ATP SYNTHESIS BY ROTARY CATALYSIS

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by

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Biological energy comes from the sun. Light energy harvested by photosynthesis in chloroplasts and phototropic bacteria, becomes stored in carbohydrates and fats. This stored energy can be released by oxidative metabolism in the form of adenosine triphosphate (ATP), and used as fuel for other biological processes. ATP is a high energy product of both photosynthesis and oxidative metabolism, and in textbooks, it is often referred to as the chemical currency of biological energy.

In the 1960s and early 1970s, the field of oxidative metabolism was dominated by a debate about the nature of the intermediate between NADH (a key product from carbohydrate and fat metabolism) and ATP itself. Many bioenergeticists believed in and sought evidence for a high energy covalent chemical intermediate. The issue was resolved by Peter Mitchell, the Nobel Laureate in Chemistry in 1978. He established that in mitochondria, energy is released from NADH via the electron transport chain and used to generate a chemical potential gradient for protons across the inner membrane of the organelle. He referred to this gradient as the proton motive force (pmf; also designated as $\Delta\mu_{H+}$). It was demonstrated that the pmf is harnessed by the ATP synthesizing enzyme (ATP synthase) to drive the synthesis of ATP from ADP and inorganic phosphate, not only in mitochondria, but also in eubacteria and chloroplasts [1].

Because of Peter Mitchell's efforts, the pmf became established as a key intermediate in biological energy conversion. In addition to being employed in ATP synthesis, it is also used by various membrane bound proteins to drive the transport of sugars, amino acids and other substrates and metabolites across biological membranes. The pmf powers the rotation of flagellae in motile bacteria. In newly born children and in hibernating animals, it is converted directly into heat by uncoupling the mitochondria in brown adipose tissue. Some bacteria that live in saline conditions generate a sodium motive force to act as an equivalent intermediate in their energy conversion processes [2]. The general notion of creating a proton (or sodium) motive force and then using it as a source of energy for other biochemical functions is known as chemiosmosis.

Today, the general outlines of chemiosmosis are well established. It is accepted that during electron transport in mitochondria, redox energy derived from NADH is used by three proton pumping enzymes called complex I

(NADH:ubiquinone oxidoreductase), complex III (ubiquinone:cytochrome c oxidoreductase) and complex IV (cytochrome c oxidase). They act consecutively and produce the pmf by ejecting protons from the matrix (the inside) of the organelle (see Figure 1). Until recently, the workings of these chemiosmotic proton pumps was obscure, but currently our understanding is being transformed by the application of modern methods of molecular biology for the analysis of their structures and functions. For example, two independent atomic resolution structures of cytochrome c oxidase isolated from bovine mitochondria [3, 4] and from the bacterium, *Paracoccus denitrificans* [5, 6], are guiding mutational and spectroscopic experiments that are providing new insights into its mechanism. Partial structures of mitochondrial complex III from two different species [7] will have a similar impact soon on our understanding of that enzmye. Complex I, the third proton pump in mitochondria, is an assembly of at least 43 different polypeptides in mammals, with a combined molecular mass in excess of 900,000 [8]. In addition, it has a non-covalently bound flavin mononucletide, and at least five iron-sulphur clusters that act as redox centres. A consequence of this extreme complexity is that the structural analysis of complex I is less advanced than those of complexes III and IV, although the general outline of the complex has been established by electron microscopy [9, 10].

THE ATP SYNTHASE

Since 1978, my colleagues and I have concentrated on analyzing the structure of the ATP synthase, another multisubunit complex from mitochondria, where it is found in the inner membrane alongside the three proton pumping enzymes (see Figure 1). Similar complexes are found in chloroplast and eubacterial membranes. Throughout our endeavours, we have been motivated by the expectation that detailed knowledge of its structure would lead to a deeper understanding of how ATP is made. A substantial part of our efforts has been directed at establishing the subunit compositions of the ATP synthesizing enzymes from various sources, and with determining the primary sequences of the subunits [11–31]. These rather extensive analyses helped to show that the overall structure of the ATP synthase, and hence the general principles governing its operation, are very similar in mitochondria, chloroplasts and eubacteria, although the enzymes from the various sources differ in the details of both their sequences and subunit compositions (see Table 1). It is also known that the ATPases from various sources differ in the mechanisms that regulate their catalytic activities [32].

In 1962, the inside surface of the inner membranes of bovine heart mitochondria was found by electron microscopic examination to be lined with mushroom shaped knobs about 100 Å in diameter (Figure 2A) [33]. Later on, similar structures were found to be associated with the thylakoid membranes of chloroplasts (Figure 2B) [34], and with the inner membranes of eubacteria (Figure 2C). At the time of their discovery, the function of these membrane bound knobs was not known, but they were thought to be proba-

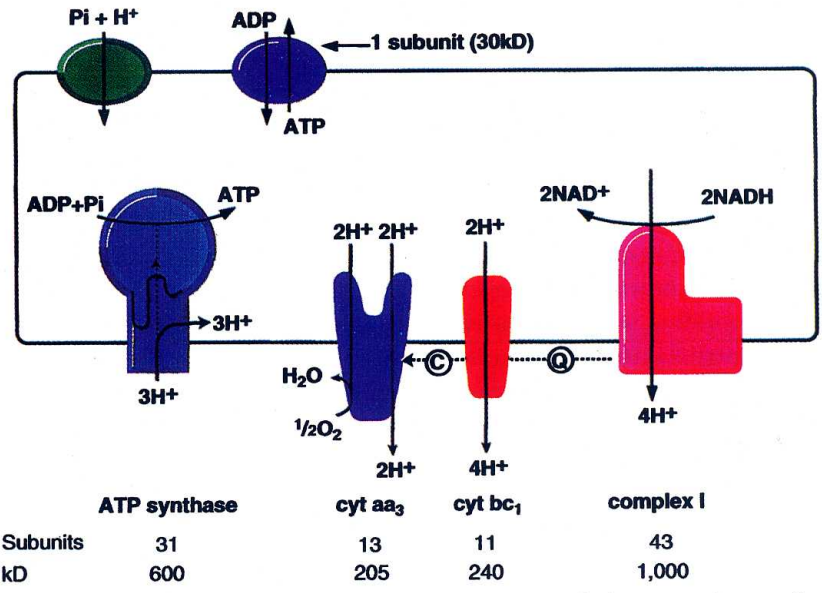

Figure 1. The enzyme complexes of oxidative phophorylation in the inner membranes of mammalian mitochondria. Three proton pumps, complex I, complex III (cytochrome bc_1) and complex IV (cytochrome aa_3) convert redox energy in NADH into the proton motive force (pmf) by ejecting protons from the matrix of the mitochondrion. The ATP synthase uses the energy of the pmf to produce ATP from ADP and phosphate. ADP and phosphate are brought into the mitochondrion by related proetin carriers. External ADP is exchanged for internal ATP, making the newly synthesized ATP available for many biological functions. Dotted lines indicate electron pathways. Q and C are the mobile electron carriers ubiquinone and cytochrome c, respectively.

Table 1. Equivalent subunits in ATP synthases in bacteria, chloroplasts, and bovine mitochondria

Type	Bacteria	Chloroplasts	Mitochondria
F_1	α	α	α
	β	β	β
	γ	γ	γ
	δ	δ	OSCP
	ε	ε	δ
	–	–	ε
F_0	a	a (or x)	a (or ATPase 6)
	b[a]	b and b′ (or I and II)	b
	c	c (or III)	c
Supernumerary	–	–	F_6
	–	–	inhibitor
	–	–	A6L
	–	–	d
	–	–	e
	–	–	f
	–	–	g

[a] ATP synthases in *E. coli* and bacterium PS 3 (both eight-subunit enzymes) have two identical copies of subunit b per complex. Purple non-sulphur bacteria and cyanobacteria appear to have nine different subunits, the extra subunits (known as b′) being a homologue of b. Similarly, chloroplast enzymes are made of nine non-identical subunits, and the chloroplast subunits known as I and II are the homologues of b and b′.

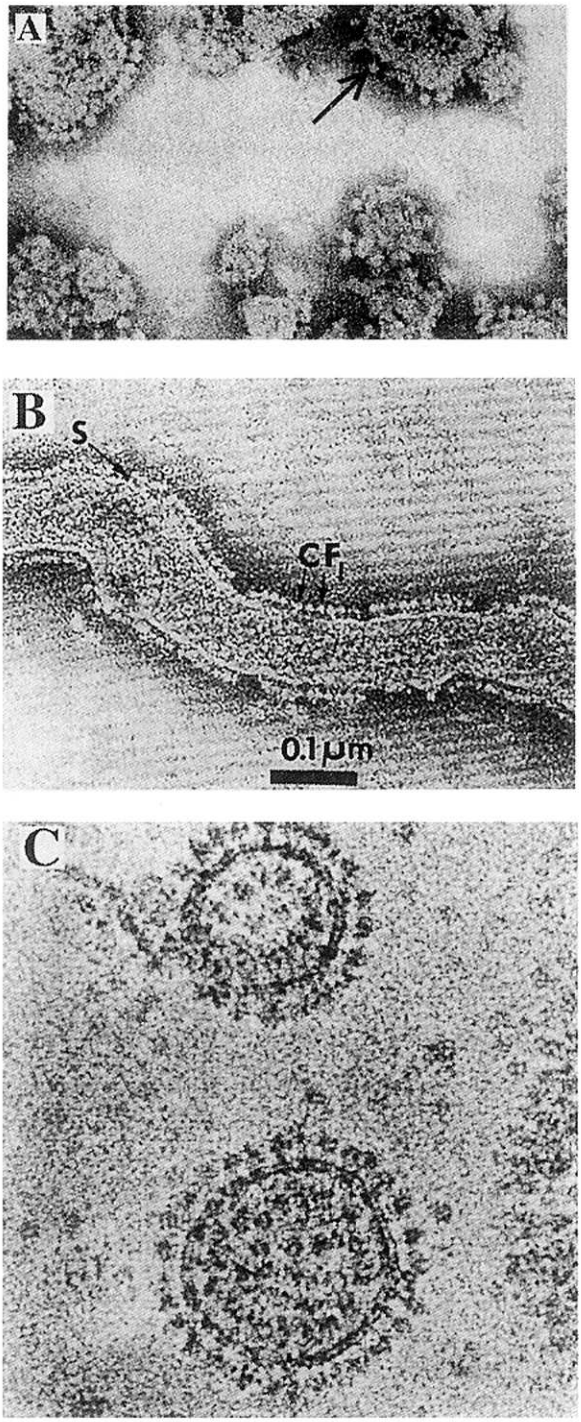

Figure 2. Knobs associated with biological membranes detected by electron microscopy in negative stain. (A), Inside-out vesicles from bovine heart mitochondria; (B), thylakoid membranes form pea chloroplasts; (C), inside-out vesicles from *E. coli*. Different magnifications have been used in parts (A)–(C), and the knobs are all about 100 Å in diameter. Reproduced with permission from references [88, 89].

bly important in biological energy conversion, and hence they were named "the fundamental particles of biology" [35]. In a brilliant series of biochemical reconstitution experiments conducted in the 1960s, Efraim Racker established that they were the ATP synthesizing enzyme complex (for example see references [36–38], and hence that these early micrographs were the first glimpses of its structure.

We now know that the head of the mushroom is a globular protein complex (known as the F_1 domain), where the catalytic sites of the ATP synthase lie. The F_1 part is attached to the membrane sector by a slender stalk about 45 Å long. The hydrophobic membrane domain (known as F_o) transports protons back through the energized membrane into the matrix, somehow releasing energy in this process and making it available to drive ATP synthesis in the catalytic F_1 domain. For some years, it has been accepted that three protons are transported back through the F_o membrane sector for each ATP molecule that is formed in F_1 [1]. However, recent experiments suggest that the chloroplast enzyme transports four protons through the membrane for each ATP that is made [39]. Therefore, it appears that either one or, less likely, both of these values of the H^+:ATP ratio are incorrect, or that the mechanisms and structures of the mitochondrial and chloroplast enzymes differ significantly in their F_o domains. This point will be elaborated later in a consideration of the possible structure and mechanism of F_o.

The general model of the mechanism of the ATP synthase that will be developed below is that the F_o membrane domain contains a rotating molecular motor fuelled by the proton motive force. It is proposed that this motor is mechanically coupled to the stalk region of the enzyme, and that the rotation of the stalk affects the catalytic domain and makes the three catalytic sites pass through a cycle of conformational states in which first, substrates are bound and sequestered, then second, ATP is formed from the sequestered substrates, and finally the newly synthesized ATP is released from the enzyme. This cycle of interconversion of the three catalytic sites is part of a binding change mechanism of ATP synthesis developed by Paul Boyer (see Figure 3)

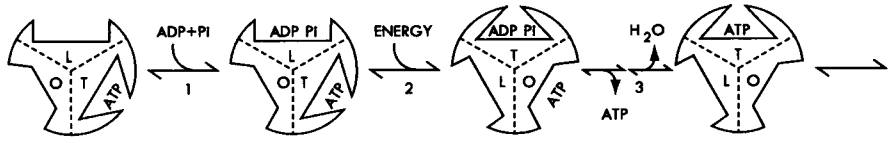

Figure 3. An energy dependent binding change mechanism of ATP synthesis. The catalytic sites in the β-subunits interact and interconvert between three forms: O, open, site with very low affinity for substrates and catalytically inactive; L, loose site, loosely binding substrates and catalytically inactive; T, tight site, tightly binding substrates and catalytically active. The proton induced conformational changes convert a T-site with bound ATP into an open site, releasing the bound nucleotide. Concomittantly, an L-site with loosely bound ADP and phosphate is converted to a T-site where substrates are bound and ATP froms. Fresh substrates bind to an O-site converting it to an L-site, and so on. One third of the catalytic cycle is illustrated. Reproduced with permission from reference [40].

[40]. One of its basic tenets is that the energy requiring steps in this cycle of ATP synthesis are in the binding of substrates and in the release of products. This mechanism also implies that the ATP synthase must be an asymmetrical structure, and structural asymmetry is also implicit in the molar ratios of the subunits of F_1-ATPase. Indeed, it is now clear from the asymmetrical features that are inherent in the enzyme's structure that there can be no structurally symmetrical states in the catalytic cycle.

THE STRUCTURE OF F_1-ATPase

An important practical point about the ATP synthase complex that has influenced the strategy for analyzing its structure is that, as Racker had shown in the 1960s, the globular catalytic domain F_1-ATPase can be detached from the membrane domain and studied separately in aqueous solution. Subsequently, Alan Senior and Harvey Penefsky purified the bovine F_1 complex [41, 42] and Penefsky demonstrated that it is an assembly of five different kinds of polypeptides, which he called α, β, γ, δ and ε [42]. Eventually, it was accepted that they were assembled in the complex in the molar ratios 3α:3β:1γ:1δ:1ε. Hence, each F_1 particle is an assembly of nine polypeptides, and in the bovine heart enzyme, their combined molecular mass is about 371,000 (see Table 2).

Table 2. The subunits of bovine F_1-ATPase

Subunit	MWt	Function
α	55,247	Nucleotide binding
β	51,705	Nucleotides, catalysis
γ	30,141	Link to F_o
δ	15,065	Stalk
ε	5,632	Stalk
α3β3γ1δ1ε1	371,694	

Both α- and β-subunits bind nucleotides, and we now know that the catalytic nucleotide binding sites lie almost entirely within the β-subunits (see Figure 4). The nucleotides bound to α-subunits remain associated during the catalytic cycle and do not participate directly in ATP synthesis. What they are doing remains mysterious, although both structural and regulatory functions have been suggested.

In 1981, we found that the sequences of the α- and β-subunits were related weakly through most of their length [12, 13], and Matti Saraste and I wondered which regions of the sequences were contributing to the nucleotide binding sites. By examination of the known primary and atomic structures of adenylate kinase [43], and of the sequence of myosin from *Caenorhabditis elegans* (at the time unpublished information, made available to me by Jonathan Karn), we were able to propose that two short degenerate sequence motifs

common to adenylate kinase, myosin and the α- and β-subunits, of F_1-ATPase were involved in helping to form their nucleotide binding pockets [44].

This proposal has had far greater consequences than we ever imagined at the time of its publication in 1982. Over the years, one of the two motifs (see

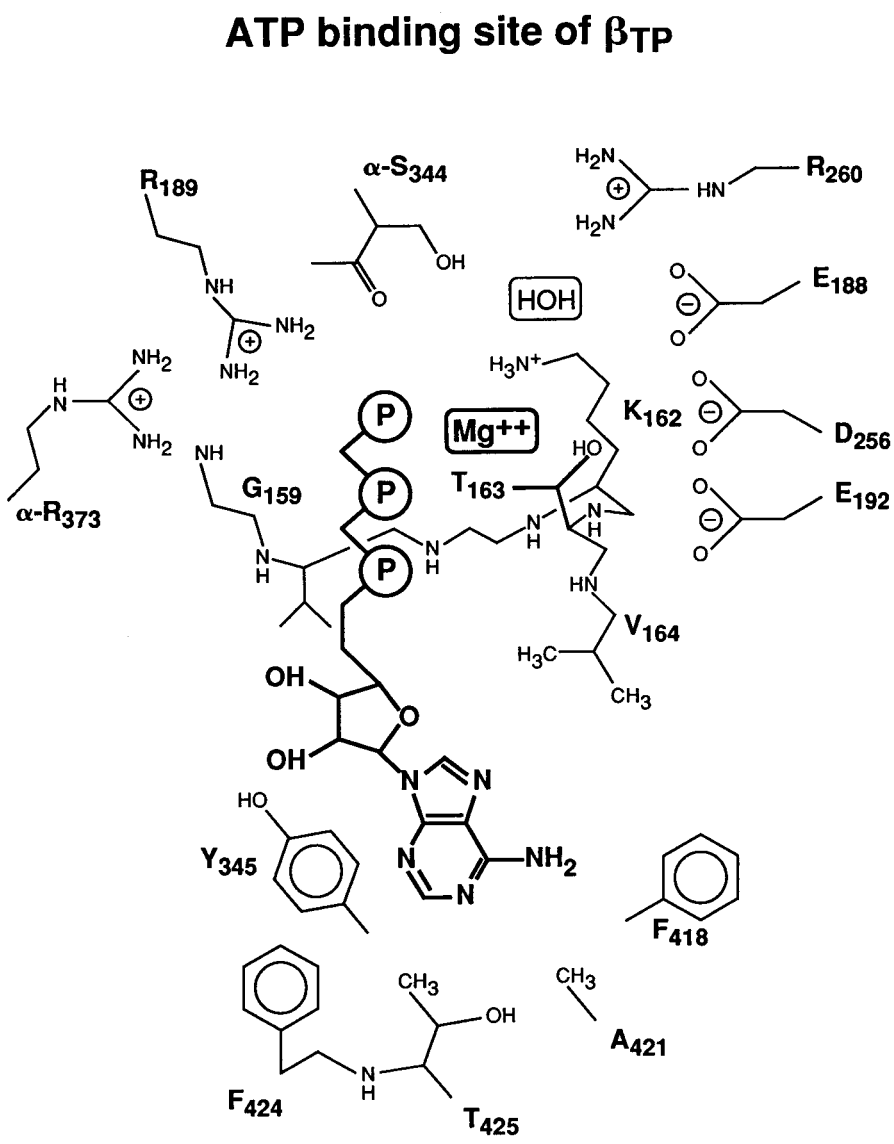

Figure 4. The nucleotide binding site in the bTP-subunit of bovine F_1-ATPase. Except for αTP-Arg373 and the main chain carbonyl and the side-chain of αTP-Ser344, all of the amino acids are in bTP. Residues 159–164 are part of the P-loop sequence. The ordered water molecule is poised for nucleophilic attack on the terminal phosphate and is activated by Glu188. As an incipient negative charge develops on the terminal phosphate in a penta-coordinate transition state of ATP hydrolysis, it will be stabilized by the guanidinium of α-Arg 373. Reproduced with permission from reference [50].

Figure 5) has become established as a reliable indicator of the presence of purine nucleotide binding sites in proteins of known sequence, but of unknown biochemical function. One spectacular demonstration of its predictive value has been in the identification of members of the widely dispersed family of ABC (adenosine binding cassette) transport proteins [45], which includes the cystic fibrosis protein and multi-drug resistance proteins. Another early success was its help in the identification of the oncogene protein p21 as a GTPase [46]. The atomic structure of F_1-ATPase described below, has shown that, as in other proteins, the two sequence motifs describe amino acids that are involved in forming the phosphate binding region of its nucleotide binding sites. For this reason, one of the sequences is often referred to as the P-loop (phosphate binding loop) sequence [47] (see Figure 5).

In the determination of the atomic structure of F_1-ATPase from bovine heart mitochondria, the key problem that had to be solved was how to grow crystals of the protein complex that would diffract X-rays to appropriately high resolution. Crystals were obtained at the beginning of our efforts, but, as often happens, they diffracted X-rays rather poorly. Therefore, over a period

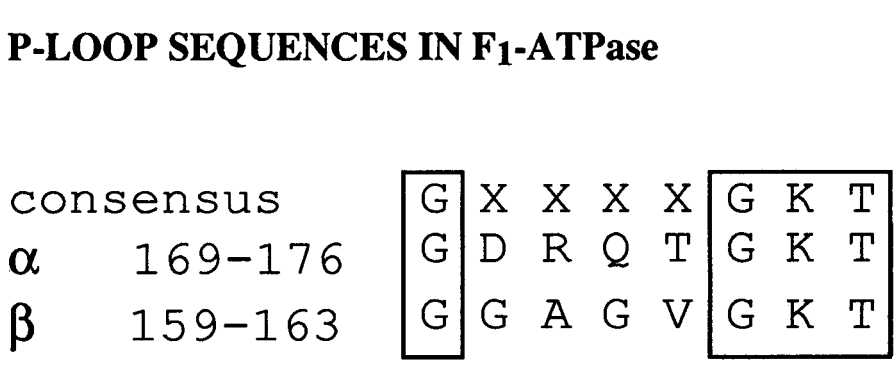

Figure 5. The phosphate binding loop sequence motif in the α- and β-subunits of bovine F_1-ATPase. In some purine binding sites, the final threonine is replaced by a serine residue.

of seven years, factors influencing crystal formation were studied systematically, and the diffraction properties of the crystals were improved gradually. Eventually, suitable cystals were obtained in 1990. In retrospect, the important factors for obtaining these crystals were to use highly pure preparations of enzyme from which trace impurities had been eliminated, to remove endogenous bound nucleotides and to replace them with a non-hydrolyzable chemical analogue of ATP, namely 5'-adenylylimidodiphosphate (AMP-PNP) which has the effect of locking the complex in a unique conformation, and to grow the crystals in the presence of deuterium oxide instead of water. By exposing the crystals to X-rays at a synchrotron source, it was demonstrated that crystals of bovine F_1-ATPase grown under these conditions diffracted to at least 2.8 Å resolution [48, 49]. Drs. René Lutter and Rose Todd were key collaborators during this critical period. At this point, for the first time the struc-

tural analysis of F_1-ATPase appeared to be a realistic possibility. Our chances of success with such a large protein complex were increased significantly by collaborating with Dr. Andrew Leslie, who is a professional protein crystallographer. Together with him and a post-doctoral visitor, Dr. J. P. Abrahams, we were able to arrive at an atomic resolution structure [50] surprisingly rapidly, given the size of the complex, and the associated problems of collecting and processing the X-ray diffraction data.

The structural model of bovine F_1-ATPase contains 2,983 amino acids. Except for short disordered stretches at their N-terminals, the sequences of the α- and β-subunits were traced in their entirety. Three α-helical segments of the γ-subunit corresponding to residues 1–45, 73–90, and 209–272 were also built into the model. The segments linking these three segments are also disordered, as are the entire δ- and ε-subunits (150 and 50 amino acids respectively). These disordered regions of the γ-, δ- and ε-subunits, comprising in total about 300 amino acids, probably lie beneath the $\alpha_3\beta_3$ sub-complex, where the γ-subunit protrudes from the structure (the γ-subunit is blue in Figures 6 and 7). The protrusion is probably a vestige of the 45 Å stalk that links the F_1 and F_o domains in the complete ATP synthase complex.

The structural model shows that the three α-subunits and the three β-subunits are arranged in alternation around a sixfold axis of pseudo-symmetry provided by an α-helical structure in the single γ-subunit (see Figures 6 and 7). Despite the large excesses of AMP-PNP and ADP in the mother liquor surrounding the crystals, five, and not six, nucleotides are bound to each enzyme complex. An AMP-PNP molecule is found in each α-subunit and a fourth one in one β-subunit. An ADP molecule is bound to the second β-subunit, and the third β-subunit has no bound nucleotide at all. A comparison of the structures of the three chemically identical β-subunits in the F_1 complex provides an explanation. The two catalytic β-subunits that have AMP-PNP and ADP bound to them (known as β_{TP} and β_{DP}, respectively; see Figures 7b and 7c) have different but rather similar conformations, whereas the structure of the third β-subunit, to which no nucleotide has bound (known as β_E; see Figure 7d), differs substantially from the other two, particularly in the central domain where the nucleotides are bound in β_{TP} and β_{DP}. In β_E, the C-terminal half of this central domain, together with the bundle of six α-helices that form the C-terminal domain, have rotated away from the sixfold axis of pseudo-symmetry. This disruption of the central domain removes the capacity of the β_E-subunit to bind nucleotides. Therefore, the crystal structure of F_1-ATPase contains the asymmetry required by the binding change mechanism, and the three different conformations of the subunits β_E, β_{TP} and β_{DP} could be interpreted as representing the "open", "loose" and "tight" states, respectively.

More recent extensive crystallographic analyses of F_1-ATPase with bound antibiotic inhibitors [51, 52], of enzyme occupied with ADP and ATP (K. Braig, M. Montgomery, A. G. W. Leslie and J. E. Walker, unpublished work), of enzyme inhibited by ADP and aluminium fluoride (K. Braig, I. Menz, M. Montgomery, A. G. W. Leslie and J. E. Walker, unpublished work), and of en-

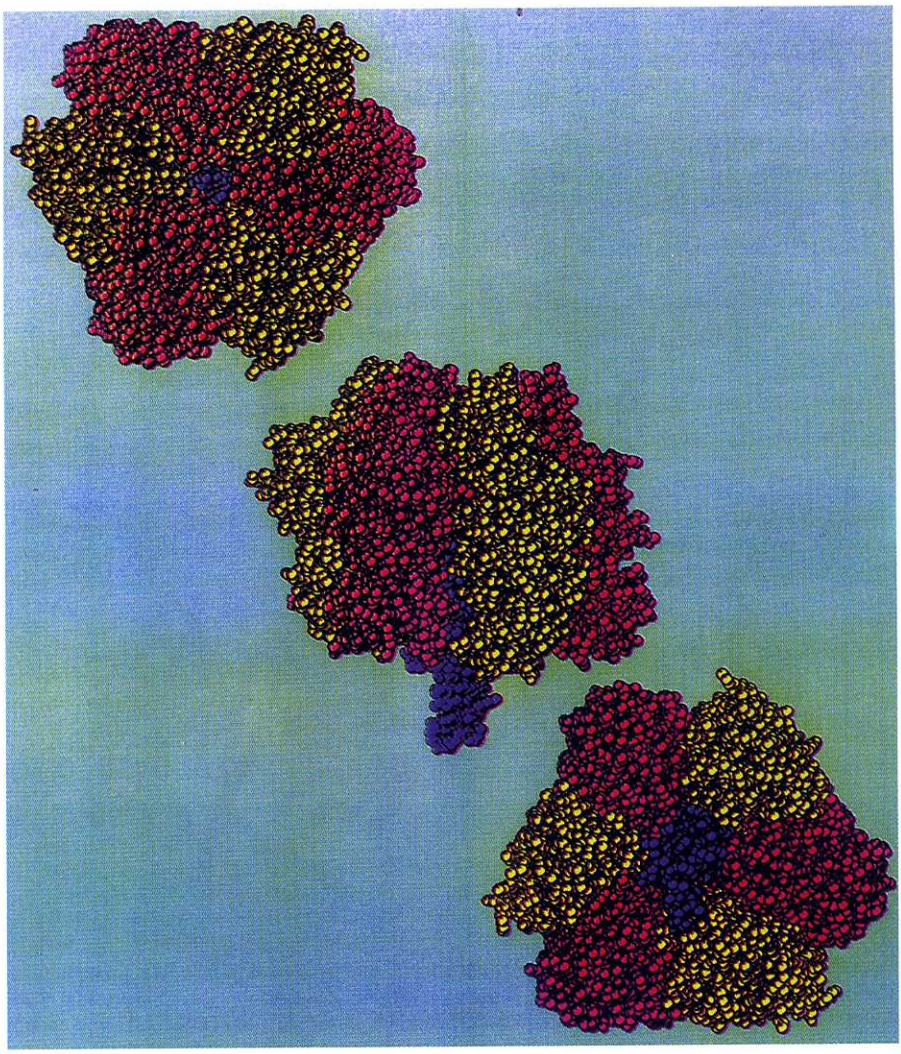

Figure 6. The three-dimensional structure of bovine F_1-ATPase shown in solid representation. The red, yellow and blue parts correspond to α-, β- and γ-subunits respecively. From top to bottom, respectively, the complex is viewed from above (towards the membrane in the intact ATP synthase), from the side, and from beneath.

zyme covalently inhibited with 4-chloro-7-nitrobenzofluorazan (G. Orriss, A. G. W. Leslie and J. E. Walker, unpublished work), leave little room for any doubt that the high resolution structure described above represents a state in the active cycle of the enzyme.

The most exciting aspect of the structure is that it suggests a mechanism for interconverting the three catalytic subunits through the cycle of conformations required by the binding change mechanism. It appears from the structure (Figure 7) that the nucleotide binding properties of the three catalytic β-subunits are modulated by the central α-helical structure in the γ-sub-

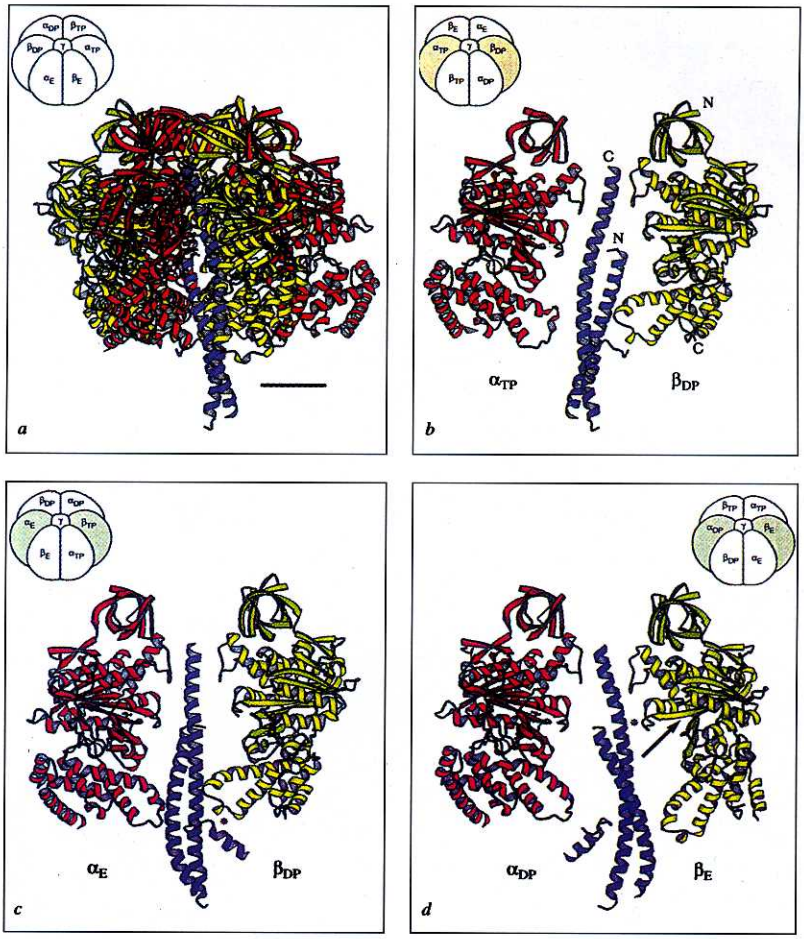

Figure 7. The three-dimensional structure of bovine F_1-ATPase shown in ribbon representation. The colour code for subunits is the same as used in Figure 6, and nucleotides are black, in a "ball-and-stick" representation. The axis of pseudo-symmetry is vertical. AMP-PNP is bound to the three α-subunits, and to the β-subunit defined as β_{TP}. Subunit β_{DP} has bound ADP and subunit BE has no associated nucleotide. Subunits αTP, αE and αDP occupy the same relative positions as the corresponding β-subunits, but are rotated by -60°. The relationships of the various α- and β-subunits to each other is summarized in the icon in the top left or right corner of parts (A)–(D). In parts (C)–(D), the shaded part of the icon shows which subunits are depicted. Subunit α_{TP} contributes to the nucleotide binding site of β_{TP}, and similarly for α_{DP} and α_E. Subunits α and γ are numbered from 1–510 and 1–272, respectively. By convention, the fifth amino acid (serine) in subunit β is residue 1 and the first four amino acids (Ala.Ala.Gln.Ala) are referred to as residues –1 to –4. The C-terminal amino acid is residue 478. (A) A view of the entire F_1 particle in which subunits α_E and β_E point towards the viewer, revealing the anti-parallel coiled-coil of the N- and C-terminal helices of the γ-subunit through the open interface between them. The bar is 20 Å long. (B) Subunits α_{TP}, γ and β_{DP} from a similar viewpoint to (A), but rotated 180° about the axis of pseudo-symmetry. The N- and C-termini of the β- and γ-subunit are shown. (C) Subunits α_E, g and β_{TP} from a similar viewpoint to (A), but rotated by -60°. The asterisk indicates an interaction of the loop containing the DELSEED sequence and the γ-subunit. (D) Subunits α_{DP}, γ, and β_E from a similar viewpoint to (A), but rotated by 60°. The arrow indicates the disruption of the β-sheet in the nucleotide binding domain. β_E-Asp 316, β_E-Thr 318 and β_E-Asp 323 in a loop of the nucleotide binding domain make H-bonds with residues γ-Arg 254 and γ-Gln 255 from the C-terminal helix, 6 Å below the hydrophobic sleeve. The asterisk indicates a loop that makes an interaction with the C-terminal part of the γ-subunit. Reproduced with permission from reference [50].

unit. This structure is curved, and its coiled-coil region is likely to have rigidity. In the crystal structure, its curvature appears to be imposing the "open state" on the β_E subunit by pushing against the C-terminal domain of the protein, forcing the nucleotide binding domain to split and hinge outwards, thereby removing its nucleotide binding capacity. Therefore, by inspection of the model, the simplest way of interconverting the three conformations of β-subunits would be to rotate this central α-helical structure. As it is rotated, the curvature of the γ-subunit moves away from the β_E conformation, allowing that subunit to close, progessively entrapping substrates and allowing ATP to form spontaneously. At the same time, the next β-subunit will be progressively opened by the effect of the curvature of the γ-subunit, allowing the ATP that has already formed in its nucleotide binding site to be released.

EVIDENCE FOR A ROTARY MECHANISM IN ATP SYNTHASE

The suggestion that ATP synthesis involves the cyclic modulation of nucleotide binding properties of the three catalytic β-subunits by rotation of the γ-subunit was attractive because it provided a reasonable structural basis for the binding change mechanism. However, the proposal was based upon the interpretation of a static atomic model, and there was no proof that the enzyme operated in this way in reality. Therefore, in the laboratories of Richard Cross, Rod Capaldi and Wolfgang Junge, various experiments were carried out to test the rotary hypothesis [53–55]. These experiments were all consistent with the rotary model, and they provided convincing evidence of the movement of the γ-subunit through 90–240°, but conclusive proof of repeated net rotations through 360° was lacking.

Early in 1997, clear evidence of continuous rotation of the γ-subunit relative to the surrounding $\alpha_3\beta_3$ subcomplex was provided by a spectacular experiment conducted at the Tokyo Institute of Technology by Masasuke Yoshida and colleagues [56]. The essence of this experiment (summarized in Figure 8) was to bind the $\alpha_3\beta_3\gamma_1$ sub-complex to a nickel coated glass surface in a unique orientation, by introducing the nickel binding sequence $(\text{histidine})_{10}$ at the N-terminals of the β-subunits. A cysteine residue, introduced by mutagenesis into the exposed tip of the γ-subunit, distal from the nickel surface, was biotinylated, thereby allowing a fluorescently labelled biotinylated actin filament to be attached via an intermediate streptavidin molecule, which has four biotin binding sites. The actin filaments were 1–3 μm long, and their ATP dependent anticlockwise rotation could be seen in a fluorescence microscope. The rate of rotation was approximately once per second, about one fiftieth of the rate anticipated from the turnover number of the fully active enzyme. The reduced activity can be attributed to the load of the actin filament and to the absence of the δ- and ε-subunits.

In addition to its impact in providing direct visual proof of the rotation of the γ-subunit, one crucially important aspect of this experiment is that it establishes the order of interconversion of the three conformations of β-sub-

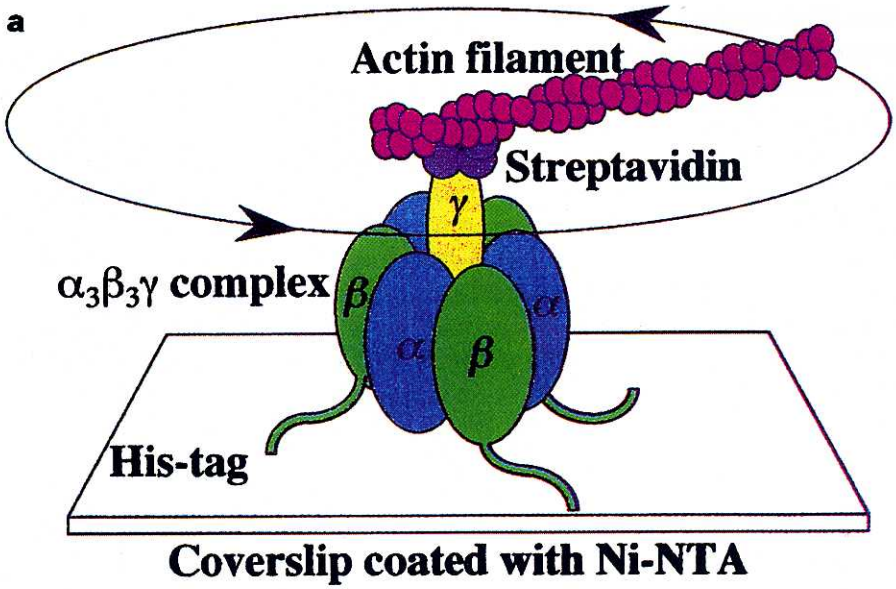

Figure 8. Experimental observation of the ATP-depenedent rotation of the γ-subunit of the $\alpha_3\beta_3\gamma_1$ sub-complex. For explanation, see the text. Reproduced with permission from reference [56].

units observed in the crystal structure. During ATP hydrolysis, the β_{DP} subunit converts into the β_E state, β_E changes to β_{TP} and β_{TP} changes to β_{DP}. During ATP synthesis, it is reasonable to assume that the γ-subunit rotates in the opposite direction, and that the order of conformational changes in the β-subunits is the reverse of those occurring during ATP hydrolysis.

GENERATION OF ROTATION BY PROTON TRANSPORT THROUGH THE F_0 MEMBRANE DOMAIN

Once convincing evidence of rotation of the γ-subunit had been obtained, among the next questions for consideration were: how is rotation generated by proton transport through F_o, and what is the nature of the connections between the F_1 and the F_o domains? High resolution structure is likely to be crucial in providing clear answers to both questions, but as yet there is no such structure for either the F_o domain or the central stalk between F_1 and F_o, except for the ordered protrusion of the γ-subunit. Structures of the isolated ε-subunit [57, 58] and of a fragment of subunit d [59] of the *E. coli* enzyme have been established. The bacterial ε-subunit (equivalent to the bovine δ-subunit [60]) probably interacts with the γ-protrusion, and the bacterial δ-subunit (equivalent of the bovine OSCP subunit [60]) appears to interact with the N-terminal region of the α-subunits (*vide infra*), but the precise locations and functions of these proteins in ATP synthase are unclear.

Despite the lack of a detailed structure of the F_o domain, evidence about the arrangement of the membrane subunits is accumulating. The simplest F_o

domain characterized so far is the one found in eubacterial enzymes exemplified by *E. coli*. It has three constituent subunits named a, b and c assembled in the molar ratios $a_1b_2c_{9-12}$ [61, 62]. The uncertainty in the number of c subunits per complex is a consequence of the experimental difficulties associated with making appropriate measurements. Secondary structural models for all three subunits have been advanced by interpretation of the sequences of the bacterial F_o subunits [63]. That of subunit a has been interpreted variously as indicating the presence of five, six or seven hydrophobic membrane spanning subunits in the protein, but recent experimental evidence indicates that the correct value is five [64]. In its C-terminal and in penultimate α-helices are found positively charged amino acids that are essential for a functional F_o. Subunit b is anchored in the membrane by a single N-terminal α-helix [65]. The remainder of this protein is highly charged and may form a homo-dimer by making a parallel α-helical coiled-coil. This polar extramembrane region interacts with subunits in F_1 [66]. The model of subunit c has two antiparallel transmembrane α-helices linked by an extra-membranous loop, and this has been shown by nmr studies to be the structure of the protein in a chloroform-methanol solvent mixture [64]. Cysteine residues introduced by mutation into the loop region and into the tip of the γ-subunit form a disulphide link under oxidizing conditions, showing that in the intact enzyme, these regions are either in contact (possibly transiently) or close to each other [67]. Similar kinds of experiment provide evidence of interactions between subunits e and c [68]. The most important feature of subunit c is the side chain carboxyl of an aspartate residue, which is buried in the membrane in the C-terminal α-helix. This carboxyl group is conserved throughout all known sequences of c-subunits, and it is required for transmembrane proton transport. [61, 63] Depending on the exact number of subunits c per complex, there are 9–12 such buried carboxyls in each ATP synthase complex.

The mitochondrial ATP synthase complex contains subunits that are equivalent to subunits a and c (see Table 1). Mitochondrial subunit b is also probably the equivalent of its bacterial homonym, although it appears to have two anti-parallel transmembrane a-helices at its N-terminus, rather than one, and there is only one b-subunit per enzyme complex. Presumably the role of the second b-subunit in the bacterial complex is performed by other subunits that are unique to the mitochondrial complex (for example subunits d and F_6), but such matters will only become clear when more detailed structural evidence becomes available for both bacterial and mitochondrial enzymes. Nonetheless, the bacterial and mitochondrial F_o domains have many features in common, and it is highly probable that they operate by very similar mechanisms.

Models have also been advanced about the association of the F_o subunits in the membrane domain. One such model suggests that the c-subunits form a ring by interactions though their C-terminal α-helices, with the N-terminal α-helices outside the ring [69], and annular structures of c-subunits have been visualized by atomic force microscopy [70, 71]. Some preliminary experimental evidence of this arrangement has also been obtained by formation of

disulphide cross links after introduction of cysteine residues at appropriate sites (N. J. Glavas & J. E. Walker, unpublished work). In addition, the atomic force microscopy images indicate that the b-subunits are placed peripheral to a ring of c-subunits [71]. Currently, there is no experimental evidence that shows whether the a subunit lies outside or within the c-annulus.

Wolfgang Junge has proposed a model of how proton transport through F_o might generate a rotary motion [72] (see Figure 9). The essence of this hypothetical rotary motor is that the essential carboxyls in the c subunits are arranged around the external circumference of the c-annulus. Part of the external surface of the annulus interacts with subunit a, and in this region the carboxyls are negatively charged. It is envisaged that subunit a has an inlet port on the external surface of the membrane which allows a proton to neutralize one of the negatively charged carboxylates. The resulting un-ionized carboxyl will find its way by thermal vibrations to its preferred environment in contact with the phospholipid bilayer. The neutralization of this carboxylate at one point of the circumference is accompanied by re-ionization of another one further around the circumference of the c-annulus, by release of the proton on the opposite side of the membrane via an exit port in subunit a, and regeneration of a negative charge in the c-annulus:subunit a interface. These protonation-deprotonation events result in a rotary movement of the c-annulus. The rotation brings the next negatively charged carboxyl to the inlet port

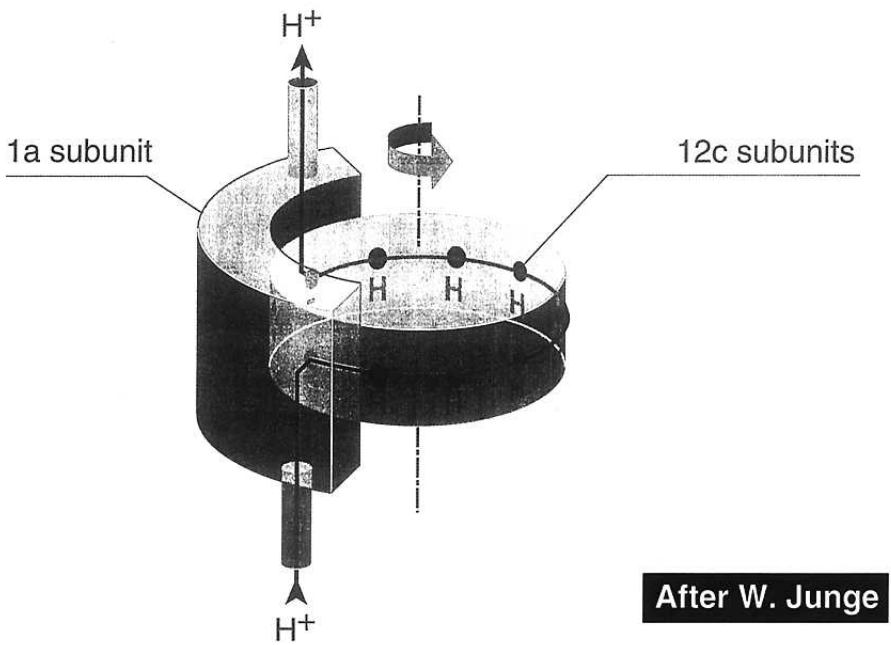

Figure 9. A hypothetical model for generation of rotation by proton transport through the F_o domain of ATP synthase. The central cylindrical part is made of c-subunits, the external curved structure is the single a subunit. The heavy black line indicates the path of the protons. For further explanation, see the text.

where in turn it is neutralized by another proton. The accompanying release of another proton via the exit port generates further rotation. It is envisaged that this rotary device is directly coupled to the γ-subunit.

The synthesis of each ATP molecule requires a rotation of the γ-subunit by 120°, and so each complete rotation of the γ-subunit in F_1 produces three ATP molecules. In a hypothetical proton motor with a c-annulus consisting of twelve c-subunits, this corresponds to the sequential neutralization of four carboxylates by proton binding (and accompanying sequential proton release from four others). In other words, a H^+: ATP ratio of four is compatible with a molar ratio of c-subunits in F_o of twelve. Likewise, a H^+:ATP ratio of three requires nine c-subunits in each F_o.

CONNECTIONS BETWEEN THE F_1 AND F_o DOMAINS OF ATP SYNTHASE

The evidence for the interaction of the central γ-subunit and the associated ε-subunit in bacterial F_1 with the loop region of a c-subunit in F_o has been described above. It is likely that these proteins are the principal components making the central interaction between F_1 and F_o, visualized as the central 45 Å stalk. There is accumulating evidence for a second link between F_1 and F_o. It is known that the bacterial δ-subunit and the equivalent bovine OSCP subunit interact with the N-terminal part of the α-subunits [73, 74], which are now known to be on top of F_1 distal from the membrane domain. Since the 1960s, it has been known that bovine OSCP is required for binding F_1 to F_o, implying that it interacts with subunits in the membrane domain. Therefore, the inescapable conclusion is that OSCP (and possibly also subunit δ in the bacterial and chloroplast enzymes) extends from the top of F_1, down its external surface, to a region associated with the membrane domain. In the bovine enzyme, the N-terminal part of OSCP interacts with the N-terminal part of the a-subunits [75, 76]. Additionally, the interactions of bovine OSCP with various F_o components have been studied by *in vitro* reconstitution experiments [77]. They indicate that OSCP binds mainly to the polar extramembranous part of subunit b (referred to as b′), and not with subunits d and F_6. However, subunits d and F_6 bind to the b′-OSCP complex to forming a stoichiometric complex containing one copy of each of the four proteins. This complex (unfortunately named as "stalk") makes no strong interactions with a complex of bovine subunits δ and ε [78], consistent with the bovine "stalk" complex being separate from the central 45 Å stalk.

By single particle analysis of negatively stained samples of monodisperse-bovine ATP synthase [79–82], Simone Karrasch has obtained electron microscopic evidence for a second peripheral stalk (see Figure 10). A similar feature has been observed independently in the *E. coli* enzyme (S. Wilkens and R. A. Capaldi, personal communication), and in a V-type ATPase (a relative of ATP synthase) from *Clostridium fervidus* [83]. The image of the bovine enzyme also contains another novel feature, seen as a disc shaped structure or collar

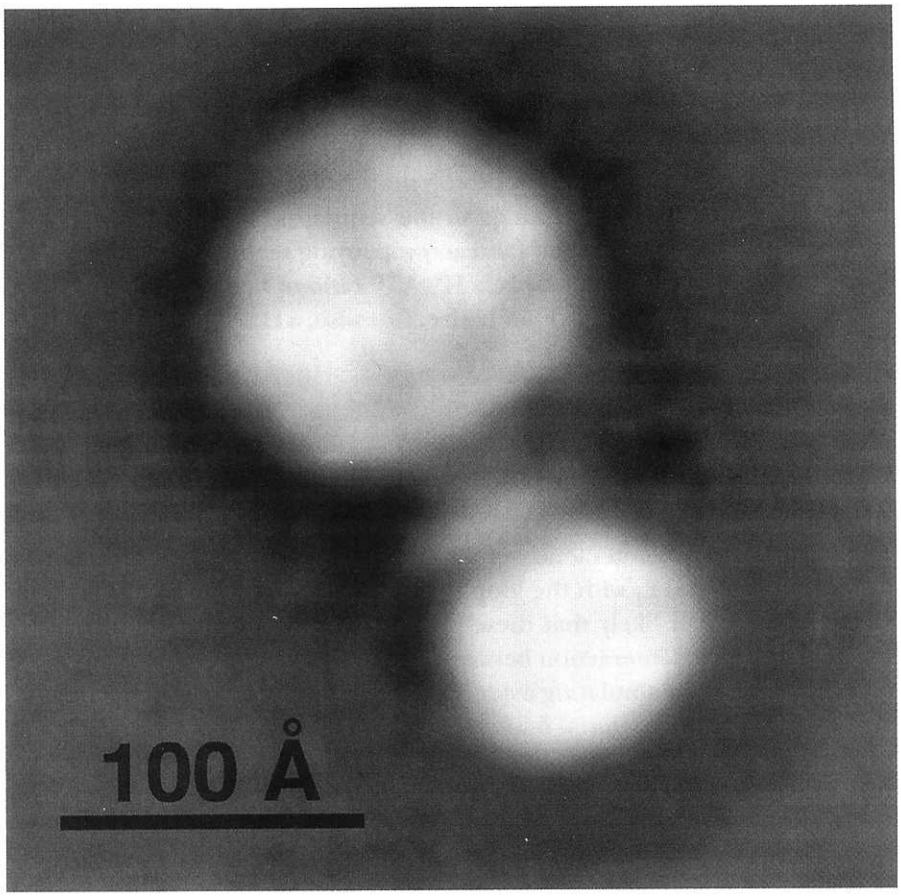

Figure 10. Image of bovine ATP synthase obtained by averaging 4940 single negatively stained particles of the enzyme. The plane of the membrane with which the assembly is associated in mitochondria, runs upwards, left to right, at an angle of 45°. The larger sphere is the F_1 catalytic domain, and the smaller sphere beneath it is F_o (or part of F_o). The new features are the collar apparently surrounding the central stalk, and, on the right, the faint peripheral connection between the collar and F_1 which may serve as a "stator". There may also be extra material on top of the F_1 domain that is not present in isolated F_1-ATPase (for more detailed discussion, see the text).

evidently sitting between the central stalk and F_o domains, and a similar collar was observed in the Clostridial enzyme. It remains to be established which subunits of the bovine enzyme are responsible for the peripheral stalk and the collar. The OSCP and subunit b are likely candidates for being components of the former, and the collar may be composed of parts of the c subunits.

At present, we can only speculate about the role of the peripheral stalk, but it may be acting as a stator to counter the tendency of the $\alpha_3\beta_3$ sub-complex to follow the rotation of the central γ-subunit. However, it is unlikely that this peripheral stalk remains bound in a unique position because the nature of the surface of the $\alpha_3\beta_3$ sub-complex must change cyclically in response to the rotating γ-subunit, and hence the preferred binding site of the peripheral

structure must also move in the same way. The role of the collar is an even bigger mystery.

EVOLUTION OF ATP SYNTHASE AND ITS RELATIONSHIP TO OTHER ENZYMES

In bacterial operons coding for the subunits of ATP synthase, the genes for F_1 and F_o subunits are often clustered separately. This arrangement suggested the possibility that the two domains have evolved separately as structural modules [84], similar to the "modular evolution" of heads and tails of bacteriophages [85, 86]. If this suggestion were correct, it might be expected that the F_1 and F_o modules would be found to fulfill other biochemical functions elsewhere in biology.

It is too early to know whether there is any significant structural and mechanistic relationship between F_o and the motors that drive the flagellae of bacteria, although this remains a distinct possibility. However, there is emerging evidence of significant similarities, both functional and structural, between the F_1 domain of ATP synthase and DNA and RNA helicases that employ energy released by hydrolysis of ATP or GTP to separate the strands of nucleic acid duplexes (for example, see reference [87]). Therefore, the rotary mechanism of ATP synthase may turn out to be the first example of a more general principle in enzyme catalysis.

CONCLUDING REMARKS

The high resolution structure of the catalytic domain of the ATP synthesizing enzyme has provided new insights into our understanding of how ATP is made in biology. Nevertheless, challenging structural experiments lie ahead in the quest to understand the generation of rotation by transmembrane proton transport through its membrane sector. A short poem written by Robert Frost provides an appropriate summary of the current state of affairs.

We dance round in a ring and suppose
The secret sits in the middle and knows

I am glad to have played a role in arriving at our present level of understanding of ATP synthesis, and in the future I hope to contribute to the revelation of the secret sitting in the middle.

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REFERENCES

1. Nicholls, D. G. & Ferguson, S. J. (1992). In *Bioenergetics 2* Academic Press, London, San Diego.
2. Dimroth, P. (1991). Na^+-coupled alternative to H^+ coupled primary tarnsport system in bacteria. *Bioessays* **13**, 463–468.
3. Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, D. & Yoshikawa, S. (1995). Structures of the metal sites of oxidized cytochrome c oxidase at 2.8 Å resolution. *Science* **269**, 1069–1074.
4. Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, D. & Yoshikawa, S. (1996). The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. *Science* **272**, 1136–1144.
5. Iwata, S., Ostermeier, C., Ludwig, B. & Michel, H. (1995). Structure at 2.8 Å resolution of cytochrome c oxidase from *Paracoccus denitrificans*. *Nature* **376**, 660–669.
6. Ostermeier, C., Harrenga, A., Ermler, U. & Michel, H. (1997). Structure at 2.7 Å of the *Paracoccus denitrificans* two-subunit cytochrome c oxidase with an antibody F_v fragment. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10547–10553.
7. Xia, D., Yu, C. A., Kih, H., Xia, J. Z., Kachurin, A. M., Zhang, L., Yu, L. & Deisenhofer, J. (1997). Crystal structure of the cytochrome bc_1 complex from bovine heart mitochondria. *Science* **277**, 60–66.
8. Walker, J. E. (1992). The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains. *Qu. Rev. Biophys.* **25**, 253–324.
9. Guénbaut, V., Vincentelli, R., Mills, D., Weiss, H. & Leonard, K. (1997). Three-dimensional structure of NADH-dehydrogenase from *Neurospora crassa* by electron microscopy and conical tilt reconstruction. *J. Mol. Biol.* **265**, 409–418.
10. Grigorieff, N. (1997). Three-dimensional structure of bovine NADH:ubiquinone oxidoreductase (complexI) at 22 Å in ice. *J. Mol. Biol.* In press.
11. Gay, N. J. & Walker, J. E. (1981). The *atp* operon: nucleotide sequence of the promoter and the genes for the membrane proteins and the δ subunit of *Escherichia coli* ATP synthase. *Nucleic Acids Res.* **9**, 3919–3926.
12. Gay, N. J. & Walker, J. E. (1981). The *atp* operon: nucleotide sequence of the region encoding the α subunit of *Escherichia coli* ATP synthase. *Nucleic Acids Res.* **9**, 2187–2194.
13. Saraste, M., Gay, N. J., Eberle, A., Runswick, M. J. & Walker, J. E. (1981). The *atp* operon: nucleotide sequence of the genes for the γ, β and ε subunits of *Escherichia coli* ATP synthase. *Nucleic Acids Res.* **9**, 5287–5296.
14. Runswick, M. J. & Walker, J. E. (1983). The amino acid sequence of the b subunit of ATP synthase from bovine heart mitochondria. *J. Biol. Chem.* **258**, 3081–3089.
15. Tybulewicz, V. L. J., Falk, G. & Walker, J. E. (1984). *Rhodopseudomonas blastica atp* operon: Nucleotide sequence and transcription. *J. Mol. Biol.* **179**, 185–214.
16. Walker, J. E., Saraste, M. & Gay, N. J. (1984). The *unc* operon: nucleotide sequence, regulation and structure of ATP synthase. *Biochim. Biophys. Acta* **768**, 164–200.
17. Falk, G., Hampe, A. & Walker, J. E. (1985). Nucleotide sequence of the *Rhodospirillum rubrum atp* operon. *Biochem. J.* **228**, 391–407.
18. Walker, J. E., Fearnley, I. M., Gay, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M. & Tybulewicz, V. L. J. (1985). Primary structure and subunit stoichiometry of F_1-ATPase from bovine mitochondria. *J. Mol. Biol.* **184**, 677–701.
19. Cozens, A. L., Walker, J. E., Phillips, A. L., Huttly, A. K. & Gray, J. C. (1986). A sixth subunit of ATP synthase, an F_0 component, is encoded in the pea chloroplast genome. *EMBO J.* **5**, 217–222.

20. Fearnley, I. M. & Walker, J. E. (1986). Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase. *EMBO J.* **5**, 2003–2008.
21. Cozens, A. L. & Walker, J. E. (1987). The organization and sequence of the genes for ATP synthase subunits in the cyanobacterium *Synechococcus* 6301: support for an endosymbiotic origin of chloroplasts. *J. Mol. Biol.* **194**, 359–383.
22. Walker, J. E., Gay, N. J., Powell, S. J., Kostina, M. & Dyer, M. R. (1987). ATP synthase from bovine mitochondria: sequences of imported precursors of oligomycin sensitivity conferral protein, factor 6 and adenosine triphosphatase inhibitor protein. *Biochemistry* **26**, 8613–8619.
23. Walker, J. E., Runswick, M. J. & Poulter, L. (1987). ATP synthase from bovine mitochondria: characterization and sequence analysis of two membrane associated subunits and of their corresponding c-DNAs. *J. Mol. Biol.* **197**, 89–100.
24. Falk, G. & Walker, J. E. (1988). DNA sequence of a gene cluster coding for subunits of the F_o membrane sector of ATP synthase in *Rhodospirillum rubrum*. *Biochem. J.* **254**, 109–122.
25. Walker, J. E., Powell, S. J., Vinas, O. & Runswick, M. J. (1989). ATP synthase from bovine mitochondria:complementary DNA sequence of the import precursor of a heart isoform of the alpha subunit. *Biochemistry* **28**, 4702–4708.
26. Runswick, M. J., Medd, S. M. & Walker, J. E. (1990). The δ subunit of ATP synthase from bovine heart mitochondria. Complementary DNA sequence of its import precursor cloned with the aid of the polymerase chain reaction. *Biochem. J.* **266**, 421–426.
27. Viñas, O., Powell, S. J., Runswick, M. J., Iacobazzi, V. & Walker, J. E. (1990). The epsilon subunit of ATP synthase from bovine heart mitochondria: complementary DNA, expression in bovine tissues and evidence of homologous sequences in man and rat. *Biochem. J.* **265**, 321–326.
28. Walker, J. E., Lutter, R., Dupuis, A. & Runswick, M. J. (1991). Identification of the subunits of F_1F_o-ATPase from bovine heart mitochondria. *Biochemistry* **30**, 5369–5378.
29. Van Walraven, H. S., Lutter, R. & Walker, J. E. (1993). Organization and sequence of genes for subunits of ATP synthase in the thermophilic cyanobacterium *Synechococcus* 6716. *Biochem. J.* **294**, 239–251.
30 Collinson, I. R., Runswick, M. J., Buchanan, S. K., Fearnley, I. M., Skehel, J. M., van Raaij, M. J., Griffiths, D. E. & Walker, J. E. (1994). The F_o membrane domain of ATP synthase from bovine heart mitochondria: purification, subunit composition and reconstitution with F_1-ATPase. *Biochemistry* **33**, 7971–7978.
31. Collinson, I. R., Skehel, J. M., Fearnley, I. M., Runswick, M. J. & Walker, J. E. (1996). The F_1F_o-ATPase complex from bovine heart mitochondria: the molar ratio of the subunits in the stalk region linking the F_1 and F_o domains. *Biochemistry* **35**, 12640–12646.
32. Walker, J. E. (1994). The regulation of catalysis in ATP synthase. *Curr. Opinion Struct. Biol.* **4**, 912–918.
33. Fernández-Morán, H. (1962). Cell-membrane ultrastructure. Low-temperature electron microscopy and x-ray diffraction studies of lipoprotein components in lamellar systems. *Circulation* **26**, 1039–1065.
34. Vambutas, V. K. & Racker, E. (1965). Partial resolution of the enzymes catalysing photophosphorylation. Stimulation of photophossphorylation by a preparation of a latent, Ca^{++}-dependent adenosine triphosphatase from chloroplasts. *J. Biol. Chem.* **240**, 2660–2667.
35. Green, D. E. (1964). The Mitochondrion. *Scientific American* **210**, 63–74.
36. Kagawa, Y. & Racker, E. (1966). Partial resolution of the enzymes catalyzing oxidative phosphorylation. Correlation of morphology and function in submitochondrial particles. *J. Biol. Chem.* **241**, 2475–2482.
37. Kagawa, Y. & Racker, E. (1966). Partial resolution of the enzymes catalyzing oxidative phosphorylation. Properties of a factor conferring oligmycin sensitivity on mitochondrial adenosine triphosphatase. *J. Biol. Chem.* **241**, 2461–2466.

38. Kagawa, Y. & Racker, E. (1966). Partial resolution of the enzymes catalyzing oxidative phosphorylation. Reconstruction of oligomycin-sensitive adenosine triphosphatase. *J. Biol. Chem.* **241**, 2467–2474.
39. Van Walraven, H., Strotmann, H., Schwartz, O. & Rumberg, B. (1996). The H^+/ATP ratio of the ATP synthase from the thiol modulated chloroplasts and two cyanobacterial strains is four. *FEBS Lett.* **379**, 309–313.
40. Boyer, P. D. (1993). The binding change mechanism for ATP synthase–some probabilities and possibilities. *Biochim. Biophys. Acta* **1140**, 215–250.
41. Brooks, J. C. & Senior, A. E. (1972). Methods for the purification of each subunit of the mitochondrial oligomycin-insensitive adenosine triphosphatase. *Biochemistry* **11**, 4675–4678.
42. Knowles, A. F. & Penefsky, H. S. (1972). The subunit structure of beef heart mitochondrial adenosine triphosphatase. *J. Biol. Chem.* **247**, 6616–6623.
43. Pai, E. F., Sachsenheimer, W., Schirmer, R. H. & Schulz, G. E. (1977). Substrate positions and induced-fit in crystalline adenylate kinase. *J. Mol. Biol.* **114**, 37–45.
44. Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982). Distantly related sequences in the α and β subunits of ATP synthase, myosin, kinases and other ATP requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**, 945–951.
45. Higgins, C. F. (1992). ABC transporters: from microorganisms to man. *Ann. Rev. Cell Biol.* **8**, 67–113.
46. Gay, N. J. & Walker, J. E. (1983). Homology between human bladder carcinoma oncogene product and mitochondrial ATP synthase. *Nature* **301**, 262–264.
47. Koonin, E. V. (1993). A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. *J. Mol. Biol.* **229**, 1165–1174.
48. Walker, J. E., Fearnley, I. M., Lutter, R., Todd, R. J. & Runswick, M. J. (1990). Structural aspects of proton pumping ATPases. *Phil. Trans. Royal Soc.* **326**, 367–378.
49. Lutter, R., Abrahams, J. P., van Raaij, M. J., Todd, R. J., Lundqvist, T., Buchanan, S. K., Leslie, A. G. W. & Walker, J. E. (1993). Crystallization of F_1–ATPase from bovine heart mitochondria. *J. Mol. Biol.* **229**, 787–790.
50. Abrahams, J. P., Leslie, A. G. W., Lutter, R. & Walker, J. E. (1994). Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. *Nature* **370**, 621–628.
51. van Raaij, M. J., Abrahams, J. P., Leslie, A. G. W. & Walker, J. E. (1996). The structure of bovine F_1-ATPase complexed with the antibiotic inhibitor aurovertin. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 6913–6917.
52. Abrahams, J. P., Buchanan, S. K., van Raaij, M. J., Fearnley, I. M., Leslie, A. G. W. & Walker, J. E. (1996). The structure of bovine F_1-ATPase complexed with the peptide antibiotic efrapeptin. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9420–9424.
53. Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L. & Cross, R. L. (1995). Rotation of subunits during catalysis by *Escherichia coli* F_1-ATPase. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 10964–10968.
54. Capaldi, R. A., Aggeler, R., Wilkens, S. & Grüber, G. (1996). Structural changes in the γ and ε subunits of the *Escherichia coli* F_1F_o-type ATPase during energy coupling. *J. Bioenerget. Biomemb.* **28**, 397–401.
55. Sabbert, D., Engelbrecht, S. & Junge, W. (1995). Intersubunit rotation in active F-ATPase. *Nature* **381**, 623–625.
56. Noji, H., Yasuda, R., Yoshida, M. & Kinosita Jr, K. (1997). Direct observation of the rotation of F_1-ATPase. *Nature* **386**, 299–302.
57. Wilkens, S., Dahlquist, F. W., McIntosh, L. P., Donaldson, L. W. & Capaldi, R. A. (1995). Structural features of the e subunit of the *Escherichia coli* ATP synthase determined by NMR spectroscopy. *Nature Struct. Biol.* **2**, 961–967.
58. Uhlin, U., Cox, G. B. & Guss, J. M. (1997). Crystal structure of the ε-subunit of the proton-translocating ATP synthase from *Escherichia coli*. *Structure* **5**, 1219–1230.
59. Wilkens, S., Dunn, S. D., Chandler, J., Dahlquist, F. W. & Capaldi, R. A. (1997). Solution structure of the N-terminal domain of the δ-subunit of the *E. coli* ATP synthase. *Nature Struct. Biol.* **4**, 198–201.

60. Walker, J. E., Runswick, M. J. & Saraste, M. (1982). Subunit equivalence in *Escherichia coli* and bovine heart mitochondrial F_1F_o ATPases. *FEBS Lett.* **146**, 393–396.
61. Fillingame, R. H. (1996). Membrane sectors of F- and V-type H^+-transporting ATPases. *Current Opinion Struct. Biol.* **6**, 491–498.
62. Foster, D. L. & Fillingame, R. H. (1982). Stoichiometry of subunits in the H^+ -ATPase complex of *Escherichia coli*. *J. Biol. Chem.* **257**, 2009–2015.
63. Fillingame, R. H. (1990). Molecular mechanics of ATP synthesis by F_1F_o-type H^+-transporting ATPases. *The Bacteria* **12**, 345–391.
64. Fillingame, R. H., Girvin, M. E., Jiang, W., Valiyaveetil, F. & Hermolin, J. (1998). Subunit interactions coupling H^+ transport and ATP synthesis in F_1F_o ATP synthase. *Acta Physiol. Scand.* In the press.
65. Walker, J. E., Saraste, M. & Gay, N. J. (1982). *E. coli* F_1-ATPase interacts with a membrane protein component of a proton channel. *Nature* **298**, 867–869.
66. Dunn, S. D. (1992). The polar domain of the b subunit of *Escherichia coli* F_1F_o-ATPase forms an elongated dimer that interacts with the F_1 sector. *J. Biol. Chem.* **267**, 7630–7636.
67. Watts, S. D., Zhang, Y., Fillingame, R. H. & Capaldi, R. A. (1995). The gamma subunit in the *Escherichia coli* ATP synthase complex (ECF_1F_o) extends through the stalk and contacts the c subunits of the F_o part. *FEBS Lett.* **368**, 235–238.
68. Zhang, Y. & Fillingame, R. H. (1995). Subunits coupling H^+ transport and ATP synthesis in the *Escherichia coli* ATP synthase: Cys-Cys crosslinking of F_1 subunit ε to the polar loop of F_o subunit c. *J. Biol. Chem.* **270**, 24609–24614.
69. Groth, G. & Walker, J. E. (1997). Model of the c-subunit oligomer in the membrane domain of F-ATPases. *FEBS Lett.* **410**, 117–123.
70. Singh, S., Turina, P., Bustamente, C. J., Keller, D. J. & Capaldi, R. A. (1996). Topographical structure of membrane-bound *Escherichia coli* F_1F_o ATP synthase in aqueous buffer. *FEBS Lett.* **397**, 30–34.
71. Takeyasu, K., Omote, H., Nettikadan, S., Tokumasu, F., Iwamoto-Kihara, A. & Futai, M. (1996). Molecular imaging of *Escherichia coli* F_oF_1-ATPase in reconstituted membranes by atomic force microscopy. *FEBS Lett.* **392**, 110–113.
72. Junge, W., Sabbert, D. & Engelbrecht, S. (1996). Rotary catalysis by F-ATPase: real time recording of intersubunit rotation. *Ber. Bunsenges. Phys. Chem.* **100**, 2014–2019.
73. Dunn, S. D., Heppel, L. A. & Fullmer, C. S. (1980). The NH_2-terminal portion of the α-subunit of the *Escherichia coli* F_1 -ATPase is required for binding the δ-subunit. *J. Biol. Chem.* **255**, 6891–6896.
74. Hundal, T., Norling, B. & Ernster, L. (1983). Lack of ability of trypsin-treated mitochondrial F_1-ATPase to bind to the oligomycin sensitivity conferring protein (OSCP). *FEBS Lett.* **162**, 5–10.
75. Joshi, S., Javed, A. A. & Gibbs, L. C. (1992). Oligomycin sensitivity-conferring protein (OSCP) of mitochondrial ATP synthase. The carboxy terminal region of OSCP is essential for the reconstitution of oligomycin-sensitive H^+-ATPase. *J. Biol. Chem.* **267**, 12860–12867.
76. Joshi, S., Pringle, M. J. & Siber, R. (1986). Topology and function of "stalk" proteins in the bovine mitochondrial H^+-ATPase. *J. Biol. Chem.* **261**, 10653–10658.
77. Collinson, I. R., van Raaij, M. J., Runswick, M. J., Fearnley, I. M., Skehel, J. M., Orriss, G., Miroux, B. & Walker, J. E. (1994). ATP synthase from bovine heart mitochondria: *in vitro* assembly of a stalk complex in the presence of F_1-ATPase and in its absence. *J. Mol. Biol.* **242**, 408–421.
78. Orriss, G. L., Runswick, M. J., Collinson, I. R., Miroux, B., Fearnley, I. M., Skehel, J. M. & Walker, J. E. (1996). The δ- and ε-subunits of bovine F_1-ATPase interact to form a heterodimeric subcomplex. *Biochem. J.* **314**, 695–700.
79. Lutter, R., Saraste, M., van Walraven, H. S., Runswick, M. J., Finel, M., Deatherage, J. F. & Walker, J. E. (1993). F_1F_o-ATPase from bovine heart mitochondria: development of the purification of a monodisperse oligomycin sensitive ATPase. *Biochem. J.* **295**, 799–806.

80. Walker, J. E., Collinson, I. R., Van Raaij, M. J. & Runswick, M. J. (1995). Structural analysis of ATP synthase (F_1F_o-ATPase) from bovine heart mitochondria. *Methods in Enzymol.* 163–190.
81. Buchanan, S. K. & Walker, J. E. (1996). Large scale chromatographic purification of F_1F_o-ATPase and complex I from bovine heart mitochondria. *Biochem. J.* **318**, 343–349.
82. Groth, G. & Walker, J. E. (1996). ATP synthase from bovine heart mitochondria: reconstitution into unilamellar phospholipid vesicles of the pure enzyme in a functional state. *Biochem. J.* **318**, 351–357.
83. Boekema, E., Ubbink-Kok, T., Lolkema, J. S., Brisson, A. & Konings, W. N. (1997). Visualization of a peripheral stalk in V-type ATPase: evidence for the stator structure essential to rotational catalysis. *Proc. Natl. Acad. Sci. U.S.A.* **94**, In the press.
84. Walker, J. E. & Cozens, A. L. (1986). Evolution of ATP synthase. *Chemica Scripta* **26B**, 263–272.
85. Botstein, D. (1980). A theory for molecular evolution of bacteriophages. *Ann. N. Y. Acad. Sci.* **354**, 484–491.
86. Casjens, S. & Hendrix, R. (1974). Comments on the arrangement of the morphogenetic genes of bacteriophage lambda. *J. Mol. Biol.* **90**, 20–23.
87. Yu, X. & Egelman, E. H. (1997). The RecA hexamer is a structural homologue of ring helicases. *Nature Struct. Biol.* **4**, 101–104.
88. Gogol, E. P., Aggeler, R., Sagerman, M. & Capaldi, R. A. (1989). Cryoelectron microscopy of *Escherichia coli* F_1 adenosine triphosphatase decorated with monoclonal antibodies to individual subunits of the complex. *Biochemistry* **28**, 4717–4724.
89. Weissman, G. & Claibourne, R. (1975). Cell Membranes: Biochemistry, Cell Biology and Pathology.