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It has been 50 years since Peter Mitchell published the Chemiosmotic Theory in one of his little monographs, and for those of you who failed to read it the first time round, there is now no excuse. The entire treatise has been reprinted.

MITCHELL P. Chemiosomotic coupling in oxidative and photosynthetic phosphorylation. BIOCHIM BIOPHYS ACTA 1807: 1507-38 (2011).

The Oct edition of the MitoAlmanac includes a "reflections" section on Mitchell. It is worth reminding everyone that the Chemiosmotic Theory took some time to be embraced, and required a meeting of the overseers of the truth along with publication of their acceptance before the heresy became law.

BOYER PD., CHANCE B., ERNSTER L., MITCHELL P., RACKER E. & SLATER EC. Oxidative phosphorylation and photophosphorylation. ANN. REV.BIOCHEM 46: 955-66 (1977).

It is interesting to look back and ruminate on the discussions that followed Mitchell's publication. Below is a summary taken from a paper published by E.C.Slater in 1967:

> The Mitchell hypothesis of chemiosmotic coupling in oxidative phosphorylation is examined in the light of experimental data on oxidative phosphorylation at present available. The following objections are brought against the theory:
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> 1. Data on the magnitude of the phosphate potential against which ATP can be synthesized by the respiratory chain require, on the basis of chemiosmotic coupling, very effective proton and/or cation extrusion and/or anion uptake by the mitochondria. Experimental evidence for this is lacking. Indeed it has been shown by Chance and Mela that mitochondria in the controlled state do not extrude protons.
> 2. Although reversible ATP-driven proton (chloroplasts) or cation (mitochondria, erythrocytes) pumps have been demonstrated, this is insufficient evidence for the postulate that the establishment of a proton gradient is the primary energy-conserving event of the chloroplast or mitochondrial respiratory chain. Indeed, it is general experience that cations are required for the extrusion of protons by mitochondria.
> 3. The stoichiometry of proton extrusion by mitochondria and of proton uptake by chloroplasts, and the kinetics of the latter process are also difficult to reconcile with the chemiosmotic hypothesis.
> 4. Further experimental evidence is presented confirming that, under the conditions of the oxygen-pulse experiments of Mitchell and Moyle, the extrusion of H+ is not associated with the oxidation of mitochondrial NADH.

5. The respiratory chain included in the chemiosmotic hypothesis is difficult to reconcile with our present knowledge of the chain.
It is concluded that the chemiosmotic theory, in its present form, is untenable.

The foregoing is a useful reminder that "testable" ideas take time to test. Their evaluation can be muddled by imprecise, naïve, and sometimes faulty studies, as well as severely constrained by limitations in available technologies.

MITOCHONDRIAL NUCLEOIDS: NEW ADVANCES.

It is not that long ago that DNA was first visualized inside mitochondria and the era of organelle genetics began. Issues such as copy number, maternal inheritance and the variation, i.e., heteroplasmy of mitochondrial DNA are now well defined. What is less clear is how the copies of mitochondrial DNA are organized within the organelle. Consensus is that mitochondria contain many (depending on total copy number of genomes) aggregates of DNA and proteins for which the term nucleoid is now regularly used. Each may have as many as 5-7 copies of the mtDNA bound by an as yet undefined number of different proteins.
Recently there has been resurgence in work on nucleoids as described below. Different aspects of the work are reviewed in three recent publications.

BOGENHAGEN DF. Mitochondrial DNA nucleoid structure. BIOCHIM BIOPHYS ACTA (2011) NOV 27 (Epub ahead of print).
SPELBRINK JN. Functional organization of mitochondrial DNA in nucleoids: history, recent developments and future. IUBMB LIFE 62: 19-32 (2010).
PRACHAR J. Mouse and human mitochondrial nucleoid—detailed structure in relation to function. GEN.PHYSIOL.BIOPHYS. 29: 160-74 (2010).

In a particularly elegant piece of work, superresolution fluorescence microscopy has been used to image nucleoids. These structures are seen to vary in size and shape with the mtDNA very condensed. Individual nucleoids appear closely associated with the inner membrane.

BROWN TA., et al. Superresolution fluorescence imaging of mitochondrial nucleoids reveal their spatial range, limits and membrane interaction. MOL.CELL.BIOL. 24: 4994-5010 (2011).

A number of the proteins involved in the nucleoid are known, including Twinkle, polymerase gamma and Tfam. A recent X-ray structure of the interaction between Tfam and promotor has shown that this protein bends the DNA to form a U-turn. The importance of this structure is discussed.

NGO HB., KAISER JT. & CHAN DC. The mitochondrial transcription and packaging factor Tfaam imposes a U turn on mitochondrial DNA. NAT STRUCT MOL BIOL 18: 1290-6 (2011).

New components are being discovered regularly. For example, Miyakawa et al recently reported that Abf2p, a high mobility group protein, is in mitochondria and associated with nucleoids.

MIYAKAWA I., et al. Morphology and protein composition of the mitochondrial nucleoids in yeast cells lacking Abf2p. J. GEN APPL. MICROBIOL. 56: 455-64 (2010).

Another protein recently described to be of importance to nucleoid structure, either as a part, or in the formation of the aggregate, is OPA1. Elachouri et al. show that silencing of the OPA1 gene leads to alteration of mtDNA distribution throughout the mitochondrial network.

ELACHOURI G., et al. OPA1 links human mitochondrial genome maintainance to mtDNA replication and distribution.
GENOME RES. 21: 12-20 (2011).

Not surprisingly there are mitochondrial diseases that result from or include altered nucleoid structure. An example has been reported by Cameron et al. They show that mutations in a protein thought to be an assembly factor for the ATP synthase, called TMEM70, which gives a familial form of ATP synthase deficiency, shows altered mitochondrial structure including altered nucleoids. The affected mitochondria show a whorled structure of their cristae. Nucleoids and respiratory chain complexes are all confined to the outer rings of these whorls.

CAMERON, et al. MITOCHONDRION 11: 191-9 (2011).

PIONEERS OF BIOENERGETICS: E.C. (BILL) SLATER.

As referenced above Bill Slater was one of the fab five who published the 1977 paper on chemiosomosis with Mitchell, referred to above, i.e. the cease-fire declaration. Bill deserved his place at the table as a contributing antagonist, and all good theories need good antagonists to evolve. If you isolate mitochondria, if you do spectroscopy of the respiratory chain, if you assay electron transfer activity, and if you use inhibitors to dissect respiration or modulate the ATP aynthase, you are using methods developed from Slater's lab in Amsterdam. What makes the man an icon at least in my mind is that he built a Department and large teaching program at the University of Amsterdam from the ground up, and more broadly was involved with expanding biochemistry and bioenergetics throughout Europe, AND YET HE DID HIS OWN EXPERIMENTS WITH THE HELP ONLY OF A TECHNICIAN. An excellent summary of Slater, the man, and his many

contributions was provided by Piet Borst in 2007 in celebration of Bill's 90th birthday. This can be found at: www.iubmb.org/index.php?id=174

If history provides lessons, what can we learn from the observation that Slater and Chance, both of whom loved the bench and the excitement and urgency of a discovery,….each lived into their 90s?