1

HISTORY

Summary

Major landmarks in the history of studies on mitochondria are presented here. Ideas do not evolve in a vacuum, and insights grow on a rich base of observations and interpretations of the past. While some may be of interest only to the historian of science, history often connects the facts as we can present them today, and seeing facts in the context of history enriches them for us.

A review by Cowdry (1918), quoted by Lehninger (1), contains more than a dozen terms referring to structures that we now identify as mitochondria: blepharoblasts, chondriokonts, chondriomites, chondrioplasts, chondriosomes, chondriospheres, fila, fuchsinophilic granules, Korner, Fadenkorper, mitogel, parabasal bodies, plasmasomes, plastochondria, plastosomes, vermicules, sarcosomes, interstitial bodies, bioblasts, and so on. Chondros (Greek), grain (English), and Korn (German) are descriptions of the morphology of distinct structures noted inside of cells by microscopists beginning about 1850. Improvements in staining yielded more accurate morphological descriptions, and the grains were in some tissues seen as threads (Faden in German, mitos in Greek), hence Fadenkorper, or mitochondria (Benda, ca. 1898). In 1888 (R. A. Kollicker), by microdissecting some of these granules out of insect muscle and observing them to swell in water, microscopists reached the conclusion that the mitochondria had a membrane.

With our current knowledge it is astonishing to learn that Altman in around 1890 referred to mitochondria as bioplasts in a book on Elementarorganismen, in which he proposed that these granules were autonomous, elemental living units, forming bacteria-like colonies in the cytoplasm of the host cell. A wild, lucky guess, or an idea ahead of its time?

For a while, in the early part of this century, cytologists were anxious to provide their geneticist colleagues with a concrete entity to which they could assign a role in the transmission of genetic information, and mitochondria were favored for a short time. Another function, challenging their role in genetics, was also proposed as early as 1912 (Kingsbury); and as retold by Lehninger, Warburg in 1913 found granular, insoluble subcellular structures to be associated with respiration. But another 30–40 years of intense and painstaking biochemical analyses were required to lead to the characterization of mitochondria as the "powerhouse of the cell." Some of the most illustrious names in the early history of Biochemistry can be found among contributors of the highlights of the evolution of this concept. The discoveries of cytochromes, iron–porphyrin compounds (heme), flavin and pyridine nucleotides, and various dye-reducing dehydrogenases fall into the period between 1920 and the 1930s, and the formulation of the citric acid cycle by Krebs was one of the crowning achievements of the study of metabolism and respiration in muscle preparations.

Although ATP was discovered in 1931 (Lohmann), it took 10 more years to demonstrate its general role beyond muscle, and during this period Warburg and Meyerhof described what is now referred to as substrate level phosphorylation (ATP synthesis coupled to the enzymatic oxidation of compounds such as glyceraldehyde phosphate), in contrast to oxidative phosphorylation, first shown by Kalckar to be firmly coupled to respiration (1937–1941).

This was also a time when biochemists proceeded to grind up tissue, filter it through cheese cloth, perhaps even centrifuge the mixture at some indeterminate speed, and then discard everything but the supernatant. Insoluble particles constituted a nuisance and an insurmountable obstacle in the purification of an enzyme, and students were urged not to "waste clean thoughts on impure enzymes." The study of mitochondria required the isolation and purification of larger quantities of the organelle, and the first attempts in this direction were doomed by the use of unsuitable buffers and media for cell suspension and breakage.

Cell Biology metamorphosed out of the older science of Cytology when two powerful new methodologies were perfected and applied to the study of biological tissues. Pioneering advances and applications were made in parallel and synergistically at the same institution during the 1940s: At the Rockefeller Institute, Claude and his colleagues began to use the centrifuge as a sophisticated analytical tool for the fractionation of subcellular structures (differential centrifugation), a technique that De Duve (2) would later describe as "exploring cells with a centrifuge." Subcellular particles were fractionated reproducibly and with increasing resolution to achieve pure fractions. At the same time,

careful biochemical characterizations were carried out. Important concepts to emerge were the recognition of the polydisperse population of particles (size variation) and the postulate of biochemical homogeneity. Microsomes and mitochondria were represented by overlapping populations of granules of different size, but at the large end were almost pure mitochondria and at the small end were almost pure microsomes (which were later resolved further into lysosomes and peroxisomes (2)). The discovery that ~0.3 M sucrose greatly stabilized mitochondria (Hogeboom, Schneider, Palade, 1948) greatly aided their isolation from liver in a morphologically intact form.

The criterion of morphological intactness could not have been applied if at the same time the groups led by Porter and Palade had not pursued the application of the electron microscope to the exploration of cells. Particles could now be viewed and compared *in situ* and after isolation by differential centrifugation, thereby increasing confidence that the particle was intact and therefore most likely completely functional.

Nevertheless, isolating and looking at a particle does not immediately give many clues about its biological function, although remarkably prescient guesses and deductions had been made from staining experiments. An approach from a different direction finally led to the full appreciation of the role of mitochondria in respiration. Lehninger (and independently Leloir and Munoz) in the period 1943–1947 had focused on the oxidation of fatty acids in liver homogenates and found the activity to be dependent on an insoluble component that was sensitive to osmotic conditions. The newly established conditions for mitochondrial isolation were applied by Kennedy and Lehninger to prove that fatty acid oxidase activity of the liver was found almost exclusively in mitochondria. The same investigators then extended the biochemical characterization of these organelles by demonstrating that (a) the reactions of the citric acid cycle can be carried out in mitochondria at a rate that can account for most, if not all, of the activity found in liver cells and (b) such reactions were accompanied by the synthesis of ATP (oxidative phosphorylation).

Just as a cell was recognized to be much more than a "bag of enzymes," it soon became clear that many of the enzymes catalyzing the biochemical reactions observed in mitochondria were not simply contained within this organelle in soluble form by the mitochondrial membrane. In fact, the potential complexity of this organelle became apparent from the early electron microscopic observations that revealed the existence of an inner and an outer membrane, with the inner membrane often highly folded (termed cristae by Palade). Topologically, one can therefore distinguish two spaces inside the mitochondrion: the intermembrane space and the matrix. However, a full appreciation of the significance of this compartmentalization was probably not achieved until much later.

The enzymes for fatty acid oxidation and for the citric acid cycle (with the exception of SDH) were found to be soluble in the mitochondrial matrix. The enzymes responsible for oxidation of NADH and electron transport to oxygen were insoluble and localized to the inner membrane (cristae). A more detailed

description of their characterization will be deferred to a later chapter, but even an abbreviated historical introduction should mention two accomplishments of the 1960s. First, the overall arrangement of the components of the electron transport chain and the flow of electrons from dehydrogenases to flavoproteins to various nonheme iron–sulfur centers and cytochromes and finally to oxygen, first glimpsed by Keilin in the 1930s, was established by a combination of spectroscopic studies and the use of specific inhibitors such as rotenone, antimycin, and cyanide in various laboratories, with that of B. Chance deserving special mention. D. E. Green was another influential investigator in the 1950s and 1960s. While his ideas about an "elementary particle" within mitochondria have not stood up to the test of time, his Institute for Enzyme Research was the training ground for a number of prominent researchers in the subsequent decades. A very informative, entertaining, and highly personalized account has been written by one of the pioneers in the field, E. Racker (3). Second, the efforts of Hatefi and colleagues culminated in the fractionation and characterization of five multisubunit complexes from the inner membrane, four of which are involved in the respiratory chain, and the fifth was identified as the site of the phosphorylation of ADP to ATP (4, 5). Among the high points in the past decades in the biochemical and structural analysis of these complexes is undoubtedly the achievement of high-resolution structures for complex II (6–8), complex III (9), complex IV (cytochrome oxidase) (10), and complex V (ATP synthase) (11–14) by X-ray diffraction and other biophysical means. A Nobel Prize in Chemistry has been awarded for the elucidation of the structure and function of the F_1-ATP synthase to J. Walker of Cambridge and P. Boyer at UCLA. After the initial breakthroughs, similar structures from different organisms and at higher resolution have quickly followed.

The distinction between substrate-level phosphorylation and oxidative phosphorylation is made the subject of examination questions for thousands of undergraduate students every year. The former is straightforward to explain in terms of enzyme kinetics and the coupling of exergonic and endergonic enzyme-catalyzed reactions. The challenge to explain oxidative phosphorylation has preoccupied some of the best biochemists for a good part of their career. An ingenious solution, offered by P. Mitchell (15), was slow to be accepted, but it eventually revolutionized our thinking about bioenergetics, membranes, membrane potentials, active transport, ion pumps, and "vectorial metabolism." A detailed understanding of the structure of membranes, along with an understanding of the relationship between lipids and integral membrane proteins, was a prerequisite (16). The chemiosmotic hypothesis has found universal acceptance in explaining not only oxidative phosphorylation in mitochondria, but also aspects of photosynthesis in chloroplasts and light-driven phosphorylations in bacteria.

While failing to live up to early expectations of mitochondria as the carriers of all hereditary information, a most important discovery was made in 1963 when one of the first definite identifications of DNA in mitochondria was

made (17). This discovery had in many ways been anticipated by the discovery of non-mendelian, cytoplasmic inheritance in yeast by the Ephrussi laboratory (18). Ramifications of this discovery are wide. It renewed and strengthened interest in the evolutionary origin of mitochondria. The problem of understanding how two genomes, nuclear and mitochondrial genes, interact in the biogenesis of this organelle is still an acute one. Changes in mitochondrial DNA sequences are believed to represent a molecular clock on a time scale that appears particularly suitable for human evolution; and provocative ideas and speculations have centered around deductions from such sequence comparisons, with implications for primate and human evolution ("the Mitochondrial Eve") and for the spread of human populations by migrations. Controversies still center around the question whether this clock is more accurate than a sundial (N. Howell). Because of the high degree of polymorphisms in human mitochondrial DNA, forensic investigations utilize comparisons of mitochondrial DNA sequences from victims and suspects. Where there is DNA, there must be mutations. An explosion of publications in the last two decades, triggered by the pioneering work of Wallace and his colleagues (19) and Holt et al. (20), has described mutations in mitochondrial DNA which are directly responsible for human genetic diseases (myopathies and neuropathies), while speculations go even further in relating accumulating defects in mitochondrial DNA to a variety of ailments accompanying aging and senescence. "Mitochondrial diseases" are now recognized to be caused by both mitochondrial and nuclear mutations.

The final chapter on mitochondria has only started to be written during the past decade when it was recognized that mitochondria are not merely the "powerhouse of the cell," but are much more intimately involved in the function, life, and death of a cell. They supply ATP, but they are also critically involved in maintaining the cellular redox potential and ionic conditions in the cytosol (e.g., Ca^{2+}). They are the target of numerous cellular signaling pathways, and in turn they can be triggered to release factors/proteins that initiate the process of apoptosis. They are the major source of reactive oxygen species (ROS); and while these can be highly injurious at high concentrations, they also have a positive regulatory function at lower concentrations. Proteomic studies have emphasized the diverse composition of mitochondria in different tissues and cell types, and understanding the tissue-specific behavior of mitochondria remains one of the challenges of the future. It is the key to understanding the many still puzzling features of mitochondrial diseases. A book published in 2005 entitled *Power, Sex, Suicide—Mitochondria and the Meaning of Life* (N. Lane, Oxford University Press) reflects the hype associated with mitochondrial studies nowadays. Mitochondria may not help in explaining the "meaning of life," but understanding them is definitely essential for understanding a healthy life and many pathological conditions. Nowadays they also occupy a prominent position in discussions on aging.

Thus, mitochondria occupy a central position in our understanding of the cell, the "basic unit of life," and from the very beginning their study not only

has contributed to more details on ATP production, but also has led to fundamental insights covering the entire spectrum of biological sciences from anthropology to biophysics to cell biology, genetics, and physiology. Their demonstrated role in disease, and their implied role in neurodegenerative developments in advanced age makes them particularly fashionable now, but a history of biochemistry is unthinkable without reference to mitochondria and the monumental discoveries made in the course of their study.

REFERENCES

1. Lehninger, A. L. (1965) *The Mitochondrion—Molecular Basis of Structure and Function*, W. A. Benjamin, New York.
2. De Duve, C. (1975) *Science* **189**, 186–194.
3. Racker, E. (1976) *A New Look at Mechanisms in Bioenergetics*, Academic Press, New York.
4. Hatefi, Y., Galante, Y. M., Stiggal, D. L., and Ragan, C. I. (1979) *Methods Enzymol.* **56**, 577–602.
5. Hatefi, Y. (1985) *Annu. Rev. Biochem.* **54**, 1015–1069.
6. Sun, F., Huo, X., Zhai, Y., Wang, A., Xu, J., Su, D., Bartlam, M., and Rao, Z. (2005) *Cell* **121**, 1043–1057.
7. Yankovskaya, V., Horsefield, R., Tornroth, S., Luna-Chavez, C., Miyoshi, H., Leger, C., Byrne, B., Cecchini, G., and Iwata, S. (2003) *Science* **299**, 700–704.
8. Cecchini, G. (2003) *Annu. Rev. Biochem.* **72**, 77–109.
9. Xia, D., Yu, C.-A., Kim, H., Xia, J.-Z., Kachurin, A. M., Zhang, L., Yu, L., Deisenhofer, J., Yu, C. A., and Xian, J. Z. (1997) *Science* **277**, 60–66.
10. Tsukihara, T., Aoyama, H., Yamashita, K., Tomizaki, T., Yamaguchi, H., Shinzawa-Iyoh, K., Nakashima, R., Yaono, R., Yoshikawa, S., Yamashita, E., and Shinzawa-Itoh, K. (1996) *Science* **272**, 1136–1144.
11. Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) *Nature* **370**, 621–628.
12. Walker, J. E., Collinson, I. R., Van Raaij, M. J., and Runswick, M. J. (1995) *Methods Enzymol.* **260**, 163–190.
13. Van Raaij, M. J., Abrahams, J. P., Leslie, A. G. W., and Walker, J. E. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 6913–6917.
14. Dickson, V. K., Silvester, J. A., Fearnley, I. M., Leslie, A. G., and Walker, J. E. (2006) *EMBO J.* **25**, 2911–2918.
15. Mitchell, P. (1961) *Nature* **191**, 144–148.
16. Singer, S. J. and Nicolson, G. L. (1972) *Science* **175**, 720–731.
17. Nass, S. and Nass, M. M. K. (1963) *J. Cell. Biol.* **19**, 593–629.
18. Slonimski, P. P. and Ephrussi, B. (1949) *Ann. Inst. Pasteur* **77**, 47.
19. Wallace, D. C., Singh, G., Lott, M. T., Hodge, J. A., Schurr, T. G., Lezza, A. M. S., Elsas, L. J., II, and Nikoskelainen, E. K. (1988) *Science* **242**, 1427–1431.
20. Holt, I. J., Harding, A. E., and Morgan Hughes, J. A. (1988) *Nature* **331**, 717–719.