Perspective

The Mitochondrion Updated

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AN INTERNATIONAL SYMPOSIUM ENTITLED "THE MITOchondrion 1986" in honor of the late Albert Lehninger (1917–1986) was held recently at Johns Hopkins University School of Medicine in Baltimore. This occasion brought together leading contributors to research on mitochondria and bioenergetics over the past four decades. Lehninger's contemporaries at the symposium provided a meaningful historical perspective for the exciting new findings presented on the molecular structure, genetics, and mechanism of action of key components of proton-driven oxidative phosphorylation and transport.

Forty years ago Albert Lehninger and his students Eugene Kennedy and Morris Friedkin (now at Harvard Medical School and University of California at San Diego, respectively) put mitochondria on the metabolic map as the "power plants" of aerobic cells—their raison d'être being energy capture [as adenosine triphosphate (ATP)] by oxidative phosphorylation. Presaging the advent of isolated intact mitochondria, Lehninger and Kennedy had observed that ATP and a catalytic amount of a tricarboxylic acid intermediate were required for the complete oxidation of fatty acids by cell-free liver preparations. Then, with amazing speed after the report in 1948 by George Palade and colleagues (at the Rockefeller Institute for Medical Research, now Rockefeller University) of a new procedure for isolating morphologically intact mitochondria in sucrose-containing media, Lehninger and Kennedy demonstrated that mitochondria isolated by this method catalyzed the complete oxidation of fatty acids to CO_2 and water. In so doing they proved that this organelle possessed all of the enzymatic machinery for the β-oxidation of fatty acids, the tricarboxylic acid cycle, and the electron transport system. Almost concurrently, Lehninger and Friedkin showed that electron transport from the reduced form of nicotinamide adenine dinucleotide (NADH) (produced from fatty acid and tricarboxylic acid cycle oxidations) to oxygen by the mitochondrion is the direct energy source for driving the phosphorylation of adenosine diphosphate (ADP) by inorganic phosphate to form ATP, the process known as respiratory chain–linked oxidative phosphorylation. This and other key contributions from Lehninger's laboratory provided great impetus for the emerging concept of cellular compartmentation of organized metabolic functions.

Lehninger was one of the first investigators of respiratory coupling to seriously consider that there might be a relation between ion transport and energy coupling in mitochondria. Early in the 1960's he and his colleagues presented the first evidence for energy-coupled accumulation of calcium by mitochondria and demonstrated its stoichiometry, two Ca^{2+} ions transported per electron pair traversing each of the three energy-conserving sites of the respiratory chain. This led to the concept, together with the chemiosmotic hypothesis of Peter Mitchell (Glynn Research Institute, Cornwall), that coupled ion movements are central to respiratory chain energy transduction into ATP and other forms.

Proton translocation has moved to center stage, where the major focus is on two key questions: how are proton gradients across biological membranes generated, and how are such proton gradients used to drive the synthesis of ATP and the transport of ions and molecules across the membrane? Impetus for much of this work stems from Mitchell's chemiosmotic hypothesis, which depicts the major oxidation-reduction (redox) complexes of the mitochondrial electron transport chain as devices that, through electron flow, generate an electrochemical gradient of protons across the membrane. This electrochemical gradient is thought to provide the driving force for ATP synthesis, for phosphate uptake (essential for ATP synthesis), and, in brown fat, for heat production.

A major recent advance established in Lehninger's laboratory shows that proton translocation by the mitochondrial redox pump is quantitatively much greater than that realized by Mitchell. Thus, John Lemasters, of the University of North Carolina, reported that the entire mitochondrial electron transport chain, from the initial complex (NADH dehydrogenase) to the terminal complex (cytochrome oxidase), catalyzes the ejection of at least 12 protons per oxygen atom, the number predicted by Lehninger's experiments, and perhaps 13 protons per oxygen atom. Mitchell has also recently acknowledged through his own experiments a higher stoichiometry than he proposed earlier for proton translocation associated with cytochrome oxidase. Proton stoichiometry is critical to, and must be taken into account in, any molecular mechanism for the generation of an electrochemical proton gradient during electron transport by the four major complexes.

Steady progress is being made on the formidable task of determining the structures and elucidating the proton translocating mechanisms of these complexes. Each complex has a subunit structure with as many as 20 different polypeptides (for example, mammalian NADH dehydrogenase) encoded in either the mitochondrial or the nuclear genome. It is likely that a combination of both biochemical and molecular genetic approaches such as those under way in the laboratories of Giuseppe Attardi (California Institute of Technology), Youssef Hatefi (Scripps Clinic and Research Foundation), and C. Ian Ragan (University of Southampton, England) will be needed to assign subunit function and ultimately their molecular mechanisms. Attardi, for example, has generated specific antibodies directed against synthetic peptides, the sequences of which were derived from nucleotide sequences of seven open reading frames that account for more than half of the protein coding capacity of human mitochondrial DNA. By this approach he has identified and assigned the function of each protein product to one of the electron transport complexes.

Another challenging area of mitochondrial research receiving considerable attention concerns the mitochondrial ATP synthase complex (F_0F_1ATPase) and mitochondrial transport proteins. These proteins are involved in the "coupling reactions" that utilize the energy of the electrochemical proton gradient, generated by redox pumps, for ATP synthesis or ion transport. The structure to 9 Å resolution of the F_1 moiety of the rat liver ATP synthase, has been completed by Mario Amzel and Peter Pedersen at Johns Hopkins University School of Medicine. Data collected at a resolution as high as 2.9 Å should soon produce a more refined three-dimensional structure. The location of specific amino acid residues within this structure will be of great interest since many investigations have implicated carboxyl, amino, phenolic, and guanidino groups in the catalytic mechanism of ATP synthesis and ATP hydrolysis.

A new development was reported by Albert Mildvan, also of

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Johns Hopkins, who, with David Fry, has provided a tentative map of the amino acid residues predicted to reside at or near the ATP binding site on the F_1-β subunit, which is presumed to be the catalytic subunit. The map, constructed by comparing amino acid sequence homologies of the F_1-β subunit with those found in adenylate kinase (described by John Walker of the Medical Research Council Laboratory of Molecular Biology at Cambridge), emphasizes the role of a flexible loop closing over the magnesium-dependent ATP (MgATP) binding site and the role of a lysine and several carboxyl residues as candidates for interaction with protons. This is of particular interest since results from Paul Boyer's group at University of California, Los Angeles, and Harvey Penefsky's laboratory at the Public Health Research Institute in New York indicate that the role of the proton gradient may be to release tightly bound ATP from the catalytic site. The specific amino acid residues that provide the proton targets have yet to be identified.

There was considerable discussion of the unusual subunit stoichiometry (that is, $\alpha_3\beta_3\gamma\delta\epsilon$) of the ATP synthase. This unique stoichiometry holds for ATP synthases from animals, bacteria, and chloroplasts and implies asymmetry, since several different approaches indicate that the smaller subunits γ, δ, and ε associate with only one of the three αβ pairs of the ATP synthase assembly, rendering it chemically unique. The asymmetry may have been introduced during evolution and may have produced a bifunctional enzyme in which one of the three αβ pairs is permanently specified for ATP synthesis, whereas the others remained for another ATP-dependent function. Alternatively, the asymmetry of one of the three αβ pairs may allow each αβ pair to participate in turn in "rotational catalysis" during ATP synthesis. The latter view is depicted in models by Boyer, by Mitchell, and by Graeme Cox of the Australian National University.

Insight has been gained into the structure and function of the F_0 moiety, the component of mitochondrial ATP synthase involved in transmitting energy from the electrochemical proton gradient to the F_1 moiety of the enzyme. Walker has succeeded in identifying the polypeptides of the eukaryotic enzyme and in elucidating their primary structures. It is now evident that eukaryotic F_0 contains at least three or four more polypeptides than F_0 of *Escherichia coli*. One of the additional peptides, long known as OSCP, is involved in binding F_0 to F_1, the interface at which the "coupling" between the electrochemical gradient and the F_1 moiety may occur. Evidence for a direct interaction of OSCP with both the α and β subunits of F_1 was provided by Pierre Vignais from the Université Scientifique et Médicale, Grenoble. One F_0 protein, apparently not present in *E. coli* and studied by D. Rao Sanadi of the Boston Biomedical Research Institute, is a dithiol protein. Catia Sorgato, of the University of Padua, presented convincing evidence for the role of dithiols in "proton coupling" between F_0 and F_1. The F_0-F_1 interface thus represents a challenging area for future research.

Important information on the coupling of the electrochemical proton gradient to ion transport across the mitochondrial (and bacterial) inner membrane was presented by several investigators. The proton gradient can be used to drive the mitochondrial uptake of cationic dyes. The observation by Lan Bo Chen of Harvard University Medical School that such molecules are retained much longer in tumor cell mitochondria has important chemotherapeutic implications. Certain cancers, including some human tumors grown in mice, can be controlled or eliminated by targeting cationic chemotherapeutic agents to the mitochondria. The prolonged retention of dyes by tumor mitochondria may be due to occlusion of the outer membrane pore protein by hexokinase, which binds to porin, as found by Richard Nakashima of Johns Hopkins University School of Medicine.

Dramatic new findings were reported by H. Ronald Kaback of the Roche Institute on the lactose permease of bacteria, one of the few proton-dependent transport systems that has been investigated at the level of site-directed mutagenesis. Mutations at amino acid residues believed to be buried in a hydrophobic transmembrane helix (helix 10) (for example, His 322 to Arg or Glu 325 to Gln) drastically inhibit proton translocation. This and other evidence strongly suggest that these neighboring functional groups may conduct protons across the membrane as components of a charge-relay system. This approach is likely to provide definitive answers on the fundamental mechanism of proton-driven transport.

Finally, several new developments, still in their early stages, show great promise for mitochondrial research of the next decade. Alexander Tzagoloff of Columbia University described his studies on the control of mitochondrial gene expression by genetic information resident in nuclear DNA. Until recently the regulatory mechanisms that coordinate an orderly output of the two physically separated genomes have been obscure. A genetic analysis of nuclear mutants of the yeast *Saccharomyces cerevisiae* has revealed at least ten different nuclear genes that affect the synthesis of the mitochondrially encoded respiratory carrier cytochrome b. These nuclear gene products exert their effects at the levels of processing of the cytochrome b pre–messenger RNA, translation of the spliced RNA, and maturation of the apocytochrome. The availability of mutants arrested at different stages of cytochrome b synthesis and of the cloned genes opens the way for detailed molecular analysis of how the two genomes interact.

Fundamental research in bioenergetics is already being effectively applied to problems in human medicine. Douglas Wallace of Emory University stressed that mutations in mitochondrial DNA, because of its maternal inheritance, play an important and disproportionate role in human evolution and hereditary disease. Several mutations have been identified by sequence analysis of the mitochondrial DNA. Since *OXPHOS* genes occur in both the nuclear DNA and mitochondrial DNA, diseases with similar phenotypes may or may not show a Mendelian inheritance pattern. Molecular diagnoses and rational therapy of genetic and acquired defects in energy metabolism are now being greatly facilitated by noninvasive ^{31}P-labeled nuclear magnetic resonance studies of the levels of phosphocreatine, MgATP, and inorganic phosphate in muscles and brains of intact animals and humans, as described by Britton Chance of the University of Pennsylvania.

Albert Lehninger would most certainly have enjoyed and participated fully in "The Mitochondrion 1986" symposium had his untimely death (4 March 1986, at the age of 69) not deprived him of this opportunity. Lehninger's extremely productive career spanned 45 years. In 1942, he received his Ph.D. degree at the University of Wisconsin. In 1945, he was appointed assistant professor of biochemistry and surgery at the University of Chicago. In 1952, he became DeLamar professor and director of the Department of Physiological Chemistry (now Biological Chemistry) at Johns Hopkins University School of Medicine. In 1977, Lehninger was appointed university professor of medical science in Johns Hopkins University School of Medicine in recognition of his many accomplishments in medical science and his service to the university. Albert Lehninger was best known among students for his lucid writings. He wrote several important books, including *The Mitochondrion, Bioenergetics, Principles of Biochemistry*, and, perhaps the most widely read textbook of biochemistry in its time, *Biochemistry*.

The symposium, "The Mitochondrion 1986," which consolidated much of our knowledge on this important subject to the present, was a fitting tribute to the memory and scholarly contributions of Albert Lehninger.