Novel features of the rotary catalytic mechanism revealed in the structure of yeast F_1 ATPase

Venkataraman Kabaleeswaran¹,
Neeti Puri¹, John E Walker^{2,*}, Andrew
GW Leslie^{3,*} and David M Mueller^{1,*}

¹Department of Biochemistry & Molecular Biology, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA, ²MRC Dunn Human Nutrition, Cambridge, UK and ³MRC Laboratory of Molecular Biology, Cambridge, UK

The crystal structure of yeast mitochondrial F_1 ATPase contains three independent copies of the complex, two of which have similar conformations while the third differs in the position of the central stalk relative to the $\alpha_3\beta_3$ subassembly. All three copies display very similar asymmetric features to those observed for the bovine enzyme, but the yeast F_1 ATPase structures provide novel information. In particular, the active site that binds ADP in bovine F_1 ATPase has an ATP analog bound and therefore this structure does not represent the ADP-inhibited form. In addition, one of the complexes binds phosphate in the nucleotide-free catalytic site, and comparison with other structures provides a picture of the movement of the phosphate group during initial binding and subsequent catalysis. The shifts in position of the central stalk between two of the three copies of yeast F_1 ATPase and when these structures are compared to those of the bovine enzyme give new insight into the conformational changes that take place during rotational catalysis.

The EMBO Journal (2006) 25, 5433–5442. doi:10.1038/sj.emboj.7601410; Published online 2 November 2006

Subject Categories: structural biology

Keywords: F_1 ATPase; ATP synthase; mitochondrion

Introduction

Mitochondrial F_1F_0 ATP synthase (EC 3.6.3.14) is responsible for the synthesis of more than 90% of cellular ATP in the eukaryotic cell under aerobic conditions. The ATP synthase is a molecular motor that converts the energy of the proton motive force across the mitochondrial membrane to a more generally usable form of energy, namely ATP. The enzyme is comprised of two functional and separable units, F_1 and F_0 , both of which can function as molecular motors. F_1 is a water-soluble component, which contains the catalytic sites

that effect the phosphorylation of ADP:Mg. F_0 is the membrane portion of the enzyme and acts as a proton turbine, utilizing the proton motive force to generate the rotation of the central stalk of F_1 .

F_1 is composed of five different subunits with stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ with a combined molecular weight of 360 000 Da. The three active sites are formed at the interfaces between the α - and β -subunits (Abrahams *et al*, 1994). Part of the γ -subunit consists of a coiled-coil that is contained within the α/β core of the enzyme. The mitochondrial δ - and ϵ -subunits are bound at the foot of the γ -subunit and the three subunits form the central stalk of the enzyme (Gibbons *et al*, 2000). The F_0 component contains a membrane rotor made up of 10 copies of the c-subunit in the yeast enzyme. The c-subunit contains an essential carboxylate group that forms part of the proton translocation pathway, which is believed to involve two half channels at the interface of the membrane-bound a-subunit and the ring of c-subunits. A peripheral stalk formed minimally by subunits b, d, h, and subunit 5 (OSCP) links the a-subunit to the periphery of the α/β core and acts as a stator, preventing the core from following the rotation of the central stalk (Kane Dickson *et al*, 2006; Walker and Kane Dickson, 2006). Additional subunits (subunits 8, e, f, g, i, and k) whose roles are currently uncertain and two regulatory proteins (Inh1, Stf1) complete the composition of F_1F_0 . Overall, the yeast mitochondrial ATP synthase is composed of 18 polypeptides and has a molecular weight of over 550 000 Da.

The binding change mechanism provides a framework for explaining how energy derived from the proton motive force is used to synthesize ATP (Boyer *et al*, 1973; Mitchell, 1977). According to this scheme, the three active sites adopt different conformations, which interconvert sequentially during catalysis. ATP synthesis occurs in the site with highest affinity for ATP, referred to as the tight site, and this step is proposed to be isoenergetic between bound ADP:Pi and ATP. Energy derived from the proton motive force is required to alter the conformation of the tight site to one with lower affinity, allowing the release of newly synthesized ATP. The crystal structure of bovine F_1 (Abrahams *et al*, 1994) shows several features that are consistent with the binding change mechanism. In this structure, two of the catalytic sites are in a closed conformation. One, at the interface of the α_{DP^-} and β_{DP^-} subunits, contains ADP (DP site), while the second, at the interface of the α_{TP^-} and β_{TP^-} subunits, contains AMP-PNP (TP site). The third catalytic site, at the interface of the α_E and β_E subunits, is in an open conformation and devoid of bound nucleotide (E site). It was proposed that DP represents the high-affinity (tight) catalytic site, TP represents the intermediate affinity nucleotide-binding site, while newly formed product will be released from the low affinity E site (Abrahams *et al*, 1994). The structure also suggested that rotation of the γ -subunit within the $\alpha_3\beta_3$ subcomplex was responsible for the interconversion of the active sites (Abrahams *et al*, 1994). ATP-dependent rotation of the γ -subunit was dramatically demonstrated by single molecule

*Corresponding authors. David M Mueller, Department of Biochemistry & Molecular Biology, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, 3333 Greenbay Road, North Chicago, IL 60064, USA, Tel.: +1 847 578 8606; Fax: +1 847 578 3240; E-mail: David.Mueller@RosalindFranklin.edu or Andrew GW Leslie, MRC Laboratory of Molecular Biology, Cambridge, UK. E-mail: andrew@mrc-lmb.cam.ac.uk or John E Walker, MRC Dunn Human Nutrition, Cambridge, UK. E-mail: walker@mrc-dunn.cam.ac.uk

Received: 7 July 2006; accepted: 6 October 2006; published online: 2 November 2006

experiments showing rotation of a fluorescently labeled actin filament covalently bound to the γ -subunit (Noji *et al*, 1997).

The electron density map of the yeast F₁c₁₀ subcomplex revealed a ring of 10 c-subunits, which is in intimate contact with the foot of the central stalk (Stock *et al*, 1999). One complete rotation of the ring results in the translocation of 10 protons (one per subunit) across the membrane, possibly by a mechanism involving two half-channels located at the interface between the a-subunit and the c-ring as first proposed by W Junge in 1993 (Junge *et al*, 1997). In the F₁ domain, a complete rotation of the γ -subunit is accompanied by the synthesis of three molecules of ATP. The stoichiometry of the c-ring dictates a nonintegral proton:ATP ratio, which requires that the synthesis of one ATP molecule is accompanied by the translocation of a mean value of 3.3 protons. This suggests that there must be some elasticity within the central stalk.

The crystal structure of yeast mitochondrial F₁-ATPase described here shows the same asymmetric features as those described for the bovine enzyme. An analysis of the differences in conformation between the three independent copies of yeast F₁ and comparisons with structures of the bovine enzyme have provided new insights into the catalytic mechanism, and in particular the details of the phosphate (Pi)-binding site.

Results

Structure determination

Yeast F₁ ATPase was crystallized in a monoclinic space group (P2₁) with three F₁ complexes (over 9000 residues) in the crystallographic asymmetric unit (Mueller *et al*, 2004a). The structure was solved by molecular replacement (Materials and methods) and refined against X-ray data to a resolution of 2.8 Å (Table I).

The quality of the electron density of the three F₁ complexes was variable. The F₁ complex with the best-defined density is referred to as yF₁I, while yF₁II and yF₁III have

progressively less-well-defined density. Electron density for the central stalk (composed of the γ -, δ -, and ϵ -subunits) was sufficiently good for yF₁I to allow building a nearly complete model, while these subunits are less complete for yF₁II and III. Details of the residues present in the final models for yF₁I, II, and III are given in Supplementary data. yF₁I and II lie 'side by side' with a similar orientation, but rotated along the axis of the γ -subunit by about 180°, while yF₁III is located above and between yF₁I and yF₁II. Thus, the crystal contacts are quite different for the three complexes (Supplementary Table 1) and this is presumably responsible for the difference in quality of the electron density.

The scheme used for naming the α - and β -subunits of the bovine enzyme will be used here (Abrahams *et al*, 1994). Yeast residue numbering will be used, with the corresponding bovine residue number, if different, in parentheses. The insertions and deletions when comparing bovine and yeast subunits are detailed in Supplementary data.

Overall structure of the yeast F₁-ATPase

The structure of yF₁I was compared to that of yF₁II and yF₁III and also to the bovine enzyme. The structure of bovine F₁ ATPase inhibited by BeF₃⁻:ADP (Kagawa *et al*, 2004) was used for comparison of the α - and β -subunits. BeF₃⁻:ADP mimics ATP and is the closest structural homolog to AMP-PNP, which is bound to both the β_{DP} - and β_{TP} -subunits in all three complexes in the yeast crystal structure. All noncatalytic nucleotide-binding sites in yF₁ contain AMP-PNP. The structure of the bovine enzyme inhibited with dicyclohexylcarbodiimide (DCCD) (Gibbons *et al*, 2000) was used for comparison of the γ -, δ -, and ϵ -subunits, because this structure has the most complete model of the central stalk.

The overall structures of all three complexes of the yeast F₁ ATPase in the crystallographic asymmetric unit are very similar (Table II). However, it is clear that the structure of yF₁I is more similar to yF₁III than to yF₁II. While the overall differences are relatively small, they lead to some significant differences in the active sites. Because yF₁I and III adopt very similar conformations, and yF₁I is more complete, the following discussion will focus on yF₁I and II.

When yF₁I is compared to bovine F₁ ATPase (Table II), the differences in α -carbon positions are less than 1 Å for individual α - and β -subunits, and 1.2 Å for the $\alpha_3\beta_3$ subcomplex, indicating that the overall structures are very similar. The

Table I Data collection and refinement statistics for the yeast F₁ ATPase

<i>Data collection</i>	
Space group	P2 ₁
Cell parameters (Å and deg)	$a = 111.5$, $b = 294.1$, $c = 190.4$, $\beta = 101.7$
Resolution (Å)	2.8
Number of reflections	293 909
$R_{\text{merge}}^{a,b}$	8.7 (37.6)
$\langle I/\sigma(I) \rangle^b$	13.9 (3.0)
Completeness ^b	99.9 (99.7)
Mean multiplicity ^b	5.1 (3.9)
<i>Refinement statistics</i>	
R -factor ^b (%)	20.7
$R_{\text{free}}^{b,c}$ (%)	24.4
<i>R.m.s. deviations from standard values</i>	
Bonds (Å)	0.011
Angles (deg)	1.3

^a $R_{\text{merge}} = \sum \sum_i |I_{hi} - \langle I_h \rangle| / \sum \sum_i I_{hi}$, where $\langle I_h \rangle$ is the weighted mean intensity for all observations of reflections h after rejection of outliers.

^bThe statistics for the highest resolution shell (2.95–2.80 Å) are given in brackets.

^c R_{free} was calculated for 5942 reflections, which were excluded from the refinement.

Table II R.m.s. differences in α -carbon positions of yF₁I compared to yF₁II, yF₁III, and bovine F₁ (Å)

Subunit	yF ₁ II	yF ₁ III	Bovine
α -DP	0.5	0.3	0.9
β -DP	0.4	0.1	0.7
α -DP/ β -DP	1.3	0.4	1.4
α -TP	0.1	0.1	0.8
β -TP	0.2	0.1	0.6
α -TP/ β -TP	0.4	0.5	1.0
α -E	0.3	0.2	1.0
β -E	0.2	0.3	0.6
α -E/ β -E	0.9	0.5	1.0
γ	0.5	0.8	2.6
δ	1.0	ND	2.1
ϵ	1.5	1.3	1.7
$\alpha_3\beta_3$	1.0	0.6	1.2

ND = not determined.

differences are significantly larger for the γ -, δ - and ϵ -subunits, but in the case of the γ -subunit decrease dramatically if the subunit is broken down into subdomains. For instance, for residues 1–30, 106–151, and 206–276, the differences are 0.8, 1.0, and 1.3 Å, respectively.

The α/β pairs that form the catalytic interfaces in yF₁I and II display larger differences than when individual subunits are compared (Table II). This is particularly true for the α_{DP}/β_{DP} pair, and reflects the more open α_{DP}/β_{DP} interface in yF₁I compared to yF₁II (Figure 1). The opening of this interface can be described as a relative rotation of 5.5° of the α_{DP} -subunit relative to the β_{DP} -subunit, and this rotation has the greatest effect on the relative positions of the C-terminal helical domains. The α_{DP}/β_{DP} interface in bovine F₁ is very similar to that in yeast yF₁II (Figure 1), so that yeast yF₁I represents the novel conformation.

Figure 1 Comparison of the α/β catalytic interfaces in bovine and yeast F₁ ATPase. Stereo image (wall-eyed) of the α -carbon traces of the α - (red) and β - (blue) subunits of the yeast DP-II overlaid on the corresponding subunits of BeF₃[−]-inhibited bovine F₁ (gray) (top) and DP-I overlaid on DP-II (salmon) (bottom). The molecules were superimposed as the α/β pair using TOP3D (Collaborative_Computational_Project, 1994). The nucleotide bound to the β_{DP} -subunit is shown in space-filling representation. The abbreviations are: DP-I, the α_{DP}/β_{DP} interface from yF₁I; DP-II, the α_{DP}/β_{DP} interface from yF₁II.

Figure 2 Comparison of the β_{DP} active sites of yeast and bovine F₁ ATPase stereo image (wall-eyed) of the active site of the yeast DP-I (gold) superimposed with the β_{DP} site of the ADP:BeF₃[−]-inhibited bovine enzyme (white) (Kagawa *et al*, 2004). The bovine residue numbering is shown in brackets when it differs from the yeast numbering. BeF₃ is colored yellow and phosphate is colored magenta. The Mg²⁺ ion is shown as a sphere, colored gold in the bovine structure and red in yF₁.

Similar but smaller differences are seen when comparing the α_E/β_E interface. Again, the interface is more open in yeast yF₁I than in yF₁II, and in this case the relative rotation of the α_E/β_E subunits is 3.7°. As with the α_{DP}/β_{DP} interface, yF₁II is very similar to the bovine structure. By contrast, the α_{TP}/β_{TP} interface is essentially the same in yeast yF₁I and II, and both closely resemble the corresponding interface in bovine F₁.

The DP and TP active sites

The catalytic sites of the yeast enzyme share many features found in structures of the bovine enzyme. The most significant difference is that the yeast β_{DP} active site has AMP-PNP:Mg bound in all three complexes rather than ADP:Mg (Supplementary Figure 2S). Based on the quality of the electron density and the refined B-factors for the nucleotide, the β_{DP} active site is fully occupied with AMP-PNP:Mg.

The arrangement of catalytic residues in the yeast DP active site is similar to that of the corresponding site in BeF₃[−]-inhibited bovine F₁ (Figure 2), although small shifts in side chain positions are observed. The side chain interactions within the active sites of the bovine and yeast enzymes are shown schematically in Supplementary Figure 3S. For simplicity, the β_{DP} active sites of yeast yF₁I, yF₁II, and yF₁III complexes will be referred to as DP-I, DP-II, and DP-III in the following discussion. Yeast β Glu189(188) acts as a general base in the catalytic reaction. The distance of the phosphorous atom in the γ -phosphate (or Be atom in BeF₃[−]), from C γ of the carboxylate of β Glu189(188) is 5.4, 5.6, and 5.2 Å in β_{DP} of the bovine enzyme, and the DP-I and DP-II sites of yeast, respectively, and 5.7 Å in the corresponding TP sites. While the reactive water molecule is not observed in the yeast model in either the DP-I or DP-II sites, there is some weak electron density in the DP-III site that may be due to the catalytic water. The apparent absence of the catalytic water in the yeast structure is probably due to the limited resolution of the X-ray data.

The structure of bovine F₁ ATPase with the transition state analog, ADP:AlF₄[−], bound to the β_{DP} and β_{TP} sites provides good evidence that α Arg375(373) stabilizes the transition state of the pentacoordinate intermediate during the reaction (Menz *et al*, 2001). Biochemical evidence supports this role for α Arg375(373) and also suggests that this residue is critical in the cooperativity of the enzyme (Le *et al*, 2000; Senior *et al*, 2002). When the structures are superimposed using the P-loop as the reference point, the guanidinium group of

Figure 3 Phosphate-binding site in the β_E -subunit of the yF_1II complex. (A) Electron density of the final $2F_o - F_c$ map for the phosphate-binding site (contoured at 1.3σ). The electron density is shown only for a radius about the phosphate to simplify the image. (B) Side chains that contribute to phosphate binding. Possible ionic interactions are shown as dotted lines, with distances in Å. (C) Superposition of the phosphate-binding region of yF_1I (green) on that of yF_1II (blue). (D) Superposition of the phosphate-binding region of the empty subunit of bovine F_1 (pink) on yF_1II (blue). The bovine residue numbering is used in this image.

$\alpha\text{Arg}375(373)$ of the yeast DP-II active site is nearly identical in position to $\alpha\text{Arg}373$ of the bovine enzyme, but in the yeast DP-I active site residue $\alpha\text{Arg}375(373)$ is 1 Å closer to the γ -phosphate of the nucleotide. The change in position of $\alpha\text{Arg}375(373)$ is due to a translation of the α -carbon atom of $\alpha\text{Arg}375(373)$ in the DP-I active site of about 1.8 Å along an axis that is parallel to the axis formed by the α - and β -phosphates of the nucleotide.

$\beta\text{Arg}190(189)$ is critical for both nucleotide binding and transition state stabilization (Le *et al*, 2000; Menz *et al*, 2001; Senior *et al*, 2002). In the yeast DP-II active site, the guanidinium group forms a bidentate salt bridge interaction with one oxygen atom on the γ -phosphate and a monodentate interaction with a second oxygen atom of the γ -phosphate (Supplementary Figure 3S). In yeast DP-I active site, this residue is displaced 0.5 Å away from the γ -phosphate resulting in the loss of the single interacting salt bridge, but the bidentate interaction is retained (Supplementary Figure 3S). The position of $\beta\text{Arg}190(189)$ relative to the nucleotide in yeast DP-II active site is very similar to that of the corresponding residue ($\beta\text{Arg}189$) in the β_{DP} -subunit of bovine F_1 with bound $\text{BeF}_3^-:\text{ADP}$ (Kagawa *et al*, 2004).

The binding pocket for the ribose moiety of the nucleotide is more solvent accessible in the α_{DP}/β_{DP} interface in the yeast complex yF_1I than in yF_1II , as a result of the relative rotation of the α - and β -subunits described above. This is reflected in the loss of a hydrogen bond between the carbonyl oxygen of $\alpha\text{Ser}374(372)$ and the $\text{O}2'$ oxygen of the ribose which are 3.0 Å apart in the yF_1II (3.1 Å in bovine F_1) but 5.3 Å apart in yF_1I .

Overall, the conformation of yeast DP-II catalytic site is more similar to bovine $\text{BeF}_3^-:\text{ADP } F_1$ than yeast DP-I. In

contrast, the β_{TP} catalytic sites are very similar in yeast yF_1I and II, and both closely resemble β_{TP} site of the bovine $\text{BeF}_3^-:\text{ADP } F_1$ structure.

Pi is bound to the β_E -subunit of yF_1II

Following initial refinement of the model, a strong electron density peak (8σ) was found in the general region of the nucleotide-binding site of the β_E -subunit of yF_1II (Figure 3A), which was modeled as P_i . The identity of the molecule is not certain, as sulfate would fit the density equally well and 0.25 mM NiSO_4 was present in the crystallization medium, but for simplicity, the bound ligand will be described as phosphate. There are multiple interactions between the phosphate oxygen atoms and side chain atoms of $\alpha\text{Arg}375(373)$, $\beta\text{Lys}163(162)$, $\beta\text{Arg}190(189)$, $\beta\text{Asp}256$, $\beta\text{Asn}257$, and $\beta\text{Arg}260$ (Figure 3B). $\beta\text{Lys}163(162)$ forms a salt bridge with the carboxylate of $\beta\text{Asp}256$, which may reduce the negative charge of the carboxylate allowing H-bonding with the proton of the phosphate oxygen. Interestingly, the P_i -binding site is far from the P-loop. If the β_{DP} -subunit of yF_1I is superimposed on the β_E -subunit of yF_1II using the P-loop as the reference, the phosphate is 7.7 Å from the γ -phosphate of AMP-PNP. A change in conformation of the β_E -subunit from the open to the closed state, presumably as a result of movement of the γ -subunit and/or binding of ADP to β_E , is required to move the phosphate group for phosphorylation of ADP during synthesis. The structure suggests that during ATP hydrolysis, the phosphate group is not released until the β_E -subunit has adopted a fully open conformation.

What are the structural differences, which account for binding of P_i to β_E of yF_1II but not yF_1I ? A superposition of

Figure 4 Structure of the central stalk of yeast F₁ ATPase. The central stalk subunits of yeast yF₁I, γ - (green), δ - (slate blue), and ϵ - (purple) subunits are superimposed on the bovine (gold) or yeast yF₁II (red). (A) The central stalks of bovine F₁ and the yeast yF₁I complex were superimposed using TOP3D to illustrate the overall similarity of the fold. (B) The central stalks of bovine F₁ and the yeast yF₁I complex were superimposed using the N-terminal β -barrel domains of the α - and β -subunits to illustrate the relative position within the core of F₁. (C) The γ -subunit from the yF₁I complex is shown superimposed on that of the yF₁II complex using the N-terminal β -barrel domains of the α - and β -subunits. (D) Comparison of the δ -subunits of the yeast and bovine enzymes. (E) Comparison of the ϵ -subunits of the yeast and bovine enzymes. (F) Stereo image (wall-eyed) illustrating the rotation of the γ -subunit from yF₁I (green) to yF₁II (magenta) showing residues 4–35 and 241–276. Stereo images of A–C are in the supplement.

the Pi-binding site in yF₁I and yF₁II (Figure 3C) indicates only small differences in the positions of the residues involved in binding Pi. The biggest differences are shifts of the guanidinium groups of α Arg375(373) and β Arg190(189) by 1.3 and 0.8 Å, respectively. In both cases, the guanidinium group is located closer to the Pi site in yF₁II than in yF₁I. These small shifts are related to the slightly more open form of α_E/β_E interface in yF₁I compared to yF₁II, which in turn is associated with the different position of the γ -subunit relative to the $\alpha_3\beta_3$ core in these two complexes.

For all the reported bovine structures, with the possible exception of the enzyme inhibited with DCCD (Gibbons *et al*, 2000), there is no evidence for Pi binding at the equivalent site in the open β_E -subunit. In several structures, there was evidence that a disordered phosphate or sulfate group was bound to the P-loop, but this was not then, and is not now, thought to be the biologically relevant Pi-binding site (Abrahams *et al*, 1994, 1996; Kagawa *et al*, 2004). In order to understand why the yF₁II complex, but not the bovine enzyme, binds Pi, the structure of this region of bovine β_E was superimposed onto that of β_E from yF₁II (Figure 3D). The differences are larger than those seen when comparing yF₁I and II. The guanidinium group of α Arg375(373) is displaced 2.6 Å and is in a different rotamer conformation in the bovine structure. The α -carbons of β Lys163(162), β Arg190(189), and β Asn257 superimpose quite well, but the side chains have different rotamer conformations. The result is that there is no well-defined Pi-binding pocket in bovine β_E .

A surface representation of the α_E/β_E interface for yF₁II reveals no obvious access to the Pi-binding site from the external surface of the F₁ complex. If ADP were bound, access would be even less favorable due to electrostatic repulsion from the nucleotide diphosphate in addition to the steric hindrance of the neighboring protein atoms. However, the phosphate site is readily accessible from the internal solvent-filled cavity of F₁. This internal cavity is connected to the external milieu by a large access channel between the C-terminal domains of the α_E - and β_E -subunits (Supplementary Figure 4S).

Conformation of the central stalk

Electron density for the central stalk region was visible in all three complexes, but the extent and quality of this density was variable. The most complete model was built for the stalk of yF₁I (Figure 4). The overall fold of the γ -, δ -, and ϵ -subunits are identical to those of the corresponding bovine subunits, with the exception of the C-terminal extension present in yeast ϵ -subunit. The three subunits also associate in a very similar way in the bovine and yeast structures. The γ -subunit contains a coiled-coil formed by an N-terminal α -helix (residues 1–56), a C-terminal α -helix (residues 208–277), and a foot composed of an α/β bundle (Figures 4A and B). The δ -subunit consists of a β -sandwich made up of two 5-stranded β -sheets (residues 13–86) followed by two α -helices (residues 99–137) (Figure 4D). The ϵ -subunit has a hairpin-like structure formed by two α -helices (residues 10–23 and 31–36) with extended regions of polypeptide at the N- and C-termini. The yeast ϵ -subunit has 10 extra amino acids at the C-terminus compared to bovine ϵ -subunit. These residues fold back and sit under the central core of the central stalk (Figure 4E).

There is a noticeable kink in the C-terminal helix of the yeast γ -subunit in the region of residue 250 (245) and a smaller kink in the N-terminal helix at residue 16. These features are associated with rather different positions of the central stalk relative to the $\alpha_3\beta_3$ subcomplex in the yeast and bovine structures. If the β -barrel domains of the α - and β -subunits are used to superimpose the bovine F₁-DCCD and yeast yF₁I structures, the lower (membrane proximal) regions of the γ -subunit are rotated counter-clockwise by about 30°, when viewed from the foot of the stalk (Figure 4B). This is the direction that the central stalk rotates during the hydrolysis of ATP. However, the rotation axis is not parallel to the pseudo three-fold axis of F₁ (deviation $\sim 16^\circ$) nor does it pass through the center of the $\alpha_3\beta_3$ subcomplex (the minimum displacement is about 5 Å), and therefore it is not clear how closely this 30° rotation models the rotation of the stalk that occurs during catalysis. The twisting of the central stalk is also not uniform: the C-terminal residues (γ 245–277) of the C-terminal α -helix superimpose very well with little or no

evidence of rotation, but the twisting becomes progressively greater towards the N-terminal region of the helix, reaching a maximum displacement of 14.6 Å in the foot of the γ -subunit. The N-terminal α -helix does not overlap well from the very beginning of the helix, but like the C-terminal α -helix, the overlap is poorer as the comparison approaches the foot of the γ -subunit. In addition, the N-terminal α -helix of the yeast enzyme is translated by about 2.5 Å parallel to the helix axis. Despite these differences, the side chain interactions between the N- and C-terminal helices within the coiled-coil are largely conserved. This conservation occurs by some changes in the rotamer conformations of side chains as well as smaller movements in the backbone of the N-terminal α -helix.

Rotation of the central stalk

The position of the central stalk relative to the $\alpha_3\beta_3$ subcomplex determines the catalytic states of the three catalytic sites. In order to compare the different bovine structures with the two distinct conformations of the yeast enzyme present in yF₁I and II, all the structures were superposed on their respective pseudo three-fold axes (defined using the α -subunits) so as to give the best superposition of the N-terminal β -barrel domains. The rotation of the central stalk was then defined as the angular rotation about the (common) pseudo three-fold axis, which provided the best superposition of residues 18–25 of the γ -subunit, as this region of the γ -subunit is involved in critical contacts with the C-terminal domain of the β_E -subunit, imposing the open conformation. The bovine reference structure (Abrahams *et al*, 1994), the ADP:AlF₄⁻-inhibited bovine structure, and the yF₁I and II structures of yeast were compared in this way. Relative to the bovine reference structure, the γ -subunit of yeast yF₁I has rotated 12° in the direction of ATP hydrolysis (counter-clockwise as viewed from the foot of the stalk), while the rotation is 4° and 16°, both in the direction of ATP synthesis, for the γ -subunits of yF₁II of yeast and ADP:AlF₄⁻-inhibited bovine F₁. These rotations, and the resulting changes at the catalytic sites, are entirely consistent with the biochemical pathway for ATP synthesis or hydrolysis. The 16° rotation (yF₁I to yF₁II, Figure 4F) is associated with Pi binding to the β_E -subunit on the ATP synthesis pathway, and the additional 12° rotation (yF₁II to ADP:AlF₄⁻ inhibited bovine) corresponds to binding of ADP and the partial closure of the catalytic site of β_E . The 12° rotation in the direction of ATP hydrolysis (bovine reference to yF₁I) results in partial opening of the α_{DP}/β_{DP} catalytic interface, in preparation for the release of the products of hydrolysis from this site.

Discussion

The crystal structure of yeast F_1 ATPase described here is the first high-resolution example of an F_1 ATPase from a source other than bovine mitochondria in which the asymmetric features of the enzyme have not been obscured by crystallographic symmetry. The presence of a crystallographic three-fold axis in structures of the rat mitochondrial and chloroplast enzymes (Bianchet *et al*, 1998; Groth and Pohl, 2001) results in an electron density map in which any difference in conformation of the α/β subunits has been averaged, making interpretation difficult. It is therefore highly significant that the three independent yeast F_1 structures show essentially the same asymmetric features as those observed

in the bovine structures, despite a completely different crystal packing of the F_1 complexes. This finding supports the view that the observed asymmetry is an essential feature of the structure of F_1 ATPase and a crucial element of the rotary catalytic mechanism.

The initial structure of the bovine enzyme was believed to represent the ADP-inhibited form (Abrahams *et al*, 1994), primarily based on the observation that Mg:ADP, and not Mg:AMP-PNP, was bound to β_{DP} . This led to speculation that the structure was of an inactive form of the enzyme that did not represent a conformation adopted on the catalytic pathway. There was also uncertainty whether the E site was competent for binding substrates. Many of the subsequent bovine structures support the view that the original structure is representative of an intermediate in the reaction pathway, most recently, the structure with ADP:BeF₃⁻ bound to two of the three active sites (Kagawa *et al*, 2004). The structures of the yeast enzyme provide two additional lines of evidence that also support this view. First, all three structures of the yeast enzyme have Mg:AMP-PNP bound to both β_{DP} and β_{TP} . Thus, the conformations of the yeast enzyme cannot be that of the ADP-inhibited form of the enzyme and yet they all have the same asymmetric features of the bovine enzyme. Secondly, β_E of yF₁II has Pi bound to a site that is consistent with biochemical data on Pi binding. Thus the structure of the enzyme represents an active intermediate in the catalytic pathway.

Why is Mg:AMP-PNP bound to the β_{DP} site of the yeast enzyme but Mg:ADP is bound to the bovine β_{DP} site? Possibly, the Mg:ADP in the bovine β_{DP} site is due to the slow hydrolysis of Mg:AMP-PNP (Tomaszek and Schuster, 1986) and small differences in the structure of the yeast enzyme make the β_{DP} site less reactive and unable to hydrolyze AMP-PNP.

Analysis of the central stalk has provided additional insight into some features of the structural architecture of the enzyme. The foot of the central stalk in the bovine structure inhibited with DCCD was rotated 30° and displaced by about 14 Å relative to that of yF₁I. Despite this large shift in the foot of the γ -subunit, the final 31 residues of the C-terminal α -helix overlap well and residues 1–40 of the N-terminal α -helix, although displaced, remain close to the position of the corresponding yeast N-terminal helix. The relative position of the final 31 residues of the C-terminal α -helix of the γ -subunit is the same regardless which structures are compared. This suggests that the proline-rich collar, first described in the native structure of the bovine enzyme, appears to be at least one structural constraint that holds this in position.

The Pi-binding site

The analysis of the Pi-binding site identified in the β_E -subunit of yF₁II provides a new understanding of the structural changes that occur during catalysis. Bound phosphate, while not observed in other structures, is not unexpected as biochemical studies have indicated that bovine F_1 ATPase has one or two high-affinity phosphate-binding sites (Penefsky, 1977). There are six amino-acids residues, α Arg375, β Lys163, β Arg190, β Asp256, β Asn257, and β Arg260, which form the Pi-binding site in the empty subunit. Of these, there is direct biochemical evidence that β Lys163, β Arg190, β Arg260, and α Arg375 are involved in binding Pi in the *Escherichia coli* enzyme (Ahmad and Senior, 2004a,b, 2005). The ATPase

activity of an *E. coli* F₁ mutant with β Asn257 (yeast numbering) mutated to alanine was protected from inhibition by NBD-Cl in the presence of 2.5 or 10 mM Pi (Ahmad and Senior, 2004b). This observation was interpreted as suggesting that β Asn257 is not directly involved in binding Pi, but can be explained equally well if, at these concentrations, Pi binding is not affected by the loss of the interaction with the asparagine.

A retrospective analysis was carried out on all published structures of bovine F₁ ATPase focusing on the region of the Pi-binding site identified in the γ F₁II complex. This revealed that in the structure of bovine F₁ inhibited with DCCD (Gibbons *et al*, 2000), there is a ligand, modeled as a sulfate, that binds in the same region. However, the position of this sulfate differs by 2.0 Å, and only three residues (β Arg189, β Arg260, and α Arg373) are involved in binding the sulfate group. It is possible that this represents an intermediate state of the Pi-binding site, with a lower affinity than the site characterized in the γ F₁II complex.

The recent structure of the bovine F₁ ATPase (Bowler *et al*, 2006) in which the inhibitor azide has been resolved suggests that azide, which was present in some of the earlier crystals of bovine F₁, may affect Pi binding. Biochemical studies indicate that azide inhibits Pi binding to bovine F₁ ATPase (Kasahara and Penefsky, 1978).

Rotation of the γ -subunit

The γ -subunit of γ F₁II complex is rotated 16° clockwise relative to that of the γ -subunit of γ F₁I (as viewed from the foot of the γ -subunit). The clockwise rotation is in the direction of ATP synthesis, which is expected if Pi is binding to the substrate-binding site. Prior studies have suggested that a proton gradient across the mitochondrial membrane is required for the efficient binding of Pi to the enzyme (Boyer, 1989; Al-Shawi and Senior, 1992; Al-Shawi *et al*, 1997), implying that the γ -subunit must be rotated in the direction of ATP synthesis. This is consistent with the structural results, although it is not clear if the differences in conformation of the γ F₁I and γ F₁II complexes are due to crystal contacts or whether there are two distinct conformations present in the crystallization medium (with and without bound Pi). Even in the former case, there is strong support from the biochemical data that the γ F₁II structure represents a physiologically relevant mode of Pi binding.

Many of the residues involved in the Pi-binding site also provide ligands for binding nucleotide or Mg²⁺ in the β _{DP} and β _{TP} catalytic sites. Of the six residues that form the Pi-binding site, α Arg375, β Lys163, and β Arg190 interact directly with the nucleotide phosphates and in the bovine enzyme, β Asp256 forms a H-bond with a water molecule, which in turn is bound to the Mg²⁺ ion. A Pi-binding site is also present in the structure of the bovine enzyme inhibited with AlF₄⁻:ADP (1H8E) (Menz *et al*, 2001). The binding of Mg:ADP and sulfate to the β _E-subunit is coincident with the rotation of the γ -subunit, relative to the γ -subunit of γ F₁I, by 28° in the direction of ATP synthesis and with the partial closing of β _E. This structure is considered to be an intermediate in the reaction pathway.

A model for the conformational changes accompanying ATP synthesis

Comparison of the structures of the β _E-subunits from γ F₁II and the bovine enzyme inhibited with AlF₄⁻:ADP, and the β _{DP}

Figure 5 Relative movement of the phosphate molecule during the catalytic cycle. The predicted path of the phosphate molecule during catalysis is marked by the position of phosphate (or sulfate) in the β _E-subunits of the γ F₁II complex (blue), the bovine AlF₄⁻:ADP-inhibited structure (Menz *et al*, 2001) (yellow), and the γ -phosphate of AMPPNP bound to the β _{DP}-subunit of the γ F₁I complex (salmon). The structures were superimposed using the P-loop and neighboring catalytic residues (β 151–177, β 330–336). The α -carbon trace of the P-loop of all three enzymes is shown along with the bound nucleotide and phosphate (or sulfate) of γ F₁II (yellow). The inset shows just the movement of the phosphate relative to the nucleotide. The phosphate bound to β _E (blue) moves to the position in the AlF₄⁻:ADP-inhibited state (yellow) and ends as the γ -phosphate of ATP in the DP site (as colored). Also shown is the movement of α Arg375 in the same path. The distances between the atoms are shown in Å.

site of γ F₁I, provides a model for the movement of phosphate during the catalytic reaction (Figure 5). The P-loop motif overlaps very well in all three structures, as do the nucleotides bound to the β _E site of the bovine enzyme and the β _{DP} site of γ F₁I. Assuming that these three structures represent different steps in the reaction mechanism, this illustrates the relative movements of the catalytic side chains and the phosphate group during catalysis.

From a combination of the structural and biochemical data, the following model of ATP synthesis can be assembled. Rotation of the γ -subunit (driven by proton translocation through F_o) results in phosphate-binding to an unliganded β _E-subunit and a partial closure of the β _E/ α _E interface. At this stage, the bound phosphate molecule is located almost 8 Å from its end point, marked by the γ -phosphate of AMP-PNP. The binding of Mg:ADP to the β _E-subunit is accompanied by further rotation of the γ -subunit and a more dramatic closure of the β _E/ α _E interface. During this stage, the phosphate has moved approximately 5 Å to the intermediate position observed in the AlF₄⁻:ADP-inhibited bovine F₁ structure. Further rotation of the γ -subunit results in the close approach of the phosphate group to the β -phosphate of ADP and positions the catalytic residues (β Lys163, β Glu189, β Arg190, α Arg375) appropriately for an in-line attack that results in the formation of ATP.

Although the model as presented above implies that Pi binds before the nucleotide (i.e. ordered binding of substrates), this is not a requirement. The formation of the nucleotide-binding site may be dependent on a small rotation of the γ -subunit, but it is not dependent on the presence of Pi. Therefore, there is no reason why the nucleotide should not bind before Pi. This raises the question of why nucleotide was not bound to the β _E-subunit in spite of the presence of both AMP-PNP and ADP in the crystallization medium. The concentration of ADP (0.025 mM) is close to the K_d for binding to

the third site and the concentration of AMP-PNP (0.5 mM) is well above the K_d (Corvest *et al*, 2005). At present there is no satisfactory explanation for this result. The presence of a channel leading to the Pi-binding site from the internal solvent-accessible cavity of F_1 is consistent with the random order of binding, or release, of ADP and Pi (Kayalar *et al*, 1977; Perez and Ferguson, 1990; Milgrom and Cross, 1997). If access to the Pi site is only possible from the external surface of the $\alpha_3\beta_3$ subcomplex, the binding of ADP or ATP would severely inhibit the subsequent binding of Pi, resulting in an ordered reaction. It should be noted that models of ordered binding, with either Pi or ADP binding first, have been suggested (Gao *et al*, 2005; Xing *et al*, 2005).

Mg:ADP and Mg:ATP or ADP and ATP bind with similar affinities to the lowest affinity site of the *E. coli* enzyme (Weber *et al*, 1993, 1994). This is consistent with the structural data, which show that the side chains that could interact with the γ -phosphate are in fact far removed from the nucleotide-binding site when the β -subunit adopts the open conformation. Similarly, because the phosphate-binding site is spatially distinct from the nucleotide-binding site, it would be predicted, as observed, that phosphate does not inhibit the binding of either ATP or ADP (Weber and Senior, 1995).

This model also provides an explanation for how the enzyme is able to make ATP under cellular conditions where the ATP/ADP ratio is from 10 to 50. ATP cannot be accommodated in the half-closed conformation of the β_E -subunit in the AlF_4^- -inhibited bovine enzyme, as this would result in severe steric clashes of the γ -phosphate with both the side chain of α Arg373 and the bound phosphate. Consequently, regardless of the order of binding of phosphate and nucleotide, the required conformational change to the half-closed state can only take place when ADP, and not ATP, is bound.

There has been some debate on whether the F_1 ATPase follows bi-site or tri-site kinetics (Milgrom *et al*, 1998; Weber and Senior, 2001; Nishizaka *et al*, 2004; Milgrom and Cross, 2005). The structure of the yF_1II complex is supportive of the tri-site kinetic model, since there are two molecules of AMP-PNP and a phosphate bound to the three catalytic sites that is a predicted intermediate during tri-site, but not bi-site catalysis. However, while supportive, this structure does not prove tri-site kinetics.

Comparison with other molecular motors

F_1 -ATPase is structurally homologous to the hexameric AAA+ family of proteins. Both contain a core nucleotide-binding domain with a RecA fold and both undergo large conformational changes in response to nucleotide binding. Comparison of these molecular motors reveals both similarities and differences. The large-scale domain motions are similar in nature and magnitude in F_1 -ATPase and the heat shock protein HslU (Wang, 2004). However, the small local re-arrangements that accompany the pseudo rigid-body domain rotations, for example those involved in the formation of the phosphate-binding site in yF_1II , are equally important. These details are likely to be specific to each individual motor. The coupling between hydrolysis events in different subunits provides another example of the differences between F_1 -ATPase and AAA+ proteins, and probably between different members of the AAA+ family. In F_1 -ATPase, the

asymmetric interactions with the γ -subunit determine the catalytic state of the β -subunits. Further insight into the relationship between the orientation of the γ -subunit and the nature of the catalytic interfaces is revealed by comparison of the yF_1I and yF_1II complexes. Rotation of the γ -subunit ensures strictly sequential hydrolysis of ATP in the β -subunits. By contrast, recent biochemical data suggest that the ClpX protease undergoes a random order of hydrolysis (Martin *et al*, 2005), while a concerted mechanism has been proposed for the SV40 helicase (Gai *et al*, 2004). While following some common general principles, there are important differences in the mode of action of these superficially similar molecular motors.

Materials and methods

Materials

A genetically engineered yeast strain, DMY301[p β NH6] was used to express and purify the F_1 ATPase. This strain has a null mutation in the gene encoding the β -subunit of F_1 ATPase and it was transformed with a vector that contains the gene encoding the β -subunit with a His6 tag just after the mitochondrial leader peptide. The expression and purification of the His₆ F_1 ATPase is described elsewhere (Mueller *et al*, 2004b).

Crystallization, data reduction, and refinement

Crystals were obtained by vapor diffusion using the sitting drop method and frozen as described (Mueller *et al*, 2004a). Data were collected from a single crystal at ID5 of the Advanced Photon Source at 100 K and processed using MOSFLM (Leslie, 1992) and SCALA (Evans, 2006). Molecular replacement was performed using the bovine structure (1E1Q), minus the nucleotides as an initial model in AMoRE (Navaza, 2001). Model building was performed with XtalView (McRee, 1999), O (Jones *et al*, 1991), and COOT (Emsley and Cowtan, 2004), and refinement was done with CNS (Brunger *et al*, 1998) and REFMAC5 (Murshudov *et al*, 1997). Noncrystallographic symmetry (NCS) restraints for main chain and side chains were applied to improve the refinement. TLS refinement (Schomaker and Trueblood, 1968; Winn *et al*, 2001) was used to account for overall anisotropic motion of the molecules. Data processing and refinement statistics are shown in Table I. The stereochemistry was assessed using PROCHECK (Laskowski *et al*, 1993). The Ramachandran plot showed that 99.5, 99.3, and 99.6% of residues are in the most favored or additionally favored regions for yF_1I , II, and III, respectively. Structure comparisons, axis rotations, and r.m.s. deviations were performed with SUPERPOSE, LSQKAB, and TOP3D, contained in the CCP4 (Collaborative Computational Project, 1994) package, EDPDB (Zhang and Matthews, 1995), and MOE (Group, 2004). Figures were generated using Pymol (DeLano, 2002), SPOCK (Christopher, 1998), GRASP (Nicholls *et al*, 1991), and O (Jones *et al*, 1991).

Coordinates

Coordinates and structure factors have been deposited in the Protein Data Bank (accession code 2HLD).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

Acknowledgements

This work was supported by grants from the National Institute of Health (NIH, R01-GM067091 and R01-GM066223) to DM Mueller and Medical Research Council support to JE Walker and AGW Leslie. DM Mueller received a Fogarty Senior Fellowship from the NIH (1F06TW002379) for part of the tenure of this project. Data were collected in part at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT ID-5A) beam lines at the Advanced Photon Source, Argonne National Laboratory with assistance from Zdzislaw Wawrzak. The DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) sector of the APS is

supported by El Dupont de Nemours & Co., the Dow Chemical Company, and the National Science Foundation through Grant DMR-9304725. Use of the APS was supported by the US

Department of Energy, Office of Science of Basic Energy Sciences, contract No. W-31-109-ENG-38. This project was aided with the use of the X-ray facility at Rosalind Franklin University.

References

- Abrahams JP, Buchanan SK, Van Raaij MJ, Fearnley IM, Leslie AGW, Walker JE (1996) The structure of bovine F₁-ATPase complexed with the peptide antibiotic efrapeptin. *Proc Natl Acad Sci USA* **93**: 9420–9424
- Abrahams JP, Leslie AGW, Lutter R, Walker JE (1994) Structure at 2.8 Å resolution of F₁-ATPase from bovine heart mitochondria. *Nature* **370**: 621–628
- Ahmad Z, Senior AE (2004a) Mutagenesis of residue βArg-246 in the phosphate-binding subdomain of catalytic sites of *E. coli* F₁-ATPase. *J Biol Chem* **279**: 31505–31513
- Ahmad Z, Senior AE (2004b) Role of βAsn-243 in the phosphate-binding subdomain of catalytic sites of *E. coli* F₁-ATPase. *J Biol Chem* **279**: 46057–46064
- Ahmad Z, Senior AE (2005) Involvement of ATP synthase residues αArg-376, βArg-182, and βLys-155 in Pi binding. *FEBS Lett* **579**: 523–528
- Al-Shawi MK, Ketchum CJ, Nakamoto RK (1997) The *E. coli* F₀F₁ γM23K uncoupling mutant has a higher K_{0.5} for P_i. Transition state analysis of this mutant and others reveals that synthesis and hydrolysis utilize the same kinetic pathway. *Biochemistry* **36**: 12961–12969
- Al-Shawi MK, Senior AE (1992) Effects of dimethyl sulfoxide on catalysis in *E. coli* F₁-ATPase. *Biochemistry* **31**: 886–891
- Bianchet MA, Hüllihien J, Pedersen PL, Amzel LM (1998) The 2.8-Å structure of rat liver F₁-ATPase: configuration of a critical intermediate in ATP synthesis/hydrolysis. *Proc Natl Acad Sci USA* **95**: 11065–11070
- Bowler MW, Montgomery MG, Leslie AGW, Walker JE (2006) How azide inhibits ATP hydrolysis by the F-ATPases. *Proc Natl Acad Sci USA* **103**: 8646–8649
- Boyer PD (1989) A perspective of the binding change mechanism for ATP synthesis. *FASEB J* **3**: 2164–2178
- Boyer PD, Cross RL, Momsen W (1973) A new concept for energy coupling in oxidative phosphorylation based on a molecular explanation of the oxygen exchange reactions. *Proc Natl Acad Sci USA* **70**: 2837–2839
- Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL (1998) Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr D* **54** (Part 5): 905–921
- Christopher J (1998) *SPOCK: The Structural Properties Observation and Calculation Kit (Program Manual)*. Texas A&M University, Center for Macromolecular Design, College Station
- Collaborative Computational Project (1994) The CCP4 suite: programs for protein crystallography. *Acta Crystallogr D* **50**: 760–763
- Corvest V, Sigalat C, Venard R, Falson P, Mueller DM, Haraux F (2005) The binding mechanism of the yeast F₁-ATPase inhibitory peptide: role of catalytic intermediates and enzyme turnover. *J Biol Chem* **280**: 9927–9936
- DeLano WL (2002) *The PyMOL Molecular Graphics System*. San Carlos: Delano Scientific
- Emsley P, Cowtan K (2004) Coot: model-building tools for molecular graphics. *Acta Crystallogr D* **60**: 2126–2132
- Evans P (2006) Scaling and assessment of data quality. *Acta Crystallogr D* **62**: 72–82
- Gai D, Zhao R, Li D, Finkielstein CV, Chen XS (2004) Mechanisms of conformational change for a replicative hexameric helicase of SV40 large tumor antigen. *Cell* **119**: 47–60
- Gao YQ, Yang W, Karplus M (2005) A structure-based model for the synthesis and hydrolysis of ATP by F₁-ATPase. *Cell* **123**: 195–205
- Gibbons C, Montgomery MG, Leslie AGW, Walker JE (2000) The structure of the central stalk in bovine F₁-ATPase at 2.4 Å resolution. *Nat Struct Biol* **7**: 1055–1061
- Groth G, Pohl E (2001) The structure of the chloroplast F₁-ATPase at 3.2 Å resolution. *J Biol Chem* **276**: 1345–1352
- Group CC (2004) *MOE: Molecular Operating Environment*. Montreal: Chemical Computing Group
- Jones TA, Zou JY, Cowan SW, Kjeldgaard M (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr A* **47** (Part 2): 110–119
- Junge W, Lill H, Engelbrecht S (1997) ATP synthase: an electrochemical transducer with rotatory mechanics. *Trends Biochem Sci* **22**: 420–423
- Kagawa R, Montgomery MG, Braig K, Leslie AGW, Walker JE (2004) The structure of bovine F₁-ATPase inhibited by ADP and beryllium fluoride. *EMBO J* **23**: 2734–2744
- Kane Dickson V, Silvester JA, Fearnley IM, Leslie AGW, Walker JE (2006) On the structure of the stator of the mitochondrial ATP synthase. *EMBO J* **25**: 2911–2918
- Kasahara M, Penefsky HS (1978) High affinity binding of monovalent Pi by beef heart mitochondrial adenosine triphosphatase. *J Biol Chem* **253**: 4180–4187
- Kayalar C, Rosing J, Boyer PD (1977) An alternating site sequence for oxidative phosphorylation suggested by measurement of substrate binding patterns and exchange reaction inhibitions. *J Biol Chem* **252**: 2486–2491
- Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program software suite for macromolecular structure determination. *J Applied Crystallogr* **26**: 283–291
- Le NP, Omote H, Wada Y, Al-Shawi MK, Nakamoto RK, Futai M (2000) *E. coli* ATP synthase α subunit Arg-376: the catalytic site arginine does not participate in the hydrolysis/synthesis reaction but is required for promotion to the steady state. *Biochemistry* **39**: 2778–2783
- Leslie AGW (1992) Joint CCP4 and ESF-EACMB. *Newsletter Protein Crystallography*. Warrington, UK: Daresbury Laboratory
- Martin A, Baker TA, Sauer RT (2005) Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines. *Nature* **437**: 1115–1120
- McRee DE (1999) XtalView/Xfit—a versatile program for manipulating atomic coordinates and electron density. *J Struct Biol* **125**: 156–165
- Menz RI, Walker JE, Leslie AGW (2001) Structure of bovine mitochondrial F₁-ATPase with nucleotide bound to all three catalytic sites. Implications for the mechanism of rotary catalysis. *Cell* **106**: 331–341
- Milgrom YM, Cross RL (1997) Nucleotide-depleted beef heart F₁-ATPase exhibits strong positive catalytic cooperativity. *J Biol Chem* **272**: 32211–32214
- Milgrom YM, Cross RL (2005) Rapid hydrolysis of ATP by mitochondrial F₁-ATPase correlates with the filling of the second of three catalytic sites. *Proc Natl Acad Sci USA* **102**: 13831–13836
- Milgrom YM, Murataliev MB, Boyer PD (1998) Bi-site activation occurs with the native and nucleotide-depleted mitochondrial F₁-ATPase. *Biochemistry J* **330**: 1037–1043
- Mitchell P (1977) Vectorial chemiosmotic processes. *Annu Rev Biochem* **46**: 996–1005
- Mueller DM, Puri N, Kabaleeswaran V, Terry C, Leslie AGW, Walker JE (2004a) Crystallization and preliminary crystallographic studies of the mitochondrial F₁-ATPase from the yeast *S. cerevisiae*. *Acta Crystallogr D* **60**: 1441–1444
- Mueller DM, Puri N, Kabaleeswaran V, Terry C, Leslie AGW, Walker JE (2004b) Ni-chelate-affinity purification and crystallization of the yeast mitochondrial F₁-ATPase. *Protein Express Purif* **37**: 479–485
- Murshudov GN, Vagin AA, Dodson EJ (1997) Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D* **53**: 240–255
- Navaza J (2001) Implementation of molecular replacement in AMoRe. *Acta Crystallogr D* **57**: 1367–1372
- Nicholls A, Sharp KA, Honig B (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins* **11**: 281–296
- Nishizaka T, Oiwa K, Noji H, Kimura S, Muneyuki E, Yoshida M, Kinosita Jr K (2004) Chemomechanical coupling in F₁-ATPase

- revealed by simultaneous observation of nucleotide kinetics and rotation. *Nat Struct Mol Biol* **11**: 142–148
- Noji H, Yasuda R, Yoshida M, Kinosita Jr K (1997) Direct observation of the rotation of F_1 -ATPase. *Nature* **386**: 299–302
- Penefsky HS (1977) Reversible binding of Pi by beef heart mitochondrial adenosine triphosphatase. *J Biol Chem* **252**: 2891–2899
- Perez JA, Ferguson SJ (1990) Kinetics of oxidative phosphorylation in *Paracoccus denitrificans*. 1. Mechanism of ATP synthesis at the active site(s) of F_0F_1 -ATPase. *Biochemistry* **29**: 10503–10518
- Schomaker V, Trueblood KN (1968) On the rigid-body motion of molecules in crystals. *Acta Crystallogr Sect B* **24**: 63–76
- Senior AE, Nadanaciva S, Weber J (2002) The molecular mechanism of ATP synthesis by F_1F_0 -ATP synthase. *Biochim Biophys Acta* **1553**: 188–211
- Stock D, Leslie AGW, Walker JE (1999) Molecular architecture of the rotary motor in ATP synthase. *Science* **286**: 1700–1705
- Tomaszek TA, Schuster SM (1986) Hydrolysis of adenylyl-5-yl imidodiphosphate by beef heart mitochondrial ATPase. *J Biol Chem* **261**: 2264–2269
- Walker JE, Dickson VK (2006) The peripheral stalk of the mitochondrial ATP synthase. *Biochim Biophys Acta* **1757**: 286–296
- Wang J (2004) Nucleotide-dependent domain motions within rings of the RecA/AAA(+) superfamily. *J Struct Biol* **148**: 259–267
- Weber J, Senior AE (1995) Location and properties of pyrophosphate-binding sites in *E. coli* F_1 -ATPase. *J Biol Chem* **270**: 12653–12658
- Weber J, Senior AE (2001) Bi-site catalysis in F_1 -ATPase: does it exist? *J Biol Chem* **12**: 12
- Weber J, Wilke-Mounts S, Lee RS, Grell E, Senior AE (1993) Specific placement of tryptophan in the catalytic sites of *E. coli* F_1 -ATPase provides a direct probe of nucleotide binding: maximal ATP hydrolysis occurs with three sites occupied. *J Biol Chem* **268**: 20126–20133
- Weber J, Wilke-Mounts S, Senior AE (1994) Cooperativity and stoichiometry of substrate binding to the catalytic sites of *Escherichia coli* F_1 -ATPase. Effects of magnesium, inhibitors, and mutation. *J Biol Chem* **269**: 20462–20467
- Winn MD, Isupov MN, Murshudov GN (2001) Use of TLS parameters to model anisotropic displacements in macromolecular refinement. *Acta Crystallogr D* **57**: 122–133
- Xing J, Liao JC, Oster G (2005) Making ATP. *Proc Natl Acad Sci USA* **102**: 16539–16546
- Zhang X-J, Matthews BW (1995) EDPDB: a multifunctional tool for protein structure analysis. *J Applied Crystallogr* **28**: 624–630