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REVIEW

Oxidative Phosphorylation at the *fin de siècle*

Matti Saraste

Mitochondria produce most of the energy in animal cells by a process called oxidative phosphorylation. Electrons are passed along a series of respiratory enzyme complexes located in the inner mitochondrial membrane, and the energy released by this electron transfer is used to pump protons across the membrane. The resultant electrochemical gradient enables another complex, adenosine 5′-triphosphate (ATP) synthase, to synthesize the energy carrier ATP. Important new mechanistic insights into oxidative phosphorylation have emerged from recent three-dimensional structural analyses of ATP synthase and two of the respiratory enzyme complexes, cytochrome bc_1 and cytochrome c oxidase. This work, and new enzymological studies of ATP synthase's unusual catalytic mechanism, are reviewed here.

Mitochondria generate most of the energy in animal cells. This occurs primarily through oxidative phosphorylation, a process in which electrons are passed along a series of carrier molecules called the electron transport chain. These electrons are generated from NADH (reduced nicotinamide adenine dinucleotide), which is produced by oxidation of nutrients such as glucose, and are ultimately transferred to molecular oxygen. The electron transport chain consists of four respiratory enzyme complexes arranged in a specific orientation in the mitochondrial inner membrane. The passage of electrons between these complexes releases energy that is stored in the form of a proton gradient across the membrane and is then used by ATP synthase to make ATP from ADP (adenosine 5′-diphosphate) and phosphate (Fig. 1).

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Our understanding of the basic principles of oxidative phosphorylation was greatly influenced by several landmark discoveries spanning nearly a century. ATP was discovered by Karl Lohmann in 1929 and its role in muscle contraction was established by Vladimir Engelhardt in 1934. Efraim Racker purified the catalytic component of the mitochondrial ATPase (F_1 or factor 1) in 1961, and in 1997, Paul Boyer and John Walker shared half of the Nobel Prize for the discovery that this enzyme functions in a novel way. Otto Warburg's characterization of "Atmungsferment," the respiratory enzyme, in 1924 established the phenomenon of cell respiration, to which ATP synthesis was linked by Herman Kaclkar in 1937. And perhaps most importantly, in 1961, Peter Mitchell proposed the general mechanistic principle of oxidative and photosynthetic phosphorylation (the chemiosmotic theory), which explains the coupling between respiration and ATP synthesis in mitochondria. This theory remained controversial until the mid-1970s, but is now a paradigm in the intellectual frame-

work of bioenergetics.

Over the last 20 years, research in mitochondrial bioenergetics has shifted from experiments with crude organelle preparations to direct molecular approaches. Three-dimensional structural information at atomic resolution is now available for two respiratory enzyme complexes and the catalytic component of ATP synthase. This information has substantiated some of the earlier thinking on how the respiratory enzymes and ATP synthase operate but, as discussed below, it has also stimulated new ideas. The crystallographic results (*1–3*) have verified that the cytochrome bc_1 complex mediates a proton-motive Q cycle, as proposed by Peter Mitchell. The mechanism of proton pumping in cytochrome oxidase has been constrained by several crystal structures (*4–8*) although the debate on it continues (*9*). And the crystal structure of the F_1-ATPase (adenosine triphosphatase) from bovine heart mitochondria (*10*) confirms the proposal (*11*) that three active sites within the F_1 head function in a rotating manner.

Key membrane protein components of the mitochondrial respiratory enzymes and the ATP synthase are encoded by genes in the mitochondrial DNA, and others are encoded in the nucleus. Directed mutagenesis of these genetically chimeric protein complexes is not yet possible because this requires an efficient means of introducing mutant genes into mitochondria. Since ATP synthase and all enzymes of the mitochondrial respiratory chain have homologs in bacteria (*12, 13*), an alternative approach is to use the bacterial systems for mutagenesis studies. This approach has produced a large amount of information that can be extrapolated to the mitochondrial enzymes. In combination, the results generated by site-directed mutagenesis and three-dimensional structural analyses have opened a new era in functional exploration of the enzymes that carry out oxidative phosphorylation.

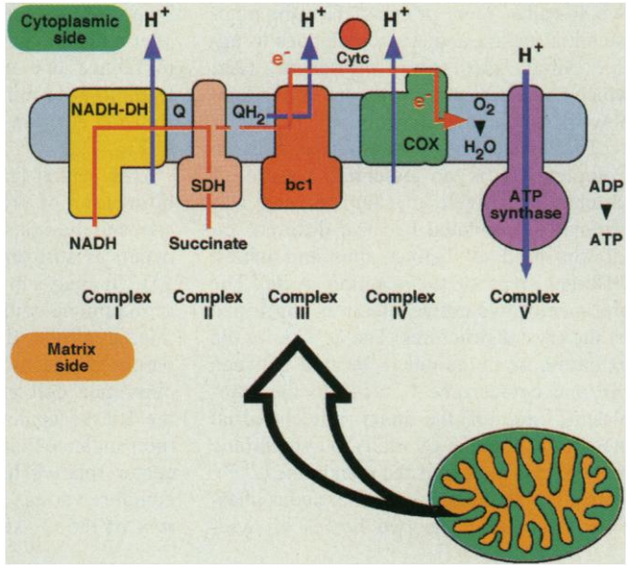

Fig. 1. The enzymes of the mitochondrial inner membrane involved in oxidative phosphorylation. NADH-dehydrogenase (yellow), succinate dehydrogenase (pink), cytochrome bc_1 (red), and cytochrome oxidase (green) form the electron transfer chain to O_2. With the exception of SDH, these enzymes translocate protons across the membrane. The proton gradient is used by ATP synthase (purple) to make ATP.

Complexes I and II

Three membrane-bound enzymes conserve energy in the mitochondrial respiratory chain by active transport of protons across the membrane (Fig. 1). Complex I, or the NADH: ubiquinone oxidoreductase, is the largest of these. The mammalian enzyme contains 42 or 43 different subunits in an unknown stoichiometry, one flavin mononucleotide, seven or eight different FeS centers, covalently bound lipid, and at least three bound quinol molecules (*14–17*). The monomeric Complex I is over 900 kilodaltons (kD), comparable in size to the protein component of the ribosome. Electron microscopy of single particles has revealed that Complex I is an L-shaped structure with two major domains separated by a thin collar (*18, 19*). Attempts have been made to model how proton translocation is coupled to electron transfer in Complex I on the basis of kinet-

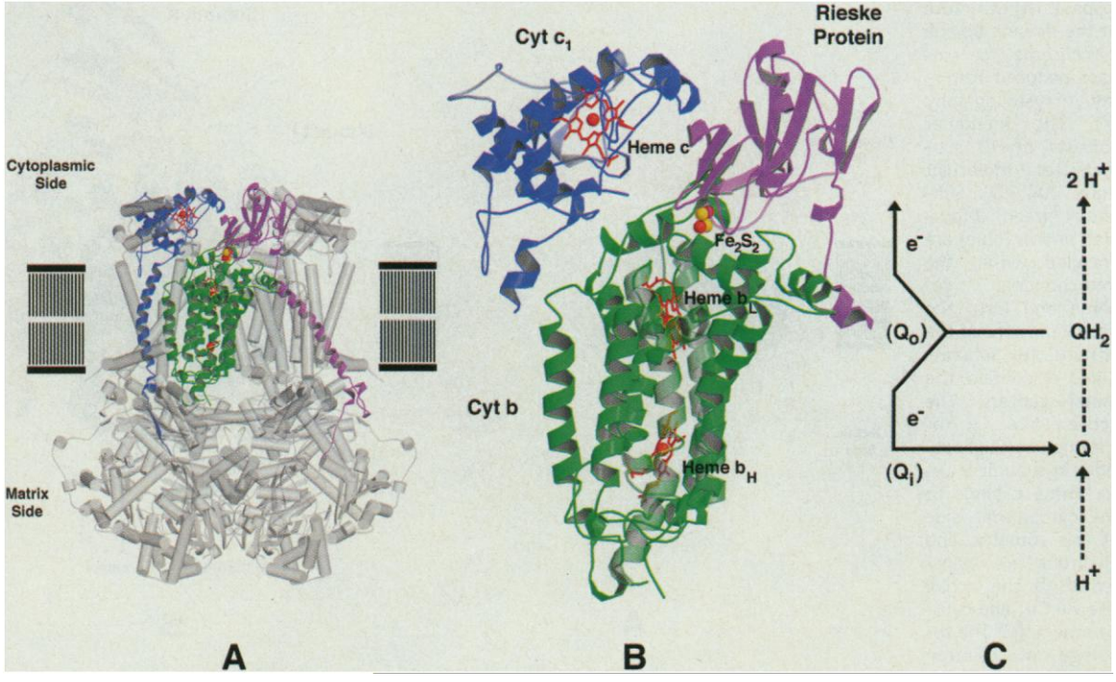

Fig. 2. Cytochrome bc_1 and the Q cycle. **(A)** Structure of the bovine mitochondrial cytochrome bc_1 deduced from x-ray crystallography (*3*). Cytochrome bc_1 is a stable dimer. Each monomer contains eleven subunits (total molecular mass of the monomer is ~240 kD). **(B)** The three subunits that form the functional core of the enzyme are cytochrome b (green), the Rieske ISP (purple), and cytochrome c_1 (blue). The FeS center in this structure is close to the Q_o site; it moves toward cytochrome c_1 after reduction. **(C)** The topology of electron and proton transfer in the Q cycle mechanism. Bifurcation of electron transfer occurs in the Q_o site.

ic, spectroscopic, and inhibitor data [for example, see (*17, 20*)], but verification of these models awaits relevant structural information.

Complex II, or succinate:ubiquinone reductase, is a component of the Krebs cycle and participates in the electron transport chain by transferring electrons from succinate to the ubiquinone pool. It contains FAD (flavin-adenine dinucleotide) and several FeS centers, and is anchored to the membrane by a b-type cytochrome. Complex II does not translocate protons, and therefore it only feeds electrons to the electron transport chain (*21*).

Cytochrome bc_1: Crossroads for Electrons

Cytochrome bc_1 (Complex III), the best understood of the respiratory enzymes, delivers electrons from ubiquinol to cytochrome c. It couples this redox reaction to the generation of a proton gradient across the membrane by a mechanism known as the Q cycle. Ubiquinol is a lipid-soluble compound that can move within the membrane. As the redox chemistry of a quinol is coupled to protonation and deprotonation, these two reactions are topologically organized such that the oxidation of quinols leads to active transport of hydrogen ions across the membrane (Fig. 2). This requires two active sites, one for the oxidation of ubiquinol and release of protons on the outer surface of membrane (Q_o), and one for the reduction of ubiquinone coupled to the uptake of protons from the inner side of the membrane (Q_i). This mechanism requires that electrons be transferred from the Q_o site to the Q_i site (Fig. 2).

The mammalian Complex III contains eleven subunits, but only three of them carry the redox centers that are used in conservation of energy, and only these three have bacterial homologs (*12*). The key subunits are cytochrome b, which has eight transmembrane helices with two hemes sandwiched between helices B and D (Fe-Fe distance 21 Å); a membrane-anchored FeS protein (ISP) carrying a Rieske-type center (Fe_2S_2); and a membrane-anchored cytochrome c_1. Most of the other eight subunits are small proteins that surround the metalloprotein nucleus, but two so-called "core proteins" face the mitochondrial matrix and are homologous to mitochondrial processing peptidases (*22*), which function in protein import. Thus, Complex III may be multifunctional.

The existence of two active sites in Complex III is an essential feature of Mitchell's Q cycle mechanism, and was originally postulated because different inhibitors bind to distinct sites and inhibit different steps of the reaction cycle. The presence of two active sites was confirmed by the crystal structures. The Q_o site for the oxidation of ubiquinol is located between ISP and cytochrome b, close to the cytoplasmic side of the inner mitochondrial membrane, and the Q_i site is in cytochrome b in the matrix side of the membrane (*1–3*). Both sites communicate with aqueous phases by channels. The two hemes of cytochrome b have different redox potentials. The Q_o site is near the b_L heme (low potential), and the Q_i site is near the b_H heme (high potential).

A quinol can donate two electrons. The electron transfer within the cytochrome bc_1 complex is bifurcated such that the first electron is transferred along a high-potential chain to the Rieske FeS center, and then to cytochrome c_1, which delivers it to the soluble cytochrome c. The second electron is transferred to the Q_i site via the hemes b_L and b_H of the cytochrome b subunit. This is an electrogenic step (it creates part of the protonmotive force) that is driven by the difference in redox potentials of the two hemes. Two electrons are transferred to the Q_i site after oxidation of two quinols in the Q_o site, to reduce one quinone. This mechanism leads to a net translocation of two protons for each electron transferred to cytochrome c (Fig. 2).

The central feature of the Q cycle is the bifurcation of the electron paths at the Q_o site, and the exact mechanism by which this occurs is still under discussion (*2, 3, 23–25*). It may involve movement of the semiquinone within the Q_o site after the release of the first electron (*24*) and, in particular, a conformational change that occurs upon delivery of the first electron to the Rieske center (*25*). The crystal structures indicate that the position of the Rieske center relative to the other metals in the complex varies, depending on the occupation of the Q_o site by inhibitors. The ISP

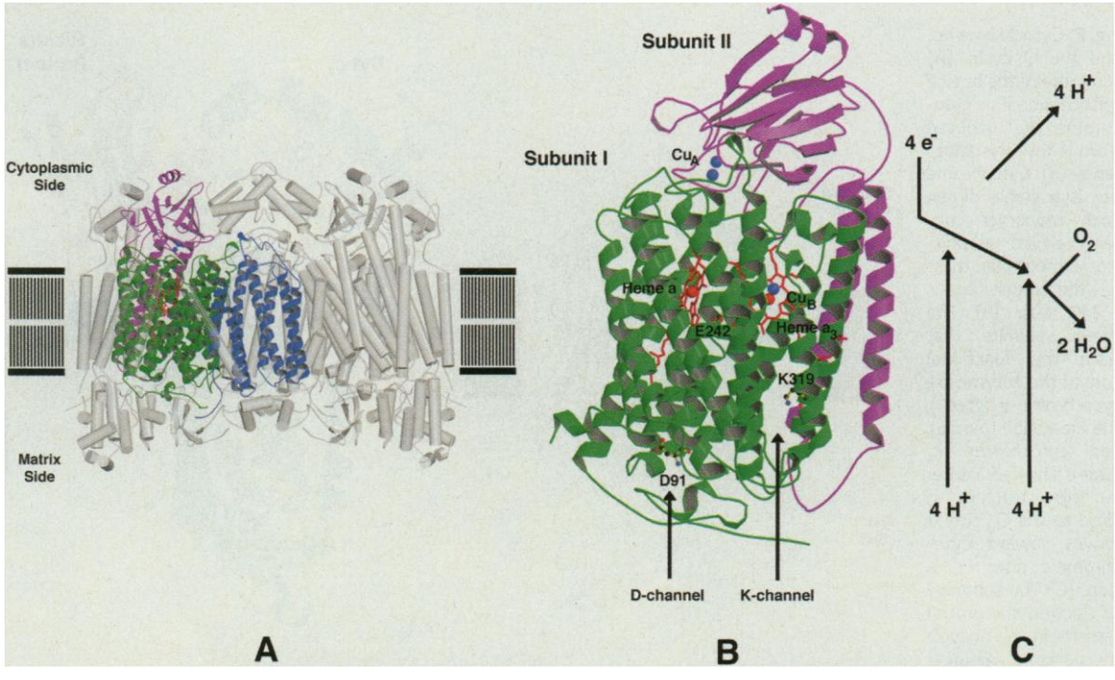

Fig. 3. Cytochrome oxidase. (**A**) Structure of the dimeric bovine cytochrome c oxidase, deduced from x-ray crystallography (*7*). The monomer consists of 13 subunits (total molecular mass 204 kD). Subunits I (green), II (purple), and III (blue) are encoded within the mitochondrial genome and form the functional core of the enzyme. (**B**) Subunits I and II contain the metal centers. The active site (cytochrome a_3/Cu_B) resides in subunit I. Cytochrome c binds to the cytoplasmic side of this complex, and electrons are transferred to the active site via Cu_A and cytochrome a. (**C**) The topology of electron and proton transfer in cytochrome oxidase. Protons that are used to reduce O_2 into water or pumped to the cytoplasmic side of the mitochondrial inner membrane are transferred through two channels (D and K) from the matrix side.

subunit has an NH_2-terminal membrane anchor that is linked by a hinge region to the headpiece domain containing the FeS center. The crystal structures (*2, 3*) suggest that the headpiece can rotate by about 60 degrees. This means that the FeS center can move about 20 Å away from the Q_o site after its reduction with the first electron and establish a better contact with cytochrome c_1 to deliver the electron. The domain movement may shuttle the electron from the Q_o site to cytochrome c_1 and it would also guarantee that the second electron goes down to the Q_i site rather than taking the same route as the first electron (*24, 25*). This type of domain movement would be unique among redox protein complexes (*2*).

Cytochrome Oxidase: A Proton Pump

Cytochrome oxidase (Complex IV) generates a transmembrane proton gradient by a different mechanism than cytochrome bc_1. Its substrate, cytochrome c, is a water-soluble hemoprotein that donates electrons on the cytoplasmic side of the mitochondrial inner membrane. These electrons are transferred to the active site, which contains a heme iron and a copper, and they are used to reduce O_2 into two water molecules. The protons needed for this reaction are taken from the mitochondrial matrix side through two channels. The same channels are used to pump one proton per electron across the membrane (Fig. 3).

The bovine cytochrome oxidase contains 13 subunits (*7, 8*). The three major subunits are coded for by mitochondrial DNA and form the functional core of the enzyme; this core is surrounded by 10 nuclear-coded small subunits. Subunit I contains the active site. Subunit II has a dinuclear, mixed valence copper center (Cu_A) (*26, 27*), which is the first site to receive electrons from cytochrome c. These electrons are transferred to a low-spin heme (cytochrome a) in subunit I, and then to the bimetallic cytochrome a_3/Cu_B active site (Fig. 3). The two hemes and Cu_B are ligated by six histidines. One of the Cu_B ligands (H240 in the bovine enzyme) forms a covalent bond with a tyrosine (Y244) from which it is separated by one helical turn (*6, 8*). The histidine-tyrosine adduct may generate a free radical that plays a role in the reduction of O_2. Subunit III contains bound phospholipids but its functional role has not been established.

In contrast to cytochrome bc_1, which exhibits dynamic behavior, cytochrome oxidase in its oxidized, reduced, and ligand-bound forms appears to be static. Although one would expect reduction to mobilize amino acids that participate in proton pumping, the bovine enzyme, in fact, undergoes only a minor conformational change that affects one loop in subunit I facing the cytoplasm (*8*). This loop is not remarkably conserved among the mitochondrial and bacterial enzymes and is not likely to participate in any general mechanism involved in energy conservation.

Two hydrophilic channels connect the active site to the aqueous phase of the mitochondrial matrix. These channels are called D and K after a conserved aspartate and lysine, respectively (*28*). A conserved glutamate (E242) in the middle of the membrane, at the end of the D channel, is essential for proton pumping activity (*28, 29*). During the reduction of oxygen, both protons that are consumed in this reaction and protons that are actively translocated enter through D and K channels. Mutational analysis has shown that both channels are essential for the full catalytic cycle (*9, 28–30*). Translocated protons have to pass through a hydrophobic barrier within the enzyme to enter the cytoplasmic aqueous phase. This passage may in part be achieved by chains of water molecules (which have not yet been localized in the structure), and is almost certainly regulated by the principle of electroneutrality (*31*), coordinated transfer of negative and positive charges (electrons and protons) during the turnover of oxygen into water.

The mechanism by which the translocated protons pass through the hydrophobic barrier is not known. Structural information initially indicated that ligand coordination around the copper atom of the active site (Cu_B) is dynamic because one of its three histidine ligands had a weak electron density in the crystal structure (*4*). This observation supported the "histidine cycle" hypothesis (*32*), which proposes that two protons are simultaneously carried through the barrier by a moving histidine. Why would one need such a mechanism?

Oxygen is reduced in a cycle that includes several intermediates. The first well-established intermediate starting from the oxidized enzyme (O) is a species (R) in which both metals of the active site are reduced after acceptance of two electrons. Oxygen is bound to R, which leads to the peroxy intermediate (P). Acceptance of the third electron results in formation of water and a ferryl intermediate (F), and finally, the fourth electron pushes the system back to the O state with release of the second water molecule (*33*). The formation of P and F intermediates can be studied within the intact mitochondrial membrane (*34*). The correlation of the occupancy of these two states with membrane and phosphorylation potentials led Mårten Wikström to propose that only the two last steps of the O - R - P - F - O cycle are involved in proton pumping, and that both steps appear to pump two protons to match the overall stoichiometry of $4H^+/4e^-$. A simple mechanism to achieve this is to have histidine do the work, because it is the only amino acid that can carry two protons.

Nevertheless, recent crystallographic studies have refuted the histidine cycle hypothesis. All histidine ligands of the metal atoms in subunit I are visible in higher resolution structures (*5, 8*) and, in particular, the metal coordination does not appear to change upon reduction of the enzyme. This has initiated a search for an alternative mechanism. Hartmut Michel has recently reanalyzed Wikström's classic experiment (*34*) and proposed a new mechanism that is based on a detailed analysis of current structural data (*9*). He suggests that there is no need to invoke a carrier that takes two protons across the hydrophobic barrier at the same time; rather, proton translocation can be achieved by an interplay between the conserved glutamate and propionates of the two hemes. Such an interaction has been demonstrated by Fourier transform

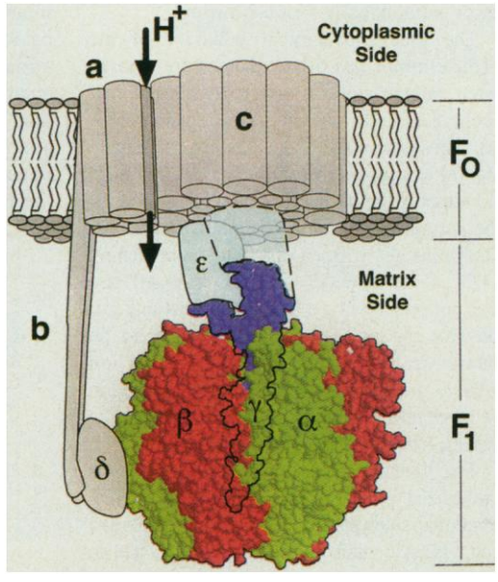

Fig. 4. ATP synthase. Essential structural features of ATP synthase deduced in part from X-ray crystallography. The green, red and purple components correspond to the crystal structure of the bovine mitochondrial F_1 ATPase (*10*). The remaining components correspond to the crystal structure of the bacterial enzyme (*37*). The membrane sector (F_0) contains an oligomer of subunit c that rotates when protons move from the cytoplasmic side of the membrane (up) to the mitochondrial matrix (down). A dodecamer of subunit c is connected to a complex of subunits γ and ε, forming the rotor. Subunit a, a dimer of subunit b, and subunit δ form the stator arm, which has an interface with the oligomeric subunit c and links with the F_1 head. The active sites are present in the three β subunits. Reproduced from *Nature* (*48*) with permission.

infrared spectrocopy (*35, 36*).

Despite the wealth of mutagenesis and structural data, the proton-pumping mechanism in cytochrome oxidase remains unclear and will likely be clarified by further structural, spectroscopic, and other biophysical studies.

ATP Synthase: The Smallest Motor

The mitochondrial ATP synthase (F_1F_0 ATPase or Complex V) is a functionally reversible enzyme—it can synthesize ATP using a protonmotive force across the membrane and it can hydrolyze ATP to pump protons against an electrochemical gradient. The bovine enzyme appears to contain 16 different proteins (*37*) and is greater than 500 kD in size. A membrane sector (F_0) contains the proton channel. It is linked to the catalytic component (F_1), located in the matrix side of the membrane, by a stalk consisting of two parallel structures (*38*) referred to as a "rotor" and a "stator" (*39*) (Fig. 4). A soluble ATPase (F_1) can be detached from the complex, and it contains five different subunits—α, β, γ, δ, and ε—in a stoichiometry 3:3:1:1:1. The α and β subunits are homologous; both bind nucleotides but only β has catalytic activity. Thus, there are three active sites within the catalytic component. According to Boyer's binding exchange mechanism (*11*), each site would pass through a cycle of three different states ("open," "loose," and "tight," corresponding to an empty state, a state with bound ADP and phosphate, and a state with tightly bound ATP), and at any given moment, the three sites would be in a different state. Boyer and others have shown that the formation of ATP does not require energy once the substrates have been separated from the aqueous solution. Energy is required for substrate binding and the release of ATP (*11*).

The crystal structure of the bovine F_1 ATPase (*10*) revealed the intrinsic asymmetry of the enzyme, a finding that supports Boyer's mechanism and suggests that the enzyme operates by rotational catalysis. The contacts between the central γ subunit and the β subunits were critical factors in the development of the rotational catalysis model. The β (and α) subunits are three-domain structures (*10, 40, 41*). Their NH_2-terminal domains form a barrel of β-sheets that keeps the hexamer together, and the nucleotide binding sites are located at the interface of the other two domains. The γ subunit contacts the COOH-terminal domain of the β subunits (*10*), helping it to open and close with respect to the middle domain. Rotation of the γ subunit inside the $\alpha_3\beta_3$ hexamer would thus facilitate binding of the substrates and release of the product.

There is now strong experimental evidence that a central structure rotates inside the F_1 ATPase during catalysis. This evidence derives from (i) measurements of anisotropy of polarized absorption after photobleaching of a fluorescent label (eosin) attached to the tip of the γ subunit within the immobilized F_1 (*42, 43*), (ii) chemical cross-linking experiments (*44*), and (iii) video microscopy of single ATPase molecules during catalysis (*45–47*).

Masasuke Yoshida and colleagues have performed rotation studies with the smallest possible assembly, the $\alpha_3\beta_3\gamma$ complex. The α and β subunits were engineered to contain NH_2-terminal polyhistidine tags and assembled with a γ subunit containing a biotinylated cysteine exposed to the solution. The assembly was immobilized on nickel-coated beads, and a fluorescent and biotinylated actin rod was attached with streptavidin to the labeled end of the γ subunit. Addition of ATP to the solution caused counter-clockwise rotation of the actin filament, which the authors recorded by video microscopy (*45, 46*).

Yoshida's experimental setup allows a detailed analysis of the rotation performed by single F_1 ATPase molecules. The angular velocity can be correlated with the length of the attached actin filament (load), and the ATP concentration can be varied. From the "load experiments," the work associated with a step of 120° can be estimated. The frictional torque per step is very close to the free energy of hydrolysis of one ATP molecule under physiological conditions, suggesting that the ATPase motor operates with almost 100% efficiency (*46, 47*). At low ATP concentrations, individual steps become visible, and backward stepping is also observed. This indicates that unidirectionality of rotation is an intrinsic property of F_1 and is due to the binding exchange mechanism. ATP hydrolysis causes a step toward an empty site, which can bind the substrate for next reaction, leaving ADP and phosphate behind to be dispelled into solution. At low concentrations of substrate, the binding of ATP to the active sites becomes rate-limiting.

The mechanochemical behavior of the ATPase motor has been modeled by George Oster and co-workers using hydrodynamic, mechanical, and structural constraints (*39, 48*). These simulations agree with the experimental results of Yoshida and colleagues, and support the high efficiency of the motor. The estimated torque (*48*) indicates that the motor operates with an efficiency approaching 100%. Oster's model proposes that ATPase does not operate like a heat engine but rather it converts energy of nucleotide binding to an elastic strain. The energy driving rotation of the γ subunit is presumed to be localized in the mechanical string at the hinge between the moving lobes of the β subunit (*48*).

The fundamental question of how ATP is synthesized by F_1F_0 remains to be answered. Rotational catalysis implies that the membrane sector must contain a structure that rotates in response to protonmotive force. One candidate structure is an oligomer formed by subunit c, a protein present in 12 copies in F_0 (*49*). This would lead to result in translocation of 4 H^+ per one ATP molecule synthesized. Each subunit c contains a conserved carboxylic acid residue in the middle of the membrane bilayer. The protonation and deprotonation of this residue may be at the heart of the rotary mechanism. However, a necessary structural contraint of such a mechanism is that other components move in the opposite direction during dynamic [electrostatic (*39*)] interactions in the membrane (Fig. 4). These components would form a stator structure that interacts with the rotor in the membrane. A key component of the stator is subunit a, which contains a conserved arginine that could counteract the moving glutamate in subunit c. The current model proposes that the dodecamer of subunit c forms the rotor with the γ and ε subunits, and the subunit a, b, and δ complex forms the stator arm. Proton movement through the interface between subunit a and the subunit c oligomer would cause a torque when the stator and rotor move in the opposite directions [see (*39, 48*) and references therein].

A better understanding of the mechanism of ATP synthesis is likely to come when the three-dimensional structure of the entire ATP synthase has been determined.

Epilogue

The term "*fin de siècle*" connotes the feeling of tiredness prevalent in certain Western cultures at the end of the last century. Although some have argued that the same spirit can be detected as we approach the 21st century (*50*), this spirit, fortunately, does not apply to modern biology. On the contrary, research on bioenergetics, for instance, has been invigorated by new approaches in enzymology, structural biology, and biophysics. Traditional research on intact mitochondria, which established the basic principles of oxidative phosphorylation, can now be complemented by mechanistic studies, which require an accuracy that can only be provided by molecular approaches. Many questions still remain unanswered, but these are challenges for the next century.

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REVIEW

The Machinery of Mitochondrial Inheritance and Behavior

Michael P. Yaffe

The distribution of mitochondria to daughter cells during cell division is an essential feature of cell proliferation. Until recently, it was commonly believed that inheritance of mitochondria and other organelles was a passive process, a consequence of their random diffusion throughout the cytoplasm. A growing recognition of the reticular morphology of mitochondria in many living cells, the association of mitochondria with the cytoskeleton, and the coordinated movements of mitochondria during cellular division and differentiation has illuminated the necessity for a cellular machinery that mediates mitochondrial behavior. Characterization of the underlying molecular components of this machinery is providing insight into mechanisms regulating mitochondrial morphology and distribution.

Mitochondria have long been recognized as prominent and vital residents of the cytoplasm of eukaryotic cells. These ubiquitous organelles were identified 50 years ago as the site of oxidative energy metabolism (*1*). Subsequent studies have uncovered myriad mitochondrial proteins that catalyze numerous biosynthetic and degradative reactions fundamental to cell function (*2*). These activities depend on a distinctive mitochondrial structure, with different enzymes and reactions localized in discrete membranes and aqueous compartments. The characteristic mitochondrial structural organization is the product of both local synthesis of macromolecules within the mitochondria and the import of proteins and lipids synthesized outside the organelle (*3*). Synthesis and import of mitochondrial components are required for mitochondrial proliferation, but rather than producing new organelles, these processes facilitate the growth of preexisting mitochondria. Because the mitochondrial membranes and the mitochondrial DNA must serve as essential templates for the growth of the organelle, mitochondrial continuity requires the transmission of mitochondria to daughter cells before every cell division.

Mitochondria display an amazing plasticity of form and distribution. Although their internal structural organization is highly conserved, the external shape of mitochondria is variable. In addition to the classic kidney bean–shaped organelles observed in electron micrographs, mitochondria are frequently found as extended reticular networks (*4*) (Fig. 1). These networks are extremely dynamic in growing cells, with tubular sections dividing in half, branching, and fusing to create a fluid tubular web (*5*). In differentiated cells, such as those found in cardiac muscle or kidney tubules, mitochondria are often localized to specific cytoplasmic regions rather than randomly distributed (*6*). Some alterations in mitochondrial shape and distribution are developmentally programmed, with characteristic mitochondrial migrations or morphological changes occurring at key stages in cellular differentiation (*7*, *8*). Additionally, alterations in mitochondrial distribution and morphology are associated with a variety of pathological conditions, including liver disease (*9*), muscular dystrophy (*10*), cardiomyopathy (*11*), and cancer (*12*).

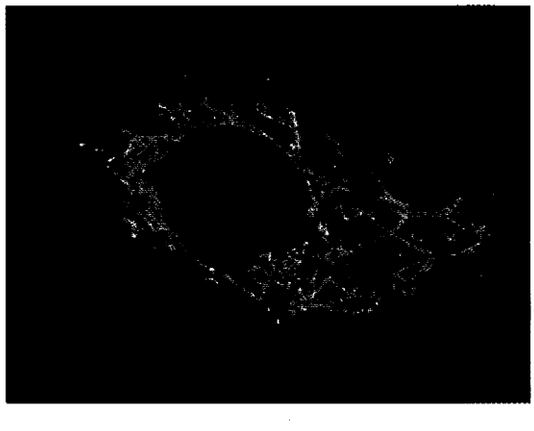

Fig. 1. Mitochondrial network in a mammalian fibroblast. A COS-7 cell labeled to visualize mitochondria (green) and microtubules (red) was analyzed by indirect immunofluorescence confocal microscopy. Mitochondria were labeled with antibodies to the β subunit of the F_1-ATPase and a rhodamine-conjugated secondary antibody. Microtubules were labeled with antibody to tubulin and a fluorescein-conjugated secondary antibody. Pseudocolor was added to the digitized image. Scale: 1 cm = 10 μm.

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