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I was discussing with a group of students how improvement in techniques have made research more efficient and opened up the chance to tackle problems that had been illusive. When I told them that I wrote my first paper on a typewriter with carbon copies they were not as impressed as I expected. When I told them that I entered my data on a stack of punch cards, walked across campus to the Computer Center where they would be loaded in THE computer, and that I MIGHT get the data within a day or two, their faces remained blank. There were now clear signs of loss of interest. The number of them with hands under the table obviously texting from their cell phones was increasing rapidly. I brought out a metal hammer and pair of metal blocks with a matching groove down one face of each, and invited them to tell me what this was for. Most suggestions cannot be printed here but at least I had their interest back. I explained that we did SDS-PAGE in cylindrical glass tubes "back in the day" that were inserted between two buffer chambers for the electrophoresis step. Then we retrieved the tubes put them into the groove of one of the metal blocks, put the second metal block on top with its groove over the gel, and then hit the assembly several times with the hammer. At least several of them had stopped tweeting or

whatever. I explained that the next step was to carefully remove the glass shards from the acrylamide without breaking the gel, which could be difficult when the acrylamide was 5%, and then dunk the gel in stain. Don't believe me? You should.

MITOCHONDRIA: ORTHODOX AND CONDENSED.

It is fun to look back sometimes, at least for the recollector (is that a word?) Not long ago, after reading yet another mitochondrial fusion/fission paper, I suddenly remembered discussions I once had with Charlie Hackenbrock on the morphology of mitochondria. Long before any findings of a mitochondrial reticulum, when we all thought the organelle was bean shaped, floating in the cytosol, he reported morphology changes in mitochondria termed the orthodox and a condensed state. The difference was in relative volume of the matrix and intermembrane space. In the condensed form the matrix shrank considerably while the intermembrane space became very much enlarged. In the orthodox state the opposite was true, the matrix took up most of the volume of the organelle.

The whole notion of condensed/orthodox states of mitochondria had been largely forgotten, but if valid which I believe it is, the implications for much of the work currently in vogue in the field are huge. Here I review the earlier observations, and invite comments of others, which is what the MIG website and correspondence over the MIG e-mail is/should be all about.

Hackenbrock's seminal paper was published in 1966.

HACKENBROCK CR. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. 1. Reversible ultrastructural changes in metabolic steady state in isolated liver mitochondria. J.CELL BIOL 30. 269-97 (1966).

There are anthropologically interesting aspects to this paper. First it is 28 pages long…can you image getting 28 pages of a journal for your work today?
Second, this is paper #1. Many thought that a project, their niche, would go on forever and that they would publish a series of papers over many years.
The concepts in the paper are well described in the abstract, which I have reproduced below;

“By means of a new "quick-sampling" method, micropellets of mouse liver mitochondria were rapidly prepared for electron microscopy during the recording of steady state metabolism. Reversible ultrastructural changes were found to accompany change in metabolic steady states. The most dramatic reversible ultrastructural change occurs when ADP is added to systems in which only phosphate acceptor is deficient, i.e., during the State IV to State III transition as defined by Chance and Williams. After 15 min in State IV, mitochondria display an "orthodox" ultrastructural appearance as is usually observed after fixation within intact tissue. On transition to State III, a dramatic change in the manner of folding of the inner membrane takes place. In addition, the electron opacity of the matrix increases as the volume of the matrix decreases, but total mitochondrial volume does not appear to change during this transition. This conformation is called "condensed." Isolated mitochondria were found to oscillate between the orthodox and condensed conformations during reversible transitions between State III and State IV. Various significant ultrastructural changes in mitochondria also occur during transitions in other functional states, e.g., when substrate or substrate and acceptor is made limiting. Internal structural flexibility is discussed with respect to structural and functional integrity of isolated mitochondria. Reversible changes in the manner of folding of the inner membrane and in the manner of packing of small granules in the matrix as respiration is activated by ADP represent an ultrastructural basis for metabolically linked mechanical activity in tightly coupled mitochondria”.

Hackenbrock and colleagues followed up the work on isolated mitochondria with cell studies, and to give justice to the work again I show the full abstract below;
HACKENBROCK CR, REHN TG. WEINBACJ EC & LEMASTERS JJ. Oxidative phosphorylation and ultrastructural transformation in the intact tumor cell.

J CELL BIOL 51 123-37 (1971)

We have examined the ultrastructure of mitochondria as it relates to energy metabolism in the intact cell. Oxidative phosphorylation was induced in ultrastructurally intact Ehrlich ascites tumor cells by rapidly generating intracellular adenosine diphosphate from endogenous adenosine triphosphate by the addition of 2-deoxyglucose. The occurrence of oxidative phosphorylation was ascertained indirectly by continuous and synchronous monitoring of respiratory rate, fluorescence of pyridine nucleotide, and 90 degrees light-scattering. Oxidative phosphorylation was confirmed by direct enzymatic analysis of intracellular adenine nucleotides and by determination of intracellular inorganic orthophosphate. Microsamples of cells rapidly fixed for electron microscopy revealed that, in addition to oxidative phosphorylation, an orthodox --> condensed ultrastructural transformation occurred in the mitochondria of all cells in less than 6 sec after the generation of adenosine diphosphate by 2-deoxyglucose. A 90 degrees light-scattering increase, which also occurs at this time, showed a $t_{(1/2)}$ of only 25 sec which agreed temporally with a slower orthodox --> maximally condensed mitochondrial transformation. Neither oxidative phosphorylation nor ultrastructural transformation could be initiated in mitochondria in intact cells by the intracellular generation of adenosine diphosphate in the presence of uncouplers of oxidative phosphorylation. Partial and complete inhibition of oxidative phosphorylation by oligomycin resulted in a positive relationship to partial and complete inhibition of 2-deoxyglucose-induced ultrastructural transformation in the mitochondria in these cells. The data presented reveal that an orthodox --> condensed ultrastructural transformation is linked to induced oxidative phosphorylation in mitochondria in the intact ascites tumor cell.

Others confirmed the morphology changes seen by Hackenbrock and colleagues. Thus, both Kwiatkowska (1975) and Latruffe et al (1981) among others confirmed the existence of the orthodox/condensed conformation and the physiological relevance of this transition.
KWIATKOWSKI M. Changes in the ultrastructure of mitochondria during the cell cycle. FOLIA HISTOCHEM. CYTOCHEM 19. 99-105 (1981)

LATRUFFE N. LENDYS D. GAUDEMER Y & BUGNON C.

Effect of various inhibitors on oxidative activity and ultrastructure of rat liver mitochondria. C.R. SEANCES SOC BIO FIL. 169. 1290-5 (1975)

Later the features of the condensed and orthodox states were more extensively characterized by freeze fracture studies and electron tomography. The freeze fracture work (a method not often used now) showed that inner membrane particles (OXPHOS complexes) were much closer together in the condensed state than in the orthodox state. HERTSENS RC & JACOB WA. Freeze fracture study of heart mitochondria in the condensed and orthodox state. BIOCHIM. BIOPHYS ACTA 894. 507-14 (1987).

In the 1990(s), the differences between orthodox and condensed states were clearly defined in a beautiful set of experiments by Mannella and colleagues. The work is best described by the abstract from one of these papers, below;

“Electron tomography indicates that the mitochondrial inner membrane is not normally comprised of baffle-like folds as depicted in textbooks. In actuality, this membrane is pleomorphic, with narrow tubular regions connecting the internal compartments (cristae) to each other and to the membrane periphery. The membrane topologies observed in condensed (matrix contracted) and orthodox (matrix expanded) mitochondria cannot be interconverted by passive folding and unfolding. Instead, transitions between these morphological states likely involve membrane fusion and fission. Formation of tubular junctions in the inner membrane appears to be energetically favored, because they form spontaneously in yeast mitochondria following large-amplitude swelling and recontraction. However, aberrant, unattached, vesicular cristae are also observed in these mitochondria, suggesting that formation of cristae junctions depends on factors (such as the distribution of key proteins and/or lipids) that are disrupted during extreme swelling. Computer modeling studies using the "Virtual Cell" program suggest that the shape of the inner membrane can influence mitochondrial function. Simulations indicate that narrow cristae junctions restrict diffusion between intracristal and external compartments, causing depletion of ADP and decreased ATP output inside the cristae”

MANNELLA CA et al. Topology of the mitochondrial inner membrane: dynamics and bioenergetics implications. IUMBM LIFE 52. 93-100 (2001).

Moreover in my laboratory we correlated the orthodox to condensed states with morphology of the mitochondrial reticulum. These studies showed that there were changes in the interconnections within the reticulum and in the volumes of the matrix and intracristal space dependent on substrate utilization (glucose versus glutamine). ROSSIGNAL R et al. Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells. CANCER RES. 64. 985-93 (2004).

SO WHAT DOES IT MEAN FOR STRUCTURE FUNCTION RELATIONSHIPS OF MITOCHONDRIA.

I am convinced of the existence of an orthodox and condensed state and transitioning between the two depending on functional state…so what is the significance

a) For the diffusion rates of proteins in the matrix space and on the inner membrane and even conformational and association changes in proteins e.g. the wildly popular super-complexes of electron transfer enzymes.
b) For apoptosis and release of mitochondrial proteins into the cytosol, particularly of cytochrome c which is in the intracrystal space.
c) Exchange of mtDNA molecules between nucleoids.
d) Fusion and fission; is there a connection?
   And other mitochondrial events.

I am particularly interested in movement of proteins in the inner membrane and matrix space of mitochondria, and whether diffusion of proteins occurs or not. This relates to

my time with David Green. His first project for me was to reconstitute the Krebs cycle, and of course, he knew how I should do this. I was provided with a lyophilized fraction of proteins released by sonication of mitochondria to make electron transfer particles. I should simply dissolve this in buffer and start measuring CO_2 release from pyruvate. A fellow post-doc at the time, George Blondin, had done a careful calculation and had come up with a protein concentration in the matrix space in the condensed form of 500mg per ml. For reference, that of a protein crystal is 500mg per ml. I wanted to impress and so began adding water to a weight of lyophilized powder in a test tube, stirring all of the time with a glass rod. By 150mg per ml all of the material was stuck to the glass rod like a pellet of chewing gum. I gave up on the project and started something else. But Green was adamant. So, I kept the glass rod coated in a bolus of protein and showed it to him whenever he came in the lab in proof of effort. It became more difficult to fool him as the blob started to turn green and then orange from mold. At least, my experiments showed that the viscosity of a protein solution in the matrix of condensed mitochondria must be highly viscous and that movements of protein within will be very restricted. (I never published this work!)

If you have ideas or experimental observations that pertain to any or all of the questions above, please communicate them to the group. There must be someone out there who has thought about these things besides me!