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THE ROYAL SOCIETY

Structural model of F_1-ATPase and the implications for rotary catalysis

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The crystal structure of bovine mitochondrial F_1-ATPase is described. Several features of the structure are consistent with the binding change mechanism of catalysis, in which binding of substrates induces conformational changes that result in a high degree of cooperativity between the three catalytic sites. Furthermore, the structure also suggests that catalysis is accompanied by a physical rotation of the centrally placed γ-subunit relative to the approximately spherical $\alpha_3\beta_3$ sub-assembly.

Keywords: F_1-ATPase; crystal structure; rotary catalysis

1. INTRODUCTION

F_1F_o ATP synthases are found in the membranes of eubacteria, the thylakoid membranes of chloroplasts, and the inner membranes of mitochondria. They use energy derived from proton movement down an electrochemical gradient to synthesize ATP from ADP and inorganic phosphate (Pi). In bacteria the enzyme is reversible, and under anaerobic conditions it can use ATP hydrolysis to generate a transmembrane proton gradient. The ATP synthase is a complex oligomeric assembly, composed of 6 different subunits in the case of the bovine mitochondrial enzyme, and eight or nine for the simpler bacterial homologues, with some subunits present in multiple copies. Morphologically the enzyme has three components; a membrane-bound sector, F_o, that contains the proton channel, which is linked by a narrow stalk to an approximately spherical assembly F_1 that contains the catalytic sites. The F_1 component can be separated from the membrane, and isolated F_1 is active as an ATPase, hence the designation F_1-ATPase.

F_1-ATPase from all characterized sources consists of five different subunits, with a stoichiometry $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$. The composition and amino-acid sequences of the subunits of bovine mitochondrial F_1-ATPase were determined by Walker and co-workers (Walker *et al.* 1985; Walker *et al.* 1991) and show significant homology to the corresponding subunits in the enzymes derived from chloroplasts and bacteria, particularly for the α- and β-subunits (Walker *et al.* 1985).

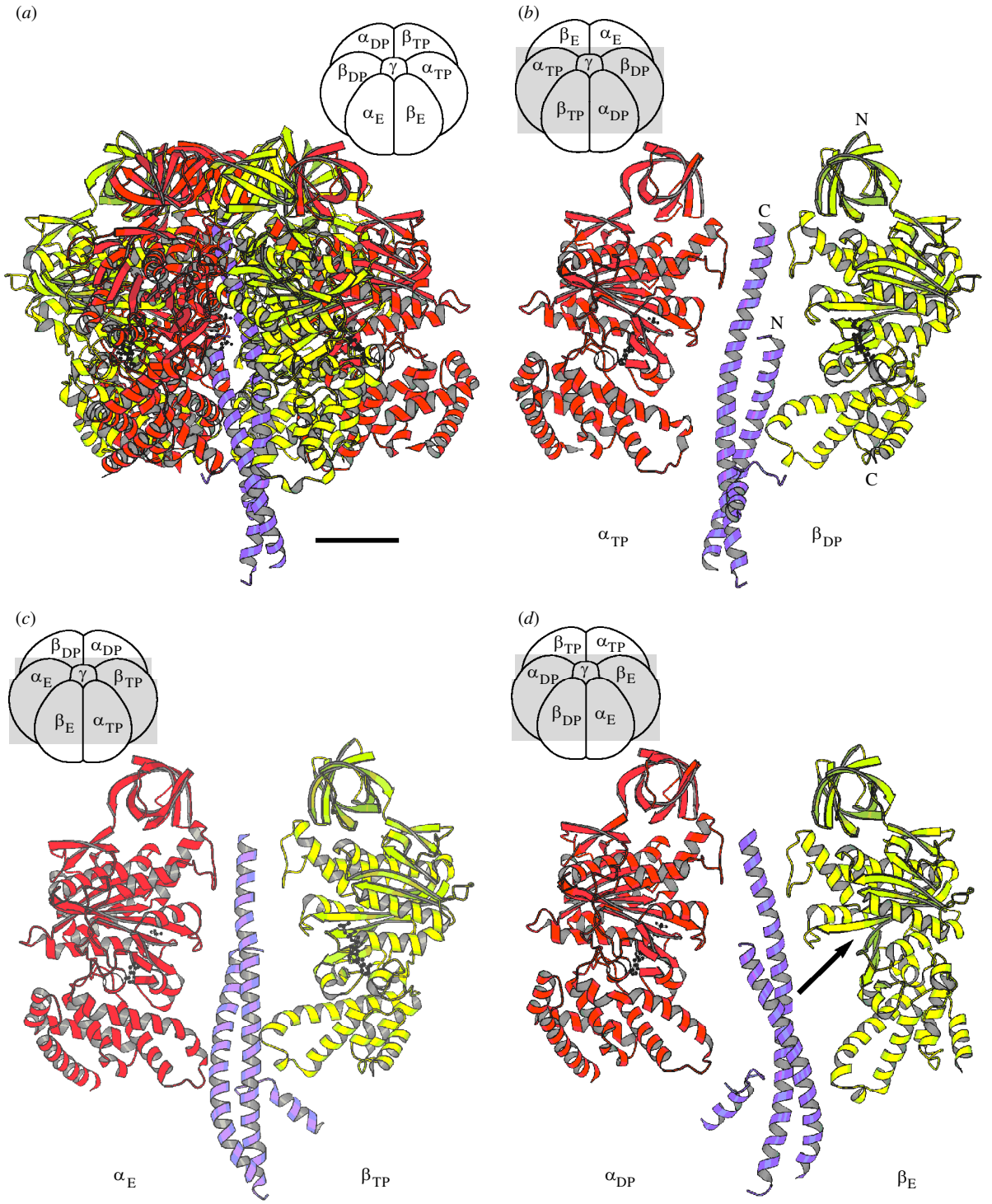
2. STRUCTURE DETERMINATION

The crystal structure of bovine mitochondrial F_1-ATPase was first determined at 2.8 Å resolution (Abrahams *et al.* 1994). The resulting structural model includes almost all of the residues in the α- and β-subunits (except a few amino acids at the N and C termini), but only about 50% of the γ-subunit and none of the δ- or ε-subunits. The missing residues are believed to be disordered in the crystal, although substoichiometry of the δ- and ε-subunits cannot be entirely ruled out. Subsequently, the structures of F_1-ATPase complexed with a variety of inhibitors and nucleotides have been determined at resolutions of between 2.5 and 3.2 Å (Abrahams *et al.* 1996; Van Raaij *et al.* 1996; Orriss *et al.* 1998; K. Braig, R. I. Menz, A. G. W. Leslie and J. E. Walker, unpublished results). It had been thought that some of these complexes might reveal significant conformational changes in F_1-ATPase that would represent different points along the catalytic pathway. However, the overall structure is remarkably similar in all of these complexes.

The crystal structure of the $\alpha_3\beta_3$ sub-assembly of F_1-ATPase from a thermophilic bacterium (PS3) has also been determined (Shirakihara *et al.* 1997). As expected on the basis of the high level of sequence identity, the structures of the bacterial α- and β-subunits are remarkably similar to those of the bovine enzyme. In the absence of bound nucleotides or the γ-, δ- and ε-subunits the $\alpha_3\beta_3$ complex shows strict threefold symmetry, in marked contrast to the asymmetry present in the mitochondrial enzyme.

The crystal structure of the rat mitochondrial F_1-ATPase has recently been reported (Bianchet *et al.* 1998). Here again, the α- and β-subunits adopt conformations very similar to those observed in the bovine structure. The crucial difference is that the rat enzyme has been crystallized in a form that imposes threefold symmetry on the electron density in the crystal. As a result, the γ-subunit is statistically disordered, making it difficult to obtain a reliable structural model. While it is possible that the three α- and three β-subunits are conformationally distinct but statistically disordered, Bianchet *et al.* have suggested that all three copies of these subunits adopt essentially the same conformation.

The γ-subunit is believed to play a crucial role in the rotary catalytic mechanism of F_1-ATPase. As this



igure 1. A schematic representation of the structure of bovine mitochondrial F_1-ATPase. Nucleotides are drawn in a 'ball and ick' representation. The relative positions of α- and β-subunits labelled as described in the text, are indicated in the icon in the pper left or upper right of the figures. (*a*) The entire F_1 assembly. Scale bar, 20 Å long. (*b*) The α_{TP}-, β_{DP}- and γ-subunits. The N and C termini of the β- and γ-subunits are shown. (*c*) The α_E-, β_{TP}- and γ-subunits. (*d*) The α_{DP}-, β_E- and γ-subunits. The arrow indicates the point at which the β-sheet of the nucleotide binding domain in disrupted in β_E. (Figures 1–3 are reproduced with permission from Abrahams *et al.* (1994), copyright (1994) Macmillan Magazines Ltd.)

ubunit is absent in the structure of the bacterial $\alpha_3\beta_3$ omplex and statistically disordered in the rat itochondrial structure, it is most appropriate to onsider the structural evidence for rotary catalysis in erms of the structure of the bovine mitochondrial nzyme.

3. STRUCTURE DESCRIPTION

The overall structure of the enzyme (Abrahams *et al.* 1994) is shown schematically in figure 1*a*. The three α- and three β-subunits associate like the segments of an orange to form a roughly spherical assembly *ca.* 100 Å

n diameter. The major part of the γ-subunit forms a eft-handed coiled-coil which runs approximately along ne axis of the $\alpha_3\beta_3$ complex, protruding from it by bout 30 Å, while the remainder forms a short α- elical segment. The overall shape and dimensions of ne structure are very similar to those determined y cryo-electron microscopy of the enzyme from *Escherichia coli* (Gogol *et al.* 1987). In particular, the rotruding γ-subunit probably corresponds to part of the entral stalk that links F_1 to the membrane-bound omponent F_o.

Both α- and β-subunits are known to contain ucleotide-binding sites, but chemical modification xperiments using nucleotide analogues have identified ne β-subunit as containing the catalytic nucleotide- inding site (reviewed in Senior 1988). In the crystal tructure, all the α-subunits bind the non-hydrolysable TP analogue AMPPNP. However, one β-subunit (β_{TP}) inds AMPPNP, the second (β_{DP}) binds ADP, and the hird (β_E) has no nucleotide bound. The nucleotide- inding sites are located at the interfaces of the α- and -subunits; the α-subunit that contributes to the β_{TP} ucleotide-binding site is therefore denoted α_{TP}, and the ther two α-subunits are similarly labelled α_{DP} and α_E see figure 1). A cross-section through the complex figure 1*b*) showing the α_{TP}-, β_{DP}- and γ-subunits, allows ne fold of the individual subunits to be seen more learly. The fold of the α- and β-subunits is very similar they have approximately 20% sequence identity). The N-terminal domain is a six-stranded β-barrel. This is ollowed by a central nucleotide-binding domain, ontaining a nine-stranded predominantly parallel -sheet with nine associated α-helices, five on one side f the sheet and four on the other. The final C-terminal omain is an α-helical bundle, made up of seven helices n the α-subunit and six in the slightly smaller -subunit.

4. CONFORMATIONAL ASYMMETRY OF THE α- AND β-SUBUNITS

The three α-subunits adopt similar conformations, lthough in the α_{TP}-subunit there is a small rigid body otation of the N-terminal domain relative to the other two omains. The two β-subunits β_{TP} and β_{DP} also adopt milar conformations, but the nucleotide-free β_E-subunit nows a dramatic conformational change, which is most mply described as a rigid body rotation of the lower part f the nucleotide-binding domain and the C-terminal omain. This rotation, almost 30° in magnitude, moves ne C-terminal domain outwards from the axis of the $_3\beta_3$ assembly. It is accompanied by a significant disrup- on in the β-sheet of the nucleotide-binding domain arrowed in figure 1*d*) with the loss of several hydrogen onds. The novel conformation of the β_E-subunit is learly associated with the asymmetrical location of the -subunit relative to the axis of the $\alpha_3\beta_3$ complex. The oiled-coil structure of the γ-subunit does not lie along nis axis, but is displaced to one side towards the $_E$-subunit. This displacement is particularly marked in ne lower region of the coiled-coil, and prevents the $_E$-subunit adopting the conformation observed in the $_{TP}$- and β_{DP}-subunits.

5. THE ACTIVE SITE

The nucleotide-binding site in the β_{TP}-subunit is shown schematically in figure 2. There is a water molecule (labelled HOH) at a distance of 4 Å from the γ-phosphate group, which hydrogen bonds to the carboxylate group of Glu188. This residue is appropriately positioned to activate the water molecule for an in-line nucleophilic attack on the terminal phosphate in the hydrolysis reaction. Lys162, Arg189 and α-Arg373 (from the adjacent α_{TP}-subunit) may stabilize the negative charge that develops in a penta-coordinated transition state. All four residues have been implicated as playing an important role in catalysis by site-directed mutagenesis of the *E. coli* and *Bacillus* PS3 enzymes (Futai *et al.* 1989; Senior & Al-Shawi 1992; Amano *et al.* 1994; Park *et al.* 1994).

6. THE BINDING CHANGE MECHANISM OF CATALYSIS

Both F_1-ATPase and ATP synthase display negative cooperativity in binding substrates but positive cooperativity in catalysis. Boyer and colleagues (reviewed in Boyer 1993) have shown that, in the case of ATP synthase, the release of ATP (the product) is greatly enhanced by the binding of ADP + Pi (the substrates) to an adjacent catalytic site. Similarly, the release of ADP + Pi is promoted by ATP binding to an adjacent catalytic site in F_1-ATPase. To account for these observations and other biochemical evidence, Boyer proposed that the three catalytic sites alternate sequentially between three different states, open, loose and tight, which have differing affinities for nucleotides (figure 3). During ATP synthesis, energy from the proton gradient is used to convert a tight site into an open site, with the release of ATP. Simultaneously, the loose site, with bound ADP + Pi is converted into a tight site, leading to ATP synthesis, while the open site, which has low affinity for nucleotides, is converted into a loose site ready to bind the substrates. This model is known as the binding change mechanism for catalysis (Boyer 1993; Cross 1981). During ATP hydrolysis in F_1-ATPase, the reverse reaction operates.

The crystal structure provides support for Boyer's hypothesis. The nature of the three catalytic sites is indeed rather different, as demonstrated by the buried surface areas at the catalytic interfaces. The α_{DP}–β_{DP} interface has a buried surface area of 3030 Å^2, the α_{TP}–β_{TP} interface 2200 Å^2 and the α_E–β_E interface 1760 Å^2. Thus the catalytic site on the β_E-subunit would represent the open conformation, with low affinity for nucleotides, β_{TP} corresponds to the loose conformation and β_{DP} to the tight conformation. At first sight, the binding of ADP to the tight site appears to be inconsistent with Boyer's scheme. However, there is a well-characterized Mg, ADP-inhibited form of the enzyme, which has ADP and Mg, but no phosphate, bound to the tight catalytic site, and the crystal structure may well represent this inhibited state. In spite of this, the similarity to the structures of the efrapeptin-inhibited (Abrahams *et al.* 1996) and aurovertin-inhibited (Van Raaij *et al.* 1996) enzymes suggests that this conformation is closely related to one of

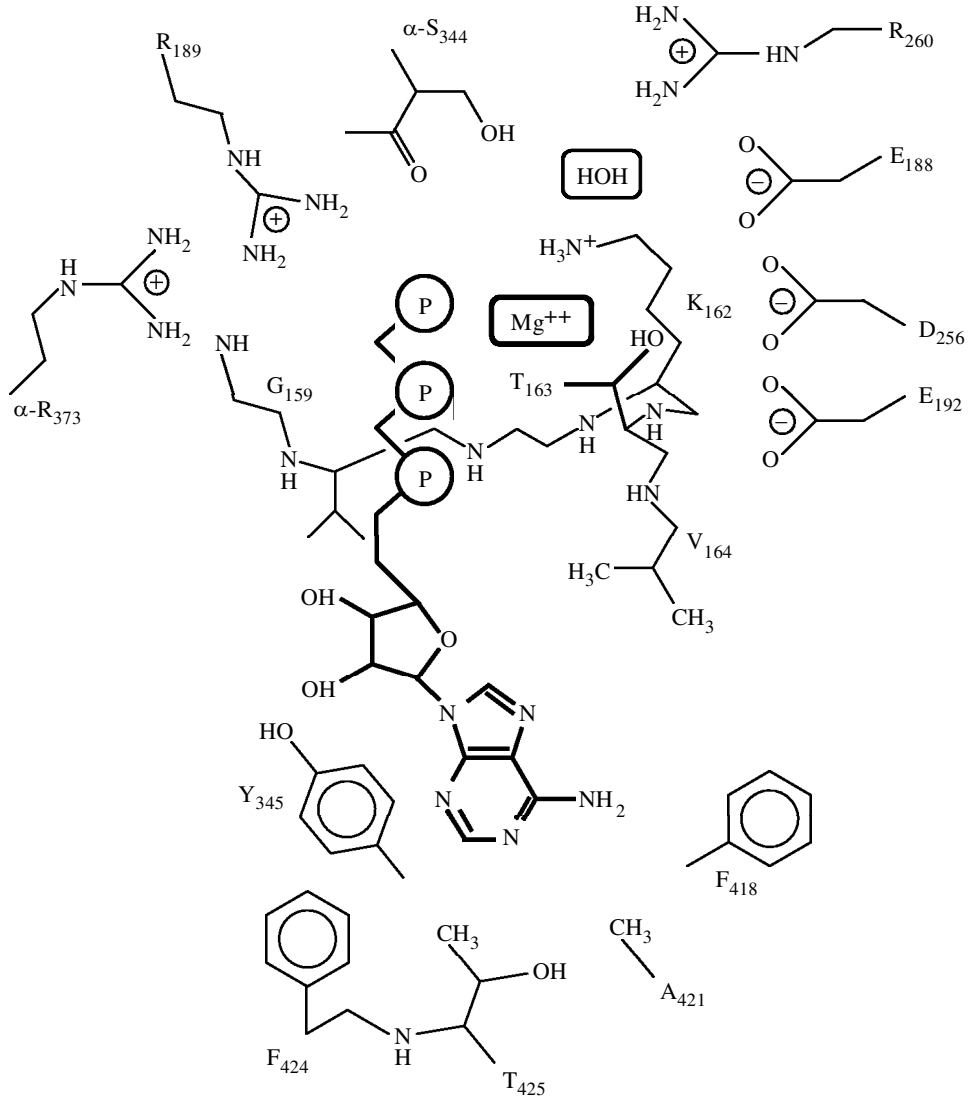


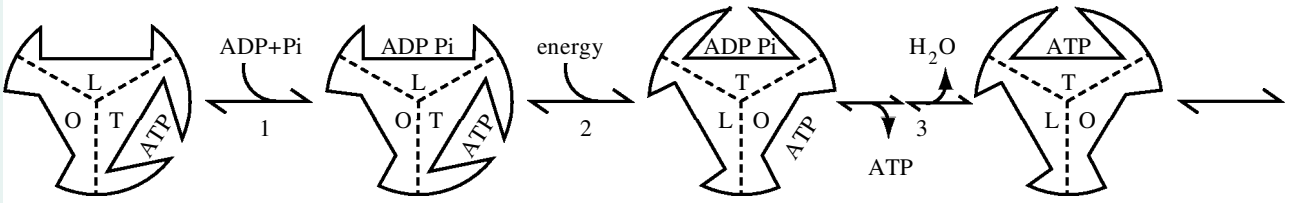
Figure 2. The nucleotide-binding site of the β_{TP}-subunit. Residues are labelled according to the single letter convention. α-R373 and α-S344 belong to the adjacent α_{TP}-subunit, all other residues are in β_{TP}. The adenine ring occupies a hydrophobic pocket formed by Y345, F418, A421 and F424. Residues G159 to T163 are part of the P-loop sequence. Mg^{++} denotes the magnesium ion, and HOH indicates the position of a water molecule that is believed to play a role in catalysis.

the states of the enzyme in the active catalytic cycle. While it is not possible to verify all aspects of the binding change mechanism on the basis of the currently available structural data, the structures certainly provide strong support for the notion of sequential conformational changes in the catalytic β-subunits that is an integral component of Boyer's hypothesis.

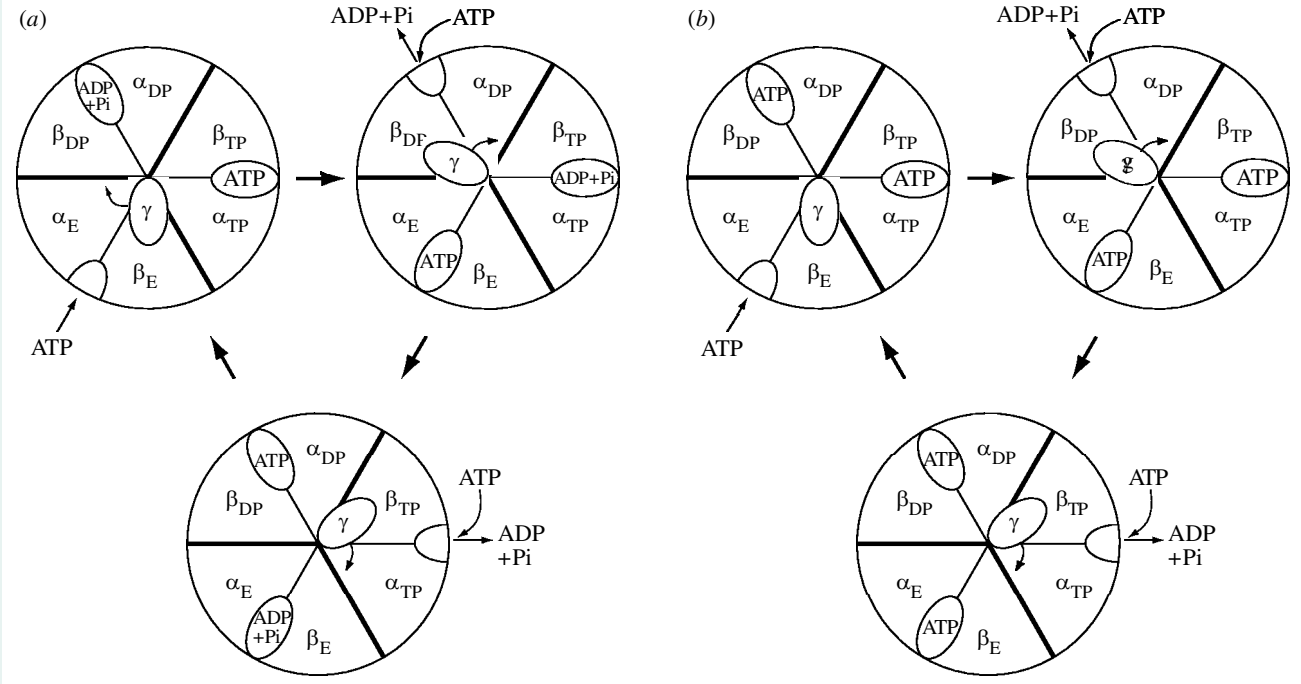
7. ROTATIONAL CATALYSIS

The subunit stoichiometry of F_1 led Boyer (1993) to suggest that the conformational changes required for interconversion between the three types of catalytic site might be achieved by rotation of the three catalytic β-subunits relative to the single copy subunits γ, δ and ε. Cross-linking studies provided some support for this suggestion. It was shown that cross-linking the γ- and α/β-subunits inhibited F_1-ATPase activity, but on reducing the S–S bond in the middle of the cross-linker, thereby breaking the link, activity was restored (Aggeler *et al.* 1993).

A number of features of the crystal structure suggest that the γ-subunit can indeed rotate relative to the $\alpha_3\beta_3$complex. The first of these is that the interaction between the C-terminal region of the γ-subunit and the α- and β-subunits (see figure 1*b–d*) has the characteristics of a molecular bearing. Six loops, one from each α- and β-subunits (figure 1), form a circular hydrophobic collar with a diameter of 15 Å and a depth of 17 Å. This 'collar' perfectly accommodates the final residues (261–271) of the C-terminal helix of the γ-subunit. These residues all have small hydrophobic side-chains, so that the whole structure resembles a shaft (formed by the γ-subunit) passing through a bearing (formed by the six loops). The second feature of interest is the presence of a large, solvent-filled cavity in the centre of the $\alpha_3\beta_3$ complex (figure 1), which is traversed by the γ-subunit. There are very few specific interactions between the central segment of the γ-subunit and the α- and β-subunits that would impede their relative rotation. Finally, the interactions between tthe lower segment of the γ-subunit and the C-terminal



igure 3. The binding change mechanism of catalysis. The three catalytic sites, labelled O (open), L (loose) and T (tight) inter-onvert sequentially during catalysis. Only the T-site is catalytically active. Energy is required for substrate binding and product elease. (After Cross 1981.)



igure 4. Two alternative schemes illustrating the hydrolytic cycle in F_1-ATPase. The subunit nomenclature is the same as in gure 1, and the view is looking down from the top of that figure. The position of the lower part of the γ-subunit, which interacts ith the C-terminal domains of the β-subunits, is shown. (*a*) Binding of ATP to the β_E-subunit induces a conformational change n that subunit which produces a 120° clockwise rotation of the γ-subunit, and also promotes ATP hydrolysis in the β_{TP}-subunit. *b*) ATP binding to the β_E-subunit results in ATP hydrolysis in the β_{DP}-subunit, which generates a large conformational change nd product release. This in turn results in the rotation of the γ-subunit as shown.

omains of the α- and β-subunits involve only hydro-hobic side-chains. Many of these residues have large emperature factors, suggesting some conformation flex-ibility, all of which supports the idea that there will be no gnificant energy barrier to rotation.

8. ADDITIONAL EVIDENCE FOR ROTATION

Subsequent to the publication of the structure, further vidence has been presented that supports the model of otational catalysis. This includes additional cross-linking udies (Duncan *et al.* 1995), an analysis based on polar-zed absorption relaxation measurements (Sabbert *et al.* 996; Sabbert & Junge 1997), and most recently the irect observation of rotation (Noji *et al.* 1997; Kinosita *et l.* 1998; Yasuda *et al.* 1998). In these last experiments, F_1-ATPase was tethered to Ni-coated beads via a His-tag at ne N-terminus of the β-subunits so that the complex was rientated 'upside down' relative to the orientation shown n figure 1. Fluorescently labelled actin filaments were hen coupled to the γ-subunit using a biotin–streptavidin linker. When the beads were attached to a cover-slip in a microscope flow cell containing ATP, the actin filaments were seen to rotate at up to 7 Hz. This rotation is slower than expected from the turnover rate of the free enzyme in solution, but the viscous drag of the actin filaments could easily account for the discrepancy.

9. THE MECHANISM OF ROTATION

Models have been proposed to explain how proton translocation in F_o results in rotation of the γ-subunit relative to the $\alpha_3\beta_3$ assembly in the intact ATP synthase (Junge *et al.* 1997; Elston *et al.* 1998) although in the absence of structural information these models are necessarily speculative. It is then not difficult to visualize how the γ-subunit could act in a manner similar to a rotating 'eccentric cam', producing sequential conformational changes in the catalytic β-subunits which correspond to the different states described in the binding change mechanism.

It is perhaps rather more difficult to visualize the reverse reaction in F_1-ATPase, where hydrolysis of ATP

esults in the rotation of the γ-subunit. Two different chemes can be envisaged (figure 4) both of which are onsistent with the direction of rotation observed by Noji *et al.* (1997). In the first, ATP binding to the empty -subunit results in a major conformational change in hat subunit, which in turn produces a clockwise rotation f the γ-subunit by 120° (as viewed from above). This onverts the β_{DP}-subunit to an 'open' conformation, with ow affinity for (ADP + Pi), which are released. Conformational changes are also transmitted to the active site f the β_{TP}-subunit (although these changes are likely to be much more subtle), promoting the hydrolysis of bound ATP to ADP + Pi. In the next step in the cycle, ATP inds to the (now empty) β_{DP}-subunit, resulting, via a urther rotation of the γ-subunit, in the release of ADP + Pi from the β_{TP}-subunit, and also to the hydrolysis f ATP on the β_E-subunit. Finally, binding of ATP to the now empty) β_{TP}-subunit, promotes release of ADP + Pi rom the β_E-subunit and hydrolysis of ATP on the β_{DP}-ubunit, and brings the whole system back to the starting onfiguration. According to this scheme, the large conformational change is primarily associated with nucleotide inding rather than ATP hydrolysis. Additionally, the resence of bound ATP favours a 'closed' conformation of he β-subunit, while bound ADP + Pi favours an 'open' onformation.

In an alternative scheme, the large conformational hange is more directly linked to ATP hydrolysis. ATP inding to the empty β_E-subunit promotes a small conformational change at the catalytic site on the β_{DP}-subunit, esulting in the hydrolysis of ATP at this site. This hydrolysis results in a large conformational change in the β_{DP}-ubunit (to an 'open' conformation), releasing the products ADP + Pi. The γ-subunit rotates 120° clockwise as a result f the conformational change in the β_{DP}-subunit, allowing he ATP·β_E-subunit to adopt a 'closed' conformation. In he next step, ATP binds to the (now empty) β_{DP}-subunit, romoting ATP hydrolysis and release of product at the $_{TP}$-subunit, and a further 120° clockwise rotation of the -subunit. Finally, ATP binding to the (now empty) β_{TP}-ubunit results in ATP hydrolysis on the β_E-subunit, hich releases ADP + Pi and brings the system back to he original state.

One important difference between these two schemes is hat nucleotide binding to the β_E-subunit promotes ydrolysis on the β_{TP}-subunit in the first scheme but on he β_{DP}-subunit in the second. The currently available tructural data slightly favour the second alternative, as he β_{DP}-subunit was identified as the catalytic subunit in he crystal structure because the active site is more buried han in the β_{TP}-subunit. However, this site contains ADP ather than ATP or ADP + Pi, and additional conformational changes due to nucleotide binding to the β_E-ubunit could modify the details of the catalytic sites on oth β_{DP}- and β_{TP}-subunits. Fluorescence measurements n the *E. coli* enzyme show that, at maximum turnover ates, on average two catalytic sites are occupied by ADP and one by ATP (Weber *et al.* 1996). This is consistent with scheme 1, but not with scheme 2, which would redict that two sites are occupied by ATP and one by ADP. In the absence of a structure with all catalytic sites ccupied by nucleotide, it is difficult to distinguish etween these two alternative schemes with any certainty. In addition, the mechanism by which a change in conformation of the β-subunit (from the open to the closed form) results in a rotation of the γ-subunit is currently not well understood.

10. CONCLUSION

There is now compelling evidence in support of a rotary catalytic mechanism in F_1-ATPase, and, by extension, in the intact ATP synthase. Although models have been proposed to explain how proton translocation in F_0 results in rotation of the γ-subunit relative to the $\alpha_3\beta_3$ assembly in F_1 (Junge *et al.* 1997; Elston *et al.* 1998) these are still speculative. Additional structural information is clearly required in order to gain a proper understanding of the structural basis of rotary catalysis.

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