A number of labs, my own included (e.g. Taylor et al Nature Biotech 21. 281-6 2003) have worked to define the mitochondrial proteome using the purified organelle. Meanwhile researchers with interest in specific cellular proteins or physiological processes continue to find a mitochondrial location for their favorite protein or sets of proteins, many of which were not picked up in comprehensive proteomic studies. It is becoming obvious why this is and it is not entirely related to the lack of resolution of the methods used. The complication is that the protein content of mitochondria depends on ongoing (temporal) cellular events, cell age, tissue type and probably the phase of the moon. More broadly it is now clear that the mitochondrial content of a cell is dynamic with respect not only to amount, morphology, distribution but also to content. There are 3 aspects to ponder in thinking about the distribution of proteins in a cell in general and in the mitochondrion in particular.
1). Some proteins may be targeted to and reside in multiple sites in the cell and provide different functions in each location.

The magnitude of the dual location of “mitochondrial” proteins has been estimated recently.

MENACHEM RB, TAI M & PINES O. A third of the yeast mitochondrial proteome is dual localized; a question of evolution PROTEOMICS Sept 9 ahead of press 2011.


2). There can be movement/trafficking of proteins in and out of mitochondria, the cytosol and other organelles as a part of cellular functioning.

THE STAT3 STORY. One of the most interesting proteins, only recently considered to be mitochondrial, is STAT3. Studies have shown that there is a pool of STAT3 in mitochondria, which controls the activity of both complex I and complex II. Over-expression of mitochondrial targeted STAT3 partly blocks electron transport through these complexes without effect on membrane potential, with no increase in ROS and no induction of apoptosis.
WEGRZYN J et al. Function of mitochondrial STAT3 in cellular respiration. SCIENCE 323. 793-7 (2009)


**TELOMERASE AS A MITOCHONDRIAL PROTEIN.**

A recent study has shown that telomerase is a mitochondrial protein in human cells, where it acts as an hTR-independent reverse transcriptase, a different function than that provided by the nuclear fraction of this protein.

SHARMA NK. Et al. Human telomerase acts as an hTR-independent reverse transcriptase in mitochondria. NUCLEIC ACIDS Sept 29 Epub before print (2011).

**INSULIN SIGNALING TO MITOCHONDRIA.**

Fusco et al have shown that G protein coupled receptor kinase 2 (GRK2) is present in mitochondria where it is able to increase ATP cellular content by enhancing mitochondrial biogenesis. Also the insulin resistance caused by cellular accumulation of GRK2 is able to antagonize ATP loss after hypoxia/reperfusion.

WHAT BRINGS DJ-1 TO MITOCHONDRIA?

DJ1 is a protein associated with Parkinsons disease and with cancer. Deletions or mutations in the gene for this protein lead to early onset Parkinsons disease. In their recent study Ren et al. show that DJ1 increases its mitochondrial distribution in response to cell stress and interacts with Bcl-X(L) but only upon oxidation of DJ1. This interaction stabilizes Bcl-X(L) by preventing its ubiquitinylation.


3) There can be sharing of proteins between organelles in contact sites.

WHERE DO MITOCHONDRIA END AND THE ENDOPLASMIC RETICULUM BEGIN?

It has been known for some time that there are functional interactions between mitochondria and the endoplasmic reticulum e.g. in Ca++ transport, and of course, in translocation of newly made proteins to the mitochondrion. Now it is clear that there are structural bridges or contact sites between the two organelles. These have been called MAMs (mitochondria associated membranes) or ERMES (endoplasmic reticulum-
mitochondria encounter structure). Proteins present in this structure include at least one ER anchoring protein and 3 mitochondrial proteins along with the calcium binding GTPase GEM1.


The link between mitochondria and ER, particularly, its role in Ca++ balance in the cell, may prove to be a target for drug treatments. For example Zampese et al. have shown that presenilin 2 mutants affect the Ca content of the ER and alter Ca++ handling by mitochondria by affecting the interaction between the two organelles. Presenilin mutants are the main cause of familial Alzheimers disease!


NEW METHODS OF MONITORING MITOCRHONDRIAL STRUCTURE AND FUNCTION: AND WILL YOU STILL BE PUBLISHING PAPERS IN YOUR 90s?

This edition of the MitoAlmanac includes a remembrance of Britton Chance who died this year in his 90s. He
continued to work and contribute seminal studies up to his death as shown in the review below on monitoring oxygen (mitochondrial utilization of) in humans.


THE PIONEERS OF MITOCHONRIAL RESEARCH REMEMBERED. 3) BRITTON CHANCE.

In a career that spanned 60 years Britton Chance developed some of the most important technology, and used this to obtain some of the most incisive observations that have been made on mitochondrial structure and functioning. Details of his scientific career are well covered in a web site describing the man and his science that has been built by his colleagues in Philadelphia

http://www.med.upenn.edu/biocbiop/chance/

As were Green and Mitchell described in previous editions of the MitoAlmanac, Brit was a larger-than-life character. Besides his scientific achievements he was a master sailor and Olympic gold medal winner in the sport. His life is well described in the above web site.

Brit got his start in enzymology in Cambridge and early on invented the now-standard stop flow device. David Green told me the story of his meeting Chance in Cambridge in those early years. Brit had the prototype stop flow machine working and needed an interesting sample to
examine. He persuaded Green to let him “use” the batch of old yellow enzyme (now called NADPH dehydrogenase) that David had spent a year in purifying. As Brit pointed out, you stick the enzyme in at one end and catch the effluent at the other end of the machine and have your enzyme back...no problem...unless you forget to use a receptacle to catch the effluent in, which Brit did. Green was not a happy man!

In the reflections part of the website cited above, Maurizio Brunori remembers a time that he visited Brit’s lab, where he collaborated on experiments using low temperature triple trapping methods to monitor intermediates in the reaction of cytochrome c oxidase. Briefly, the enzyme was saturated with CO in the active site and then the CO was removed by a light flash to allow oxygen binding, the rate of which was slow enough at the very low temperatures, that intermediates in the oxygen binding reaction could be detected and characterized. Brunori remembers the enthusiasm Brit always had for doing experiments himself. I took the same pilgrimage, and did the same experiments in the early 1980s. The thing I remember most is going into Brit’s office and seeing his notebooks of research, all beautifully bound, covering all of the walls. I also remember going into the spectrophotometer room through black curtains to the sound of animals afraid of the light scurrying to their lairs. I only felt comfortable in there by wearing two pairs of socks with my trousers tucked in the outer set. Chance later focused his efforts on developing approaches for monitoring non-invasively the metabolism of living tissues. This included his making major contributions to the use of phosphorus NMR.
Chance’s was one of the first labs to conduct P31NMR studies of the changes in levels of creatine kinase, ATP and ADP during exercise, including on patients with mitochondrial disease. Early on, my colleague Nancy Kennaway took a patient to Philadelphia to have her evaluated by Brit using this technique. The story of this event has grown with time but the facts as I remember them are as follows: the young woman had a mutation in cytochrome b, which left her an invalid with little energy and subject to bouts of acidic lactidosis. Brit and his colleagues confirmed the serious energy deficit with Pi-NMR and then treated her with vitamin K and vitamin C to (theoretically) bypass the dysfunctional cytochrome b. There was no doubt that her functioning, again measured in the NMR machine, was dramatic. Whether she went down town with her mother the next day and walked around the shops may be something I made up!
