going to have subclassifications of people, and so we're going to be able to identify who fits into what bucket. And the different buckets are, in fact, going to have different responses. And so, we'll probably be much better able to use these biomarkers when we figure out that overall structure and how we can define which bucket we need to put a person into in terms of their metabolic and genetic characteristics, so then we can usefully use those biomarkers that are currently not very accurate. I think the precision will be much better when we get improved subclassification structures.

Morten Scheibye-Knudsen: Morten Scheibye-Knudsen at the National Institute on Aging. I have a question for Jan, mostly. So this is about aging in relationship to DNA repair in the context of proliferating and nonproliferating tissue. So what is the, is there any difference in the correlation between aging rate and the mutational load in cells that divide compared to cells that do not divide. So, for example, you could imagine that it would be more detrimental for dividing cells to have mutations because that carries on.

Dr. Vijg: That's actually a very good question. We looked at mutation accumulation during aging in different organs and tissues. For example, in the intestine we see a pretty dramatic increase in mutations, they're all point mutations. In the spleen which is also an organ with a lot of actively proliferating cells, we do not see that at all. And that, I always explain this, but again, I have no real evidence for that, the level of apoptosis in spleen cells, splenocytes, is probably much higher so that's why you will not see the mutations.

So yes, of course, under certain circumstances it's very important to repair quickly, but at the end of the day the price you pay for that could actually be mutations, of course, we don't really know that. But that's certainly a possibility.

Now having said that, you can induce mutations in both actively dividing cells and in cells that do not divide. You actually compare that directly. But in cells that are actively proliferating, if you, for example, treat them with ultra violet light, you see many point mutations. Those are undoubtedly replication errors. But if you treat the same cells when they are quiescent and not dividing and you treat them with UV you see much less mutations, which is the reason why I said it's probably replication errors.

But on the other hand, if you treat them as a classigen so like hydrogen peroxide or bleomycin you see lots of structural variations, even more than when the same cells are actively dividing. So you can really in both post mitotic cells and actively proliferating cells, you see mutations.

Brian Kennedy, PhD: Brian Kennedy, Buck Institute. I wanted to go back and come back to Rich's point a little bit and broaden it because I think this is really one of the big problems in the field right now. We have had two really interesting and great lines of investigation. One of them is find mutations that are conserved that extend lifespan, more or less unbiased.

The other is let's look at particular models we have for what might be driving aging, DNA damage, protein misfolding, inflammation. You choose, I'm agnostic. The problem is it's been very hard to connect those two things and the conserved aging mutants we find can be linked to multiple of these pathways. And so I guess the question is, is the only way, and I don't have the answer, how do we connect those two lines of investigation? Is it the only way to extend lifespan, to hit multiple forms of these events that are driving aging? And we're hitting these nodes like TOR and insulin and sirtuins, and whatever. Or is it that there are just a couple of ones that are really driving the process and we just don't have the tools to really go in and specifically tweak those to get the effects.

This is something I think about a lot and I don't have an answer for. I wonder what your thoughts are on that.

Dr. Vijg: I thought that the whole argument about the systems, like dampening TOR, dampening IGF and so on that you really, in one way or the other, begin to effect a whole host of defense systems. And I think although there's not a lot of evidence for that, some evidence, at least, is there. For example, when we knock down FOXO, for example, and we then tweak the cells, knock down FOXO with a particular DNA damaging agent, we do see many more mutations than when FOXO was fully active. That's evidence and I think, and Anne Brunet already showed that, that FOXO is important in DNA damage response.

The same way I just told Rich, we do actually see an effect of this IGF mutants on mutations. So mutation rate is really lower in those organs. And again, I'm sure that, they've demonstrated many other effects for a whole host of different cell defenses. So I really think what they do is they really effect a broad range of cellular defenses. Exactly as I believe it's generally felt in the field. But it's not, of course, demonstrated for everything.

Dr. Richardson: Not quite on your question but. certainly. we've been confronted when we were looking at trying to test the oxidative stress theory of aging and it was very frustrating because we had made animal models where we had to increase oxidative stress, because there was always a pretty tight correlation between damage and dwarf mice and this sort of thing. And it's really important, which both you and Rich raised, t go in and to manipulate the system and we did manipulate the system so we were able to get more damage or less damage, and the upshot was we didn't see any difference in lifespan.

Now, on the other side, I'm a little bit skeptical about whether our model systems are maybe the most relevant because if we do the same genetic manipulations that had no effect on lifespan and we do it in a disease model, either Alzheimer's or cardiovascular disease, or so on, we see a difference, as you would be predicted from the standpoint of more damage is bad for you, less damage. And I'm wondering in the mouse model system, we're using the longest live strain and we're doing it under perfect conditions, and I'm wondering under those conditions if when you do change the oxidative damage or DNA damage around, that it's not going to be a major player in what's affecting lifespan.

So we're, I've never been able to hit on this, but I think that it would be very interesting to look at a model that was at least slightly stressed and to see if manipulations that would maybe not work and extend lifespan would be, under this condition.

And the other thing is also possible, and certainly with TOR, is would these manipulations work in a stress population? They might not.

Dr. Yaffe: I just want to make two points directly related to what Arlan said. One is I think we have to keep in mind that genetic models and acute inhibition by drugs are not the same thing and you may or may not see an effect in one that you that you can reproduce with the other. I think the other thing to keep in mind is that many, it's a lot of crosstalk between pathways and so I think your point of trying to ask, should we be hitting one pathway or multiple pathways and how to figure that out, is the big question. And I would just caution that if you inhibit one pathway you'll get compensatory upregulation of signaling through other pathways. And so we have to think about some of this in a dynamic way. It's probably easier in aging than it is in cancer because the cell types presumably aren't hypermutagenic to begin with and so they're less likely to evolve a bypass mechanism.

I would argue that it's probably going to be some type of combination therapy or combination treatment that's likely to be the most effective, but that of course, also raises the question of what happens to the other cells that aren't damaged when you do the same types of treatment?

Dr. Richardson: I agree. I think that trying to figure a new way of thinking about how to solve this problem is one of the critical issues and I don't know what the answer is.

Speaker: And I would just go maybe even further than Michael and say there really are no such thing as pathways, they're only networks. And to use these simple constructs and think we really understand how

these things are integrated without looking at the system more holistically, and it's not just looking at known signaling pathways and these known things like mTOR and so on and so forth. Ten years ago we didn't really even know what micro RNAs were, and once those were discovered look at how it changed the regulatory landscape. We haven't sequenced, 10% of the genome has not even been sequenced. The ability to get at structural variations just now starting to come to light.

So all of these missing pieces they have to be organized in the kind of constructs that Michael was talking about in a more systems level way and to get away from this idea that these things are simple linearly ordered pathways, signaling pathways that are determining things because that's not true. These things are operating in very, very complex environments, thousands and hundreds of thousands of variables at play, and we have to get our heads around that if we to be successful.

Dr. Richardson(?): Let me just say one other thing. The point that you brought up and this is one of the few things that aging and being older is good for, is that, and George probably remembers this. If you go back about 30 or 40 years, people would argue that you wouldn't be able to do anything with aging by a single drug or a single drug or a single genetic manipulation. And I think it's amazing that these gene manipulations have been, that there's so many that alter and that we have something like rapamycin, theoretically, one drug, although it's probably affecting many targets.

Speaker: I just wanted to add an example of how crosstalk can be surprising in some ways and it concerns ATM, which, of course, has been the protein that is for double-strand breaks and DNA damage but Tanya Paul found that, actually, it is also involved in ROS levels in the cell. ATM has got a sulfide in it which gets oxidized or reduced under the conditions. You can knockout the residues that have to do with DNA damage, it still works. So here you've got the same protein doing two totally different things.

James F. Nelson, PhD: Jim Nelson, Barshop Institute, San Antonio. I think Rich's question and then the follow up by Brian gets to the major issue with respect to the question of, does macro molecular damage that's seen during aging really, how do you find out whether that really has an impact on either age longevity or specific aspects of age related lifespan?

I think there are two examples that have addressed this question successfully. And the first one hasn't been raised here, is one concerning the age-related increase in DNA damage that occurs in the germ line. Chris Walter has studied this very beautifully for the last 15 or 20 years using a mouse model, a male, and identified a specific endonuclease that is downregulated with age and correlates with the increased DNA damage that you see in sperm. And she went beyond that, as one has to, to find out whether this is significant with two interventional approaches. The first one was to make a mouse heterozygous for a knockout to this enzyme. And she showed she accelerated the DNA damage in the sperm. And then she overexpressed the same enzyme and showed she decelerated the damage. So that's the kind of interventional approach that's needed.

This was, perhaps, a low hanging fruit because this is an example of a situation where only a subset of sperm cells are damaged, much akin to what Jan is suggesting with respect to somatic mutations being very anecdotal or cell specific.

But I would ask the question, perhaps one could think about other systems in the body that might be similarly affected and one of those might be the stem cell lineages which might have similar effects of DNA damage and could ramify in deleterious ways, and one might want to look at that particular system with respect to such interventions.

Dr. Vijg: Stem cells, actually, there a number of paper showing that stem cells have a particularly low spontaneous mutation frequency. Apparently there is a very low rate of mitotic recombination. So a very low number of lots of heterozygosity events. And that may, of course, be related to the fact that you are, that preservation of genome integrity in stem cells is particularly important. And I think there are similar evidence for germ cells. For example, from Chris Walters group, as you just said. There's an important question of how to sort of improve, or actually how to demonstrate that this whole constellation of damage

could actually really have a functional effect, I think is more something for Michael to answer because I really totally agree that this is a systems biology approach with a lot of modeling involved.

But it's very essential that if you first get a good handle on what actually happens in different organs and tissues of a living animal during aging. But if we have made a systematic inventory of what's all going wrong in terms of damage accumulation, then I think you can move into modeling, into this sort of approach that Michael was talking about. And I'm sure he wants to comment on that.

Dr. Yaffe: I think Jan's completely correct. I think that there is data, as you are probably aware, that stem cells seem to have enhanced DNA repair capabilities as well. So something is different about them. There's some data that it might be higher levels of ATM activation or higher activity of certain endonucleases. But I think that we really have to come up with some way to be able to look in a tissue at the extent of damage over time. And to my knowledge, there's really no good way to do that now. Maybe we need to come up with better model organisms or better in vivo assays.

Dr. Martin: George Martin, again, University of Washington. So I'm trying to think about putting together some aspects of the session we heard on epigenetics and *vis*-à-*vis* epi mutation. And so with Stuart Kim's nice presentation where he showed some, what seemed to be systematic changes in gene expression with aging and one interpretation might be with aging, there is a concerted compensation, large scale compensation for physiological decline. So upregulate this, downregulate that, so it may be protective. But in so doing, if there's a lot of that remodeling going on, would you think, Jan, and Dr. Bonner that maybe that increases the probability of epi mutations?

Is it getting a lot of reassessment and remodeling of chromin.

Dr. Vijg: Yes. I didn't really explicitly mention that but there's an enormous amount of epigenomic remodeling going on simply before and after the actual enzymes have done their DNA damage repair work. And many of those responses are showing up as consistent changes. Look for example, at _____ H2X this whole layer of _____ H2X, this whole painting of the double-strand break and this is a very large area. And, as Judy showed, some of these events, some of these foci, they stay around in senescence cells. So they could be like a marker for senescence cells. And that would be a consequence of epigenomic changes and those will show up then in fairly consistent way as part of the whole senescent cell household.

There are probably many of those changes; David also mentioned this relocation of sirtuins and that also happens in response to purely stochastic forms of DNA damage. So I really believe that there's a tight interaction there.

Dr. Bonner: I had one quick comment on that, is just that these epigenetic markers, this aspect, I don't remember it coming up, but can go to the next generation, too. I mean, studies of women smoking, having grandchildren that had higher levels of asthma evidently, so this could happen with aging, too. Our sensitivity to various types of damage.

Dr. Yaffe: I'd like to comment on this just for a second. I think this is really one of the arguments that I feel is very strong for us going back and trying to systematically understand human exposures because we have exposures that are highly variable in terms of childhood infections, in terms of dietary changes, in terms of activity levels, in terms of environmental exposures to pesticides, and so forth. These are really very highly variable and if they, in fact, modify the epigenome, as the evidence really is accumulating to show that they do, then we really have to think about this and we have to systematically think about this in terms of all of our aging and age-related disease investigation.

Dr. Bonner: Yeah, a really important point. I absolutely agree.

Dr. Levine: George's comment and question prompts me to mention that there's another kind of epigenetic change, it also occurs on proteins but it's the oxidative one. If a cell or an animal is exposed to

elevated oxidative stress, say chronic inflammation, then the proteins that are most susceptible to oxidative modification have an increased frequency of oxidation. And as I already mentioned, if that oxidation is site specific, say at the active site where a metal is bound, then you have a dysfunctional enzyme or structural protein whose dysfunction is exactly the same as you get from an inherited DNA mutation.

Dr. Richardson: It's kind of a little bit of follow-up but it's back to Nelson's question, where we were talking about damage. And I think Jan made a good point of that when he was talking about DNA, he was talking about where you had a lot of damage and yet it was repaired, but the key was going into mutations. And I've talked to Rod about this, but I think from the standpoint of oxidative damage, when we're talking about damage, most of it is repaired, and Rod pointed out that a lot of the things in proteins may actually be repairable.

So I think what I'm thinking now, based on our studies that were rather negative, is I think that they key maybe to damage is not the level of damage because it's repairable, but when you have enough damage that you tip and make a cell, and I think this is where Judy's studies on cell senescence are very attractive because I think that once you get enough damage that you could tip a cell to become senescent you've captured the cell just like you captured with the mutation.

And so, in other words, it may be that the damage that we're measuring is not the key thing, it would be those things that would make it a key, whether it's cell senescence or a change in chromatin methylation or this sort of thing.

Dr. Yaffe: Right in line with what Arlan said. I just want to stress that when you look at the epigenetic changes and epigenomic modification in the setting of DNA damage, you have to distinguish whether you're seeing a difference in the amount of damage or a difference in the response. So if you treat cells, we recently published that if you treat cells with HDAC inhibitors, for example, or bromodomain modulators that recognize acetylation, if you treat cells with HDAC inhibitors you see a much more profound gamma H2X response but if you actually use pulse field gel electrophoresis, to quantify the breaks, there's no increase in breaks. What you're seeing is an enhanced response because the chromin is open and better able to load larger stretches of H2X rather than an increase in the amount of damage, per se.

Christian Sell, PhD: Hi, Chris Sell from Philadelphia. I just wanted to insert into the discussion, to hear you guys talk about the issue of timing of repair. And so timing, we haven't talked about here at all in the sessions and I thought, earlier in the metabolic sessions we'd hear about it because we know developmental rate is tied to longevity. We know selection independently for delayed development increases longevity. And now at the cellular level it would be interesting to think about the timing of repair relative to cell cycle progression and how that has to be coordinated and its relationship to longevity, species longevity and perhaps individual longevity as well.

Dr. Levine(?): Well I think it's an excellent point and we haven't thought about it enough. Taking, as an example, what Arlan just mentioned, if you can repair the damaged macro molecules fast enough you may not be tipped into senescence. If you can't you end up with a senescent cell.

Dr. Sell I agree but I think you also have to think about the differences between different species in terms of their repair and in terms of their ability to arrest to repair. We don't think about cell cycle repair in terms of the macro molecules, or the relationship between macro molecule damage and cell cycle progression but I think we should. We always think about it in terms of DNA repair. But I think that it's important to consider all of these things need to be repaired before the cell can properly go through the cell cycle in order to avoid unwanted consequences.

I just think that the systems biology here is the place to start. Systems biology of the cell biology of repair. We see the networks you've laid out. I guess I'm looking at it as a systems biology expert. Can you model this for us?

Dr. Yaffe: I think you're completely correct. Part of it may have to do with the cells that we choose to look at. It's possible that the bulk of the cells that we study are really terminally differentiated and although they may accumulate a lot of DNA and protein damage, that may be relatively irrelevant to what happens. It may be a precursor population or a stem cell population in which the timing between repair and where they are in the cell cycle is really critical for predicting whether we get successful repair or pathological repair. So I totally agree with you but I think we also have to think about whether we should be studying the cell types that are easy to study because they're the bulk of the population or the minority, which may be the ones responsible for the aging phenotype.

Dr. Schadt: Why don't we do two more questions and then I think we're going to be out of time.

Joseph Gorse: Hi, my name is Joe Gorse. I'm from the University of Akron. I'm a research engineer. So I remember the slide we kind of started off with Felipe's car, and I think that from the engineering perspective, this type of thing, I'm very interested in the classes of damage and perhaps this is what Dean was hinting at a little bit with the exposome. Sort of, has anybody enumerated all of the classes damage and then potential therapies for intervening in those classes of damage for where the body is, the natural repair mechanisms are insufficient as we're seeing. And then, also, maybe the failure mode, so senescence cellular senescence looks like the main failure mode as Judy Campisi has shown, on this type of thing.

And I think that lends into systems biology. But, at the same time, I want to sort of caution that we don't need to look for complexity, it will find us within that type of thing. So we don't necessarily need to look at maybe the asperities on the ball bearings and types of things in order to know that we need a new ball bearing. And then prioritization, like, what would be the most important damage and that type of thing. Thanks.

Dr. Richardson: So I think this is an important question because part of it is limited technology and measuring damage is very difficult to do. Mainly because a lot of the damage is very low levels. And so what you have in most cases, is in individual measures, one kind of damage, whether it's in DNA or in protein. Carbonyls were a big deal to measure. And so we don't have that. And I think, as I had mentioned before, it could be that it's not when we're measuring one type of damage. We're not getting a good snapshot.

The assumption is if we measure isoprostanes or carbonyls or DNA oxidation, we get the same results. And that's just three assays. And I don't know anybody that's done that even though we have had the ability to do it, is some of them are just so difficult that we haven't done it. But this comes back to a question I raised to Rich before about talking about stress resistance and why we see, in many of our long lived animals this resistance to stress but yet you have animals that you make resistant to stress, then you don't see any difference. And I think the possibility is there's types of damage that may be important that we're not picking up. And so my feeling is that we need to do this but it's going to be very difficult and it's not particularly inexpensive.

Speaker: So I will comment on this. It's actually a humbling experience. There's a study that Maria Kadiiska at NIEHS conducted looking at oxidative stress in a mouse model and it was really a brilliantly designed experiment to give carbon tetrachloride, which is known free radical inducing oxidant, and follow that with collecting samples and sending them out to at least a half a dozen different laboratories who were absolutely expert in all of the different measurements, the glutathione, the isoprostanes, and so forth.

What happened was truly remarkable because when we got the data back everything was blinded, it was really done rigorously. When we got the results back, what the results showed was that the most sensitive marker was an increase in glutathione in the blood. And it turned out that the damage to the liver was such that it killed the liver, releasing the antioxidants into circulation. And so even though it was an absolutely major oxidative insult, the most sensitive indicator was exactly the opposite of what you would get.

And so it illustrates the point that no matter how clever we are in terms of our design and sophistication in terms of how we measure things that we're still limited in these analytical models and I think that's why I argue that we really need to come back to humans and as best we can, develop the methods for dealing with the complexity in humans and being able to evaluate that in terms of the aging process.

Speaker: So would it be fair to summarize real quick that it's measurement in human beings that we're looking to go forward on that.

Speaker: Well that would be my argument, is the best way if we're going to deal with age associated disease and healthy longevity in humans.

Dr. Schadt: All right. Unfortunately, our time's up. Ron's going to take over.

Dr. Kohanski: I'd like to thank the Co-Chairs, the panelists, and you, the audience for a fine session. So now we will break for lunch and be back at 1:00 pm for the next session on proteostasis.

Lunch
Session VII: Proteostasis

Henry Rodriguez: If folks could start coming in to be taking their seats, we're going to begin the next session to keep on track. Okay, so hopefully, to keep in the spirit of all the great talks that we've been hearing, not just yesterday but also today in the morning, let me just say good afternoon to everyone and welcome to the first session in the afternoon, which is going to focus on proteostasis. My name is Henry Rodriguez, and I'm the director of the Office of Cancer Clinical Proteomics at the National Cancer Institute. My co-organizer for this session is Bradley Wise, who is the chief of Neurobiology of Aging branch at the National Institute on Aging.

So for the session on proteostasis, we have two cochairs. One is Richard Morimoto, and the other one is Ana Maria Cuervo. So Dr. Morimoto, he happens to be the director of the Rice Institute for Biomedical Research at Northwestern University. He's also the Bill and Gayle Cook Professor of Biology within the school's Department of Biochemistry, Molecular Biology, and Cell Biology. So Rick is going to first give an introductory overview of the topic for today's session.

Now the other cochair is Dr. Ana Maria Cuervo. So Dr. Cuervo, she's the codirector of the Institute for Aging Research at Albert Einstein College of Medicine; and also along with, as her distinguished colleague, Dr. Morimoto, she is also a Robert and Renee Belfer Chair for the Study of Neurodegenerative Diseases and is a professor in the Department of Developmental and Molecular Biology. So Ana Maria is going to direct the actual discussion which follows right after the speakers, and she's going to give a summary of the overall of the session.

So at this point, please welcome Dr. Morimoto.

Introduction

Dr. Morimoto: Good afternoon. Welcome back. We have a wonderful session for you on proteostasis. We're going to hear from Fred Goldberg on how does aging impair proteostasis, Roberta Gottlieb on mitochondrial quality control, Judith Frydman on proteostatic mechanisms, Randy Kaufman and the unfolded protein response, and Jeff Kelly on therapeutics.

So a little bit of background so we can all come together. One of the fundamentals in all of life, of course, is the making of a protein. A lot of what you've already heard from others are how different types of damage impinge on proteins. What we're going to be talking about are powerful cell stress mechanisms that detect balance and the importance of the proteostasis mechanism to make sure that every protein that's made is intact.

So every protein starts off on your ribosomes as unfolded. They go towards a native state. The problem is the environment, mutations, error-prone synthesis shift the equilibrium towards damage, ligamers and aggregates.

From a biophysical perspective, this is an energy landscape diagram. Proteins enter the pool as unfolded. If you only have intramolecular context, the protein folds to its lowest free energy state, that's good. The problem is mutations, environment expansions of polyQ; Alzheimer's disease with tau and Abeta; Huntington, alpha synuclein, these types of proteins, when they shift into an alternate confirmation, form morphous aggregates, ligamers and amyloid fibers. The problem is for every protein in our cell, there is a risk for a shift between intramolecular, normal native states, and intermolecular. The problem for the cell is how to deal with this.

Fortunately, what comes to the rescue are molecular chaperones as you're going to hear about and clearance mechanisms. The chaperones play the critical role to make sure that all of our proteins are properly folded.

Together with the clearance mechanisms, the proteasome and autophagy, the chaperones make sure that these off pathway intermediates that can accumulate and interfere don't cause damage to the cell.

Of course, the problem over time is that we accumulate because of mutations you've already heard about that, biosynthetic errors is huge. There's dozens of steps from the time that DNA is transcribed to the point that the protein is actually folded. And in every one of these steps, there is error-prone events that occur. And so the consequences, the accumulation of protein damage, aging, of course, it's a major issue.

Proteostasis, then, represents the integration. It's the network of molecular interactions, the stress responses, that determine the balance between function in our cells and dysfunction. When things go awry, for example, in aging or in diseases of protein confirmation, the neurodegenerative diseases, adult-onset diabetes, many cancers, and metabolic diseases, you now have an imbalance no pushing the system.

From a cellular level, this example of a cell, this proteostasis network then plays a critical role to take our proteins despite the polymorphisms that we all encode and allow normal proteins to be made.

Aging, with mistranslation, accumulation of protein damage from proteotoxic stress, of course, challenges this system. In fact, it sets up competition for the proteostasis network. You now have an influx of mismanaged proteins.

Then you have another challenge that occurs with disease-associated mutations. These are the diseases which now cause proteostatic illnesses. The lead to increase in misfolded proteins, which then generate toxic species. One now has an entire shift in the equilibrium, the balance in ourselves, our tissues, and the organism is altered leading to cellular dysfunction. And it's not going to be just one protein. It's literally hundreds of proteins that become mismanaged; and they collect over time. And as you'll hear, they challenge both the folding and the clearance apparatus.

Genetic interactions then play a critical role because, obviously, all we need to do is look around any population of humans to realize some people are well protected; others are increasingly at risk.

So the critical thing is protein function. Around this are pathways. We've already heard about many of these pathways. Synthesis; post translational modifications, oxidation, for example; degradation pathways. Around this are signaling pathways, and you've already heard about some of these signaling pathways: you have the insulin signalizing pathway, which was discovered to have a critical role in regulating proteostasis; organelle or calcium; mitochondrial with oxidative stress; inflammatory pathways; caloric restriction; signaling pathways such as the heat shock response; and you'll hear about the unfolded protein response.

These then sit around the genetic modifiers, genetics, epigenetics, HDAC and HATs, metabolites and physiological stress literally dictate which proteins are made and their function at any particular moment.

Just to remind you, this is a remarkably conserved system that's present in every organism in life, this ability to take a polypeptide and to partition it to a folded state to prevent aggregation. This represents the chaperone networks, the different chaperone proteins that then, in blue, prevent aggregation from occurring or allow folding.

And then, finally, these signaling pathways that sit around the cells to literally detect changes in the environment. The consequences, you have a single cell like in a prokaryote or in a single cell, eukaryote, that functions and senses stress.

Somewhere between three and four billion years ago, the cell changed. Somewhere after that, you had a simple metazoan, in this case, *C. elegans*. What becomes important here is that this represents the evolution and adaptation of cell autonomous networks to cell nonautonomous because in an organism with 959 cells, you still have a specification of the sensory stress apparatus. So in *C. elegans*, it's the neurons that initially sense the heat shock stress. But other tissues recognize an imbalance in proteostasis. Mismanagement of protein in an intestine or the muscle or the neuron send signals between the tissues to restore balance. So this critical point that we heard earlier about information flow, hormones, and signaling molecules also occurs to maintain proteostasis to ensure functionality.

What we'll also hear about is how the cellular challenge and cellular toxicity really has a therapeutic opportunity. If one understands the threshold, the bandwidth between challenge and toxicity, it gives us a remarkable space and many opportunities to think about therapeutics.

And with this, I'd like to now move to the first speaker in the session. Ana Maria, you want to introduce them?

Ana Maria Cuervo, MD, PhD: My name is Ana Maria Cuervo, and we are going to introduce now the first speaker for the session. So he doesn't need introduction. He doesn't need introduction, but it's Dr. Fred Goldberg from Harvard Medical School; and he's going to try to answer how does aging alter proteostasis at the cellular level using the main focus of his lab that is the proteasome system.

Aging and the selective degradation of abnormal proteins

Alfred Goldberg, PhD: As Rick Morimoto's eloquent introduction emphasized, evolution of higher organisms and lower organisms of all organisms has required the development of major systems to eliminate misfolded, potentially toxic proteins. And the most fundamental one is shown in the first slide.

Today, we're going to focus on, really, the effects and the role of the intracellular proteolytic system in eliminating such proteins. And by misfolded proteins, we mean a list of- Well this slide is a little bit of protein sociology indicating the kinds of proteins that are rapidly degraded in basically all cells and even within organelles such as within the mitochondrial matrix.

And wherever it's been looked at incomplete proteins, such as a rise by nonsense mutations, mistakes in RNA processing, also proteolytic cleavages by, say, TASpases triggers rapid degradation.

About 20% of the missense mutations affecting, say, hemoglobin, generate missense proteins that are rapidly degraded. It only looks like those proteins that most perturb overall confirmation trigger the selective degradation.

A big source of substrates for this system are subunits of multimeric complexes that may be free, not incorporated into multimers. We usually think of multimers as being expressed in exact stoichiometry. In fact, that's not true. The free excess subunits get rapidly eliminated in all cells where it's been examined.

Now you've heard a lot- This field of aging has long been focused on post-synthetic damage to proteins. We still don't know how often this happens, what forms are most toxic. Certainly, the forms that you've heard about this morning that generate stable proteins that organic chemists can assay, these are not the proteins that evolution is worried about eliminating rapidly; and many free radicals trigger rapid degradation.

In addition, of course, there's intracellular denaturation happening in a stochastic manner all the time. The common feature seems to be the misfolded state, which is a very ill-defined phenomena.

I could have put this slide up thirty years ago and probably did. At that time and until today, we really do not know the detail about how these proteins, in any detailed manner, how these proteins are recognized.

What's clear in mammalian cells, is that this pathway, the ubiquitin-proteasome pathway, is a major defense against such proteins leading to their rapid elimination.

Now this is the undergraduate version of the pathway. What this pathway is meant to indicate is that, for the great bulk of proteins in the cell, at least three quarters under optimal conditions, there's degradation through their selective ubiquitination, the proteins- The ubiquitin is a small heat-stable cofactor that gets activated and linked to substrate molecules through isopeptide bonds. And when there's more than four ubiquitins in a row, the proteins are rapidly degraded by the proteasome.

Now this is a very proteasomocentric view of the cell. Most people talking on that – and unfortunately I don't have time to do so – would spend a lot of time talking about the ubiquitination enzymes because there are about 700 different enzymes in this process in the human genome, about 20 to 40 different ubiquitin-conjugating enzymes and probably 600 enzymes that recognize substrates and can attach ubiquitin to them. They're usually substrate specific, involved in the regulation of the cell cycle or different cellular responses.

Which of these enzymes targets misfolded proteins in mammalian cells is still not clear. We know about two on the ER that get proteins in the secretory pathway and lead to their degradation, cytosolic proteasomes. There's one well-characterized ubiquitin ligase called CHIP that recognizes misfolded proteins because they have chaperones, Hsp70 and 90 bound to them.

There have got to be many other systems. Quite a few are known in yeast. We still do not understand or know about the quality control mechanisms, therefore, we can't study the effects of aging.

Now about 2% of the cell proteins in most of our cells are this structure called the proteasome, 26S by its size. And this is a 60 subunit molecular machine that binds the ubiquitinated proteins and degrades them to small peptides, 2 to 20 residues long.

Now 99% of these peptides will get converted to amino acids within ten seconds. Peptides in the cytosol are very dangerous and are quickly eliminated. A small fraction, perhaps 1%, get taken up into the ER and get displayed on the cell surface on MHC class I molecules. And this is the source of information that the immune system, cytotoxic T-cells, uses to find virus infected and cancerous cells.

So the proteasome screens the system, screens intracellular space just like the lysosome and endosomal system screens extracellular space for nonnative proteins; and it's an important role of the system, also, when we think about aging.

Now let me focus, because this is going to be a proteasomocentric view, on this structure because our view of its function, especially in clearance of misfolded proteins, has changed dramatically in the last couple years.

This is a more realistic but not quite up to date figure, and that emphasizes that the core 20S proteasome is the degradative structure. Inside its central chamber, proteins are cut up by six active sites. So the

proteolytic enzymes are not free in the cytosol; they're isolated. And proteins to be degraded get bound to the 19S regulatory particle and get injected into the central core through a small opening at either end. And this process is catalyzed by six ATPases that form a ring and are involved in the decision of whether a protein gets deubiquitinated and lives longer or gets translocated in and gets destroyed. This is definitely a life/death decision for each protein, and it's now clear that it is regulated.

This step of degradation is one that has had a big medical application recently. As many of you know, people studying this pathway are now able to use proteasome inhibitors such as MG132 as reagents to dissect how important this process is in different homeostatic mechanisms.

About 20 years ago, we put in some effort to develop such molecules for clinical applications. And now two proteasome inhibitors are widely used drugs. Velcade is now a $2 billion drug used by at least 400,000 patients. It blocks the degradation of misfolded proteins. And, in certain cancers, especially multiple myeloma, sends the cells into apoptosis, the opposite of what we want to do considering aging. It's probably what you want to do to eliminate certain cancers which are associated with large amounts of misfolded proteins.

A challenge in thinking about aging is to enhance the proteasome's efficiency. And I think that is now turning out to be practical. And let me show you a more sophisticated view that's just really emerged in say the last year or so about proteasome function.

It turns out the proteasome was more intelligent than it looks, more intelligent than we had long believed, even those of us working on it; and it's making decisions of a key kind.

The 19S regulatory particle recognizes substrates in two steps. One, it recognizes the ubiquitin chain and binds them to two main ubiquitin receptors. That itself is the initial and easily-reversible reaction.

Then the ubiquitin-degrading enzymes, the deubiquitinating enzymes, start chopping up the ubiquitin so it can be recycled. It turns out the major enzyme doing this, USP14, is also a key regulator of the rest of this machinery. In fact, this machinery in the cell is in an off state until substrates bind and they interact with USP14 which activates the proteasome.

It opens a gate here. It changes the several different reactions. But, most importantly, the proteins will continue to get deubiquitinated unless they have a loose end on the substrate. This comes from work from Matouschek and Finley. The main point is that this is a two-step reaction, and only proteins are destroyed if they have a loose end; and this seems to be an important point in quality control. If they're aggregated, the proteasome has a major challenge, and we don't know if it really can or cannot disaggregate the kinds of proteins we associate with disease.

So let me end with a critical question that you're probably asking and I wish I could answer. First of all, we have many steps in this pathway. How does aging affect them? Well, basically, for all the critical first steps, which are rate limiting for most proteins, we simply don't know. We don't know the effects of aging on the cytosolic ligases. One has been characterized. The others, we don't know what they are or how they change.

The process of quality control in the secretory pathway is well characterized. The enzymes are known. The effects of aging has not been studied, and there are ribosome-associated ligases that carry quality control out of the nascent proteins, as shown by Dr. Frydman. This, the effects of aging is unknown.

For the proteasome, in the last few-, last year, probably, critical information has arisen that really is convincing, in my view, that in the brain with normal aging, there are decreases in the capacity of the ubiquitinate proteins to be degraded by the proteasome. Pathogenic proteins like tau as well as PrP scrapie, the prion protein, can definitely interfere with the 26S regulatory components. This is not an essential part of aging. It's maybe an essential part of the disease because, in the same mice, if you look

in the muscle, the proteasomes are working fine and even re, the pathway is probably enhanced and accelerated in sarcopenia, the wasting of muscle that we've talked about.

And there's one other step we haven't even talked about, and that's the p97 ATPase complex, which helps in the disassembly of many large complexes, probably aggregates too; and no one has studied its precise function in diseases or aging. I wish I could answer more on the effects of aging.

Dr. Cuervo: Thank you very much, Fred. So our next speaker is going to be Roberta Gottlieb from the Cedars-Sinai Medical Center, and she's going to try to address the interplay between proteostasis in the cytosol and in organelles using, as a model, her favorite organelle, that is the mitochondria and how it is removed by autophagy or mitophagy.

Mitophagy: impact of organelle quality control in Proteostasis

Roberta Gottlieb, MD: I'd like to thank the organizers for including me in this; and I'm going to really offer, you know, in the spirit of the preaching that we heard earlier, I'm going to offer the concept that mitochondria are the center of the issue and clearance by autophagy is the key to this process.

So let me put this first in an organismal context because the organs that are most profoundly affected by aging are those that have long lived cells. So the heart and the brain and, I guess, another organ that you might consider would be the retina. These are all cells that have a very high metabolic demand, but the cells themselves turn over with a very slow rate if at all. And so it's important for the cell to be able to maintain all of its organelles in optimal working condition.

And so this is quite a challenge for a long-lived cell. You can't replace that cell. You need to replace the component parts. And up until now, we haven't heard much about autophagy as a mechanism of clearing the organelles; but this is the primary, in the case of the mitochondria, probably the only means by which you can eliminate damaged mitochondria or other damaged organelles.

And so here I've listed kind of all of the bad things that can happen when dysfunctional mitochondria or even old, beat up, tired mitochondria are not cleared in a timely fashion.

And so, obviously, mitochondria are there to provide ATP for the cell. And as they deteriorate in their functional capacity, then ATP levels drift down. This can impact, for instance, the pancreatic beta cell, which requires very high ATP levels for insulin secretion; but it has its impact in many other cell types as well. And a limitation of ATP is going to impact the function of other ATP-dependent enzyme systems within the cell including the proteasome.

So the additional effect of accumulating damaged mitochondria is the production of excessive reactive oxygen species. And although this by itself may not be a problem because of the abundant detoxification systems that exist within the cell, it does put an additional burden on the cell to meet the demands of detoxification.

Finally, one thing that we haven't talked about very much but which is really important is the connection to innate immunity and inflammation. Mitochondria are essentially free-swimming bacteria living within the host cell, and they're really recognized by the cell as foreigners. And, particularly, the mitochondrial DNA, notably, when it's oxidized mitochondrial DNA, is recognized by the NLRP3 inflammasome and by TLR9; and this can actually drive inflammasome assembly and production of cytokines including IL-1 beta and IL-18. So this is a very important aspect, and then the reactive oxygen species only facilitate this by contributing to NF-kappa B signaling.

Finally, in cells where it's really important for those cells to stay alive, damaged mitochondria have a lower threshold for releasing cytochrome C, which would trigger programmed cell death. So it's important to eliminate those mitochondria before they reach the threshold of releasing their cytochrome C and triggering cell death or having permeability transition pore opening and triggering necrosis.

And mitochondria are very important in dealing with calcium and quenching the calcium fluxes. And so impairment of mitochondrial function will have its impact on a variety of calcium-dependent signaling pathways.

So you can imagine then, that the mitochondria might become damaged and depolarized and that initially they would, before depolarizing, might emit a lot more reactive oxygen species; and this would activate NF-kappa B signaling and be a costimulatory signal for NLRP3.

As the mitochondria further deteriorate, mitochondrial DNA may be released into the cytosol where it can interact with either NLRP3 or TLR9; and this drives, then, the production of inflammatory cytokines. Not only is this cell at risk, but neighboring cells then would be impacted by the presence of these inflammatory cytokines.

The cure for this is mitophagy. So the use of an autophagosome to engulf the mitochondria before these bad things happen then is a key cellular response that's engaged in a variety of different cells in response to cellular stress, and it's part of the normal housekeeping process in any cell to tightly control the number of mitochondria and their functionality. And it's elicited by a decrease in mitochondrial membrane potential and recruitment of a protein called parkin, which is a ubiquitin ligase that signals recruitment of p62 and then the autophagosome to engulf these damaged mitochondria.

So mitophagy is a subset of the autophagy system, macroautophagy. And that system is induced by starvation, but it's suppressed by overnutrition; and you've heard a little bit about the impact of metabolic syndrome and caloric excess. So there are a variety of situations where autophagy is impaired including advanced age and also the mutations in the parkin and PINK1 pathway that are associated with Parkinson's and other neurodegenerative disorders.

So the importance of clearing the mitochondria then is really this essential elimination of these damaged elements. And metabolic syndrome is something that's very important in terms of suppressing autophagy. There are a number of studies that have shown that metabolic syndrome decreases autophagic flux and clearance of target proteins. We've used transient ischemic stress or ischemic preconditioning in the heart to look at the protective effects, and this depends on mitophagy and autophagy; and the protective effect is lost in animal models of metabolic syndrome where there is no longer the ability to clear those damaged organelles and to replace them.

I think the other important aspect of this is that not only is mitophagy required, but this is coupled to biogenesis; and this may be relevant in, for instance, skeletal muscle where mitochondrial biogenesis is important for opposing the effects of sarcopenia and restoring things.

So I see I'm staying right on time here. And so the key then is how can we enhance or harness the autophagy/mitophagy pathway. Obviously, there are many benefits as listed here. But there are a number of agents that have been identified to activate mitophagy; and these include rapamycin, statins, and chloramphenicol, in part because they elicit the mitochondrial unfolded protein response.

And there are a variety of different agents that have been shown to prolong lifespan, and work by Jonathan Ar_____ has shown that those agents that prolong lifespan also induce the mitochondrial unfolded protein response; and, by guilt by association, also induce mitophagy.

So this is going to have benefits not only for the target cell but also for associated cells. As reactive oxygen species and cytokines are produced, this is going to impact endothelial function by consuming bioavailable nitric oxide and also by increasing inflammatory signaling of those adjacent target cells. And so you can see that in any tissue where mitochondrial dysfunction is ongoing, that it'll have impact on adjacent cell types.

So there's a caveat to this that excess mitophagy may not always be a good thing because you have to have a balance between degradation of the mitochondria and their replacement by biogenesis. And it

may be, in certain settings, for instance, nutritional deprivation or the very elderly, where biogenesis can't match the mitophagy. And one possible setting of that is in the elderly patients receiving statin therapy who actually don't do very well with that, we hypothesize, because they're unable to regenerate their mitochondria. So with that I will thank you for your attention.

Dr. Cuervo: So our next speaker is going to be Dr. Judith Frydman that is coming from Stanford University, and she's going to try to answer the question of how all these proteostatic networks are regulated by illustrating the gain or the interplay between the difference chaperones.

Consequences of the aging-associated decline in cellular protein homeostasis: the chaperone network.

Judith Frydman, PhD: Okay. Well thank you very much for inviting me to this really exciting summit. So as we've heard from the previous speakers and from many other speakers this morning and yesterday, proteins are important. And in order for proteins to function, they have to be folded into their unique three-dimensional state. And when they are not folded in their unique three-dimensional state, they can either lose function or gain an additional toxic function; and this is very deleterious for the cell.

And it has become very clear through work from a number of labs particularly Rick Morimoto and Andy Dillon and Jeff Kelly that protein homeostasis, the machinery that helps proteins folds is impaired progressively with aging in ways that we don't quite understand. And I'll try to give you a flavor of the questions and aspects of this problem today.

So a number of diseases that are characterized as increasing in incidence in aging are associated with protein misfolding with defective protein homeostasis recolluded to the amyloid deposits such as those that form in Alzheimer, ALS, Huntington, prions. These are proteins that have nothing to do with each other. They don't resemble, at all, each other when they are folded. But when they are forming these amyloid structures, they form these very stereotypical beta sheet reach aggregates that are the hallmark of affected neurons in old age.

Mutations, we heard today that, with age, there is an increasing incidence of mutations that can lead to cancer, metabolic diseases. These very often also impair the ability of a protein to fold or to remain stable. We've also heard of damage such as that that occurs during proteotoxic stress such as ischemia and stroke.

So with aging, there is a decline in protein function, increased protein aggregation, increase susceptibility to disease; and we can ask why is this happening with age? And the culprit here is most likely the protein homeostasis machinery. This is a very complex set of enzymes that all together maintain the health and functionality of the proteome. And there are almost 2,000 components in the protein homeostasis machinery. So the question is why is it so complicated? What are all these components doing, and which and how are they affected with age?

So what is the proteostasis machinery? It's, in a way, a collection of enzymes. Many of them are chaperones. Many are components of the ubiquitin-proteasome pathway that can recognize nonnative proteins, and they recognize different aspects of nonnative proteins and then somehow facilitate folding, assembly, and disassembly of oligomeric complexes, stabilization and degradation.

So chaperones have a biochemical cycle that they use, for instance, for folding. And some chaperones interact with the translational apparatus, which is very intimately linked to the protein homeostasis machinery to promote the folding of aggregation-sensitive proteins.

Chaperones can also promote quality control, as Fred mentioned in his very nice talk. And, actually, here, where I'm looking at the pathways of folding and quality control of a tumor suppressor protein called the Von Hippel–Lindau tumor suppressor where mutants lead to cancer, you can see that different

chaperones will either lead to folding such Hsp70 and TrIP or to degradation such as also Hsp70 and other downstream chaperones.

So where does the complexity come from? Why is there such complexity, and why do different chaperones do different things to proteins? Kind of very elementary-school level way of thinking about this is schematized here. So the chaperone machinery is structured in such a way where there is a motor chaperone, for instance, Hsp70; and there is a long list of cofactors and cochaperones that regulate the activity of 70. And what we want to think about pharmacologically targeting these machinery, it will be much more effective to target the specific cofactors, let's say, for aggregation or for refolding, than to target the motor that may have other more pleiotropic functions.

So what do we need to do to understand the protein homeostasis machinery and its regulation during disease? We need to understand the functional networks. How are chaperones organized in the cell? What's their division of labor?

We need to understand their physical labor. So I mentioned that some chaperones interact with ribosomes. Others interact with mitochondria and keep mitochondria functional. They, in fact, communicate, the proteins that are made-, transcribed in the nucleus and made in the cytosol, they take them to the mitochondria; and they are a big part in the mitochondrial nuclear communication as well as the cytoskeleton and proteasomes.

We need to understand the regulatory network. So there has been a lot of talk here about stress, but different types of stresses induce or repress different types of chaperones. And this diversity and nuanced nature of the stress responses may explain some of the paradox that we have heard about here, and we need to really understand how stress regulates chaperone networks.

And an emerging concept that has actually been quite surprising is that chaperones don't operate like they would in a test tube. They are not floating around and encountering their substrates. There is a lot of spatial organization to protein homeostasis and quality control; and that raises the question of what determines the spatial localization, and what is the cell biology, in a way, of protein homeostasis?

And, of course, a very important challenge is what are the assays, readouts, and approaches that we need to address this.

So very quickly give you a flavor of how people in the field, including my lab, are trying to address these issues; and I want to make some points that I think are important in moving forward to understand this machinery.

So, for instance, when we talk about chaperone networks, work from a number of labs including my own have found that in eukaryotic cells there are distinct chaperone networks, one that works on protein biosynthesis and interacts physically and functionally with the ribosome; and we call this CLIPS for chaperones linked to protein synthesis. And there is a stress-inducible chaperone network composed of the so-called Hsps or heat shock proteins that protects the proteome from stress.

Interestingly, while stress, many stresses, all stresses, actually, induce chaperones of this family to varying degrees, stress represses the chaperones of this network. And, therefore, stress will also have a nuanced effect on the available chaperones to the cell.

Okay, and there are a number of big questions to understand, for instance, the cotranslational acting network. And one important thing is that these two networks are connected, and Rick had a very nice paper very recently showing that one of the key chaperones in the cotranslational acting network is sequestered by amyloid aggregates which really would suggest that having aggregates can affect a very down upstream process that is unrelated to stress and misfolding.

So we think about this network having several hierarchical levels that will enhance fidelity while retaining plasticity. We want a network that is robust. And, in fact, one of the problems with aging is that it stops being robust; and it becomes very sensitive to misfolding.

Now the other aspect of the organization is what happens once you have a protein in the cell and it is misfolded. And Fred had a very nice summary of all the bad things that can happen to proteins in the cell.

So now the cell has to decide whether to refold it, degrade it, or sequester it in an inclusion; and we really need to understand, it's very important that we understand the logic of this decision. And it turns out that it is emerging that there are pathways that connect these three _____tions at specific subcellular locations.

So it has been known for many times that amyloids form aggregates or inclusions in the cell. But work, again, from a number of labs including my own has shown that quality-control pathways are spatially localized. And one interesting aspect of the spatial localization is that there are distinct quality control compartments where different misfolded cytosolic proteins are concentrated.

So we've characterized this system in yeast, but actually it also happens in mammalian cells and worms. So one of the pathways that we call JUNQ for juxtanuclear quality control concentrates soluble misfolded proteins. You can think of it as a holding cell that concentrates misfolded proteins until the cell decides whether to refold them or degrade them whereas the IPOD, or insoluble protein deposit, sequesters terminally insoluble aggregate such as Huntington and prion amyloid proteins.

And the interesting thing is that although chaperones are involved in these two pathways, they are involved in different ways in these two pathways. Ubiquitination status is also very important for sorting of protein to these cellular compartments. And interestingly, the different degradation pathways interface differently with these compartments. The proteasome seems to act primarily on JUNQ-directed proteins, whereas the IPOD seems to be linked to autophagy. Okay?

The other interesting thing is that we have shown recently that the JUNQ pathway declines with aging in simple model systems so that the flux through the JUNQ is actually impaired in aged cells; and it's also regulated by signaling pathways and, in particular, treatment with rapamycin increases the flux of proteins, of misfolded proteins through this pathway. So rapamycin doesn't only work on autophagy or only work on initiation of translation. It seems to affect, generally, the protein homeostasis machinery.

Okay, so I'll skip to this. I just want to make two additional points. I'm over, right? Okay, two additional points. I'll make them very briefly. One additional point. Two half points! Okay, one of them is that post-translational modifications are also very important for regulating chaperone activity. So when we think about the regulation of protein homeostasis, we also need to think beyond transcriptional responses.

Okay, and the other point I want to make is that we heard about mutations. So when we think about mutations such as those that accumulate with age to cause cancer or that happen during viral infections, we also need to think about the chaperone machinery because chaperones are very important in either refunctionalizing or managing mutations such as those that drive these two different types of processes. Okay, so that's my point. And, of course, we want to understand how these are modified with aging.

Dr. Cuervo: Okay, thank you very much Judith. So our next speaker is Randy Kaufman who is coming from the Burnham Institute, and he's going to be illustrating going back to organelle quality control but how that plays in the interplay among cells and in the whole organism. And he's going to be mostly focusing in ER distress.

The unfolding protein response at the organismal level

Randal Kaufman, PhD: Well thank you very much, and I'd like to thank the advisors Maria and Rick for letting me talk to you today. What I'd like to really describe to you is the process of protein folding in the endoplasmic reticulum and describe how that is different than folding in the cytosol. It's quite a challenging environment for protein folding.

So basically, the endoplasmic reticulum is a site for calcium storage. It's a major site for calcium storage in the cell, and it's released in response to growth factor stimulation or hormonal stimulation. Obviously, that tells you it's going to be somewhere important in signaling.

Calcium forms a buffer for protein folding in the ER, and most chaperones in the ER require calcium for their function in promoting proper folding. Protein folding is oxidative. That means that disulfide bonds are formed, and it's very dependent on the redox potential of the ER. So anything that changes redox potential alters disulfide bond formation and disrupts proper pairing of disulfide bonds.

There's quality control where only those properly folded proteins are able to traffic to the Golgi, and those that are misfolded are retained in the ER and eventually degraded through either ER-associated protein degradation by the proteasome or through autophagy. This is a very tight surveillance system.

It's the site for lipid and sterile biosynthesis. So it's basically the site that determines how much lipid the cell has. And you can see here is a pana cell, extensive amounts of ER; but things are very important to note-, or the ER are very integrated with the structure of mitochondria. You find that the membranes are basically juxtaposed.

And that, basically, from early studies, suggested that mitochondria as being the source of ATP that maybe the protein folding or protein synthesis at the ER membrane requires energy. And that's one of the main things I want to tell you is that protein folding in the ER is probably one of the most energy-requiring steps in the cell. Protein folding itself is the most error-prone step in gene expression.

What's the impact of protein misfolding in one cell versus another cell in the body? Well you can imagine. All proteins that go through the secretory pathway have some aspect- One third of our genome encodes proteins that go through the secretory pathway, and many of those are secreted and have effects either through endocrine or exocrine functions; the synthesis of steroids, all the way from corticosterols; the synthesis of synaptic transmitters. So you can imagine that protein misfolding can affect a lot of intracellular and intercellular functions within the organism.

So when proteins come in, they're in an unfolded state. They're glycosylated. These large end linked glycosylations are very important for interaction with protein chaperones that recognize specific structures that promote the proper folding as well as trafficking out of the cell. Disulfide bonds form and they're rearranged, and it's the properly folded form that's recognized for trafficking out.

Anything that disrupts ER homeostasis, and this could be a wide variety of inducers such as calcium depletion; altered glycosylation as if you have a glucose deprivation; nutrient deprivation as well as nutrient excess, cholesterol excess, lipid excess; redox stress; as well as protein synthesis. Just an increase in protein synthesis is going to increase the amount of protein misfolding. So an activation of a TOR-signaling pathway will result in more ER-misfolded protein.

The misfolded protein itself has basically several destinations. It could be refolded. We don't understand the machinery involved in this; but it's very complex, and it does happen. But what are those enzymes? And we really don't know to date.

Alternatively, it's degraded, I mentioned, through either autophagy or ERAD. If you remove these functions from the cell, the accumulation of that protein will kill the cell. So it's the toxic accumulation of these proteins.

There's an adaptive response when this accumulates. It's called the unfolded protein response, and that is basically an adaptive response to resolve protein misfolding. It's carried out at three levels. One is you decreased protein synthesis because you don't want to synthesize protein if you can't fold the protein. You activate transcription of several thousand genes. At least 50% of them provide functions that are all depicted here: ER-associated degradation, trafficking, translocation, end folding.

And finally, if these are not sufficient to resolve the protein-folding stress, cells activate an apoptotic response. One of the major signals in the apoptotic response is reactive oxygen species.

So what signals the response. There are basically three transducers that have primarily been discovered and characterized, and quite well, through deletion studies and overexpression studies.

Just briefly, two of them are protein kinases. They get activated by dimerization, and they go through basically a specific linear pathway, very specific. Ire1, a kinase and a nuclease initiate splicing of one mRNA that makes an active transcription factor that activates adaptive function, primarily ERAD, protein chaperones, and lipid biosynthesis.

ATF6 is a transcription factor that activates by cleavage as it traffics to the Golgi upon release from a stressed ER, and that activates a complementary set of genes. These are primarily adaptive; but, importantly, is that the Ire1 xbp pathway is very important for differentiation of highly-secretory cells. Immunoglobulin-producing plasma cells absolutely require this pathway. This pathway is required for beta cell differentiation and for a number of other highly-secretory cell types. And if you knock this out, it's lethal in embryonic life.

ATF6, in contrast, has no real phenotype in the mouse when you knock it out, but it's very important for adaptation to an acute stress.

In contrast, PERK is basically the signal that basically determines whether the cell will survive or die. And when it gets activated, you basically shut down protein synthesis transiently because this is a reversible process. And that basically ensures that protein synthesis is coupled with protein folding.

Paradoxically, some mRNAs are preferentially translated; and one of them is ATF4 that activates a larger set of genes. Importantly, it activates autophagy. It activates amino acid biosynthesis, metabolism, and it activates this transcription factor CHOP, which is a major apoptotic factor. This is the pathway that is primarily determining whether the cell will survive or die, and it's the expression of CHOP that's important in that decision. Expression of CHOP correlates with production of reactive oxygen species.

So how does this occur? Well if we envision the ER, it's juxtaposed to mitochondria. Protein is spooled in the ER, and they aggregate. They bind to a protein chaperone called BiP. This is a major ATPase. This is probably one of the most ATP-dependent steps in protein folding in the ER. ATP hydrolysis and binding are required for release. Disulfide bond formation requires electron transport onto oxygen which forms hydrogen peroxide as a reactive oxygen species.

At the same time, when unfolded proteins accumulate, there's calcium leak out of the ER. The mechanism for this is not really well defined. It's either through things like IP3 receptors or through BAK SPAAK channels or possibly through the Sec61 channel. But the calcium leak results in a mitochondrial loading. And it's the mitochondrial loading of this calcium that actually activates dehydrogenases and activates ATP synthesis.

So in a way to say that as you're using up ATP in the ER, you're stimulating electron transport and oxidative phosphorylation and mitochondria. We think in this way that the ER has evolved to be an ATP sensor. However, when excessive protein accumulates in the ER, you get excessive loading of mitochondria. And that actually results in toxic responses that result in superoxide production.

In addition, ATP is required for other aspects, and that's for reloading calcium into the ER. So you can see that there are a number of steps that are all very immensely intimately related that revolve around ATP utilization, calcium trafficking in and out, and production of ATP and production of superoxide.

Well, what are the reasons that cells make the superoxide? And, indeed, if we put the antioxidants on cells, we can prevent the cell death and we can improve the protein folding. So, obviously, those events are related. Where are the superoxides coming from? Well, it appears that we think that most of them are coming from mitochondrial function. I think most evident from that is studies in which we've looked at insulin resistance in the mouse. And, basically, insulin resistance by either diet-induced obesity causes the beta-cell to increase insulin production through biosynthesis increasing translation of insulin put load on the ER. That activates the UPR. Eight hundred million people in the world that are insulin resistant, two-thirds of those will go on and compensate. Their beta cells will make more insulin and they'll never develop a diabetes.

The one-third that do develop diabetes developed the SER to ER. You can see the insulin is misfolded. They have fewer granules and they actually activate all the events of oxidative stress, apoptosis, and that means you have haploinsufficiency of UPR signaling. You're prone to this particular beta-cell failure. And an antioxidant can protect all of that.

So, finally, I just want to leave you with this slide without going through it. And that's basically the interaction between protein misfolding in the ER and how both protein aggregates and oxidative damage are associated with a wide variety of diseases from metabolic disease, immune inflammatory responses, cancer, as well as neurodegenerative diseases. Thank you for your attention.

Dr. Cuervo: Thank you very much, Randal. And our last speaker of this session is going to be Jeff Kelly from the Scripps Institute and he is going to address the possibility of targeting proteostasis as a therapeutic approach. So he's telling us about all the drugs that they are developing.

Novel therapeutic strategies against proteotoxicity

Jeffrey Kelly, PhD: Start my timer so I don't get in trouble with Felipe.

Okay, great. So its a real privilege to be at this summit and to talk about a subject that's near and dear to my heart. The underlying hypothesis being that it's now possible to adapt proteostasis that you've heard a lot about in this session to ameliorate diseases linked to both aging and aggregation.

And in the first short part of this, I'll talk about decidedly chemical strategies that have been, at least in one case, realized. In the second part, I'll talk about what I think is a much more general strategy, a biological approach to adapt proteostasis that should be more general.

So work from a number of investigators' labs have now taught us that when polypeptide chains come off the ribosome, there's a kinetic competition between folding, misfolding also exhibiting unimolecular kinetics, and concentration-dependent aggregation. And, of course, when aggregation dominates, that can lead to degenerative diseases like Alzheimer's and Parkinson's and Huntington's and the transthyretin amyloid diseases that I'm going to talk about some more.

Now the other thing that's important is that when proteins fold in the ER that you just heard about from Randy, and when they're secreted, they can still in the extracellular space sample unfolded states that can drive aggregation. And that's the big problem with neurodegenerative diseases because the proteostasis network in the extracellular space we know very little about, but it's clear that it's nowhere near as sophisticated as that in a cell.

Okay, so our first chemical strategy is pretty simple. The idea is that you can populate a folded confirmation, say in a mutated or aggregation prone protein. And if you can craft a small molecule that

binds with high affinity to that misfolding-prone protein, then you can prevent conformational excursions that drive aggregation.

So we decided to test that concept in the context of the transthyretin amyloid diseases that are quite representative of human amyloid diseases. The huge advantage here is that you can do a clinical trial without worrying about the brain because these diseases destroy the peripheral and autonomic nervous system and the heart. Okay.

So this protein transthyretin is secreted from the liver as a tetramer. When it's in the extracellular space, it aggregates into a whole bunch of structures, including amyloid fibrils for which these diseases are named. And this process of aggregation drives the ultimate degradation of these tissues that don't easily regenerate. We now have, I think, incredibly strong data that the process of aggregation drives these diseases. What we don't understand is how does that couple to the loss of postmitotic tissue. That's a big unanswered question.

Okay, so specifically what do we know? We know that the tetramer dissociates in a rate-limiting step in order to drive aggregation. So we reason that if we could fashion small molecules that could bind to these small molecule binding sites, then we could stabilize the protein enough so that the protein would no longer aggregate; realizing that when people become symptomatic, they have all these structures in the plasma. So the idea is if you can stop further aggregation, you can stop the pathology.

And while I don't want you to stare at the details of the data, the bottom line is the patients get worse when the data go up the Y axis. The treated group is in the filled symbols and the untreated group is in the unfilled. It's clear, very clear that this drug works. And, therefore, it's been approved in Europe and in Japan and, hopefully, soon by the FDA.

What's the problem? The problem is that you have to develop a small molecule for every misfolding-prone protein. There are 50 of them. This took us 15 years to do. We can probably do it in 10 now, but still a big problem, right? We need a more general strategy.

So the more general strategy is clear. We should be able to tap the protein homeostasis network to adapt it to keep us free from disease when we get older. This system is amazing at preventing degenerative diseases when we're young, right? Nobody gets Parkinson's disease when they're 25. No one gets the transthyretin amyloidosis when they're 25. They carry all the mutations. So what protects them? It's this system.

So how do we adapt this system? Now the way that I think about the proteostasis network is quite simpleminded perhaps. But as the polypeptide chain comes off the ribosome, the critical decision is do you fold the protein or do you degrade it? And we're going to take advantage of that central decision that the proteostasis network makes in coming up with a strategy for these degenerative aggregation associated diseases.

Now, of course, what hasn't been mentioned but has been implied is that there's a proteostasis network for every subcellular compartment; they're different, right? And each one of them is regulated by a different stress-responsive signaling pathway. And I'm going to talk more about the unfolded protein response which regulates the proteostasis capacity in the endoplasmic reticulum. And we're going to do that again in the context of the transthyretin amyloid diseases.

I want to point out something new on this slide you've already seen. So in the familial diseases, of course, the patients are heterozygotes. So one allele codes for the wild type protein. The other allele for the mutant. Therefore, the tetramers that are secreted from the liver are mixtures of wild type and mutant allele. And so wouldn't it be wonderful if we could somehow deplete the tetramers that are secreted of the misfolding-prone mutant subunits.

So our thinking is as follows, right? If we can tune up the endoplasmic reticulum to have a higher level of quality control, when mutant proteins are injected into the endoplasmic reticulum through the translocon, the desire would be to have them to be degraded by the proteasome of, yeah, the process of ERAD that Randy mentioned while the wild type protein could still be secreted. Of course, the situation would be now the tetramers would be mostly wild type, which are much more stable than those incorporating mutants.

Since these stress responsive signaling pathways that regulate the proteostasis capacity of the ER all end in transcription factors, we simply decided to express these transcription factors at physiologic levels using a trick that we just published in *Cell Reports* to ask whether activation of one or more of these pathways would affect the kind of quality control we want so that a morph of stable protein would be secreted. Turns out that this really works fantastically both for the transthyretin amyloidosis, so when you activate ATF6 in this pulse-chase experiment, you go from here to here. So about 50% of the mutant protein is eliminated. And, similarly, with light chain.

So I would just close in saying that we have an awful lot to learn about the protein homeostasis network, but I think it's already clear through fairly subtle alterations of the proteostasis network that we can alter processes of aggregation that lead to degeneration, processes of misfolding that lead to loss of function diseases like cystic fibrosis and so on.

So I think I'll stop there. Hopefully, I've given you some food for thought and some optimism that perhaps we can discuss further in the discussion period. Thank you very much.

Wrap-up and Discussion

Dr. Cuervo: Thank you very much. Thank you to all the speakers to keep on time. So we are going to move into the table for the discussion. So if you guys can please come to the table. And while you do that, I'm just going to summarize a little what we hear today. So let's see.

So, basically, the whole idea, as you saw, is that there are different levels in which you can modulate proteostasis. So but there are things that we already learn, and I think we are in a good situation as you hear from the speakers; but there are many things that we still need to know.

So when you think about the chaperones on the proteolytic systems scale that we have a good understanding of the molecular mechanism. And we just hear how you can drug these systems and make them work better. But we still have many questions regarding how this interplay with protein repair that we hear this morning in the session and also how are interplayed with damaging other molecules. We've been talking about proteins. So what about the DNA damage that we hear this morning?

Then when you move out of the cytosol into the organelles, I think we got a good feeling of how much we know about the ER stress as well as the mitochondrial stress and how these pathways crosstalk, but we still don't have a very clear idea how these other compartments, and one of them that we are really missing is nuclear. We hear about DNA damage. And homeostasis in the nuclear is not very well understand. It is not clear how these compartments can dump the garbage from one to each other and which is the strategy to do that; as well as when you start modulating chemically one of these compartments or the proteostasis in one compartment, what is going to happen to the others.

Then if we move to cell-to-cell communication, it's very clear that the proteostasis in one cell is going to affect the neighbor ones; and there are examples in the different systems. But what is not very clear is that how that communication occur. Is this from cell-to-cell interaction? We didn't hear about exosomes, but we hear yesterday is this a way to communicate the proteostasis of the cell? And is this something that is continuously communicating or only happens during stress?

And this really pins into the importance of protein homeostasis in the idea of prion-like proteins using these proteins as a seeding to generate aggregates in another protein. That is something that in neurodegeneration we have heard a lot.

And then the last part, and this is the one that we understand less, is how the proteostasis in one organ affect the other. We hear some very elegant models from Dr. Morimoto in *C. elegans*, so how when you're affecting one system it's going to alter in a different organ, the brain to the gut, and vice versa. But is this something that we can use? Can we interfere with the proteostasis in one peripheral organ to affect the brain? And we hope that that's going to happen.

And then all this communication is happening through hormonal and it's happening through the blood. So how does it affect it by other things that we have here that happen in the circulation such as the inflammation and nutrients. How is all this put in place?

So we are going to open this for discussion. Please identify yourself before asking the question.

Dr. Morimoto: Let's start over here. Thank you.

Dr. Evans: Bill Evans, GSK. Fred, maybe you didn't get to it in your talk but some years ago we did a study where we biopsied 90-year-old people. And it's an exercise study. But the pre-exercise samples showed a tremendous amount of disorganization in muscles, z-line streaming and just it looked like there was a tremendous amount of damage that was going unrepaired in the muscle of these extremely old people. And I'm wondering if you can say something about why that might be and what is it about senescent muscle that may not activate the proteasome appropriately.

Dr. Goldberg: Bill, I wish, again, I could answer these questions. There's no question, if you ever saw me on the tennis court, age decreases muscle performance. And anyone who has looked at it, and I guess your data in humans is similar to data we've seen in mice — I mean, we meaning the community — is there is a loss of classic architecture. You see splitting of myofibers, so quality control mechanisms are reduced.

Now whether that's in ubiquitination, the assembly, the response to let's say little bit of exercise that a 92-year-old person does, all of those questions we don't know about. All I can say in, unfortunately, the time we had, is certain of the components we can't measure because we just don't know enough about ubiquitination and recognition.

For methods that have been developed in the last couple of years, it should be straightforward to assay proteasome function and also p97 is this critical complex that pulls subunits out of larger structures out of the ER and, now we know, out of the myofibril. Whether they're functional, we don't know about. We don't know about their production.

It is interesting, if you inhibit the proteasomes as we say with the pharmacological drugs, cells do upregulate and produce new proteasomes through a transcription factor called NRF1. They upregulate p97 and they also, to some extent, upregulate p62 and factors that feed ubiquitinated proteins into autophagy. Be very interested to see if that transcriptional response is altered in the aged populations.

I think the answer to your question is we only now know the terms at which to ask the questions that you've got a very specific example for.

Dr. Morimoto: And, Judith, we'll give you one minute.

Dr. Frydman: Yeah, even less than that. So I wanted to comment on work that maybe you're not familiar with from Jörg Höhfeld in Germany. So, as you know, all the proteins in the myofibrils are very large and very complicated to fold. And there is a number of people that have looked at the distribution of chaperones and the folding of these proteins in muscles. And the work from Jörg is particularly nice

because he's found that the proteins are folded on the z-line and there are a number of chaperone cofactors that help those proteins fold that are localized, their muscle specific and they are localized to the nascent myofibrils that are kind of folded in situ. And when he knocks out these chaperone cofactors, the z-line is completely disorganized. The muscles from Drosophila look like what you described for 90-year-olds. So this could also be a folding issue.

Dr. Morimoto: Okay, let's go over here.

Dr. Kotsanis: Constantine Kotsanis from the Kotsanis Institute. The past month we have heard quite a bit about tau and NFL injury to the head from repeated exposures. Two questions. What's the mechanism of the formation of tau? And question number two, what can we do to prevent or reverse such trauma?

Dr. Cuervo: I can take I guess. So, tau, the main problem with the protein, it's a protein that is going to be always in your brain. It's a protein of the cytoskeleton. It's very abundant. But then undergoes post-translational modifications and this is one of the examples of how these post-translational modifications can alter the proteostasis. So when it gets hyperphosphorylated, you start having these aggregates or these fibers that occur in the cell.

But the interesting thing, and that's something that has been very recently understood now, is that these proteins propagate. So this is the idea that you can propagate the disease from one cell in the brain to another for transmission. And we know very little about the mechanism but has been reproduced *in vitro*. And it's probably the trauma is increasing or is somehow compromising the clearance intracellularly and then it has to pass to the next one, and this is accelerating. So it's probably a combination of what we hear about inflammation, oxidative stress damage. They go to these regions and make them very vulnerable for propagation.

Dr. Morimoto: Fred, did you want to say something?

Dr. Goldberg: I think the physical effects of trauma that initiate what Ana Maria has just mentioned is a very open question. We're used to stresses of heat and chemical nature, but physical trauma to the brain is a little cruder than cell biologists and biochemists want to do. Even toxicologists don't want to hit mice in the head with hammers. And not surprisingly, we can't say anything very intelligent about what initiates that except that it sounds like an inflammatory stimulus. And once you start macrophages, neutrophils, glia generating free radicals, all these sequelae will happen.

Dr. Gottlieb: Well, to some extent, the traumatic brain injury is an ischemic injury because with the swelling and the transient clotting that takes place, there's ischemic injury and with that you're going to have damage to mitochondria. This is going to limit then the ATP available for protein refolding or protein repair, and so that could also be an inciting trigger.

Dr. Kotsanis: What would you do preventively?

Dr. Gottlieb: Helmets. Don't play football.

Speaker: Yeah, don't play football.

Dr. Morimoto: _____.

Speaker: Yeah. This is another question for Fred. I think you mentioned if you have inappropriate stoichiometry, you have some heteromultimeric proteins with extra subunits hanging around, you zap those in the proteasome. I was wondering is there any information about those heteromultimeric proteins where stoichiometry is altered where that doesn't work so well? I was thinking in mitochondrial that proteins where most of the guys are coming from the nucleus and a few from mitochondria, you imagine

you get into trouble about stoichiometry. And is anything known about that, that that is a problem in dealing with that?

Dr. Goldberg: Absolutely, going back to let's say the best characterized cases might be thalassemias. If you have excess alpha subunits or excess beta subunits, then they tend, unlike normal tetramers, to precipitate out, distort the red cells, need to be clearing. So that's one extreme medically relevant example.

In mitochondria, if you just — and this is an experiment we did in the early '70s, I mean, '80s. If you put in an inhibitor of mitochondrial gene expression, such as chloramphenicol, you will find that the imported subunits are rapidly degrading. Or if you take isolated mitochondria and you follow the fate of the gene products made there, when they don't get the subunits from the cytosol, they're all rapidly degraded within the matrix. So there are many, many examples that you can show *in vitro*. If you stop ribosomal RNA production, all of the proteasome subunits are rapidly degraded.

So I think this is a very common event. The comments that you heard from some of the other speakers, Judith, that chaperones are working at the ribosome on newly synthesized proteins and they may be different from the ones that maintain the proteome of the mature proteins, that just emphasizes that the challenge of newly synthesized proteins, until they get folded and find their other mates in larger structures, they're very susceptible to causing problems. And presence of things like amyloid in the cells also interferes with that process, as others on this panel have shown. So I think it's a very important aspect of the challenges for the proteostasis that hasn't been emphasized enough.

Speaker: Thank you.

Dr. Morimoto: Judith.

Dr. Frydman: Yeah, so, of course, because of the danger of unassembled subunits, the genome has tried to balance transcription and translation of subunits of oligomeric complexes. And perhaps the most dramatic example of when that is disrupted is during aneuploidy in cancer cells or trisomy of chromosomes because there you disrupt the natural balance of subunits. And there is nice work, for instance, from Angelika Amon showing that ribosomal proteins are all in different chromosomes. If you have one more or one less of one of the chromosomes, then you have an imbalance of all the other subunits. And I think this is one of the reasons why cancer cells are so dependent on having a robust stress response for survival.

Speaker: So that's happening in Down Syndrome presumably.

Speaker: Exactly. Exactly. The same reason.

Dr. Gottlieb: That was an interesting hypothesis. Yeah.

Speaker: That's interesting.

Dr. Goldberg: In that context, I didn't get a chance to mention it, one of the exciting new developments in this area on the proteasome work is work from Finley and King who have shown that if you alter the proteasomal reactions that I quickly went through, you can activate breakdown of proteins. And in Angelika Amon's work, this is particularly effective in the cancers that have polyploidy that's inappropriate in aneuploidy.

And so I think this is a very interesting connection between everything that's going on here and hasn't been studied enough in cancer.

Dr. Morimoto: Over here.

Dr. Buffenstein: Rochelle Buffenstein, Barshop Institute. This question is also to Fred Goldberg. It pertains to your disparate findings between the brain and the muscle in terms of proteasome function. I'm quite fascinated by one being elevated with aging and the other being decreased with aging. And I wondered if that had anything to do with the influence of maybe inflammation or oxidative stress being activated and phenotypic plasticity of the proteasome and, in particular, the immunoproteasome. Would you like to comment?

Dr. Goldberg: Yes. We're fascinated by the same phenomena, but I had a footnote on that slide which he didn't go into saying everything I was saying was based on one paper that is really rigorous because only recently have people been isolating the 26S proteasome and really studying it with physiological substrates.

Assuming those two studies are valid, I think what I was trying to emphasize is that not all organs are responding the same way. And when you think about it, there is a muscle wasting phenomena, there's an increase in ubiquitin conjugates in the muscles of elderly animals as well as humans called sarcopenia. I mean, the proteasome and the ubiquitin pathway in brain has a very different role. And, clearly, it's mainly involved in quality control, not in the kinds of protein balance issues that say were talked about by Bill Evans. That's very important in peripheral tissues, especially muscle, in addition to quality control.

So the answer to your question is I think there are big differences. We have no reason to believe that what we talked about has anything to do with immune response. I presume it's more related to aging and it's independent of the phenomena of immunoproteasomes. Immunoproteasomes go up with aging, but they do not affect the recognition of misfolded proteins. That occurs through ubiquitination in the 19S. The immunoproteasomes, which is a variant in the active sites, influences the efficiency of generating peptides that are appropriate for antigen presentation. That's rather important, but it's a little different from the phenomena that I was referring to.

Dr. Morimoto: Thank you.

Dr. Buffenstein: Thank you.

Dr. Morimoto: Over here.

Melissa Harris, PhD: Hi, Melissa Harris, I'm at the NIH at the National Human Genome Research Institute. So I've gotten the idea through this symposium that we have a lot of global dysregulation in a number of processes, so here proteostasis. And maybe it's because of the idea of the TED talks. But I was struck by a comment I think from Stuart Kim, yesterday, where he talked about gene expression changes can be very dramatic with age from young to old, but there are some tissues where you don't see these dramatic changes.

So what I wanted to pose to this panel is how much do we know about dysregulation of proteostasis in all different cell types, so stem versus terminally differentiated and all different cell lineages? And is it really an organismal phenomenon or are there certain cells or certain tissues that are more driving that have dysregulation of proteostasis that contribute to the aging phenomenon?

Dr. Gottlieb: So there's work from Phyllis Linton that has looked at the induction of autophagy and the ability to respond to a starvation stimulus in different tissues as a function of age. And although in many tissues the process of autophagy diminishes, this isn't consistent across every tissue and the response to starvation is not consistent across different tissues.

I think when we think about autophagy or many of these cellular process in a whole animal, there are a number of caveats that we have to take into consideration. If we're using mouse models, a strain of mouse can impact very significantly the functionality of the autophagy pathway and likely other cellular

homeostatic mechanisms — time of day, sex of the mouse, and feeding status. So all of these different factors can impact the rate of proteostasis in different tissues.

Dr. Morimoto: Let me add a specific experiment that will highlight quite dramatically how the proteostasis network changes in aging in a model system. So this is back to *C. elegans.* If one takes these animals and they have a series of folding sensors, proteins in different compartments, that you can monitor in real time and they all have a very strict phenotype — myosin, paramyosin, RAS, for example, acetylcholine — their misfolding occurs between day 2 and day 6 of adulthood. These are all temperature-sensitive proteins so they're not massively altered. If you then look at the heat shock response that you've heard about or the unfolded protein response, they shut down precisely between 8 and 12 hours of adulthood.

Now the reason why this is interesting is that the animal has already set in stage its major function of life, fecundity. *C. elegans* are hermaphrodites; they make the eggs and the sperm. And so, essentially, that's accomplished. And this is a very precise genetic reprogramming that's occurring early in life that then dictates what's happening in the out years.

So as to whether one tissue is affected more than another, we don't know at this point. But it's not random, it occurs at a precise moment, and all of the different stress responses are being shut down simultaneously. And at this point, we think it's almost entirely epigenetic control.

So I think it's highly specific and rather catastrophic in at least the model systems. And I've heard that there's observations in Drosophila that are very similar to suggest at least in the invertebrate models there's going to be a similar process.

Over here.

Dr. Musi: Yes, Nick Musi from the Barshop Institute. So I'm interested in the opinion of the panel regarding what is the effect of aging on the expression and activity of ubiquitin ligases, such as MURF-1 and acrogen and if they are affected, what are the physiologic consequences?

Dr. Goldberg: Yes. Maybe I should just fill in the background for the audience. I mentioned there were 600 or so ubiquitin ligases. There are a group of them that are associated with muscle wasting. Say if you denervate a muscle or fast or have sepsis, cancer cachexia, and we call these atrogens, including MURF which takes apart the thick filament and atrogen which affects nuclear proteins and protein synthesis.

Those are definitely induced much more dramatically in young animals than in old animals. This is the work of others. If you give a specific stimulus such as glucocorticoids, for some reason in older rats and mice, it's not induced as dramatically.

On the other hand, work from ourselves and David Glass finds that MURF is hard in sarcopenic animals and a lot of that may be post-transcriptional. Exactly what that means we're not certain of, but it probably means excessive myofibril ubiquitination and breakdown. But there are ongoing experiments addressing that.

In this business, in terms of the previous question, what your question raises is the idea that ubiquitin ligases, that early part of the pathway is not ubiquitous. Ubiquitination is not ubiquitous. There are organ-specific differences — dividing cells are different from nondividing cells, muscle is different from neuron. There are a whole group of neuron-specific ubiquitin ligases. And so the biology of that part of the pathway as compared to the proteasome is very specific to the physiological context or the pathological context.

Dr. Morimoto: Over here, please.

Joseph Gorse: Joe Gorse, University of Akron. My question is for Roberta. So could you talk a little bit more about maybe a strategy for enhancing the autophagosome. I imagine, you know, how where maybe the first places to look, whether you'd think human cells versus the mouse model, that type of thing, but how you would go about that.

Dr. Gottlieb: So we've looked at autophagy and mitophagy in everything from cell models to actually human samples and we see a fairly consistent theme that this is a response to ischemic stress and is induced by nutritional deprivation suppressed by nutritional excess.

In the setting of humans, we've looked at autophagy in the human heart during surgical, the stress of ischemia during cardiac surgery. And there autophagy is mounted as part of a protective response. In animals we know that it's a protective response because if we block it, the magnitude of injury is much greater. So there are a number of approaches that are possible to look at autophagy.

I think the other question was ways of inducing it.

Joseph Gorse: Yes.

Dr. Gottlieb: And so the question is on a chronic basis. Certainly, there are lots of pharmacologic agents that induce autophagy and probably induce a whole host of other proteostatic pathways, and maybe that's a good thing to induce all of them in concert so that they can keep each other in balance. But I'll tell you a story from work done with Phyllis Linton where mice were subjected to fasting twice a week starting at the age of ten months and continuing for a year. And at the end of that point, what we found was their autophagy capacity was much more intact than their age ad-lib litter mates. And their cardiac reserve in terms of response to ischemia was much better and their immunologic function was better. So that's one mechanism of this intermittent fasting. Potentially exercise would elicit the same set of pathways to upregulate autophagy on a physiologic chronic basis.

Dr. Cuervo: So just to add a little about the upregulation of the modulation of autophagy in neurodegeneration because that's something that has interested a lot. There are enough evidence that in conditions like Alzheimer's, Parkinson's, Huntington's, you have a compromise of the autophagy system. So in that case, something that we have discussed many times, it's very important to know where is the problem.

There are some times that you might not want to put more of these autophagosomes, as Roberta described very nicely, because you have a problem to eliminate them. So I think in those cases it's very important, rather than giving rapamycin to everybody, start to see where is the problem and do much more a specific treatment that I think we are reaching at this stage that that's going to be possible.

Dr. Morimoto: Good, thank you. Gordon.

Dr. Lithgow: Gordon Lithgow, Buck Institute. I'd like to go back to large assemblies and I'm curious as to what we know and don't know about repair or removal of single subunits in assemblies. So if there's irreparable damage to a single subunit in electron transport chain complex, can you repair or remove that subunit? Do you have to take down the entire complex or, in fact, do you have to take down the entire mitochondria?

Dr. Gottlieb: I think we don't have good answers to that yet, partly because of the limitation of the tools. There are AAA proteases that are abundant in the mitochondria as well as proteasomes. And so there are mechanisms for degrading individual components. But whether they can access the components that are in these almost crystalline arrays of the super complexes isn't clear. But if we imagine that these structures kind of breathe and reassemble dynamically, then you have a chance for misfolded components to not be reincorporated as these structures reassemble. And you can imagine that they might sort of then get shoved along the membrane to some region that's not so packed with these. And that might be the place where, as the mitochondria undergo fission, that junk gets butted off and is

removed for autophagy because when mitochondria grow through fission, what you get is two asymmetric mitochondria; one with high membrane potential highly functional and one that's not so good. And that was is targeted for disposal.

Dr. Morimoto: We have about five minutes so we're going to have to have shorter questions and very succinct answers.

Dr. Franceschi: Claudio Franceschi. I have a comment more than a question. I would like to draw the attention of the panelists to another protein modification that has not been mentioned. And this is related to the glycomic. So the alteration of n-glycans and o-glycans. And these are a part that these alteration are involved in inflammation and other pathways. But this offer, for example, the accumulation of galactosylated n-glycans in the proteins in IGG and in other liver proteins offered us some of the most powerful biomarkers of aging. So I think that this should be added to the list of alterations.

Dr. Morimoto: Well, actually, we'll ask Randy and Jeff because it turns out that the addition of glycans is a major signal in the unfolded protein response.

Dr. Kaufman: So the n-glycans are put on in the ER and their modifications involve trimming and then rebuilding up through the Golgi stacks. Those glycans in the ER are essential for interaction with lectin chaperones and for trafficking through vesicle mediated traffic to the Golgi where there are other glycosyl transferases that modify them, and eventually sialic acid is put on and they're secreted.

For the o-glycans, those are primarily put on in the Golgi compartment and they tend to be particularly enlarged in tumor cells, in particular mucins of the epithelial, gut epithelial tract. What you find are that tumors in those tissues, they have excessive large amounts of mucins which are thought to be part of a way to evade the immune system.

But in terms of aging, I actually looked for this before coming here, and I really couldn't find any evidence that the expression anatomy of these glycosyl transferases is altered with aging.

Dr. Franceschi: No, but there are. We published several papers that there is this alteration of n-glycans. And there are many papers, so.

Dr. Morimoto: Okay, thank you. Rich.

Dr. Franceschi: You can give a look, yes.

Dr. Miller: Rich Miller from the University of Michigan. The NIA Interventions Testing Program is always interested in trying to find drugs that might slow aging and extend lifespan by improving the ability of cells within the mice to deal with protein misfolding. Whenever I hear a talk for the last five years about this topic, there is always one such compound that does it. It's called compound 1. And so I was wondering if there are drugs that are not compound 1 that could any of you recommend for administration to mice for testing to see whether lifespan and healthspan can be improved by a pharmacological improvement of the protein folding apparatus.

Speaker: The answer is yes.

Dr. Kaufman: So there are chemical chaperones that basically buffer protein aggregation and these, most classical are phenylbutyric acid or taurine deoxycholic acid, which is a cholic acid derivative. And they're in the clinic, actually, for probably over a dozen different diseases now all associated with protein misfolding. Phenylbutyric acid has been approved for orocecal disorders and TUDCA has been approved for bile obstruction diseases. But they're in the clinic for Parkinson's and they've been shown to improve insulin sensitivity in humans. The number of studies in animal models show that most diseases of protein folding associated with misfolding in the endoplasmic reticulum can be protected by these extremely high

doses, grams per day. In some of our experiments, we can actually delete a molecular chaperone and completely correct the phenotype by adding a chemical chaperone to their diet.

Dr. Morimoto: Jeff.

Dr. Kelly: So there was just a paper from a group in Finland showing that about 1% of humans have a mutation in transthyretin that works strictly analogously to the drug that we introduced that Pfizer now sells. And what's interesting about those people is that they live 5 to 10 years longer than the other 70,000 people in that population that was studied for a median of 32 years. And the authors attributed that lifespan extension to a decrease in vascular disease, and that was very clear from their data. So I think there are drugs that will extend lifespan by ameliorating disease. And vascular disease is a big one.

Dr. Gottlieb: To the extent that inducing mitophagy is going to be beneficial, and I think that at least as long as the autophagy flux pathway is intact, then that can be a beneficial thing. There are already drugs that are available that are on the market and these include rapamycin, statins, chloramphenicol. But I think that because we have this balance of stimuli that are both pro and anti autophagy going on in each person, it's not clear. For instance, sympathetic stimulation is going to tend to suppress autophagy beta blockers are beneficial. So many of these benefits may already be active in the patient population.

Dr. Goldberg: I would just add there are enough ages being studied at the moment that your question is a little premature. When the pharmacodynamics are ready, I'm sure several people will be phoning you to test them. But when you think about it, rapamycin, aside from activating autophagy, well we have now found it activates ubiquitination generally, so the two main degradative pathways are activated. And anything that affects the IGF pathway and/or DAF-2 is affecting FOXO. And aside from metabolic effects, FOXO is inducing the transcription of all the autophagy genes I know and it also is inducing major components of the ubiquitin proteasome pathway and other proteostatic mechanisms. So without knowing it, you may be the leader in introducing manipulations of the ubiquitin proteasome pathway.

Dr. Wise: Okay, if there's no other questions, I'm Brad Wise from the National Institute on Aging. And to close the session, I'd like to thank the cochairs, the panelists, and the audience for a very interesting and informative session. We will break now and reconvene at 3:15 for the last session of the day.

Thank you.

Break
Session VIII: Stem Cells and Regeneration

Leslie Frieden, PhD: Good afternoon and welcome to the session on Stem Cells and Regeneration. We're now in the home stretch. My name is Leslie Frieden and I am from the National Institute of Dental and Craniofacial Research. My co-organizers for this session are Young Oh from the National Heart, Lung, and Blood Institute and Ron Kohanski from the National Institute on Aging.

Our cochairs for this session are Thomas Rando and Tony Wyss-Coray. Dr. Rando is a Professor in the Department of Neurology and Neurological Sciences and the Glenn Laboratories for the Biology of Aging at Stanford University School of Medicine. He will give an introductory overview of the topic for this session.

Dr. Wyss-Coray is Professor in the Department of Neurology and Neurological Sciences of Stanford University School of Medicine and Associate Director of the Center for Tissue Regeneration, Repair and Regeneration at the Veterans Administration, Palo Alto Healthcare Systems. He will be directing the discussion which follows the last speaker and giving a summary of the session. Thank you.

Introduction

Thomas A. Rando, MD, PhD: Great. Thanks, Leslie, and thanks all of you for sticking around. I am from Stanford but I grew up in New England and I did most of my schooling in Boston, which I just wanted to mention because it's also the home of the world champion Boston Red Sox.

So this is the last session of the summit and this is a session titled Stem Cells and Regeneration. So you'll hear from speakers about different aspects of stem cells and regenerative biology, but I just wanted to point out this really is a different kind of session in a way from the others that we've heard about so far because this is just the titles of the other sessions. And these are either really kind of cell extrinsic influences on any cells or cell intrinsic processes that occur all related to aging, whereas in this session we're actually talking about a particular cell type. And we have chosen to talk about stem cells; we could talk about any cell. And so really the question is why are stem cells in a way unique or special in terms of the biology of aging when really we'll hear about all of these aspects of processes that go on in aging in stem cells.

And I would just kind of throw out a couple of ideas of why we would talk about stem cells in terms of the biology of aging as opposed to hepatocytes or neurons or any other cell that might be interesting, the cells of the immune system. And, clearly, there's a relationship that conceptually in terms of stem cells being important for tissue homeostasis, and the same way there are a lot of these processes that are important for cellular homeostasis. So, clearly, you have to have important control of proteins to maintain a cell. We have to have important control of the tissue components of a tissue to maintain that tissue. So there's really this homeostatic component.

There's also, obviously, kind of a therapeutic aspect of stem cells playing a role in aging thinking about using stem cells to treat aging and age-related diseases. But in terms of the kind of biology of aging, the stem cells I think do have kind of a unique role in thinking about how cells in the body age or they don't age. And in one sense, the kind of cells we'll talk about, somatic stem cells, are in a way intermediate between the kind of immortal germ line cells and the mortal soma cells. So there are these aspects of what are unique about somatic stem cells that give them the ability throughout the life of organism to maintain that tissue. So I think there's this interesting intermediate between the immortality of the germ line and the mortality of the soma.

And then there are also, I think, probably important unique aspects of stem cells that play a role in thinking about the processes of aging. And we'll mention many of them during this summit. I want to mention two in particular. And one is the idea that stem cells can undergo this asymmetric cell division typically giving rise to daughters with two fates. Often one of those daughters is a self-renewing stem cell and the other daughter goes on to become a more differentiated cell.

Now in that process of asymmetric cell division, there's a possibility, at least, of asymmetrically segregating aspects of the biology of aging, whether it's defective proteins or damaged DNA. So I think there's something important there in terms of aging with regard to asymmetric cell division.

And the other aspect that I think is very important is the idea that many stem cells can reside in the quiescent state. And I just wanted to show this is an illustration of something interesting about stem cells. And we've talked about different aspects of aging in this summit in two ways. We talked about chronologic aging in terms of cells that exist, say neurons or cardiomyocytes, throughout the life of an organism and, therefore, experience the challenges of prolonged existence without replenishment.

We've talked a lot about cell senescence or replicative of aging that occurs as cells undergo multiple cell divisions and many stem cells actually exist in both states. So they will stay in a quiescent state for a long time and then divide so they experience both chronological aging and replicative aging.

Okay so, clearly, there are a lot of different types of stem cells, and I actually thank Ron Kohanski for his stimulating the idea for this slide, is thinking about stem cells from different tissues really having different roles. So there are stem cells in the tissues that turnover continuously like skin, intestine, blood, where the cells are always replenishing the more differentiated cells. And then there are cells in tissues which really are there much more for repair so the cells are predominantly quiescent but they have the ability to

activate, proliferate, differentiate in response to stimuli and so forth. So there are really different types of stem cells, so it's hard to talk about this almost as a single cell type because these really have different biology.

So I'm going to pose at least one theoretical question that will come up, I think, in different ways in the talks. And this idea is kind of a dichotomy of how stem cells play a role in aging and in the sense do stem cells cause aging or do stem cells prevent aging? And I think one can really think of these, and in either way it's important for us to think which of these the data supports. And I'm going to start with this idea that stem cells cause aging, and this is from a review from Ned Sharpless and Ron DePinho from a few years ago, in which they actually titled the review something like How Stem Cells Age and Why This Makes Us Grow Old, or something like that.

So really the idea that stem cells are conveying the aging information to the rest of the body. And the way they illustrated that is you have these young stem cells which give rise to a healthy array of differentiated cells. And then with age, these stem cells change. And when they differentiate, they give rise to a different array of cells that aren't quite normal. They're not the same as young.

So in this sense, it's really the problem is that the stem cells are aging. But is this really true? And I think we have to kind of view this question and ask if this is really supported by the data. And I'll just present a couple of ideas of how we might think of this.

So this is work from Derrick Rossi when he was a postdoc and these are hematopoietic stem cells from young or old individuals. And what he's looking at here is evidence of DNA damage. So in the young cells, there's very little evidence of DNA damage shown by these gamma H2X foci staining whereas in the old cells, there's a lot of evidence of damage.

So this would suggest, consistent with this model, that basically the stem cells themselves are experiencing some kind of chronological and replicative aging and are carrying information in terms of DNA damage, which is different than young cells.

But I'll just point to this as that this may not be the simplest answer because what many groups have shown is that even though if you see these foci damage — and here is actually a skeletal muscle fiber with four cells shown here showing DAPI, and these are stained for gamma H2X. Here's one, two, three. But this fourth stem cell which is a progeny of a division of one stem cell now into two, one of these cells exhibits a lot of DNA damage and the other cell exhibits almost none. So something happened here asymmetrically in terms of the cell division in terms of the fate where this damage that was observed in the stem cell actually is ameliorated in the process of this division.

So on the other end of the spectrum is the idea that, okay, maybe stem cells are actually the guardians of youthfulness. That what the stem cells are are these pristine cells which have very little DNA damage, very little protein aggregation. And that what they do is they live in this environment in their niche, the tissue, the systemic influence, external influences and these are the cells that are actually maintaining the health of the tissue by maintaining themselves in this pristine state. And is it possible, in fact, that old stem cells are really perfectly fine and just as good as young stem cells; but when they exist in an aged environment they behave less effectively?

And I'll present just one piece of data kind of in support of this and this is work from my cochair Tony Wyss-Coray's lab. And this is using the technique of heterochronic parabiosis to think about how the aging influence, the external influence influences the behavior of stem cells. And here we're looking at markers of neurogenesis shown here in the black label and it's known from a young mouse to an old mouse that neurogenesis declines precipitously. So a marked decrease in neurogenesis with age.

But when mice that are old are exposed to the young environment of a young animal through heterochronic parabiosis, there's kind of a rejuvenation of the aged stem cells so they behave like young

stem cells. So maybe the aged stem cells are perfectly fine and when given the right environment, they now behave perfectly fine, just as well as young stem cells.

So that's sort of the other end of the spectrum. And, clearly, it doesn't have to be black and white and there could be an intermediate between stem cells may age but, in fact, the environment in which they live also has a profound influence on how well they function. In that sense, it's really a question of with this kind of parabiotic technique and other heterochronic approaches, can old stem cells in a sense be reprogrammed? And this is Dolly, the sheep, but simply put up as an idea that the reprogramming we know very well in terms of cellular differentiation going from a differentiated cell to a less differentiated cell, very prominent in the stem cell field. Is this also true of aging? Can we think about what happens when we've heard about some rejuvenation studies and rejuvenation interventions, Stuart mentioned yesterday, things that can really make an old cell look like a young cell? Is it really something akin to a kind of epigenetic rejuvenation? So we'll get to this idea of epigenetics during the talk too.

So I just wanted to end with this one slide and this is from a takeoff on a review I wrote with Howard Chang who's a colleague at Stanford with the idea that if you have this metaphor where the genome carries the information, shown here as the film, that has to be passed through a lens, which is the epigenome, to give you a clear picture or a healthy cell. Now with age, you have damage to DNA, you have disruption of the chromatin, and you end up with a cell that is not as effective, not as efficient, and not as healthy.

But then with these rejuvenation interventions, what are doing? We're probably acting at the level of the epigenome. We're not correcting the DNA mutations, but we're restoring the lens through which this information has passed to obtain cells, and perhaps stem cells, that look particularly effective and work particularly effective in terms of their function.

Okay, so I'll end there. These are the questions. I won't read them. You'll hear them from each of the individual speakers. And these are the speakers who will cover these topics. Just want to point out the first two really kind of speak to this idea of what happens inside the cells mostly and the last three really addresses the issue of what happens outside the cell in the stem cell compartment that can really influence stem cell function?

So with that, I'll turn the mike over to Tony Wyss-Coray who will introduce our first speaker. Thank you.

Tony Wyss-Coray, PhD: So our first speaker is Danica Chen. She's from UC Berkeley. She's interesting in signaling pathways that control stem cell activity, especially with aging and she has worked on how sirtuins might be responsible for some of the caloric restriction effects. And she will address the question how stem cells change intrinsically with age and how this could be exploited for therapeutics.

How do stem cells change intrinsically with age and what are the implications for stem cell-based therapeutics?

Danica Chen, PhD: Okay, thank you, Tom; thank you, Tony.

Tom has already introduced the stem cell theory of aging and I will just go ahead and present some evidence that supports this theory.

How does stem cell function change with age? Now use the blood system as an example. The regenerative capacity of a hematopoietic stem cell or the blood stem cells decreases with age. And, broadly speaking, hematopoietic stem cells can differentiate into the myeloid lineage and the lymphoid lineage. And with age, hematopoietic stem cells tend to differentiate towards the myeloid lineage and away from the lymphoid lineage.

And this is consistent with the physiological changes in the aging hematopoietic system, for example, compromising immunity and elevated inflammation. And this is also consistent with the pathological changes in the aged hematopoietic system. Childhood leukemia tend to be of lymphoid region and aged people tend to develop leukemias of myeloid region. And stem cell functional changes with age in other tissues, such as the brain and muscles, are also consistent with the degeneration and dysfunction in aged tissues.

The stem cell deterioration accelerates aging. A good example is the human progeria disease or premature aging disease. These patients have a very short lifespan, about 10 to 20 years. Mesenchymal stem cells in these patients are compromised and they have a reduced differentiation toward adipocytes and increased differentiation towards osteoblast. And consistently in these patients, the main tissues affected are all from mesenchymal region. For example, they have an increased turnover in the bone and a loss of subcutaneous fat.

Can stem cell manipulation lead to lifespan extension? There's some emerging evidence suggest that stem cell manipulation can extend lifespan. For example, in flies, manipulation of several molecular pathways in intestinal stem cells leads to lifespan extension.

Now what about mammals? I haven't seen any data for physiological aging; but in a progeria mouse model, injection of muscle stem cells almost doubled the lifespan.

We have learned a great deal about organismal aging and stem cell aging at the molecular level. If stem cell deterioration is, indeed, a major contributor to organismal aging, then stem cell aging and organismal aging should share common molecular regulators.

Recent data is also consistent with the idea that longevity pathways regulate stem cell function. And I listed here the three common conservative longevity pathways and they have all been tested. It's important to point out that FOXO, a major component in the insulin IGF pathway and SIRT3, a mitochondrial sirtuin, have been shown to be highly enriched in the hematopoietic stem and progenitor cells and preferentially regulate stem progenitor cells but not the differentiated progeny. So the enrichment of longevity factors, specifically in the stem cell compartment, provides further support to the stem cell theory of aging.

Next I will discuss how do stem cells age? As Tom just mentioned, stem cells mostly reside in the quiescent state which is metabolically inactive. And we thought that this is to reduce the production of metabolic byproducts, reactive oxygen species or ROS. However, ROS levels still increase with age in stem cells.

There are a number of animal models with defective management of oxidative stress and they all have compromised stem cell functions. Conversely, antioxidant increases the regenerative capacity of stem cells. This is likely to be a conservative mechanism. It has been tested in different species and in mammals has been tested in different tissues.

Another contributing factor is a telomere attrition. Most human cells do not express telomerase, the enzyme that replenish shortened telomeres. However, stem cells express high levels of a telomerase. Despite the presence of a telomerase, telomeres do shorten with age in stem cells. Telomerase deficient mice have defects in stem cell function but only after three generations. And this is likely because mice have extended telomeres.

In humans, telomere attrition is also consistent with compromised stem cell function. Dyskeratosis congenital disease in many ways resembles premature aging. And patients have a short in the telomeres and they have impaired regeneration of proliferative tissue such as bone marrow and skin.

So, finally, can stem cell aging be targeted to combat aging-associated degenerative diseases? As I just discussed that stem cell aging is in a way viewed as accumulation of cell damage to various cell activity

throughout the life. In response to cell damage, tumor suppressors are supposed to be activated resulting in apoptosis, senescence, or cell cycle arrest.

So, indeed, stem cell aging is regulated by tumor suppressors. Suppressing tumor suppressor P16 rescues stem cell aging. So in a way, stem cell aging is viewed as an evolved mechanism to oppose cancer, and I think Anne also brought up this point yesterday.

So idea is that targeting tumor suppressor to prevent stem cell aging is possible to increase tumorigenesis. So idea of stem cell-based therapies should aim to reduce the accumulation of cell damage in order to prevent both stem cell aging and cancer. However, if the accumulation of cellular damage is intrinsic to cell activity throughout the life, so the question really is, is it possible to reduce cellular damage?

For example, the production, the accumulation of ROS in aged cells is felt to be a random passive process, so free radicals are produced due to respiration. So it's a byproduct of respiration. And this causes mitochondrial DNA mutation and a defective mitochondria electron transport chain. And this further increases the production of ROS.

However, a recent study suggests that this is rather a regulated process with SIRT3 playing a role. So SIRT3 regulates a stress response in hematopoietic stem cells to reduce oxidative stress. And this protective mechanism is suppressed in aged hematopoietic stem cells and this may contribute to the build up of cell loss in aged stem cells. And, interestingly, reintroduction of SIRT3 to aged hematopoietic stem cells improves the functional capacity of aged hematopoietic stem cells. So in a way we can view it as a rejuvenation of aged stem cells.

So I think it's important to understand which types of cell damage is a regulated process and which types of cell damage have the effects of stem cell function that is reversible. And this may provide the basis to rejuvenate aged stem cells in order to combat aging associated-degenerative diseases. Thank you.

Dr. Wyss-Coray: Our next speaker is Hartmut Geiger. Hartmut is from the Cincinnati Children's Hospital and from the University of Ulm in Germany. He's interested in hematopoietic stem cells and signaling pathways that control fate and particularly in how changes in the local environment in the so-called niche affect the function of these stem cells with age.

How does aging alter the local stem cell niche and does this contribute to chronic disease progression?

Hartmut Geiger, PhD: Thank you Ron, and Tom for the invitation to present our data. As was said, my lab is interested in stem cell biology, stem cell aging, stem cell rejuvenation and other niche influences on aging.

This is just my introductory slide and I always like to show that because it was on *National Geographic* this month. And it should just remind us that we're talking about a global problem in terms of aging. I think initially it was shown but I'd like to emphasize this one more time. That's not just restricted to Western countries or something like that.

So you have heard a lot about stem cells. And, as Tom said, there is a di— I cannot even pronounce it, okay, diurnal thing, that can be good or bad whether stem cell ages and whether they protect us from becoming aged. Stem cells are frequently as single units which is good in terms of cell biology, but like human beings they are not alone. So they like to each other. They talk to themselves and other people. So they have other cells and other systems around that they influence them. And we call this niches, as Tom said, or stroma cells or systemic factors. So we should never forget that. It's important to remember.

There's always a crosstalk and for the rest of the talk, this means niche can be also for larger systems that look like cells. For some of the _____ it also refers to cell part of the niche. And they interact with the stem cells and this regulates their behavior. They also interact with other stem cells and they also interact with other nonstem cells that might be from the same tissue and origin.

So why is stem cell aging interesting? And as Thomas has showed in his slide, there is the assumption that highly proliferative tissues which depend on stem cell activity all of the time like blood, intestine — so I would even put muscle there and skin and the germ lines — that stem cell aging because they're on top of this hierarchy of tissue maintenance, if they age, the tissue ages. And if there's high turnover and I think in testing it's ten days. In the blood system, we talk two or three months. But they depend on that. So it's very important to understand that stem cell age and what actually contributes to myofunctioning of stem cells or is it anything like manufacturing of stem cells?

We work primarily with the hematopoietic system which is as organized in hierarchy so the stem cells can sort of renew as all stem cells give rise to progenitor cells and then make all of the different type of blood cells. And this happens in the bone marrow usually. And there is a good slide from Mei Ling Lee long time ago, but still very nice to show that there's certain niche cells in the bone marrow — in either endothelial cells like vessel structures or bone lining cells. And the assumption is that these stroma cells interact with hematopoietic stem cells and determine, at least in part, the outcome of cell renewal and differentiation. Again, stem cells are not doing that in the middle of nowhere. They always have their friends around which stroma cells are systemic factors. In vivo you cannot take them away.

So the question is we have heard about stem cells age. That happens on the hematopoietic system and there's strong evidence there's a lot of stem cell intrinsic component in aging metabolic system where the stroma cells do actually change with time too. This is from our laboratory. Other laboratories have similar data. That's actually in and published.

We just made a very simple experiment. We took these niche cells out of a young and aged mouse and checked which kind of cytokines do they secrete because I think cytokines affect desmoid influence. And what you can see a lot of these cytokines go up upon aging. _____ has been also published by Peggy Goodell's laboratory and she could actually show that this is most likely also involved in this myeloid lymphoid shift and there might be causative change by adventis. We have not checked all of the other ones. This is just to emphasize, yes, a very simple assay and yes there are changes. The interesting part is most of them go up.

Let's also talk about MIP inhibitors, so it might be interesting here. We don't know the function on the causative relationship, but I guess there's changes.

So the other question was diseases. So, as you all know, leukemias are age associated. This is for MDS. It's probably myeloid dysplastic syndrome these incident rates and this is probably one of the most aging associated leukemia kind of things that has been found.

So the question is, is there anything coming from stem cell niches? Also forgot to mention MDS is thought to be a disease that's initiated by stem cells, malignant stem cells in the bone marrow. And the question was we asked just to make sure— One short example is whether an aged microenvironment or niche might actually support that. So far the thinking is that stem cell ages and that will lead to mutations, epimutations and that will drive these leukemia type diseases.

And we actually made a very simple experiment. I think people should do more of these. Not because just I like them, but because somebody talked yesterday about it. You should test in aged mice what's going on because a lot of these leukemia or other cancer models are done in young mice. The hematopoietic system has the advantage. We can do transplantation systems which allow us to change niches in all of that. This would be also something other stem cell systems need to develop a little bit more to be able to dissect aging of stem cells from aging from niches. But what we can do is we can transplant preleukemic cells. And I'm sorry I needed to take the data out, but I'll explain it to you.

Transplant at preleukemic cells that we can generate by retroviral transduction with an oncogene. In that case it's AML-ETO. That's one that a lot of people use in the field and this gives you a certain preleukemia in a young mouse. And we simply ask if you take the same cells, young cells in that case, and just put them in an aged mouse as a recipient, what do you see in terms of progression of this preleukemia in an aged mouse versus a young mouse?

And the data was pretty clear that you could see is that in this aged mouse you have a fast expansion of the leukemic clone. It doesn't convert it to disease yet, but you can clearly measure a fast expansion which means this aged niche or system is less suppressive I guess or more promoting this disease clone.

So what can happen is that aging will probably not change the disease itself, but the progression and allows further expansion of these clones that they hit earlier or faster this paradigm of clinical diagnosis which is based on blast numbers, leukemic clones, and peripheral blood.

So similar example has been done by Chuck ____ et al. where they looked at aging of skeletal muscle and niches and which is usually in the adult skeletal muscle you see the stem cells are quiescent, coming back to Tom's paradigm, and they are highly reactivated. And if they're activated, they're different shape. This is done by high FGF2 and there's a notch inhibitor that allows not to differentiate them too much to become activated or differentiating too much.

In the aged one, nicely shown and it's causative that FGF is going further up and starting with more activation. The notch which normally inhibits that is not doing that anymore and what you can see is activation and differentiation but it's not a futile— We've got differentiation type of thing because then the muscle fibers become more fibrotic.

So that would summarize and there's other data from other systems, again, that systemic factors — I think we will hear about that more in niches — do change with age. They secrete different factors and that can influence how stem cells perform.

So that's already the summary slide. Three points to make. That's what I wanted to say. So if you want to understand aging of stem cells better, we should not forget about niches and systemic factors. Stem cells are not alone. They're always exposed to these factors. And if they age, they will influence partially aging of stem cells.

The interesting part is that you can argue either they have a loss of suppressive activity of the stem cell or they have a loss of activation activity. Most data we have so far, and I find it interesting, summarizing that actually that age niches lose suppression activity. So that would mean stem cells, in general, would like to go forward, do something either differentiate or replicate, and that niches do suppress that. And if it's lost upon aging and you have hyperactivity and certain diseases associated with that, more autoimmunity, cancer, and enhanced stem cell differentiation because it looks like they _____ correctly.

For sure, this might be also loss of acute activation activity which then would lead to hyperactivity and chronic wasting of certain tissues. So that will be important for the differentiation because therapeutic strategies would be very, very different in one or the other setting. And with that I would like to conclude and thank you for your attention.

Dr. Wyss-Coray: Thank you, Hartmut. Our next speaker is Emmanuelle Passegué from the University of California San Francisco. Her lab is interested in how cell intrinsic damage in stem cells can affect stem cell survival and possibly lead to dysfunction and tumorigenesis. And she will ask the question specifically how DNA damage can lead to cell dysfunction.

How does DNA damage affect aging and function of stem cells?

Emmanuelle Passegué, PhD: Thanks Tony, Thanks Tom for the invitation for this fantastic summit. So, as the previous speaker, I'm a stem cell biologist, and I want to look at one of these most old and

convincing principle of aging DNA damage from the lens of stem cell biology and the effect on the specific cell populations, so stem cell, which maintain tissue homeostasis.

So stem cells are like every other cell in the body exposed to a whole range of damaging insult which are either coming from the environment cell intrinsic insult or coming from the cells themselves, cell intrinsic insult. And as we have learned over the previous sessions, these are cumulative damage which affect the cell which are maintained over time.

And to ensure to this kind of damage, as every other cell in the body, stem cell can use the whole panel of DNA repair mechanism to cope with various type of lesions to their DNA. And I'm not going to go over this mechanism. Just to point out that while the majority of them have a fairly high fidelity of repair, one of those mechanisms, nonhomologous end joining, is very well known to be highly error prone, meaning double strand breaks, which are one of the most damaging DNA insult which can act on DNA, is actually can be fixed especially in noncycling cell which are either in quiescent stage or in G1 phase of the cell cycle with a mechanism which just glue back together the broken strand of DNA. And you see deletion or various change including translocation, therefore, being by itself a very mutagenic event.

So why is that important? It's because the consequence of the type of repair, the consequence of the response to the cell to a DNA damage response which is survival death of senescence together with the fidelity of this repair is what will dictate, at the end of the day, the biology of the tissue. If a stem cell encounters DNA damage and survive this DNA damage and repair DNA damage with high fidelity, there is no consequence. The stem cell kill, keep self-renew, and producing ____ difference here to protein ensuring tissue homeostasis.

If the same stem cell survives the DNA damage but then repair it with an error prone mechanism and acquired mutation, this can lead to the production, literally to the production of progeny which carries this mutation and, therefore, can contribute to both cancer development or aging in the context of chronic disease and age-related malignancy.

The other aspect can be as damaging for tissue maintenance and for aging which is when the stem cell encounters DNA damage, it doesn't survive. It dies. And you lose your stem cell population, you lose your differentiated progeny, and you lose maintenance of the tissue leading to organ failure which is as well one of component of aging. So that's why we care about the DNA damage response within the stem cell population.

So my system, like those of the previous speaker, is a blood system. And there is no mystery about that because hematopoietic stem cell, the mother of all blood cells, is the most known stem cell population where we know the marker to identify it, we can take it out, we can transplant it, we can manipulate, we can functionally test the biology of the stem's hematopoietic stem cell population. And it is really been on the forefront of a lot of discovery in the stem cell field.

So what I just want to say about this hematopoietic blood-forming stem cell, which stands both for human and mouse, with a slight difference. But the general principle all true in both species is that they are essential for maintenance of production of all the blood cell, including cell of the _____ stains that we have talked about are very important in the context of aging. They self-renew and differentiate and what's very important is that in an adult organism — I'm not talking about development or establishment of the blood system. I'm talking about maintenance in adult. These stem cells are kept in a quiescent stage of the cell cycle, which is a low metabolic rate, which is basically enforced by the bone marrow niche, as described in the previous talk; but the CAP systems are in a very poised situation ready to fire and ready to differentiate, ready to proliferate and produce progenys.

Little bit like the runner in the starting block. Maintain quiescence based on environment but ready to proliferate and produce blood cell. And the fact that these stem cells are quiescent is highly relevant in the ways that they cope with double strand break and DNA damage. And that's a work we published a few years back from my lab where we show that this quiescent stem cell survives irradiation. And in this case we use, and I forgot to put it in the slide, exposure to ionizing radiation and using double strand

break, low doses because the blood is one of the most radiosensitive tissue in the body. Low doses of radiation, double strand break these cells survive. They are quiescent in the bone marrow niche. They can only use this error prone nonhomologous mediated type of repair to fix the damage, and this is associated with accumulation, acquisition of mutation. And what we show loss of function. And we imagine that upon further accumulation of mutation, malignant transformation.

And it's certainly because the cell maintain quiescence and we force them to proliferate. They still survive. This survival mechanism is high level of prosurvival factor which really buffer induction of the proapoptotic machinery by the P53 pathway. It's the same in this proliferative stem cell, but now they can use homologous recombination to repair the double strand break which is much more futile and prevents this damaging cycle of accumulation of mutation. And that's very specific to the stem cell compartment since the more differentiated progeny they don't do that because upon DNA damage, they are basically eliminated.

So and this principle, this predisposition to mutation upon DNA damage in this quiescent stem cell population might be relevant to a lot of other contexts. Might be a general property of all quiescent stem cell population, which are not every stem cell you have in your body but are a large part of stem cell in the muscle, in the hair follicle, cancer stem cells, which are driving cancer development. It might explain the stem cell origin of a lot of chronic disease or cancer because there are the cell and it's a little bit contraintuitive to the dogma of cancer development, but this quiescent center which do not proliferating, they are mutating upon accusation of DNA damage. And that clearly can contribute to the loss of function with age.

And that's a summary of what has already been shown by the previous speaker. The stem cell, the hematopoietic stem cell is pretty much the only blood cell that age because its process over the lifetime and in an old organism, both human and mice, there is a whole array of problems associated with this aging stem cell. And the most striking feature is that they accumulate with age. In other tissue, stem cell population decrease. In the blood system, all stem cell accumulate but they are highly dysfunctional, as already mentioned by the previous speaker.

So there is clear evidence of DNA damage accumulation in this expanded age population. First in the mouse, all deletion of repair mechanism mostly it's single strand break, not double strand break as mentioned in the previous session. And your stem cell function decline, which is similar to what is observed during physiological aging.

Related as well as to a question which occurred in the last session, there is now very, very sound and very strong argument coming from human sequencing data which shows that perform on relapse acute myeloid leukemia patient over time. We show that in addition of driving mutation which really are in genes the fuel for other disease, there is over time an accumulation of mutation which persists which suggests that this mutation, this low background mutation — and we are talking between 500 to 1,000 mutations accumulating in these patients — happen over time.

And what Tom already showed, accumulation of gamma H2X foci in this old stem cell which reflects DNA damage either coming from increased production of ROS hydrolysis of micromolecule and work done in my lab showing that it's in large part due to replication error.

So how can we prevent that accumulation of DNA damage. In a young cell, basically it's exactly the principle of an antagonistic pleiotropic strategy. The cells survive, produce blood accumulation mutation. It's a side effect of this at all cost survival mechanism.

In an old organism, which I check the survival property of a young stem cell, we see accumulation of a pool of dysfunctional stem cell which have DNA damage and which can repair using this mutagenic mechanism to go on and contribute to chronic disease.

And that leave us for my last slide with maybe very naïve way to try to intervene with this accumulation of DNA damage in all cells by preventing the survival and accumulation of these old stem cell and trying to reduce the occurrence of this error prone repair mechanism. Thank you.

Dr. Wyss-Coray: Thank you Emmanuelle. Our next speaker is Richard Lee from Harvard Medical School. He's studying cardiovascular disease and how targeting stem cell may be useful in regenerating or possibly rejuvenating the heart tissue. And he will ask the question how does repair of the whole organ be achieved in aging?

How do you repair whole organs in an aging system?

Richard T. Lee, MD: Okay, thank you, Tony. So I knew I was coming at the end of a long two-day meeting and I'm going to speak very conceptually with essentially no data at all. It may be a little bit inflammatory, so hopefully you'll stay awake. The question then is what do we want to do? We would like to grow kidneys, limbs, hearts, things for organs that we really have great disease problems for. So I'm going to start by making a comparison that you're going to think is ridiculous, but if you bear with me for a second.

Let's just say that you wanted to go to the moon. And that's just going to sound completely ridiculous to you because we already did that half a century ago. But let's just think for a second that you didn't know that, that you'd done that 50 years ago, 45 years ago. And you go out tonight and you are looking out at the moon and thinking, we can go there. It's a ridiculous concept, right? I mean, it really is almost mind boggling that they did this.

Now how could they do this? It took a lot of determination, it took a lot of teamwork, it took a lot of courage, it took a functioning government. So I'm probably never going to be invited back here again. It actually wasn't the best technology. It wasn't anything near what we have available to us today.

So this is my iPhone. It has 2,000 times the computing capacity of the Apollo 11 spacecraft. That's this iPhone and this isn't even the up-to-date iPhone because my cellular plan won't let me upgrade for like another year. So our technology these days really is not the problem.

How did they actually do this? It's actually fun if you go back and you look at the flight plans for the Apollo 11 spacecraft because they were remarkable. This is just a snapshot here. But they did this by having the humility to understand everything. They really understood the problem. They understood every little detail. If you go back through all of the things on the NASA website, you can see that they knew everything that was going to happen at every second. That's remarkable. And that's actually how they pulled it off with what we would call stone age technology from 45 years ago.

So the comparison that I would like to make is that if we want to do some of these dramatic things with regenerative medicine, we have to approach it the same way. We can't actually go into these things not knowing what the heck is going to go on. And this is a slide I use a lot. It's actually from Tom Rando's wonderful *Nature* review back in 2006 but I think it's still very much up to date. And Tom made the point that there are many cells that we understand quite well that have a lot of cellular regenerative potential. And these are places where we have things in clinical use for regeneration.

On the other hand, a lot of the tissues that we're interested in, like the heart or the retina or the brain or the lung, are areas where they're controversial but it seems to be there's relatively low regenerative potential and we actually don't understand those tissues very well.

So just in my tissue, the heart, there have been many thousands of patients treated with cell therapy based on the concept that there's stem cells in the heart. But many studies are now suggesting that those concepts were completely erroneous. So we're thousands of patients into human trials without understanding the fundaments. Can you just imagine if the Nassau guys looked at that and said like, "Wow, you guys didn't even think about how far away the moon was."

So I believe that we really need to understand the fundamentals. That the basic investigation with what's going on in these tissues is crucial to the translation. So this is something that you all know. This is US life expectancy over the past century and you see that it's gone up almost a doubling. And the concept is something that we all know which is that for most of us who are playing on the back line of life now, we are dealing with an environment that is new, that is biologically new.

Just to make a point here. This big dip here around 1917 this was not World War I. If this were a graph of France, then that big dip would be World War I. In the United States this was Spanish flu. So that's just a reminder to get your flu shot this year. And you might have noticed that there's some data that came out just a few days ago that, for reasons that are not clear, if you get a flu shot, it decreases your risk of a heart attack and a stroke. So if it wasn't enough to incite you to prevent from getting sick as all get out with influenza, that it may actually seem to prevent a stroke.

But the concept I want to point out here is that the aging environment is a new problem and it's something that we really don't understand. You heard over and over today that there are many studies that are performed in metabolism, regenerative medicine; and they're mostly done on ten-week old mice. The number of studies where we look at these problems in mice that are two years old, which is reflecting the sort of environment that we really are dealing with most of the time, they're actually relatively small except for the lab specifically studying aging.

So what should we do? What are the kinds of things that I believe we need to do? So you all know that you can take cells out. We can make iPS cells, we can really fashion regenerative organs, we can do 3D printing, we have scaffolds, we have lots of matrix tools, we have ways of doing regeneration in the laboratory. And I think that the capacity to grow limbs is going to actually come about. In Boston, that's a big topic this year.

But what we need to do is to think about these things not in terms of just doing them in ten-week old mice, we need to think about the context of the problem. We need to think about them in the context of the fundamentals. And that's diseases like diabetes where the milieu is totally different and a lot of regenerative medicine, for example, in limbs and in the heart they have to be considered in the context of the diabetic patient because that's what we're facing. And the aging environment these studies are hard because they're expensive, but all of those clinical trials that I was telling you about in the heart, they were based largely on 10-, 12-week-old mice and some young rats. Very, very rarely are they based upon animal models that are reflective of the type of patients that we're actually trying to take care of.

So, ultimately, what we need to do is recognize that although we want to take regenerative medicine forward as fast as we can, if we do that but we don't understand the simplest fundamentals, like how cells are actually being replaced, what's maintaining homeostasis, what does the system actually look like, then we're probably going to make a lot of mistakes.

So thank you very much.

Dr. Wyss-Coray: Thank you, Richard. Our last speaker is Irina Conboy. She's from the University of California in Berkeley. Her lab studies tissue aging and how stem cell activity may help regenerate tissues. And she's going to ask whether this will be a useful approach for the treatment of chronic diseases.

Is stem cell rejuvenation a likely candidate for therapeutics against chronic disease?

Irina Conboy, PhD: Okay, so I'll skip actually this one and go to the next one. So aging is equivalent with the disease. There is no such thing as aging or disease. Aging is basically just a generic term, one term for all of these and many more horrible diseases. And there is no such thing, by the way, as healthy aging. Healthy aging simply means that the person does not succumb to all of these horrible diseases but only to some of them. So, yeah, sorry to be so pessimistic.

So age-related diseases are mostly degenerative and these slides were given to me by Judy Campisi because it was very difficult for me to make a ten-minute talk and Judy and I think a lot in the same way. So Judy usually excludes cancer from the list of degenerative diseases and makes a very nice, elegant connection to senescence; but from my stem cell scientist lens, I perceive that cancer, actually, development of cancer is fueled by immune dysfunctions which Irv Weissman is now working on very successfully at Stanford. And so cancer also could be perceived as the degenerative disease of the immune system.

So age-related diseases arise exponentially with age and this is the breaking point. And so why is that? Well, the logical surface explanation or intuitive explanation is that because damage accumulates with age. But then two-year–old human you'll be somewhere here and two-year-old mouse you'll be somewhere there. So why is that? Why is there more DNA damage in two-year-old mouse as compared to two-year-old human and why two-year-old mouse has cataracts and many other horrible things that two-year-old human does not?

Well some people say because mouse is small and human is big and also mice are very metabolically active. But then three-year-old rat will be here. You'll have many age-related diseases. You'll be aged. And three-year-old squirrel, which is basically the same rat with bushy tail, you'll be here. And squirrels actually live 40 years.

So accumulation of damage to anything — micromolecules or tissues or anything — cannot really explain the aging. What can explain the aging is the abandonment of tissue maintenance and repair. So there must be basic processes that drive aging and those tease out in most or all of age-related diseases. And in my lab, we have identified some of these key biochemical processes that cause abandonment of organ maintenance and repair or collectively aging.

So some people ask me, "So if there is common cause to aging of multiple tissues, why then some people when they grow old they are bald but have excellent eye sight, but other people cannot see anything but have beautiful head of hair?" So just to give you an example if now, today, all of us abandon maintenance and repair of our automobiles, it does not mean that transmission will fail on all of them on the same day. But what it means is that sooner or later, some of the key parts will fail and the car will die.

Treating basic aging process can prevent many or all age-related diseases, all of them. And what I cannot understand is that why there is not more investment from society and finding into aging because we can then eliminate diseases and their cause. And it will be zillion dollars less costly than trying to cure diseases as they develop. Some of them are incurable, we don't know what to do with them; and with the changing demographics, it will be really impractical to cure all of them.

So what I was thinking is that why people do not really invest in proverbially speaking parachute when they know they are going to jump off the plane sooner or later is that they simply do not believe that there could be one fundamental cause of aging of multiple tissues and one fundamental cure.

So I'll give you an example. If just very recently, maybe 150 years ago, you told somebody that completely differing diseases, such as, for example, tuberculosis and meningitis could be cured by fundamentally similar process very quickly, people would not have believed you. They would say, "No, those are different diseases. For tuberculosis, we send patients, if they can afford it, to high altitude resort and for meningitis, well, we cannot do anything. We can just hope that our diagnosis is wrong."

But then when we discovered that those diseases are caused by bacteria and bacteria and eukaryotes have fundamentally different translational machinery and developed antibiotics, now this whole class of diseases could be eliminated. So what I firmly believe is that the same fundamental underlying process for tissue aging exists and we are actually very close to understanding it and using this understanding for shift in our paradigm such as that all of these diseases could be eliminated before their development or etiology.

So too, in my last slide, I'm going to give you some illustration of that. So in a renal congruent group, we tried to globally reverse tissue aging by defined molecules. And what you can see here is the skeletal muscle that has been injured experimentally. In a young mouse or human, it will be regenerated in five days very rapidly by healthy tissue; but in the old mouse or human, it will be replaced by scarring and fibrosis which causes, of course, lack of function and also promotes other diseases of aging such as inflammation, development of senescent cells, and so forth. However, old muscle of the same chronological age of a mouse can be rejuvenated very rapidly to resemble the young one.

How do we know how to rejuvenate muscle and other tissues? The understanding of that came from experiments such as heterochronic parabiosis or heterochronic tissue transplant which told us two main things. One is that old stem cells do not really age intrinsically very much. And when they find themselves in a young environment, they are completely competent and can regenerate young, healthy tissues. And the second lesson was that the behavior of stem cells is regulated by biochemical pathways and resetting key biochemical pathways to the young signal or strength is sufficient to make old muscle to be regenerated as efficiently as young by the resident of stem cells.

What I would like to emphasize is that heterochronic parabiosis when blood circulation of animals are shared is not the same as connecting dirty bag of water with clean bag of water. Old parabiont benefits from young set of lungs, young heart, young immune system, and many other organ systems which collectively rejuvenate stem cell behavior and it is completely and categorically incorrect to assume that there will be one molecule in circulation which makes us to grow old or one molecule which we are lacking and can make us grow young. But the important point is that you can rejuvenate organ stem cells within weeks and make young, healthy tissues. And that it's specific biochemical signaling which needs to be changed to achieve this effect.

So that brings me to this part which is beyond parabiosis. If you know that all you have to do is calibrate key biochemical pathways to the youthful signaling strengths, why not do just that and not torture poor mice by connecting them to each other? And, actually, by the way, this illustrates that in 1957 they connected one poor old rat to six young animals and aging prevailed long term and there was no extension in lifespan. So parabiosis just tells us something. It is not really translational approach.

So in the paper, which is now in review in *New England Journal* with my collaborator from Berkley, David Schaffer, we introduced one small molecule into circulation of old mouse. And in about couple of weeks, simultaneously enhanced myogenesis and supercampal neurogenesis by youthful calibration of one on the same signal transduction pathway. So this mouse will not have sarcopenia and will not have lack of cognition due to old age.

And this is the very last part which tells that, well, there are many diseases which are not associated with aging, for example, juvenile diabetes. But, very interestingly, tissue aging becomes prematurely obvious in juvenile diabetes. So what you can see here, decline, rapid decline in muscle repair of young mice with ablated beta cells. And our ability to rejuvenate muscle repair in this Type 1 diabetes model not only by insulin but also by inhibitors of the _____ signaling pathway.

So, once again, aging and disease are coupled and normally during physiological aging, the perceived aging is development of many disease. And when disease occurs in a young animal or young human, tissue regeneration becomes aged prematurely. Thank you so much.

Wrap-up and Discussion

Dr. Wyss-Coray: Thank you, Irina. And maybe can I ask all the speakers to come to the podium and also the chair, and I'll make a few comments.

I think as Tom alluded already in his introductory remarks, the aging of stem cells can really be seen as an integrative hallmark of aging. As we've seen in all the previous sessions, the fundamentals of the biology of aging, apply very much to the aging of stem cells.

And to just summarize a few of the main points that we wanted to make in this session, stem cells come in different flavors. There are stem cells with a high turnover that produce cells continuously such as in the blood, there are stem cells that give function in tissue repair and then there's stem cells with very specialized function such as those, for example, in the adult brain that give rise to neurons involved in memory processes.

We also learned that stem cell activity declines dramatically with age in almost all tissues that as far as we know. We know that the stem cell activity is governed both by cell intrinsic as well as extrinsic effects. And the intrinsic effects can both lead to dysfunction of stem cells or tumor formation, but they can also promote aging of tissues. And on the upswing, beneficial effects, for example in the environment but also within the cell, can help in repairing tissues and possibly rejuvenating them. And I think this is one of the exciting aspects of this field.

And so why don't we take questions now.

Benjamin Best: Ben Best, Life Extension Foundation. I'd like to ask if there's any tissue for which stem cell function declines because of declining telomerase activity?

Dr. Conboy: Okay, so I can just tell you for muscle that stem cell function does not decline because of declining telomerase activity and that we published a paper a couple of years back saying that, at least in the mouse, telomerase activity in muscle stem cells remain high, does not significantly decline, but stem cells fail in tissue regeneration. So they age without decline of telomerase activity.

Dr. Passague: So and I can add to that. Again, with data on the mouse system where passage, transplantation of blood forming stem cell which have high telomerase activity and long telomere when you passage them for more than five times and force them to undergo a lot of replication stress, you see erosion of telomere and a crush of stem cell activity. So it does happen but it does happen in the extended context compared to physiological aging and the function of the stem cell in an old organism.

Dr. Rando(?): Actually, I would just add one thing which is you can obviously deplete stem cells by inhibiting telo—, or knocking out telomerase. You can make stem cells worse. But the question you're asking really is if you were to be able to maintain telomeres say in humans, would there be a stem cell population that would be—? We, obviously, can't do that so we don't know the answer to that, but at least the evidence so far, and certainly evidence from mice where the telomeres are long, is that there isn't a depletion of stem cells because of a shortening of telomeres.

Speaker: (INAUDIBLE)

Dr. Geiger: So there have been experiments published when you re-establish telomerase you do not really make the stem cells live longer in the mouse system. This was Irv Weissman's lab long time ago. So it's highly unlikely that telomerase in initial rounds is playing a major role in aging of stem cells.

Dr. Franceschi: Okay, Claudio Franceschi. I understand that you are all interested in stem cell biology, but I am also interested in the other part of the body. So the question for you is the systemic milieu can impinge upon the aging phenotype of somatic cells. So in other words, an aged environment can have not only effect on young stem cells but also on other somatic cells so that these could be in favor of what I presented the first day, this crazy idea that aging is propagating among the cells and the tissues of the body.

Dr. Wyss-Coray: Absolutely, and maybe I can take this from the point of the brain. We know that cells in the brain, immune cells, microglia, are affected by systemic changes in the environment and the activation of these microglial cells then has negative effects on the stem cells in the brain.

Dr. Geiger: So, in general, I would say for most of the systems I think there's always a balance between the stem cell and the nonstem cell part in aging. I think that's what I wanted to say. And it's not a black

and white. Is that what you were asking? So to have somatic differentiated cells that age and for sure they influence stem cell aging but also vice versa.

Dr. Martin: George Martin, University of Washington. Irina, you emphasized a single agent made a difference both with neural stem cell regeneration and skeletal muscle regeneration. So you didn't tell us what it was, but I was thinking generally this tremendous selective pressure I would think even during reproductive phase to have a back-up system would be a really gamble to just put your money on the single agent. So how do you respond to that?

Dr. Conboy: So I cannot tell you what it is because it's a collaborative work, otherwise I definitely would and it is just now in review. But if you just follow my publication record, you can easily guess because we focus on several canonical biochemical pathways which change with age to inhibit tissue repair and we try to calibrate them to young levels.

Now I don't think it will be one single agent, right, which they are using as the small molecule which calibrates signaling strength precisely to young levels or close to young levels, but I don't think that we need hundreds of molecules to reverse or delay tissue aging. I think we need somewhere between three and ten.

Dr. Martin: Yeah, I wish, actually, if you want to help my high frequency hearing loss maybe with your small molecule because I had trouble understanding you. Maybe Tom could—

Dr. Conboy: Sorry, yes, and my accent too. I apologize for that.

Tom, did you want to expand on that?

Dr. Wyss-Coray: No, maybe I can just mention also I think the point is that if you have factors, you may not need multiple factors. If you have one factor, that may be sufficient to help regenerate a tissue. That doesn't exclude that there are many other ones that can do the same thing. But given at a sufficient concentration, one factor may have a significant impact.

Dr. Geiger: So maybe I might comment on that too. So we have recently published that you can actually rejuvenate stem cells, hematopoietic stem cells with one factor. I think it, obviously, depends on what kind of problem you want to fix or whether the underlying problem is more complex or you have a relative simple mechanism. It looks like sometimes mechanisms can be relatively simple and one factor could be a very interesting solution to that problem.

It goes back to what Richard said. We really need to understand, I think, in detail, what we are targeting and what aging is. And maybe it's sometimes simpler than we think, especially in the stem cell world. I think that's the take-home message. Maybe it's really not that complicated because I would say it's more a developmental part and it's probably, as somebody else said, stem cells seem to be very protective of a couple of things that would lead to aging in differentiated cells. There was talk about DNA damage is probably a little bit differently handled. As well someone else said probably the proteome stuff is a little bit differently handled so it might come more to simple decisions in stem cells. Could be.

Dr. Wyss-Coray: Over here.

Patrizia D'Alessio: Patrizia D'Alessio, University of Paris. The question is to Geiger, Hartmut Geiger. He mentioned the chronic wasting of some tissue following hypo or hyperactivity and it reminded me couple of papers of Goodell. And she was claiming that inflammatory stress was able to degenerate the niche components and she identified with other authors in several other articles that followed between 2007 and 2009 that inflammatory cytokines could be stocked into adipose tissue or muscle and contribute to their functional progressive inactivity, which I think is a subject which has been an issue, has been addressed by previous sessions but just reminded me that this could be a mechanism. So what do you think?

Dr. Geiger: So you're saying some of the cytokines that we showed being up or Peggy had shown that are inflammatory? Is that the question?

Patrizia D'Alessio: For adipose tissue this is demonstrated.

Dr. Geiger: Yes.

Patrizia D'Alessio: I mean, doesn't need to be demonstrated, but maybe muscle.

Dr. Geiger: Yeah, yeah. No. Muscle would be a question on this side. So I would answer like that. So there are probably parameters of inflammation that might be seen in bone marrow with aging, but you can also interpret a little bit differently with the set of cytokines we have. I'm always struggling in my laboratory too with that. Is this a pure inflammatory response or is this just response to the inflammatory response that you see with the cytokines? There's a small difference. Sounds simple, but there's a small difference. Like with DNA damage, do you see DNA damage or do you see the response to DNA damage and this mimics as like something else. I don't know that currently, there might be something. We are currently investigating that. I'm looking forward to the answer. I don't know it yet, but maybe something from you for muscle.

Dr. Rando: I guess I'm not quite sure I understand the question, but I would say this that if we look at old stem cells from muscle and compare them to young stem cells, there's a prominent signature of inflammation in cells. So the cells are clearly responding to some environmental influence that is whatever we've talked about here in terms of inflammation. Is that what you're asking?

Patrizia D'Alessio: No, it was not exactly what I was asking. But, okay, the idea was to say that inflammatory cytokines may contribute to stem cell aging basically.

Dr. Rando: So I think that's what I'm saying, that the signature in the cells suggests that there is an inflammatory component to the transcriptome of aged stem cells. Now whether that's— And, again, if you add cytokines, you can show inhibition of function. Whether what components of this cytokine profile are actually contributing *in vivo* to the stem cell aging, would have to be one by one blocking them and seeing what effects you get in terms of beneficial rejuvenation of the stem cell function.

Dr. Conboy: And I would also like to add one point which is antagonist pleiotropy, that there is no such cytokine or molecule which makes us grow old. All of them are very, very important and allow us to survive and it's only deregulation in their levels which then contribute to tissue aging. So you cannot turn something off and become younger. You cannot add something and become younger. You need to establish the levels. So inflammation and muscle regeneration overlap in space and time and inflammatory response is very important for tissue remodeling. But when becomes excessive, then the situation is bad.

Dr. Wyss-Coray: Okay, the next question over there.

Zorina Galis, PhD: I'm Zorina Galis from the Heart, Lung, and Blood Institute. I want to congratulate the organizers and the speakers for the spirit of the conference. It was very engaging and interesting, thought provoking. So in that vein, I'm going to ask my first question. I always wanted to ask, it and because today is Halloween, I want to ask whether parabiosis is basically just the scientific basis for Dracula's old trick?

Dr. Wyss-Coray: Yes.

Dr. Galis: And so I was wondering whether blood transplantation can really bring some of the benefits? But I do have another question and it's probably so simple that may be almost philosophical and it's triggered by Rich's technology comparison. And so I think, as most people in the room, I have a personal interest in delaying aging and age-related diseases and, obviously, a scientific interest. However, nature

designed us as any good oiled machine, at some point we do have to quit. So the question is whether we should try to figure out what would be the most efficient shutdown procedure, speaking also about shutdown, and not just an abrupt crash, like we experienced recently.

But so what do you think it's potentially an organized shutdown procedure. I know Irina said that there is no healthy aging, but I think there is. I hope there is. I do want to shut down at some point. I don't want to crash. So I came with a few spare parts, the stem cells, and sometimes I will run out of them and what's next?

Dr. Lee: Maybe I can just— In terms of this concept that there are systemic things that can perhaps rejuvenate or systemic things of youth, that actually appeared in the first scientific journal in 1666 in *Philosophical Transactions*, the very first journal.

Dr. Galis: The Count of Dracula.

Dr. Lee: Pardon?

Dr. Galis: Dracula was 1400.

Dr. Lee: Yeah, yeah, but Dracula didn't publish it. So this is since the beginning of the scientific literature this concept that there were hormonal factors that could control youth and aging has appeared. And so I think now we're starting to get to the point where we can really figure out what some of those are, both from proteomics technology and from deep sequencing and molecular fingerprints. And so it is going to be exciting, but it's hard to think about these things and work on them without thinking about healthy aging and how long do we want to live.

Dr. Conboy: Yes, go ahead.

Dr. Rando: I was just going to say, I think Tony could address the question, your first part of your question which was the blood transfusions. So do you want to.

Dr. Wyss-Coray: Yes. So we have experiments that show that we can take plasma from young animals and rejuvenate brain activity and cognition in old animals. And my son is playing Dracula for Halloween.

Dr. Conboy: Can I add something quickly? So I actually want to give plug to Science Foundation and Life Extension Foundation in this regard because they are funding very cool project in our lab on actually replacing this gruesome parabiosis procedure with something which is much better controlled which is blood anastomosis and plasmapheresis so you can see how long parabiotic effect lasts. You can connect and disconnect animals in a precise manner. You can remove and add components to plasma. And so we see it as a next generation experiment to look at multiorgan rejuvenation, but I would like to caution everybody that to think that there is some young components in blood releasing young components, parabiosis is a connection of different organ sets. And in culture when you mix young cells with old blood, old blood prevails and ages them.

Dr. Kim: Stuart Kim from Stanford. Do stem cells age with the same mechanism and do they age with the same rate? Different stem cells.

Dr. Rando: Oh different stem cells. Well, if you look at, for example, markers of DNA damage, you can see these in quiescent stem cells from different compartments. So, again, it would depend a little bit on how you define what an aging stem cell is and how you would then compare them across different stem cell populations. But using the kind of paradigms we've talked about at this meeting, if we look at cellular functions or cellular characteristics, I think it's always hard to talk about rates of aging in different tissues because their manifestations are what we measure as the aging process.

Dr. Kim: Well if you look at your satellite stem cell function compared to the Derrick Rossi myeloid switch, I mean are they aging at the same rate?

Dr. Rando: So let me give a more dramatic example. So hair follicle stem cells and other stem cells will decline much more rapidly. Neural stem cells decline much more rapidly than what we see in the muscle or certainly in what one sees in the intestine or in the blood. So there will be different kinetics depending on what you measure. So I would say from what we have measures for, there are different rates of aging in different stem cell populations for whatever, you know, for different reasons. So that's one thing.

But then you asked about whether they age with the same mechanisms. Again, I think this gets down to the basic topic of the summit of what are the mechanisms of aging and then can we apply that to different stem cell compartments?

Dr. Conboy: So I can tell you that for muscle and brain, stem cells age at least with one fundamentally identical mechanism. And that is our paper under review and I will be happy to discuss with you.

Dr. Passague: So and to add to that I think you're asking a very loaded question in the sense of stem cell biology because it relate as well how you define stem cell. In the blood field, stem cell, blood stem cell are defined functionally by their ability to transplant, regenerate the blood, and regenerate balanced production of myeloid/lymphoid. So this functional definition doesn't hold in a niche context because the production isn't there. So are you still looking at the stem cell or are you just using marker and it's not a stem cell anymore? So that's still a very difficult question in the field which has not been answered.

Dr. Geiger: And I would like to add so because stem cell aging is not the least expensive one, a lot of us tend to look at the beginning at the end of the game. So it is a young mouse and there's a mouse, for example, with the aged stem cell. The kinetics are expensive to look at and that's my lab included. Sometimes we do not do these kinetics too often. I can give a you a mouse example, for example, of stem cell aging in mice and we can compare inbred strains, there's some data available, and even in identical inbred strains little bit kinetics of behavior upon the stem cell aging. Like 6SH is a little bit different from DBA/2s, but even there we don't have precise kinetics on that. so it would be interesting to look more precisely at this is a lot of work because the stem cell functional assays are not easy to run, taking a lot of mice, a lot of aged mice and things like that. But it's definitely a question one should look at in more detail if there's more hands and more dollars to it.

Dr. Chen: Yeah, and I can just add a little bit to that. And I think there is a great deal of interest in the instance of researchers to compare the mechanisms that control different stem cells in different tissues. And at least so far there are mechanisms that have been tested in different tissues and they do share same— Some mechanisms are shared and some mechanisms still needs to be tested. So it has been tested in one tissue and then remains to be tested in others. For example, oxidative stress has been tested in a number of tissues so that's one example that they do share a common mechanism.

Dr. Wyss-Coray: Next question.

Dr. Richardson: Alan Richardson from San Antonio. So with respect to the niche, has anybody looked at — and this goes back to the question a little bit different on the inflammatory cytokines. Has anybody looked at the niche with respect to I don't know whether you can do it in vivo where you, in vitro where you would look at the response of stem cells to an environment where you would have senescent cells or cells producing the senescent secretory phenotype? In other words, would a tissue—? It seems to me from what we've said, which is very interesting, is that the milieu for old tissue would be different than from a young animal. And I like the point where the fact that most of the experimenters in this area are kind of forgetting that working with young mice. And I was wondering from an old animal perspective, would the build up of senescent cells have an effect with respect to the milieu that the stem cells could respond to?

Dr. Conboy: Yeah, that is a great idea which the type of ideas I would expect of the type of person who ask this question. And Judy and I have discussed very cool experiments how to answer this question. I'm pretty sure that, thus far, the connection between senescence and aging of circulatory milieu has not been well answered. But Judy Campisi and our laboratory are going to collaborate and we have perfect experimental system to answer this question.

Dr. Geiger: So experiments have been done in our lab and other labs. And if you ask this question also for normal stem cell aging, it just showed leukemia type of experiments, correct? You can easily see that in vivo and ex vivo that aged stroma cells — and we do primary experiments. We take it out, keep it in literally, but all we do in vivo transplants does influence stem cell behavior. Whether this is the aged secretory phenotype what I've shown here, I don't know. The only part has been shown so far is this _____ test, but there's much more going on. Whether it's aged cell/cell contact, I don't know either. This is the problem we have in our thing. And trans _____ sometimes don't work very well because stem cells like to have sometime cell/cell contact so you cannot really separate it from if everything goes down it doesn't help you. So people are getting at that. It's not that simple because we would like to have them causal relationships. And then modifying the niches for your protein, as Tom said, is not the most fun experiment to do and it's not a quick one because you might have to go the genetic route and this is not simple. But we are slowly moving somewhat towards that and trying to figure out what's going on.

Dr. Passague: And to add to that, as much as stem cell biology is relatively a recent field, understanding the niche where these stem cell reside and their interplay between the stem cells and niche and niche cell and the stem cell is even more on the frontier.

So not related to aging but related to malignancy and disease development, we just recently showed, for example, that production of myeloid cell, which have different secretion of factor act on mesenchymal stem cells, change their production of osteoblastic lineage cell which accumulate at fibrotic tissue which is very refractory to stem cell maintenance in the niche. So there is this kind of feedback mechanism which will come more and more into play between changing the niche, acting on the stem cell, and the stem cells themselves and their progeny acting on the niche.

And what's known as well about the blood forming stem cell niche in the bone marrow is that adipocyte are very refractory to maintenance of stem cell. And an old bone marrow is a very adipocytic environment. So there is a lot of players which will come to play and impact on stem cell maintenance and stem cell function.

Dr. Conboy: And now just to end up this discussion, I think that we are benefiting on this panel from people in the completely different areas of stem cell research. So for hematopoietic system, yeah, the niche stem cell interaction is a frontier. For muscle system, a lot of unknown but it's less of a frontier because niche is much more easier to access and is much better defined. And I just want to emphasize, again, that we do not know if the young blood turns into old blood molecularly and functionally because of the contribution of senescent cells. These experiments are very important.

Speaker: I just wanted to say that older muscle that I don't know what older muscle looks like in mice, but in older individuals frequently the muscle is very heavily infiltrated with fat and it changes, I believe that it changes the association with the vasculature and with the nervous system as well. And I don't think people have really been talking very much about the heterogeneity in the older population. We see this primarily in people who have a great deal of disuse of the muscle and I think that it's an important issue especially when you talk about adipocytes affecting the niche.

Dr. Chen: I think there has been some study addressing that and in there what they've been suggesting is that adipocytes can actually act as part of the niche and they negatively impact stem cell function. And that has been done in hematopoietic stem cells so they have done at least— There are a couple of evidence. So one is they have compared different parts of the animal and there's a correlation where if there's increased adipocytes and the stem cell function is decreased. And, of course, they also use genetic modified animal model where the adipocyte is manipulated and then they also saw a difference in stem cell function. I think that that's the best evidence I have seen so far.

Speaker: Thank you.

Dr. Geiger: So there would be also an approach that you engineer, these thoughts reversed. So if you take out old stroma and you see these differences, it would be very interesting for us, which we're trying also to do is where do this difference come from. So I didn't talk about cellular changes yet there are for sure some of that and you can quantify them. The question is what does it mean and where do these changes in cytokines, for example, come from? What is the contributing factor in cell type, for example? This is not simple to solve. That's I think what you're saying, correct?

Speaker: Yes. And also there was a very interesting paper presented at the bone meeting this year by Cliff Rosen showing that there's a tremendous amount of adiponectin produced by bone marrow fat and suggesting in population studies we see an increase in adiponectin so that it's both a paracrine but it's also an endocrine function in older animals and also a parabiosis experiment showing rejuvenation of osteocytes as well.

Dr. Geiger: Yeah, the question is still if it's correlative or if it's causative. This is what I want to get at. Correlative it's straightforward. Causative is, unfortunately, not that simple, but as Richard said, we need to know what we're doing before we move forward. So we look forward to identify causative changes, but it's a little bit harder to get it.

Speaker: I agree and I think it would be really nice if we had ways to get at human muscle.

Dr. Wyss-Coray: Okay, next question. Or last question.

Speaker: It was a great session, thank you. So I just wanted to go back to the issue of telomerase because the panel seemed to be quite uniform in the idea that perhaps telomerase isn't so important for maintenance of stem cells. So Maria Blasco did publish a few years ago that I think higher levels of telomerase in cancer resistant mice does improve the health of some renewable tissues, I think the skin and the intestine, and also extend the lifespan of the mice. So would you propose then that that's just a stem cell independent phenotype?

Dr. Rando: I think the experiments that have to be done is overexpressing or maintaining high levels of telomerase in stem cell specific compartments. I think until we do that, we just don't know the answer. I mean, I think that's a provocative experiment but I think it's, again, not directly looking at— So I think the jury is out.

Speaker: Yeah, I think it's true. I don't think she did. I don't recall, but I don't think she did show that there was any specific, any improved maintenance of stem cells specifically. So I think you're right about that.

Dr. Passague: And stem cells themselves have high level of telomerase activity and that's why this parallel between cancer cell and cancer stem cell has been met as well in the first place.

Speaker: Right. But I guess it then just raises the interesting question if there's a stem cell independent effect of telomerase, then what is that? That's quite interesting.

Dr. Passague: Well stem cells do show telomerase exertion when you really force them to undergo lots of replication. So it's not that they are immortal cells or equivalent of a cancer fully transformed cell. They have high level what I view as a protective strategy. You want to maintain your stem cell pool because you want to maintain your tissue function, but that all came into organ physiology where the niche, the systemic factor, the cell/cell interaction all of that contributes to maintain the balance and the functionality of the stem cell. And when something goes wrong, there is consequence for what the stem cell do and all the stem cell maintain the tissue. And that's aging.

Dr. Rando: But I think your point is well taken that we don't know whether telomerase expression is any way rate limiting

Speaker: Yeah.

Dr. Rando: For stem cell we don't know that. And so I think that we can do only with genetic experiments.

Speaker: Yeah.

Dr. Wyss-Coray: Great.

Dr. Geiger: Again, for the hematopoietic system, there's a paper 2007 published by Alsip et al., it's Irv Weissman's lab. And they did overexpress telomerase. In the hematopoietic system, I think there was no beneficial effect long term with serotransplants. It's a long time. And for sure we know that telomerase has telomere independent functions that's coming slowly to light. So it could be well that there is in differentiated cells functions of telomerase that do not have to do with telomeres because of the first one and that could contribute.

And not excluding that would also contribute to stem cell maintenance. But I think this telomerase telomere length for a normal lifetime is probably not the major factor.

Speaker: Yeah, that's true, but I think we also need to recognize that, I mean, I think there's a— I'm not in the telomere field, but I think there is a feeling that it's not just telomere length which is important. I mean, you can have a dysfunctional telomere which is seemingly a long telomere.

Dr. Geiger: Yeah, I would agree on that, but, again, it's not that easy to conclude that telomerase is the rejuvenation factor in that sense.

Speaker: Right, okay. Thanks very much.

Dr. Wyss-Coray: Okay, maybe we have one more question.

Robert Brooke: Thanks. Robert Brooke. I just wanted to know, do you find any merits with any of the offshore stem cell clinics or any stem cell clinics in the US? I know it's pretty controversial. But then if you don't, you know, do you see any way or experiments you would want to do with them to take more advantage of the people that do go to them?

Dr. Rando: This is, obviously, a very hot topic and the International Society for Stem Research is very involved in trying to look at these stem cell clinics and get a sense of which clinics are based on science and which ones are not. And clearly there are many that are not. So I'm not going to speak for the group or the society in terms of what that is. But so in California, the California Institute for Regenerative Medicine is setting up, hopefully within the next couple of years, clinics within California that will be really set up to identify diseases that can be approached with stem cell therapies based on the science that's been supported by research in the US and around the world. And other than that, we all know the problems of such clinics around the world and people will spend a lot of money to go there. I don't think it's because more of a political issue than a scientific issue in terms of what to do about it.

Dr. Wyss-Coray: Okay, I think we have to wrap up. Thank you to the panelists again.

Dr. Rando (?): If NIH rules permit me, I'd like to make a statement and then a follow-up. I'd like to thank the cochairs, panelists, and the audience and people who have traveled from around the world to be here for an exciting final session and for all the thoughtful presentations and discussions over the last two days. Thank you.

Now it's my pleasure to introduce Dr. Richard Hodes to give the closing remarks. Dr. Hodes received his MD from Harvard Medical School, etc. Here's Dr. Hodes.

Closing Remarks

Richard Hodes, MD: Thank you. At the end of these two days, I appreciate an opportunity just to recap a little bit what it's been about and then spend the most of the few last minutes with you here thanking people who deserve that gratitude.

It's been interesting over the last years to see a conviction growing among many of us, I hope evidence based, that questions of aging and the mechanisms that underlie aging are both interesting and tractable. And with that notion has come the conviction that the relevance of the biology of aging to processes studied by many who identify, self-identify their focus with particular organs, diseases, or the case of NIH, institutes, really demands a breadth of commitment for the sake of the science and bringing together the perspectives that will best serve our understanding of aging and so that the understanding of aging can in turn best serve its application to the many components of the NIH mission to health.

And I think this meeting has been a particularly important juncture in that regard. In a sense, we've been talking out of conviction about the importance and relevance of involvement of many. We've taken that conviction I think at NIH and through the actions of all of you in the scientific community with the creation of geroscience. I guess the word began in 2007 from the Buck Institute and we give credit for the word. But it's been picked up congressionally by NIH and now with this Geroscience Interest Group which just in the way of briefest history began through efforts among those I'll thank, certainly Felipe Sierra's being paramount with a get together, in fact, in this building in a room associated with another meeting we were having, about 5 or 6 institute directors to talk about their willingness to commit themselves to such an effort. It was successful and now expanded to 20 at current tally of the institutes who were committed, again, not just in name but in effort, to making a commitment to pursuing the science here.

So we may well ask, most importantly, what is this going to lead to? I think, in fact, what has already happened at this meeting is an important part of what it has and will lead to. The very evident, vibrant conversations that have happened among people with various backgrounds has already, I hope, served to stimulate us and you to pursue science and accelerate the direction of science that we're taking.

From our own side at NIH, in these next days beginning with a session tomorrow, we'll be putting together trying to assimilate the very complex and vibrant inputs that have come from many of you. I take it it will be stimulating your own scientific interests. It will stimulate ours as well and I encourage you to maintain contact with those of us at NIA and all the institutes that have been collaborating here to make sure that we make a go of this. Whether there will be specific areas to identify that are worthy of initiatives in a time when we're all struggling to support all of you as best we can with budget constraints as they are, we will see. But we're certainly not going to be bashful about following scientific imperatives.

So let me turn from that to just giving thanks and congratulations to those who have made this possible. The organizers of the meeting, Felipe, Ron Kohanski, Kevin Howcroft in particular, a round of applause for them I'd say.

In addition to the representatives of the many institutions you've seen here helping to organize sessions, to the chairs who did a marvelous job overall in watching for the red lights and enforcing when need be the constraints on people. I think it led to an extraordinarily good balance of presentations, as well as very, very active interactions from many of you who've been here through these two days.

I would also like to thank all of you, above all, for taking the time to come here and be with us. And if there's anybody I'll ask to stand up it's our partners who haven't been recognized maybe as publicly, Dan Perry, Sue Peschin, if James Appleby is here, but folks from the Alliance for Aging Research and the Gerontological Society of America who were the partners that made this possible in many ways. And not just this meeting, but these are the folks who've been talking across institutes with NIH, who have been

talking on the Hill and responsible for a number of us being there to speak to a senate subcommittee yesterday; all part of an effort that has made geroscience just not only a name of an interest group here at NIH but a landmark, a hallmark for what's happening in science. It's in congressional language. It's in the minds of all of us. And, most importantly, I think we need to make it a reality of a rigorous and legitimate science as we are seeing its evolution happen now.

So thank you all. Thank you for being here and safe travels home and we look forward to interactions in the future with you enriched and informed by these last two days.

Thank you.

END OF MEETING