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Molecular Switch of F_0F_1-ATP Synthase, G-Protein, and Other ATP-Driven Enzymes

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Exchange-out of amide tritium from labeled γ-subunit of $\alpha_3\beta_3\gamma$ complex of F_0F_1-ATP synthase was not accelerated by ATP, suggesting that hemagglutinin-type transition of coiled-coil structure did not occur in γ-subunit. Local topology of nucleotide binding site and "switch II" region of G-protein α resemble those of F_1-β subunit and other proteins which catalyze ATP-triggered reactions. Probably, binding of nucleotide to F_0F_1-ATP synthase induces conformational change of the switch II-like region with transforming β subunit structure from "open" to "closed" form and this transformation results in loss of hydrogen bonds with the γ subunit, thus enabling the γ subunit to move.

KEY WORDS: F_0F_1-ATP synthase; G-protein; P-loop; molecular switch; SecA; RecA; NifH.

INTRODUCTION

How Can Two Distant Active Sites Couple?

F_0F_1-ATP synthase couples down/up-hill proton translocation across membrane and ATP synthesis/hydrolysis (Mitchell, 1966). What is unique for this enzyme is that the catalytic sites of both reactions are physically far apart from each other (about 80 Å); proton translocation is mediated mainly by *c* subunits of membrane-embedded subcomplex F_0 (Hermolin and Fillingame, 1989) and ATP synthesis is catalyzed mainly by β subunits of membrane-peripheral subcomplex F_1 (Boyer, 1993). Direct interaction between these sites is impossible. Then, how is energy transferred from F_0-*c* subunits to F_1-β subunit through a long distance? Another unique feature of F_0F_1-ATP synthase is the peculiar subunit stoichiometry. The numbers of F_1-β subunit and F_0-*c* subunit in a single F_0F_1-ATP synthase are three (Pedersen and Amzel, 1993; Senior, 1990; Yoshida *et al.*, 1979) and about ten (Foster and Fillingame, 1982), respectively. In addition, there are several kinds of single-copy subunits including F_0-*a*, F_1-γ, −δ, and −ε subunits. Does each of the catalytic subunits play an equivalent role in catalysis and what are the roles of other subunits? At the moment, we cannot give a definite answer to these questions but the crystal structure of bovine mitochondrial F_1 (MF_1) (Abrahams *et al.*, 1994) seems to add more attractiveness to the binding change mechanism which now assumes physical rotation of the central part of the enzyme (Boyer, 1993). In this review, we discuss the conformational change in secondary, tertiary, and quarternary structures which may connect proton translocation and ATP synthesis.

TRANSITION OF COILED-COIL STRUCTURE

Does Drastic Change of Coiled-Coil Structure Like Hemagglutinin Occur in the γ Subunit?

Change in secondary structure means rearrangement of α-helices or β-strands. One of the most dynamic rearrangements is the transition of coiled-coil structure of two α-helices. A viral membrane protein, hemagglutinin, undergoes such confor-

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mational change (Bullough *et al.*, 1994). When influenza virus particles enter into the host cell and the endocytotic vesicles fuse with the lysosomes, acidic pH shift triggers transformation of coiled-coil structure and converts a previous loop region to the new coiled-coil structure. As a consequence of this change, the peptide region responsible for membrane fusion moves to a position accessible to the vesicle membrane and the resulting membrane fusion enables viral DNA to get into the cellular cytosol (Wiley and Skehel, 1987). The crystal structure of mitochondrial F_1 (MF_1) revealed that the γ subunit has a typical coiled-coil structure of the twisting two α-helices of the N-terminus and C-terminus which penetrates the central cavity surrounded by the hexagonal ring structure of $\alpha_3\beta_3$ (Abrahams *et al.*, 1994). If ATP hydrolysis triggers transition of coiled-coil structure of the γ subunit, this large conformational change should be transmitted to the F_0-*c* subunit since direct contact of both subunits was demonstrated by cross-linking experiment (Watts *et al.*, 1995). To know if this is really the case, we carried out a tritium exchange experiment. Jagendorf and his colleagues reported long time ago that hydrogen atoms of chloroplast F_1 molecules underwent a significant exchange with tritium in the medium upon illumination of the thylakoid membranes (Ryrie and Jagendorf, 1972). Since protons exchanged under these conditions should be the slow exchanging ones and hence mostly amide protons involved in the secondary structure, the implication of this result was that destabilization (or rearrangement) of the secondary structure occurs at the F_1 portion of the chloroplast F_0F_1-ATP synthase during ATP synthesis. One may speculate that the subunit that is most responsible for change of secondary structure is the γ subunit, though this was not specified by their experiment.

Tritium Exchange Experiment

To test if large-scale rearrangement of secondary structure really occurs, the release of tritium from F_1 in which only the γ subunit was labeled by tritium (exchange-out) during ATP hydrolysis was measured. Taking advantage of F_1 from thermophilic *Bacillus* PS3 (TF_1), which can be reconstituted from each of the isolated subunits, we labeled the isolated γ subunit by tritium in advance, reconstituted it into the $\alpha_3\beta_3\gamma$ complex, and measured the discharge of the slow-releasing tritium in the presence and absence of substrate ATP. The $\alpha_3\beta_3\gamma$ complex is the minimum complex that possesses essential features of TF_1 (Kaibara *et al.*, 1996). Tritium labeling was carried out by incubating the native γ subunit at 60°C for 1 hour or by incubating the urea-denatured γ subunit at 25°C for 1 hour. As shown in Table I, no enhancement of tritium discharge from the γ subunit by ATP was observed. Exchange-in experiment was also performed. TF_1 was allowed to hydrolyze ATP in the buffer containing tritium water, and uptake of tritium into TF_1 was measured. In this experiment the subunit into which tritium was taken up was not identified (a method to isolate each subunit from TF_1 without affecting stability of secondary structure has not been found). ATP did not accelerate incorporation of tritium and, rather, it slightly stabilized the secondary structure of TF_1 (Table I). Since previous deuterium exchange experiments showed that the α and β subunits in TF_1 were stabilized by ATP, the stabilization observed here can be attributed to the stabilization of the α and β subunits (Ohta *et al.*, 1980). Although we continue to have reservations until the same type of experiment is carried out for TF_0F_1-ATP synthase because it is not certain that the $\alpha_3\beta_3\gamma$ complex and TF_1 undergo the same conformational change as TF_0F_1-ATP synthase, the above results suggest that drastic rearrangement of secondary structure as seen in hemagglutinin may not occur in the γ subunit during ATP hydrolysis.

LOOP 7G IN F_1-β AND SWITCH II IN G-PROTEIN α

Loop 7G Is Most Influenced by "Open-Closed" Transition of F_1-β

Given that no large-scale rearrangement of secondary structure takes place in the coupling process of proton translocation and ATP synthesis, the relative motion of one subunit to another might be responsible for long-range transmission of energy in F_0F_1-ATP synthase. The motion has to be triggered and regulated by the change of tertiary structure within the key subunits. Then, what is the initial conformational event which leads to relative motion of subunits? In the crystal structure of MF_1 (Abrahams *et al.*, 1994), each of three β subunits takes one of two forms; "open form" or "closed form" in which the nucleotide binding site is empty, or occupied by either AMP-PNP or ADP, respectively. Although the possibility remains that each β subunit is fixed to one of these forms, it is believed to be more likely that transition from one form to the

Table I. Tritium Exchange during ATP Hydrolysis[a]

	Labeled subunit	Condition	Radioactivity of protein	Hydrogen atom/molecule[b]
			dpm/μg	Residual
1. Exchange-out	γ Subunit	+ATP, 10 min	19.2	52.9
		−ATP, 10 min	20.0	55.1
				Incorporated
2. Exchange-in	Whole TF_1	+ATP, 10 min	10.1	209
		−ATP, 10 min	11.9	246

[a] Procedures. *Exchange-out* experiment. The lyophilized γ subunit was suspended in 100 μl of 50 mM Tris-HCl, pH 7.0, 200 mM NaCl in tritium water. The radioactivity of the buffer solution was 1.06×10^9 dpm/ml. The tritiation of the γ subunit was carried out by incubating the solution at 60°C for 1 hour. The γ subunit thus treated can be reconstituted into $\alpha_3\beta_3\gamma$ complex with normal ATPase activity. The tritiated γ subunit was separated from tritium water by centrifuge at 6000 rpm for 5 min and resuspended in the buffer without tritium. This procedure was repeated three times to remove residual tritium water. The solutions containing α and β subunits were added to the tritiated γ subunit solution at the ratio 3:1 (w/w) and incubated at 25°C for 6 hours to reconstitute $\alpha_3\beta_3\gamma$ complex. The rest of γ subunit which failed to reconstitute was removed by the centrifuge column of Sephadex G-50 DNA grade (Pharmacia). The mixture containing $\alpha_3\beta_3\gamma$ complex was preincubated at 25°C for 3 min and ATP-Mg was added to a final concentration of 5 mM. For a control experiment, the same volume of the buffer was added. After incubation at 25°C for 10 min, the samples were applied to the centrifuge column of Sephadex G-50 DNA grade. A half of the elution from the column was taken out and radioactivity was determined. Another half of the elution was used to determine protein concentration. *Exchange-in* experiment. TF_1 was dissolved in 130 μl of the buffer in tritium water and incubated at 25°C for 10 min in the presence or absence of 5 mM ATP-Mg. The radioactivity of the buffer solution was 1.71×10^9 dpm/ml. Other procedures were the same as described above. The same experiments were carried out three (exchange-in) and four (exchange-out) times and gave essentially the same results.

[b] Residual (exchange-out) and incorporated (exchange-in) hydrogen atom per molecule were calculated according to Ryrie and Jagendorf (1972). One cubic centimeter of water contains 1/9 g atom of hydrogen. When radioactivity of buffer solution was 1.06×10^9, there would be $9 \times 1.06 \times 10^9$ dpm per g atom of hydrogen. Thus each 1 dpm in protein represents the incorporation of $1/(9 \times 1.06 \times 10^9)$ g atom of hydrogen. The hydrogen atom per molecule in line 1 (+ATP) was obtained by $19.2 \times 1/(9 \times 1.06 \times 10^9) \times 10^6 \times 31778$ (M_r of γ subunit)/1.21(the isotope effect of tritium). In experiment 2, 385300 was used as M_r of TF_1.

other occurs in each β subunit during catalytic turnover. When open and closed forms are compared, the N-terminal β-barrel and C-terminal helical bundle of the MF_1-β subunit are almost unchanged. The most pronounced change occurs at the β-strands 3 and 7 in the central α-β domain; the interaction between the two strands is disrupted in the open form (notation of secondary structure is according to Abrahams *et al.*, 1994) (Fig. 1A–1C). The β-strand 3 is followed by the P-loop (Walker's motif A) and a central helix B (Walker *et al.*, 1982). The β-strand 7 is followed by a loop containing 7-chloro-4-nitrobenzofurazan (Nbf-Cl)-reactive Tyr-311 (this loop is called as loop 7G in this article) and then helix G. Nbf-Cl has been known to inactivate F_1 in a single-hit manner (Ferguson *et al.*, 1975).

Loop 7G in F_1-β Resembles a Loop in G-Protein α (Gα) Switch II Region

We have noticed that the topology of the loop 7G relative to the P-loop in the MF_1-β subunit is surprisingly similar to the topology of the so-called switch II region relative to the P-loop in eukaryotic G-protein α (Gα) even though the peptide chain connectivity is different (Fig. 1). In addition, both proteins have "helical domains" and a bound nucleotide is sandwiched in the crevice formed between the helical domain and the "α-β domain." The mechanism of molecular switch of G-proteins has been extensively studied based on the crystal structures of heterotrimeric G-proteins of bovine transducin G_t and of rat G_i. Comparisons of structures of the GDP-bound and GTPγS-bound Gα indicate that the γ-phosphate of GTP promotes significant conformational changes in three or four "switch" regions (Lambrigh *et al.*, 1994; Mixon *et al.*, 1995). The switch II region comprises half of β-strand 3 (corresponding to β-strand 7 of the MF_1-β subunit), α-helix 2 (α-helix G of MF_1-β subunit), and a loop connecting them (loop 7G of MF_1-β subunit) (shown by an asterisk in Fig. 1D). This region is vitally important because it serves as a critical interface mediating the changes induced by GTP binding into effecter recognition (Mitta *et al.*, 1996). Furthermore, recent unraveling of the heterotrimeric $G_{\alpha\beta\gamma}$ protein structures of G_t and G_i clearly showed that the most extensive interface of G_α to bind G_β are the residues in the switch

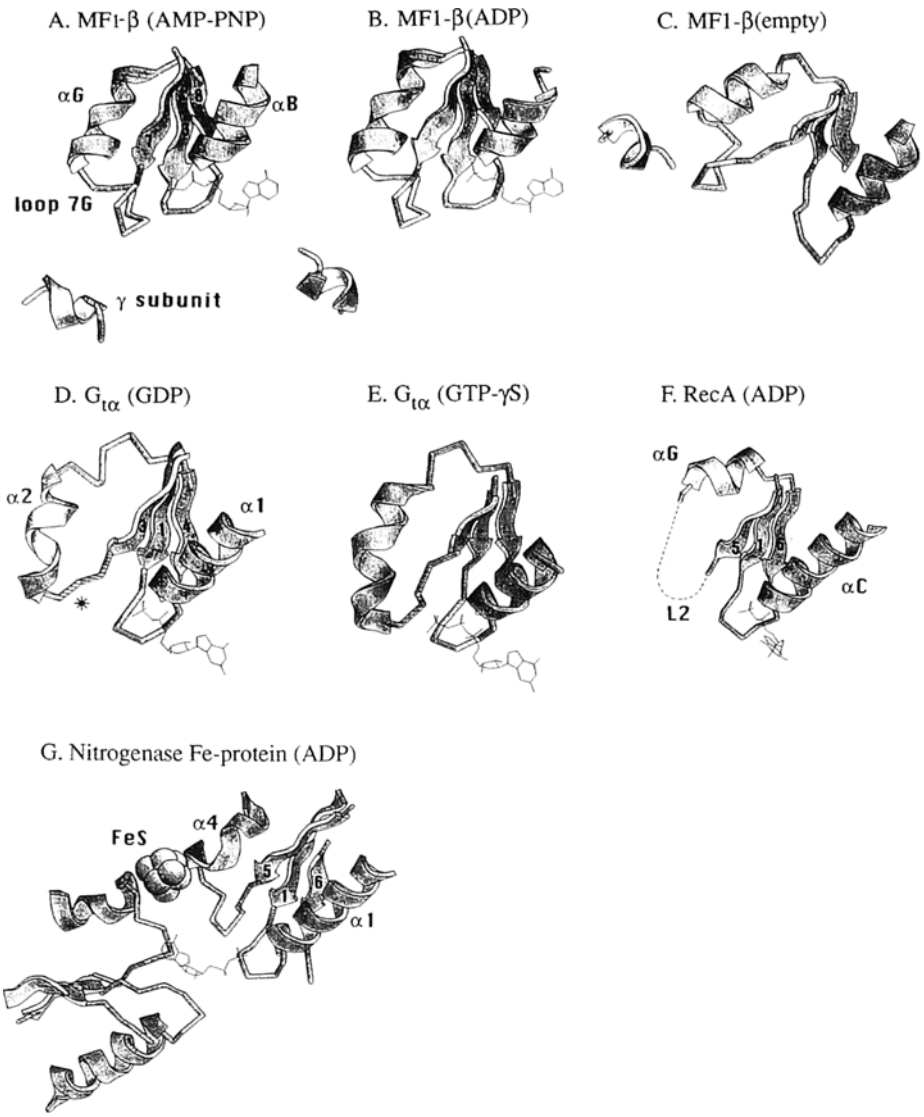

Fig. 1. Comparison of local folding topology of nucleotide-binding domains of various proteins. (A) Bovine MF1-β subunit with bound AMP-PNP (β_T). (B) MF1-β subunit with bound ADP (β_D). (C) MF1-β subunit with no bound nucleotide (β_E). (D) Bovine transducin α with bound GDP ($G_{t\alpha}$·GDP). (E) Bovine transducin α with bound GTPγS ($G_{t\alpha}$·GTPγS). (F) *E. coli* RecA with bound ADP. (G) *A. vinelandii* nitrogenase Fe-protein. Notation of secondary structures is according to the original papers indicated in the text. Asterisk (*) in Fig. 1D shows the loop included in the "switch II" region of G-protein.

II region (Lambright *et al.*, 1996; Wall *et al.*, 1995). The spatial equivalence of the region around the loop 7G of MF_1-β subunit and switch II of G_α is an indication of their similar switching mechanism. GTP hydrolysis induces partial unzipping of β-strand 3 and β-strand 1 in switch II of $G_{t\alpha}$ (Fig. 1D and 1E). The loop β3-α2 becomes more extended ($G_{t\alpha}$) or disordered ($G_{i\alpha}$). Similarly, when the MF_1-β subunit transforms from open form to closed one, partial unzipping of β-strand 7 and β-strand 3 occurs. It is apparent that binding/release of AT(D)P to/from F_0F_1-ATP synthase, rather than ATP hydrolysis/synthesis occurring at catalytic sites by itself, can induce conformational change in the switch II-like region of the MF_1-β subunit because, contrary to the remarkable difference of structure in this region between the open and closed forms, there is little difference between the two closed forms (ADP-bound and AMP-PNP-bound β subunits; com-

pare Fig. 1A and 1B). The affinity for ATP and ADP may be differentiated by mechanism other than open-closed transition, probably by a small rearrangement of side chains. The MF_1-β subunit in the open form binds to the γ subunit through three hydrogen bonds formed between residues in loop 7G of the β subunit and those of the C-terminal α-helix of the γ subunit. Conversion of the open form to the closed form results in loss of all of these hydrogen bonds, and motion of the γ subunit relative to the β subunit may become possible.

SWITCH II-LIKE REGION PLAYS A CRUCIAL ROLE IN PROTEINS WHICH CATALYZE ATP-TRIGGERED REACTIONS

A Common Fold in Proteins That Has Motifs A, B, and Catalytic Glu/Asp

Before determining the crystal structure of MF_1, we predicted that the structure of the catalytic site in F_1 would be similar to the nucleotide-binding site of *Escherichia coli* RecA protein whose structure was solved previously (Story and Steiz, 1992; Story *et al.*, 1992). Based on the crystal structure, Glu-96 of RecA was proposed as a general base to activate a water molecule for an attack of γ-phosphate during ATP hydrolysis and is located at 24 residues from Lys-72 of Walker's motif A sequence (GXXXXGKT, X can be varied, P-loop) (Walker *et al.*, 1982). We had identified Glu-190 of the TF_1-β subunit as an essential residue from experiments of dicyclohexylcarbodiimide labeling (Yoshida and Allison, 1983; Yoshida *et al.*, 1981) and mutagenesis (Amano *et al.*, 1994a; Ohtsubo *et al.*, 1987), and the location of the Glu-190 is 26 residues from Lys-164 of the motif A sequence. The similar location of Glu ("catalytic carboxylate") of the two enzymes prompted us to propose the RecA-like structure of the nucleotide-binding site of F_1-β (Amano *et al.*, 1994b). The crystal structure of MF_1 verified our prediction and, we extended this prediction to other proteins catalyzing ATP-triggered reactions that have the motif A, motif B (ZZZZD; Z is the hydrophobic residue), and conserved Glu (or Asp) at around 24 residues from the Lys of motif A (Yoshida and Amano, 1994). The list of the proteins includes NifH (nitrogen fixation), MidD (cell division inhibitor), ArsA (As pump), FrxC (chlorophyll synthesis), IncC (plasmid maintenance and replication), SecA (protein secretion), Rho protein (transcription termination), and DnaB (DNA replication). Although their primary structures are different from each other, these proteins may have common folding topology at their nucleotide-binding domains, that is, six β strands surrounding the central helix. The common domain structure suggests the presence of common functional step(s) in propagation of ATP-triggered reactions catalyzed by them. We will explain three examples, SecA, RecA, and NifH (nitrogenase Fe-protein), and try to postulate the possible mechanism.

SecA; Membrane Insertion and Deinsertion

SecA is a peripheral membrane protein and promotes presecretory protein translocation across the bacterial membrane by undergoing ATP-driven cycles of membrane insertion and deinsertion (Economou *et al.*, 1996). The structure of SecA has not been solved. The energy of ATP binding drives membrane insertion of a 30-kDa domain of secA which directly couples to the translocation of the 20–30 peptide segment of the presecretory protein. We predicted that Asp-133 of *E. coli* SecA is a catalytic carboxylate, and indeed mutation replacing this residue by Asn resulted in drastic decrease of protein translocation and translocation-coupled ATPase (unpublished observation). Interestingly, cross-linking studies showed that a peptide stretch 267–340 of SecA is involved in binding of the presecretory protein (Kimura *et al.*, 1991). The distance of this stretch from Asp-209 in motif B is about 60 residues, roughly agreeing with the distance between Asp-256 in motif B and loop 7G in MF_1-β subunit. We speculate that secA catches presecretory protein at the switch II-like region and carries it across membrane.

RecA; ATP-Dependent DNA Binding during Recombination

RecA mediates a genetic recombination coupled with ATP hydrolysis. During the genetic recombination, RecA polymerizes along a single-strand DNA to the direction from 5′ to 3′. The ATP-bound form of RecA binds DNA tightly and ATP hydrolysis stimulates the release of RecA from DNA (Kurumizawa and Shibata, 1996). The nucleotide binding domain in RecA is almost identical to that of the MF_1-β subunit, and a loop in the switch II-like region (loop L2), as well as another loop on top of the switch II-like region

(loop L1, not shown in Fig. 1F), comprise the DNA binding domain (Fig. 1F). In the crystal structure of the ADP-bound form of RecA (Story and Steiz, 1992), loop L2 and loop L1 are disordered and not seen. Relatively, the loop of switch II and the loop on top of it (switch I) of the ADP-bound form of rat $G_{i\alpha}$ are also disordered (Mixon *et al.*, 1995). The structure of the ATPγS-bound form of RecA has not been solved but it is likely that, when ATP is bound to RecA, loop L2 and loop L1 undergo a structural adjustment which is optimized to bind DNA. It has been proposed that Gln-194 of RecA stabilizes loop L2 when it interacts with γ-phosphate of ATP (Story and Steiz, 1992; Story *et al.*, 1992). Location of this residue in the switch II-like region in RecA is very similar to Gly-199 in $G_{t\alpha}$ which triggers conformational changes in the switch II region through an interaction with the γ-phosphate of GTP and Gln-200 which stabilizes the transition state for GTP hydrolysis.

Nitrogenase Fe-Protein; Enforcing One-Way Flow of Electron

Nitrogenase catalyzes the ATP-dependent reduction of N_2 to ammonia during the process of nitrogen fixation and it consists of two proteins, Fe-protein and MoFe-protein. Fe-protein receives an electron from ferredoxin or flavodoxin, transfers it to MoFe-protein in an ATP-dependent manner, and MoFe-protein reduces N_2. Fe-protein is a homodimer of 30 kDa subunits that contains one [4Fe-4S] cluster (Normand and Bousquet, 1989). According to the crystal structure of *Azotobacter vinelandii* Fe-protein, the shape of the overall fold is described as a "butterfly" with each subunit, [4Fe-4S] cluster, and bound ADP representing the wing, the head, and the heart, respectively (Georgiadis *et al.*, 1992). The structure of the nucleotide-binding domain is similar to that of the F_1-β subunit with the modification that the β-strand 5 preceding the switch II-like region contains the motif B sequence (corresponding to β-strand 6 of MF_1-β) (Fig. 1G). The switch II-like region encompasses loop β5-α4 and α-helix 4. The loop in the switch II-like region and the adjacent loop (loop β2-α2, not shown in Fig. 1G) of one subunit face those of other subunits at the subunit interface, and one ADP is bound between them. It is known that ATP hydrolysis occurs only when the MoFe-protein is associated. Presumably, ATP binding triggers the conformational change in the switch II-like subdomain and loop β_2-α_2 resulting in dislocation or movement of both α-helices 3 and 4 in which cysteine residues liganding to the [4Fe-4S] cluster exist. MoFe-protein can recognize Fe-protein in this conformational state and gain specific access to reduced [4Fe-4S] cluster. Thus, the affinity of Fe-protein for electron acceptor (MoFe-protein) becomes much stronger than the oxidized electron donor molecule, enforcing proper one-way flow of electron by preventing back reaction. Subsequent ATP hydrolysis may stimulate dissociation of Fe-protein from MoFe-protein.

SUMMARY

It appears that a protein fold, six β-strands surrounding the central α-helix, is adopted by various proteins which catalyze ATP-triggered reactions for the purpose of propagating signals generated by ATP hydrolysis (or subsequent release of ADP) at a crevice of the catalytic site into a certain protein surface which is (or becomes) a binding interface with other macromolecules (subunit, protein, or DNA). In other words, switching between alternate conformational states is regulated by nucleotide binding and hydrolysis. In the case of F_0F_1-ATP synthase, the interaction of the β subunit with the γ-subunit may be weakened by the transition of an open-form β subunit (β_E) (Abrahams *et al.*, 1994) to the closed form (β_D or β_T). This makes motion of the γ-subunit possible. The motion can be dislocation, precession, or rotation. The binding change mechanism favors rotation, and a supportive result using cross-linking was reported (Duncan *et al.*, 1995). The protein machinery that rotates is not completely novel. In addition to a well-known example, bacterial flagella, it is worth pointing out that many proteins interacting with DNA actually rotate around the double helix of DNA at a rate faster than 50 turns per sec. Finally, what is needed now is physical evidence for rotation or other motion of the γ-subunit in the F_0F_1-ATP synthase at work.

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