Chapter 2
F_1-ATPase: A Prototypical Rotary Molecular Motor

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Abstract F_1-ATPase, the soluble portion of ATP synthase, has been shown to be a rotary molecular motor in which the central γ subunit rotates inside the cylinder made of $\alpha_3\beta_3$ subunits. The rotation is powered by ATP hydrolysis in three catalytic sites, and reverse rotation of the γ subunit by an external force leads to ATP synthesis in the catalytic sites. Here I look back how our lab became involved in the study of this marvelous rotary machine, and discuss some aspects of its rotary mechanism while confessing we are far from understanding. This article is a very personal essay, not a scientific review, for this otherwise viral machines book.

2.1 A Rotary Molecular Machine

To my common sense (in a bad sense), rotation within a molecule seemed impossible, because I was taught that a protein molecule is made on the basis of the lock-and-key principle, or that any part of a molecule is complementary to the other (Fig. 2.1). A distortion is alright, or even a shift by one tooth unit or two along a well-designed interface may be possible. Rotation, however, would require continuous shift of one part against the other, over 360°.

So, at about the year 1980 when Oosawa began to preach that F_1-ATPase must rotate and begged young Japanese biophysicists to show him the rotation experimentally, I did not listen to him. I was not aware that Boyer (Boyer and Kohlbrenner 1981) and other eminent scientists also proposed that F_1, or the ATP synthase of which F_1 is a part, may well rotate. Oosawa's theory was published much later (Oosawa and Hayashi 1986), to which I did not pay much attention at the time. In retrospect, I could have been more imaginative, already knowing that myosin slides past actin for many reaction cycles (Huxley 1957; Huxley 1969) and that bacterial flagellar motor, a gigantic machinery, rotates (a review by Berg 2003). If a linear molecular motor such as myosin can shift on a substrate track continually, all one needs to make a rotary motor is to roll the track into a ring (Kinosita et al. 1998).

Earlier in 1977, I happened to listen to a lecture by Kagawa, who showed that active F_1-ATPase can be reconstituted from purified subunits of thermophilic origin (Yoshida et al. 1977). I was deeply impressed, and dreamed of future collaboration. Oosawa's proposal in 1980, however, failed to push

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M.G. Rossmann and V.B. Rao (eds.), *Viral Molecular Machines*,
Advances in Experimental Medicine and Biology 726, DOI 10.1007/978-1-4614-0980-9_2,

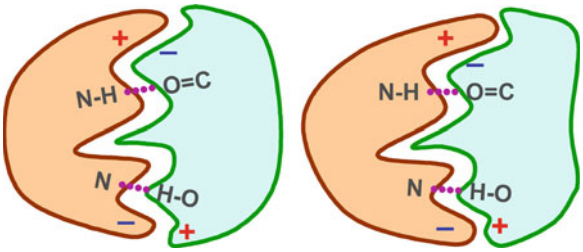

Fig. 2.1 Lock-and-key principle of protein construction

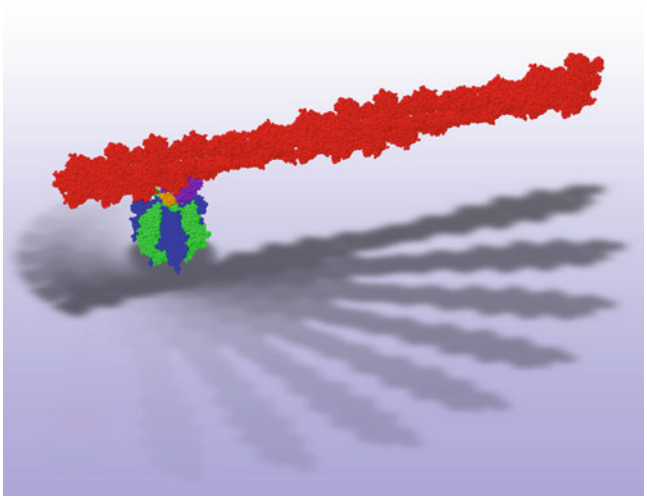

Fig. 2.2 Rotation of F_1-ATPase, a part of the enzyme ATP synthase. The rotary molecular motor consists of six stator subunits (three β in *green* and three α in *blue*) and a central rotor subunit γ (*orange*). Its rotation, powered by ATP hydrolysis, has been videotaped under an optical microscope by attaching a long actin filament (*red*; actual length far exceeds the image size) to the rotor through streptavidin (*purple*)

me into real collaboration. I was simply full of timid common sense. It was only after young associates in our lab, excluding myself, successfully began single-molecule work that the long-dreamed collaboration materialized (Noji et al. 1997), with the team of Yoshida, a former associate of Kagawa. People involved in the collaboration have different stories of how the collaboration began. Indeed, several members of both teams had thought about this collaboration, including those who eventually could not join. From my standpoint it was my admiration for the work of Kagawa's team that led me to talk to Yoshida. More important, it was no longer timid myself who would venture into the rotation experiment.

The collaboration started in 1996 and immediately bore fruit. By that time, a crystal structure of F_1 had been solved (Abrahams et al. 1994), showing that the putative rotor, the γ subunit, deeply penetrates the stator cylinder made of $\alpha_3\beta_3$ subunits. The tip of the rotor was made mostly of hydrophobic residues and the portion of the stator that surrounds the tip was also hydrophobic. The oily, or waxy, residues would act as a "molecular bearing" (Abrahams et al. 1994), strongly suggesting rotation. Both Yoshida and I were still highly suspicious, but were willing to bet on young associates. The actual bet we agreed upon, with young Noji and Yasuda, was not whether F_1 would rotate. We betted upon the sense of rotation, right or left. That is the way single-molecule physiologists must adopt.

Yasuda found a rotating F_1 molecule (actually a rotating actin filament that was supposedly attached to the rotor subunit) in the very first observation chamber he made for microscopic observation (Fig. 2.2). This sample was the first mutant that Noji prepared, among many he had designed. These two were the luckiest people whom I have known personally. I cannot understand why, but I am certain that being loved by Lady Luck is a talent. The talent I desperately lack.

Another rotating filament was soon found, and we were ready to toast after a third. But we had to wait for 2 months for the third. That's our single-molecule physiology.

2.2 Rotary Mechanism

Various aspects of the rotary mechanism of F_1 and experimental evidence leading to our tentative views have been discussed (Adachi et al. 2010, 2011). Below I present my current, personal views on some topics.

2.2.1 Symmetry Considerations

The primary reason why rotation in F_1-ATPase was proposed in early days was that there are three catalytic sites that are equivalent in function and there is only one γ subunit that was known to be crucial to catalysis and to be devoid of a threefold symmetry. The only way for the asymmetric γ to interact with the three catalytic sites impartially would be to rotate (not necessarily in a unique direction).

Physicists tend to believe that Nature likes symmetry, or at least physicists are the people who love the idea of symmetry. The Monod–Wyman–Changeux model of allosteric transitions (Monod et al. 1965), for example, was very popular among biophysicists of which I was one. At present, though, I think that the experimental distinction between this concerted model and the induced-fit theory (Koshland 1958) is very difficult, or rather, the truths are somewhere in between.

Shortly after I was shown the F_1 rotation, I began to think about how it may rotate. The crystal structure solved by Abrahams et al. (1994) had shown that the three catalytic sites reside at β–α interfaces, hosted primarily by β (Fig. 2.3a, b). As seen in Fig. 2.3a, the upper portion of the β subunit is bent toward γ when ATP (analog) or ADP is bound, whereas it is unbent in the nucleotide-free state, suggesting that these nucleotide-dependent bending and unbending drive γ rotation (Wang and Oster 1998). To correlate the nucleotide states and γ rotation, I drew a diagram like one in Fig. 2.3c, where γ was represented by a triangle. The simplification itself should have been okay, but somehow the triangle fooled me into thinking that the interaction of γ with a β subunit would basically (though not exactly) be threefold symmetric (Fig. 2.3d). This led me nowhere into rotation, which requires broken symmetry. Soon I learned that previous workers (Oosawa and Hayashi 1986; Wang and Oster 1998) correctly assumed non-threefold interactions such as one in Fig. 2.3e.

Symmetry consideration may turn out to be useful in predictions and explanations, but Nature does not always respect it. In vivo, the F_1 motor is connected to another rotary motor F_o, which is driven by proton flow, to constitute the ATP synthase (Fig. 2.4). The proton motive force under physiological conditions operates in the direction from top to bottom in Fig. 2.4, which drives F_o clockwise when viewed from above. This rotary direction is opposite to that of F_1 which rotates counterclockwise when it hydrolyzes ATP. The rotors of the two motors are supposed to be fused to each other, and hence clockwise rotation of F_o drives the F_1 motor in its reverse direction, resulting in the reversal of the ATP hydrolysis reaction in the three catalytic sites of F_1. This is the accepted view of the mechanism of ATP synthesis. A small problem from a physicist's view is that the rotor of the F_o motor comprises a ring of several *c* subunits, to which the asymmetric γ rotor of F_1 is attached via ε. Symmetry would predict that γε would rotate against the *c* ring, but then ATP synthesis would fail. I think it is still possible that the interface between γε and the *c* ring serves as a kind of clutch, which may disengage when sufficient ATP has been accumulated. Another small uneasiness that I have been having is that the basically symmetric stator of the F_1 motor ($\alpha_3\beta_3$ ring) is

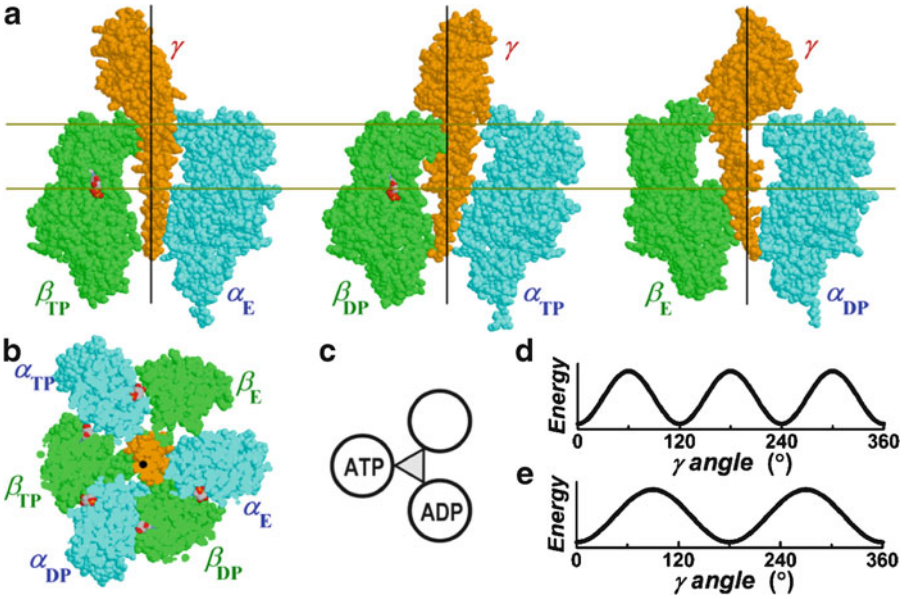

Fig. 2.3 Structure of F_1-ATPase and schematic diagrams. (**a**, **b**) An atomic structure of bovine mitochondrial F_1-ATPase (Gibbons et al. 2000). The γ rotor and an opposing pair of α and β subunits are shown in (**a**), and the bottom view of the section between the *gold lines* is shown in (**b**). Nucleotides are shown in CPK colors. The stator subunits are designated according to the nucleotides in catalytic sites found in the original structure (Abrahams et al. 1994): TP site between β_{TP} and α_{TP}, largely in β_{TP}, bound an ATP analog (AMPPNP), DP site ADP, and E site none, while non-catalytic sites in the other three interfaces bound the ATP analog. The *vertical line* in (**a**) and the *black dot* in (**b**) represent the putative rotation axis (Wang and Oster 1998). (**c**) A schematic diagram showing the relationship between catalytic nucleotides and γ orientation. (**d**) A possible diagram showing the interaction energy between γ and a β subunit. (**e**) Another possible energy diagram

connected to the single-unit F_o stator (a) via δb_2. In the whole ATP synthase, the three catalytic sites of F_1 may not be entirely equivalent.

Linear molecular motors such as kinesin and myosin have two identical legs (no distinction between right and left feet) that are joined in twofold symmetry as opposed to the mirror symmetry in humans (Kinosita et al. 2005). The most natural way for these molecular motors to walk is to rotate 180° every step, as in a dance (Howard 1996). Several attempts to observe this all failed (Kinosita et al. 2005) showing that the motors, under the experimental constraints, can walk disregarding the twofold symmetry. But recently Komori et al. (2007) have succeeded in showing rotation in either way randomly, indicating that molecular motors want to observe symmetry principles. I still hope to observe rotation in a unique direction.

Beauty is the prime goal of (theoretical) physics. In the biological world of individualism, there are many different beauties, and a beauty is not always a charm. C'est la vie, which I enjoy.

2.2.2 *Kinetics of Chemo-Mechanical Coupling*

The kinetic scheme we had arrived at by 2007 is summarized in Fig. 2.5, and evidence supporting the scheme has been discussed (Adachi et al. 2010). In brief, ATP binding initiates and drives rotation from 0° (an ATP-waiting angle; the angle descriptions below refer to the pink, or the lower left, catalytic site in Fig. 2.5b or c) to 80°. After 200° of rotation since the ATP binding, that ATP is split into ADP and

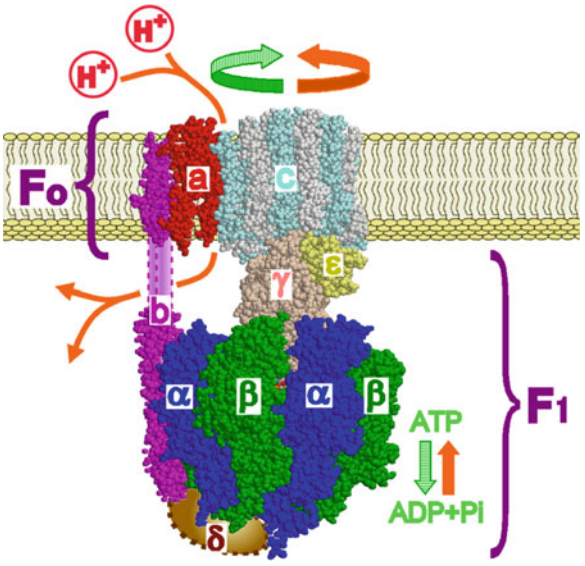

Fig. 2.4 Subunit assembly of ATP synthase. The minimal composition inferred from bacterial enzymes is $\alpha_3\beta_3\gamma\delta\varepsilon$ for F_1 and ab_2c_{8-15} for F_o (Yoshida et al. 2001; Junge et al. 2009; Watt et al. 2010). F_1 is a rotary motor driven by ATP hydrolysis (the subcomplex $\alpha_3\beta_3\gamma$ suffices for rotation in isolation), and F_o is another rotary motor driven by proton flow (Diez et al. 2004). The two motors are connected and coupled, such that the common rotor consists of $\gamma\varepsilon c_{8-15}$ and the stator assembly of $\alpha_3\beta_3\delta ab_2$. When the free energy obtained from proton flow (*curved orange arrows*) is greater than that from ATP hydrolysis, the F_o motor gains control and the rotor rotates in the *orange* direction, resulting in ATP synthesis in F_1. When F_1 wins, rotation in the *green* direction occurs by ATP hydrolysis and protons are pumped back. The atomic structures shown, arbitrarily arranged, are from Gibbons et al. (2000), Stock et al. (1999), Rastogi and Girvin (1999), Dmitriev et al. (1999), and del Rizzo et al. (2002)

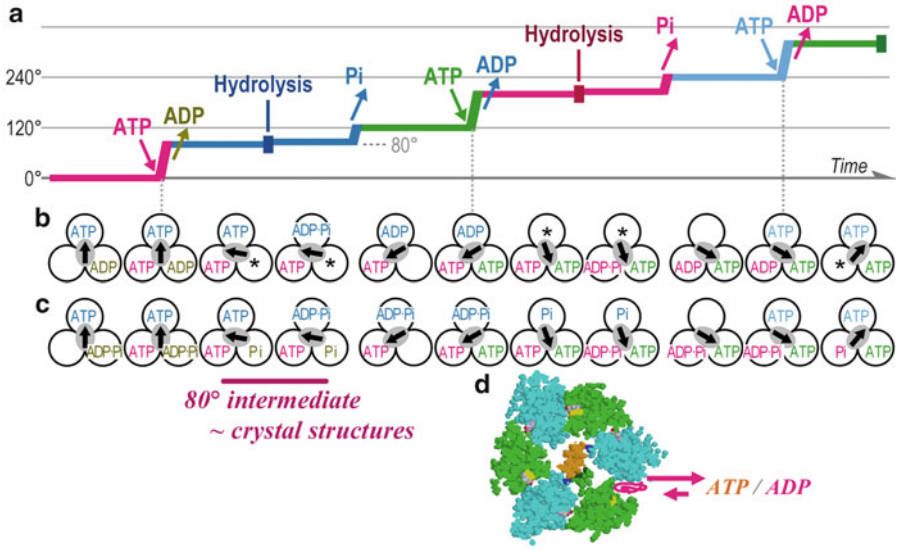

Fig. 2.5 Proposed scheme for coupling between catalysis and rotation. (**a**) Schematic time course of rotation. Colors indicate the site at which the rate-limiting reaction is to occur in (**b**). Angles quoted in the text refer to events in the *pink* (*lower left*) site. (**b**, **c**) Alternative schemes suggested by Adachi et al. (2007), differing in the timing of phosphate release by 120°. Chemical states of three catalytic sites (*circles*) and γ orientation (*central arrows*) are shown. (**d**) Filling of the third catalytic site by a medium nucleotide (Shimo-Kon et al. 2010). The *asterisked* site in (**b**) is freely accessible to medium nucleotide, which, upon entry, quenches the fluorescence of the reporter tryptophan (*yellow*). The crystal structure shown is from Gibbons et al. (2000), where one catalytic site is empty and is fully open

inorganic phosphate. The phosphate is either immediately released to drive rotation from 200° to 240° (Fig. 2.5b), or remains bound for another 120° rotation to drive the last rotation from 320° to 360° (Fig. 2.5c). ADP is released after a third ATP is bound, during rotation from 240° to 320°. The ADP release likely contributes to the 80° rotation in addition to the third ATP binding (Adachi et al. 2007).

We still had a few problems. The timing of phosphate release, either Fig. 2.5b or c, had to be solved. Also, either of the schemes indicates that the site occupancy, the number of nucleotides bound to the three catalytic sites, remains two for most of the time except for the brief moment of rotation over 80°. The occupancy number of two was at odds over tryptophan quenching studies including our own. Earlier, Weber et al. (1993) have introduced a reporter tryptophan residue in each catalytic site (yellow in Fig. 2.5d) to show that the tryptophan fluorescence is quenched when a nucleotide is bound. With *Escherichia coli* F_1, they have shown that the time-averaged site occupancy goes up to three when the ATP concentration is high and the enzyme is exerting its full activity. Dou et al. (1998) have confirmed the results with the thermophilic F_1 that we work with, and we were also getting similar results. Because the nucleotide scheme in Fig. 2.5 was derived from experiments using a fluorescent ATP analog (Cy3-ATP), we thought that the behavior of the analog may be different from that of unlabeled ATP used in the tryptophan studies.

The discrepancy in the site occupancy was resolved when Shimo-Kon noticed that active F_1 undergoing catalysis and inactive F_1 either in an inhibited state or binding ADP alone show the same occupancy pattern for the third site, as probed by tryptophan quenching (Shimo-Kon et al. 2010). The implication is that the third nucleotide is not necessarily the nucleotide being catalyzed; it may come from the medium. In Fig. 2.5b, the asterisked site that has been vacated by leaving ADP can be filled by a medium nucleotide, ATP or ADP (Fig. 2.5d). The Cy3-ATP experiments that led to Fig. 2.5b or c were made at very low (nanomolar) concentrations of the nucleotide, and thus the third site was not filled. This view that the third nucleotide comes mainly from the medium is consistent with the crystal structures solved by the Walker group. Of the many structures solved to date, only one structure binds three catalytic nucleotides (Menz et al. 2001), another one no nucleotide (Kabaleeswaran et al. 2009), and all the rest bind two nucleotides and the third site is fully open. We think that the two-nucleotide crystal structures resemble the 80° intermediate in the kinetic scheme (Fig. 2.5) and thus the intermediate would readily accommodate a medium nucleotide in the open site.

I have to confess that I liked the bi-site mechanism of rotation that Boyer has been championing (Boyer 1998, 2002). The site occupancy alternates between one and two in the bi-site mode, warranting maximal asymmetry that, I thought, would assure powerful rotation. So, it was not without my personal regret that we eliminated almost completely the possibility of bi-site catalysis in our enzyme working with unlabeled ATP (Shimo-Kon et al. 2010).

An added bonus of the tryptophan study (Shimo-Kon et al. 2010) was the settlement of the timing of phosphate release. Because the asterisked site in Fig. 2.5b allowed binding of a medium ATP, the site cannot hold phosphate as in Fig. 2.5c. When we added phosphate in the medium, binding of ATP to the asterisked site was hindered.

I was to stop here, to conclude that the kinetic coupling scheme is now complete. The site occupancy problem that had been lingering in my mind for years is finally gone. Watanabe et al. (2010), however, have recently reported intricate experiments that indicate, at least for the slow-hydrolysis mutant they used, phosphate release at 200° is too slow to be compatible with the scheme in Fig. 2.5b. The authors suggest Fig. 2.5c. To reconcile with the observation above that the third site can accommodate a medium ATP, the authors also suggest that the phosphate retained by 320° would immediately be released upon reaching 320°, leaving the 320° site open for most of the 320° dwell (the asterisked site in Fig. 2.5 will also be open). But then the 40° rotation from 320° to 360° cannot be driven by phosphate release, as opposed to our contention that phosphate release confers driving torque for the 40° rotation, whether from 200° to 240° or from 320° to 360° (Adachi et al. 2007). Something, or somethings, must be wrong, or the F_1-ATPase allows different kinetic pathways and chooses one (or more) depending on the reaction conditions and/or mutations.

Thus, at this moment, I no longer have a clear-cut view of F_1 kinetics. All aspects of the kinetics may have to be re-examined. In this regard, I note that, under a certain circumstance, ATP hydrolysis may take place at 120° (Shimabukuro et al. 2006), not at 200° as shown in Fig. 2.5b or c. Also, in a related enzyme V_1-ATPase of *Thermus thermophilus*, rotation proceeds in steps of 120°, without the 80° and 40° substeps as in F_1; all chemical reaction steps, at least ATP binding, hydrolysis, and another rate-limiting reaction likely ADP or phosphate release, take place at the same angle (Furuike et al. 2011). Why, then, does F_1 bother to divide them into two angles? Possibly to produce torque over a wide range of angles and to synthesize ATP efficiently? If so, why does the *Thermus* V_1, which also participates in efficient ATP synthesis when combined with V_o (Toei et al. 2007), not adopt the same strategy?

2.2.3 Binding Changes and ATP Synthesis

Boyer has proposed that binding changes are the essence of the function of ATP synthase (Boyer 1998). The final step of the synthesis, for example, is the release of a tightly bound ATP, or a decrease in the binding constant. The binding change results from a conformational change of the catalytic site, and the conformational change is driven by mechanical rotation. Other chemical steps, bindings of ADP and phosphate to the catalytic site and linking of phosphate to ADP in the catalytic site, all occur through rotation-driven binding changes.

Isolated F_1 alone has been shown to catalyze ATP synthesis when the γ rotor is forced to rotate in reverse by an external, artificial force (Itoh et al. 2004; Rondelez et al. 2005), implying that the binding changes can be effected by γ rotation alone, or that the γ angle determines the equilibrium constants of each catalytic site for ADP binding, phosphate binding, ATP synthesis/hydrolysis, and ATP unbinding (a "γ-dictator," or "γ-controlled," mechanism). In the rotation driven by ATP hydrolysis, the central γ will coordinate the chemical steps in the three catalytic sites.

A general framework of how the mechanical work of rotation (the mechanical energy liberated in the hydrolysis-driven rotation, or the energy input required for synthesis rotation) is coupled to the binding changes (shifts of the equilibrium constants of the catalytic sites) has been discussed and used to build models of F_1 rotation (Oosawa and Hayashi 1986; Wang and Oster 1998; Kinosita et al. 2004). All models treat the equilibrium constants as a continuous function of the γ angle, and hence the energy involved, or the torque as the derivative of the energy, is also a continuous function of the γ angle. This is in accord with the observation that, apparently, F_1 generates torque at all angles except when it is waiting for ATP binding and phosphate release (Kinosita et al. 2000; Pänke et al. 2001; Palanisami and Okamoto 2010). (This "power stroke" view, though, represents only one side of a coin; see Adachi et al. 2010, and discussion below).

Angle-dependent binding change has been quantified for phosphate around the phosphate release angle: the association constant for phosphate decreases by a factor well above 10^4 upon rotation from 200° to 240° (Adachi et al. 2007). Experiments have also indicated that the association constant for ATP (or the rate of ATP binding) increases around the ATP-binding angle (Watanabe-Nakayama et al. 2008; Iko et al. 2009). Using Cy3-ATP and Cy3-ADP, we have been measuring the binding changes for ATP and ADP over all angles and under various conditions. Years of analyses are now almost complete, which we will report in the near future. One hint from the analyses is that ATP synthesis in F_1 appears to be basically the reverse of hydrolysis: when forced to rotate in the reverse direction, catalytic events seem to occur from right to left in Fig. 2.5a.

Our current view, yet abstract, is that binding changes and protein conformational changes (including γ rotation) are two sides of the same coin (Fig. 2.6). ATP, for example, binds through the process of "induced fit" (Koshland 1958), whereby the catalytic site adapts itself to better accommodate ATP, increasing the number of weak bonds (blue dots in Fig. 2.6) that hold ATP. Thus, if ATP

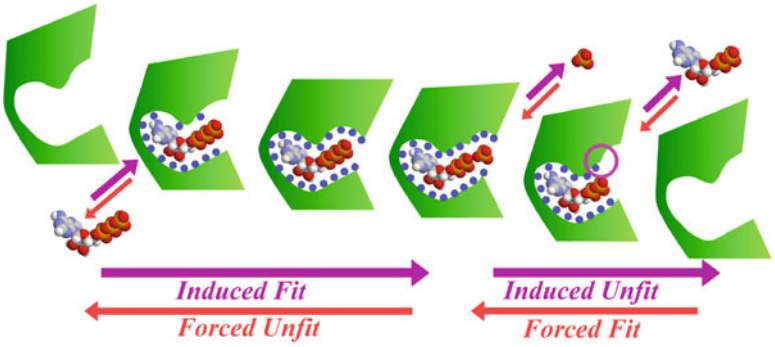

Fig. 2.6 Chemo-mechanical and mechano-chemical energy conversion through the processes of "induced fit" and "induced unfit." *Violet arrows* show reactions driven by free energy liberated by ATP hydrolysis; *red arrows*, reverse reactions driven by an external force. *Green blocks* show conformations of the protein part in a highly schematized fashion, their vertical locations representing the free energy level in an environment favoring hydrolysis and in the absence of an external force. *Small blue dots* surrounding a nucleotide represent weak bonds (mainly hydrogen bonds) that hold the nucleotide in the catalytic site. Through progressive formation of these bonds a binding ligand (ATP) pulls the protein toward a conformation that better fits the ligand (induced fit). A leaving nucleotide promotes a reverse process, which we call "induced unfit." Both induced fit and induced unfit can be reversed by an external force on the protein part, resulting in forced binding changes. The *pink circle* highlights the small protrusion that would hinder ATP binding (but not ADP and phosphate separately); ATP binding should somehow be hindered for efficient synthesis

binding drives a conformational change, that particular conformational change, when induced thermally or by an external force, will increase the affinity of the catalytic site for ATP. Likewise, phosphate or ADP release goes through "induced unfit," and the conformational change accompanying the release process will decrease the affinity. To state that binding/unbinding drives a conformational change is the so-called power stroke view. An alternative view is that a thermal fluctuation into a proper conformation induces and stabilizes binding/unbinding ("conformational selection"). Again, truths are always somewhere in between, I suppose (Adachi et al. 2010).

The induced fit and induced unfit are both basically downhill, toward lower free energy. An external force applied to the protein part can reverse these processes, by forcing fit and unfit. This is how we view ATP synthesis on the protein machine (Fig. 2.6, from right to left). For efficient synthesis, forced fit for ADP must not allow ATP binding, and the little projection in the pink circle in Fig. 2.6 is a highly schematic representation of this distinction mechanism, one possibility being preoccupation by phosphate which must be present in the medium for ATP syntheses. In the last step of synthesis, it must be ATP and not ADP that is released into the medium. For this, we conjecture that a small clockwise rotation of γ would shift the equilibrium between ADP + phosphate and ATP toward the synthesis side. A corollary, then, is that ATP hydrolysis in the catalytic site would accompany a small counterclockwise rotation of γ, which seems natural to me.

2.2.4 Structural Basis of Rotation

Wang and Oster (1998) made movies of rotating F_1 by interpolating the crystal structure (Abrahams et al. 1994) in which the three β subunits adopt different conformations as though the conformational changes directly drive γ rotation. The movies were pretty much impressive to me, and I thought that the basic mechanism would be the push and pull of bending and unbending β against γ (Fig. 2.3a) as these authors suggested.

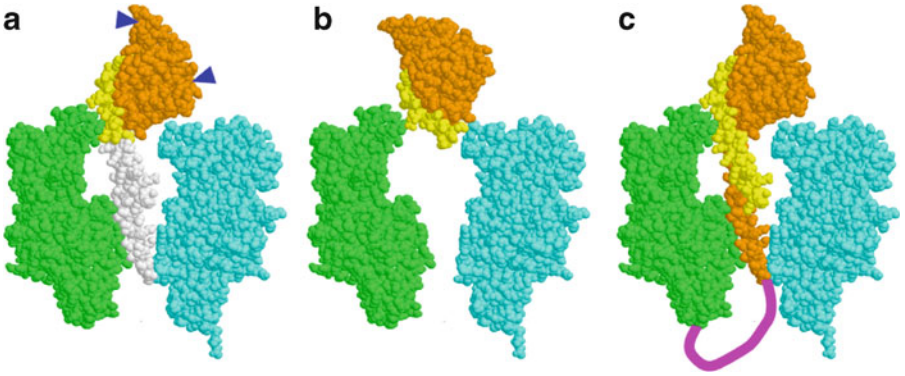

Fig. 2.7 Truncations of the γ rotor. (**a**) An axle-less construct in which the *white* portion is deleted (Furuike et al. 2008). *Arrowheads* indicate approximate positions of the cysteine residues introduced for the attachment of a rotation marker. (**b**) The rotor head in (**a**) appears to occasionally adopt such orientations where the marker (a *gold bead*) would be right above the rotor. (**c**) A construct (Kohori et al. 2011) devoid of the entire amino-terminal α helix (*yellow*). The carboxyl terminus is connected to β via a short peptide linker (*magenta*)

When Hossain joined us as a postdoc of physics background, I suggested if he might wish to redo the γ truncation work of Müller et al. (2002) as biology training. Thus started a never-ending story. Truncation of γ in the direction from bottom to top led him to the final product shown in Fig. 2.7a. This axle-less construct rotated in the correct direction for >100 revolutions, albeit slowly (Furuike et al. 2008). The rotation was visualized by attaching a gold bead to the two cysteine residues near the arrowheads in Fig. 2.7a, and the obliquely attached bead moved in a circle. Occasionally, though, the bead moved to the center of the circular trace, suggesting an upright orientation as in Fig. 2.7b. That the remaining rotor head in Fig. 2.7a continued to rotate without being carried away by thermal diffusion is already a surprise, and the orientation in Fig. 2.7b still clinging is more than amazing. The push–pull action requires a relatively rigid axle pivoted at the bottom. Obviously, the axle-less construct rotates by a different mechanism, which is totally a mystery to me.

An undergraduate student Kohori attempted a sideways truncation, removing the entire amino-terminal α helix of γ (yellow in Fig. 2.7c). The remaining carboxyl terminal helix was connected to a β subunit via a short peptide liner. This construct rotated at a quarter of the wild-type speed and produced approximately half the wild-type torque (Kohori et al. 2011). Truncation from top is now under way, and the preliminary indication is that the entire γ head is dispensable. If we take AND of all constructs, a conclusion seems inevitable that none of the γ residues are needed for rotation.

Is this the end of the story? That rotation does not depend on specific interactions between γ and the stator has led us to propose that F_1 may have evolved from a motor that can rotate anything (other than an object with threefold symmetry) albeit inefficiently. Evolution, though, cannot be proved, being one-time, irreproducible experiment that God is playing at. So, I am suggesting young colleagues to resort to a "creationist approach," to create F_1 that indeed rotates an unrelated object such as DNA.

2.3 Spiral Rotations and Viral Machines

Now I come to the viral packaging machine, and my interest is whether it rotates.

God did not invent too many different kinds of proteins and nucleotides, and he often resorts to symmetry for building larger structures, ending in helical filaments, disks, and spheres. Repetitive

interactions with a helix (or a disk) should lead to rotation, as indeed observed. A dynein and some kinesins spirally rotate a microtubule (Vale and Toyoshima 1988; Walker et al. 1990; Yajima et al. 2008), myosin or formin rotates an actin filament (Nishizaka et al. 1993; Sase et al. 1997; Mizuno et al. 2011), and RNA polymerase rotates DNA (Harada et al. 2001). Conversely, myosin, when unconstrained, spirals around an actin filament (Ali et al. 2002, 2004).

These spiral motions often disregard the helical pitch of the filament. The pitch of spiral motion and the structural pitch of the helix will agree when movement during one interaction cycle is shorter than half the helical pitch, as for DNA polymerase and formin above and regular kinesin (Howard 1996). Linear molecular motors that walk with long strides disobey the helical pitch, and some appear to produce lateral force to go sideways.

There seems to be no a priori reason why the helical DNA being actively packaged into a viral capsid should not rotate. Although rotation of the portal motor has been denied (Hugel et al. 2007), the stepwise interaction of DNA with the packaging motor (Moffitt et al. 2009) suggests rotation. Extensive DNA rotation, however, would hinder its packaging, and thus if DNA does rotate, its torsion would have to be relaxed by occasional slippage in the motor or, say, by a topoisomerase action. As an ex-physicist, I do not care if DNA really rotates during packaging in vivo. If the machine has the potential of rotation, however, I would like to see it. If the machine is not willing, why not let it go, by artificially preventing slippage if needed. By the time this book appears, someone hopefully will have shown me a movie of rotation.

Acknowledgments I thank members of Kinosita and Yoshida labs for collaboration and discussion, R. Kanda-Terada for technical support, and S. Takahashi, K. Sakamaki, M. Fukatsu, and H. Umezawa for encouragement and lab management. This work was supported by Grants-in-Aids for Specially Promoted Research from the Ministry of Education, Sports, Culture, Science and Technology, Japan.

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http://www.springer.com/978-1-4614-0979-3

Viral Molecular Machines
(Eds.)M.G. Rossmann; V.B. Rao
2012, XIV, 687 p. 221 illus., 192 in color., Hardcover
ISBN: 978-1-4614-0979-3