The catalytic cycle of ATP synthesis by means of a torsional mechanism

Sunil Nath, Hirdesh Rohatgi and Anjanabha Saha*

ATP synthase (or F_1F_0 ATPase) is the universal enzyme in biological energy conversion, synthesizing ATP from ADP and inorganic phosphate using the free energy derived from a transmembrane ionmotive gradient. Recent research indicates that a central 'shaft' rotates in a static 'barrel' in ATP synthase, making it the world's smallest molecular machine. Recently, we have shown how the ionmotive force generates a torque in the F_0 portion of ATP synthase. We have also shown how the ionmotive torque is transmitted a biologically long distance of about 10 nm in ATP synthase and how energy is stored in this remarkable mechanoelectrochemical enzyme. Here we focus on the catalytic cycle of the ATP synthase. We elucidate the entire sequence of events for one cycle of ATP synthesis from ADP and P_i, providing the first motion picture of ATP synthase at work, and the dynamics of its interacting parts. We dissect ATP synthesis into its fundamental elements and illustrate the specific elementary chemical process taking place in each catalytic site conformation. Taken together, our work presents the molecular mechanism of coupling ion translocation to ATP synthesis.

Adenosine triphosphate synthase (ATP synthase or F_1F_0 ATPase) is the universal enzyme in biological energy conversion that is almost three billion years old and is present in the membranes of mitochondria, chloroplasts and bacteria with amazingly similar structure and function in different species. This large enzyme complex (with an overall molecular weight of 520,000 in *Escherichia coli*) consists of two major parts: a membrane-extrinsic, hydrophilic F_1 containing three α, three β, and one copy each of the γ, δ, and ε subunits, and a membrane-embedded, hydrophobic F_0 composed of one a, two b, and twelve c subunits. The F_0 and F_1 domains are linked by two slender stalks[1–6]. The central stalk is formed by the ε subunit and part of the γ subunit, while the peripheral stalk is constituted by the hydrophilic portions of the two b subunits of F_0 and the δ subunit of F_1. The ion channel is formed by the interacting regions of a and c subunits in F_0, while the catalytic binding sites are predominantly in the β subunits of F_1 at the α–β interface[1–5]. The molecular mechanism of coupling ion translocation through F_0 to ATP synthesis in F_1 is unknown. Great interest has been generated in this field after direct observation of rotation of the central stalk by innovative techniques. ATP synthesis takes place by conformational changes at the catalytic binding sites. Recent structural[1,2], biochemical[3,4,7], spectroscopic[8,9], and microscopic[10,11] studies indicate that these conformational changes arise from rotation of the γ–ε subunit in a static barrel of the $\alpha_3\beta_3$ subunits in ATP synthase, making it the world's smallest molecular machine (rotor radius ≈ 1 nm).

Several models have recently addressed the issue of torque generation in ATP synthase[12–15]. However, no detailed molecular mechanism has been proposed to explain the subsequent events leading to ATP synthesis. We address the following questions in detail in this paper: how does energy transmission cause conformational changes in ATP synthase, and, most important, what does the catalytic cycle of ATP synthase look like?

According to the torsional mechanism of energy transmission in ATP synthase[16], the bottom of the central stalk of ATP synthase rotates in twelve discrete 30° steps, while the top of the γ subunit rotates in three discrete 120° steps. The apparent disparity at the top and bottom of the stalk can be understood by considering it as a shaft that is free to rotate at the bottom but is constrained at the top. These constraints are the interactions of the γ subunit with the catalytic sites. The bottom of the stalk rotates relative to the top, causing torsion in the shaft, leading to generation of a torsional strain that is proportional to the angle of twist and storage of torsional energy in the shaft. Thus, the energy of the proton flux is stored as torsional energy in the γ subunit. This torsional energy is further used to cause conformational changes at the catalytic sites, which lead to ATP synthesis. The translocation of protons increases this strain and torsional energy. When they are large enough, the constraints are broken, and the top of the shaft rotates to a new angular position where there is zero torsional strain[16]. This breakthrough stress is attained upon translocation of four protons, in accord with the H^+/ATP ratio[17].

The nucleotide-free $\alpha_3\beta_3$ subcomplex of ATP synthase is a symmetric trimer[2]. However, in F_1-ATPase, the three catalytic sites possess different conformations at any instant of time as seen in the Walker crystal structure[1]. One of the sites in this structure binds the ATP analog AMP–PNP and is designated as β_{TP}; another site binds ADP and is denoted by β_{DP}, while the third site is empty and distorted and is called β_E. In our view, the β subunits adopt these different conformations due to asymmetric interactions with the γ and ε subunits. The asymmetry in the γ subunit can be understood by considering it to have three faces interacting asymmetrically with the catalytic sites, and we have recently made the key proposal that the unique ε subunit (in *E. coli*) is always closest to the catalytic site with the *least* affinity for ATP (β_E)[12]. As the γ and ε subunits rotate relative to $\alpha_3\beta_3$, their interactions with a particular catalytic binding site change, thereby causing conformational changes at that site. These conformational changes lead to the synthesis of ATP.

The catalytic site of a β subunit contains three major sub-domains of interest. The adenine-binding sub-domain consists of the amino acid residues

*For correspondence
(e-mail: sunath@dbeb.iitd.ernet.in)

Tyr 345, Phe 418, Ala 421, Phe 424, Thr 425, Pro 346, Val 164, and Gly 161. The phosphate binding sub-domain is made up of the following residues of the β subunit: Lys 162, Thr 163, Val 164, Leu 165, Gly 161, Val 160, Gly 159, and Arg 189. The amino acid residues Thr 163, Glu 192, and Asp 256 of the β subunit contribute to coordination with the Mg^{2+} and form the third sub-domain. In our view, a β subunit which has no interactions with either γ or ε, and contains no bound nucleotide or Mg^{2+} adopts an open conformation (β_E). In such a β subunit, none of the sub-domains is in the correct conformation to bind the substrate MgADP. Therefore, while MgADP can diffuse in, it cannot bind properly. First, Mg^{2+} interacts with its ligands and makes the sub-domain acquire the correct conformation for binding. Now Mg^{2+} binds and the binding energy is used to force the adenine-binding sub-domain into its correct conformation. Thus, the adenine ring of the nucleotide binds, which further promotes the binding of the phosphate group. As a result of substrate binding, the lower portion of the β subunit moves up. This movement causes the β subunit to adopt a new conformation which we refer to as β_C (closed). Although the β subunit now contains bound Mg^{2+} and nucleotide, in the absence of any interaction with γ or ε, there is insufficient space at the catalytic site to accommodate both MgADP and P_i simultaneously as separate entities.

Thus, some interactions are required to greatly enhance the access of P_i to the catalytic site. This, we propose, takes place when the β subunit changes its conformation from β_C to β_{TP}. One face of the γ subunit (Lys 87 and Lys 90) interacts with the DELSEED sequence (394–400; the residue numbers for the F_1 domain refer to mitochondria, unless specifically mentioned) and causes the binding site to open slightly, which allows the P_i to enter and come in proximity to MgADP. We consider this as the loose site. Figure 1 shows the conformation of the catalytic site in the β_{TP} conformation of the Walker structure[1] (although the catalytic site should be occupied by bound MgADP, it contains the MgATP analog, MgAMP–PNP). The γ subunit now rotates and the above interactions break, leading to the β_{DP} conformation which we refer to as the tight conformation. As the new face of γ has no interactions with the catalytic site, the subunit again has insufficient space to accommodate both MgADP and P_i as separate entities. This forces the P_i to move towards MgADP. Also, there is a change in conformation of the phosphate sub-domain; the positively charged atoms of the key catalytic residues move closer to the O2B oxygen atom of the MgADP (e.g. the distance of the O2B oxygen to the N and NZ atoms of the critical catalytic residue Lys 162 reduces from 2.81 Å and 3.28 Å respectively to 2.50 Å and 2.73 Å respectively). Further, the Mg^{2+} interacts with the O1B oxygen of the substrate. These interactions lead to the development of a better, more effective ADP–O^- nucleophile. The π electrons of the phosphorus–oxygen double bond in P_i (which exists primarily in its monoprotonated form HPO_4^{2-} at physiological pH) can be considered to be localized more on the higher electronegative oxygen atom, resulting in a δ^+ charge on the central P atom of P_i. Moreover, the Mg^{2+} now interacts with one of the two negatively charged oxygen atoms of the approaching P_i, causing a change in the coordination state of the Mg^{2+} and a corresponding rearrangement in the conformation of the ligands coordinating with it, and leading *to electron withdrawal from the P atom* of P_i and the further development (and stabilization) of the δ^+ charge on the P atom, thus making it more susceptible to a nucleophilic attack. Both effects (increased nucleophilicity of ADP–O^- and increased susceptibility of P_i to a nucleophilic attack) are important and contribute to the driving force for ATP synthesis. We envisage the nucleophilic attack to be perpendicular to (and in the center of) the essentially planar and triangular face of the oxygens of the tetrahedral P_i, and at an angle of 180° to the leaving group (i.e. apical and in-line). The MgADP serves as the nucleophilic acceptor of a phosphoryl group, and a pentacoordinate transition state is formed. This transition state is stabilized by the critical catalytic residues Lys 162 and Arg 189. The transition state leads to formation of MgATP with inversion at the γ-phosphorous atom and a concomitant loss of the OH^- from P_i to form water by taking H^+ from the medium. The *essential catalytic residues Lys 162 and* Arg 189 (the NH1 nitrogen of the guanidinium group of Arg 189 lies at a distance of 3.36 Å and 3.15 Å from the O2G and O3G oxygens of the γ-phosphate respectively, while the NH2 nitrogen lies 3.04 Å from the O3G atom) provide binding energy for MgATP by interacting with the negatively charged oxygens of the γ-phosphate (Figure 1). Thus, bond formation and water elimination occur simultaneously in this direct, in-line nucleophilic displacement reaction mechanism without the formation of any *phosphorylated intermediate*.

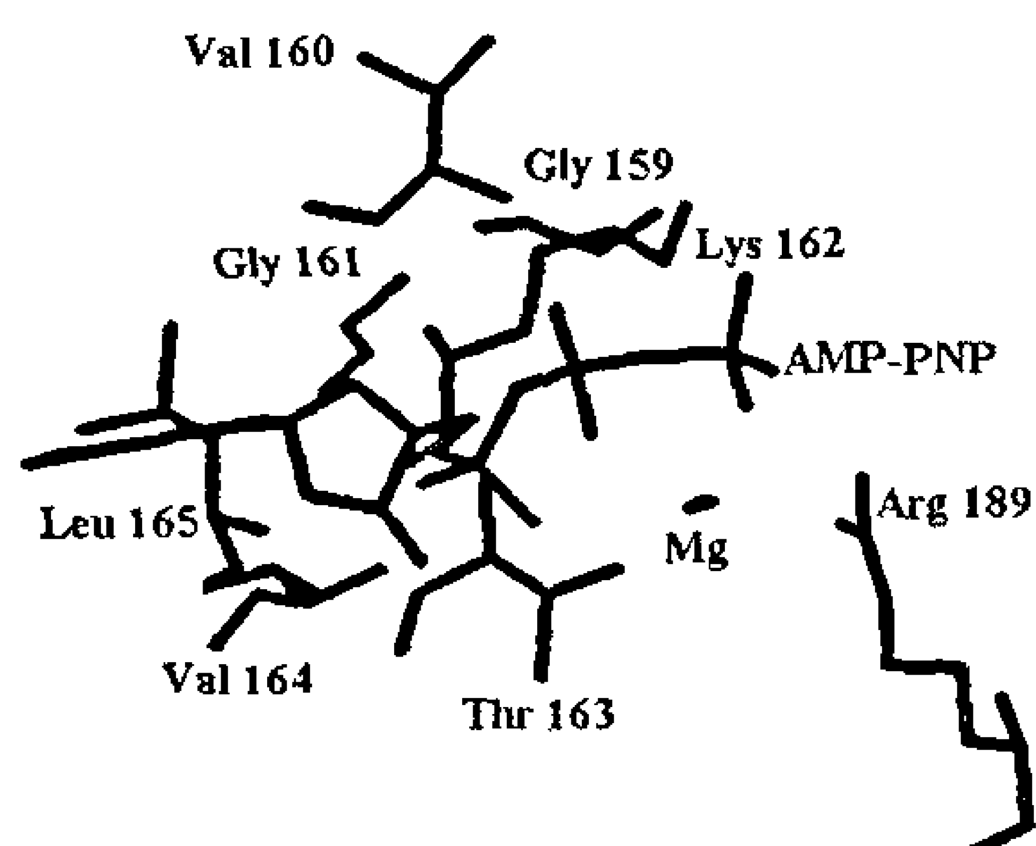

Figure 1. Catalytic binding domain containing ATP analog. The Mg^{2+} lies between the β- and the γ-phosphates and stabilizes the O2B and O2G oxygens of the analog. The amino group of Lys 162 interacts with the O1B and O1G oxygens. The O3G oxygen atom of the ATP analog is stabilized by the nitrogen atoms of the guanidinium group of Arg 189.

The above sequence of steps is followed by the conformation β_E in which the C terminal of the γ subunit (Arg 254 and Gln 255) interacts with the Asp 316, Thr 318 and Asp 319 of β_E. Further, Ser 108 (*E. coli* numbering) of the rotating ε subunit interacts covalently via an ester linkage[18] with Glu 381 (*E. coli* numbering, corresponding to Glu 395 in mitochondria) of β_E, as a result of which the lower half of β_E is hinged out, leading to the opening of the catalytic site and release of the bound MgATP in this open conformation. The upper half of β_E is held in place by its interactions with the γ subunit. The interaction of ε causes a conformational change in the ligands coordinated to the Mg^{2+} such that it can no longer bind to them. This unbinding causes change in conformation in the other two sub-domains and the MgATP can no longer remain bound, resulting in its release. We expect that the Lys 162 and Arg 189 residues move away, thereby weakening their interaction with the γ-phosphate of MgATP. The interaction of the ε subunit with the β subunit is critical because without this interaction, the β subunit cannot achieve the open conformation as long as the Mg^{2+} remains

bound to it. Moreover, as long as the ε subunit is interacting with the β subunit, the substrate cannot bind, as Mg^{2+} binding is the initiation step for substrate binding. Therefore MgADP can enter in β_E, but bind only when the ε–β interaction breaks. As ion translocation takes place in the F_0 subunit, our mechanism predicts that the *bottom* of the shaft (the ε subunit and the bottom of the γ subunit) rotates, while the top remains stationary (due to the constraints arising from its interactions with the catalytic sites). This causes the interaction of the ε subunit and the Glu 381 of β_E to break, resulting in substrate binding and the entire cycle repeats. Thus, during ATP synthesis, the order of conformations for a particular β subunit according to the mechanism proposed above is $O \rightarrow C \rightarrow L \rightarrow T$ (Figure 2). In the case of free rotation, there would exist no β_C conformation, and the β subunit will go directly from the open conformation (β_E) to the loose conformation (β_{TP}). However, as β_{TP} contains bound MgADP + P_i, and β_E contains no bound nucleotide or P_i, it is difficult for us to conceive how both the binding steps can take place simultaneously in β_{TP}, especially keeping in mind that the binding of P_i is not spontaneous and requires energy. Such difficulties do not arise in our proposed mechanism because the presence of torsion in the γ subunit ensures that another conformation (β_C) is attained between β_E and β_{TP} where MgADP gets bound.

Extending the above sequence of events proposed by our mechanism of ATP synthesis at a single β subunit to the entire ATP synthase enzyme, we see that the enzyme switches from the two-nucleotide state to the three-nucleotide state and back again. Consider an instant in time when the F_1 subunit is in the two-nucleotide state. At this instant, the conformations adopted by the three β subunits are: loose (β_{TP}), tight (β_{DP}), and open (β_E). MgADP can enter in β_E, but in the absence of a binding site, it cannot bind to it. As ion translocation takes place in the F_0 subunit, the bottom of the shaft, consisting of the ε subunit and the bottom of the γ subunit rotates. This causes the interaction of the ε subunit and the Glu 381 (*E. coli* numbering; Glu 395 in mitochondria) of β_E to break, allowing the MgADP to bind. The catalytic site now adopts the closed conformation (β_C). The top of the shaft remains stationary in this interval of time; consequently, the conformations of the β_{TP} and β_{DP} sites remain unchanged. Thus, at this particular instant, all three β subunits contain bound nucleotides. This is the three-nucleotide state reported recently[21]. With further rotation of the bottom caused by the ionmotive force, the constraints at the top are broken, and the entire shaft rotates to a new position, where ε interacts with the Glu 381 of β_{DP} and converts it to the open conformation, leading to the release of MgATP. The change in γ–β interactions converts the β_C site to the loose conformation and the β_{TP} site to the tight conformation. Thus, the enzyme regains the two-nucleotide state and the cycle starts all over again. Thus, in our proposed mechanism of ATP synthesis, binding of substrate converts the enzyme from the two-nucleotide state to the three-nucleotide state. *Catalysis takes place in the three-nucleotide state*, which converts back to the two-nucleotide state with release of product. One-third of the ATP synthase catalytic cycle is depicted in Figure 3. According to our mechanism, the bound nucleotide occupancies of the catalytic sites during ATP synthesis are: MgADP + P_i in β_{TP} (loose), MgATP in β_{DP} (tight), no bound nucleotide in β_E (open), and MgADP in β_C (closed) (Figure 3). Therefore, both the two-nucleotide state and the three-nucleotide state are forms of the ATP synthase enzyme occurring at *different* instants of time.

The conformations of the catalytic sites in F_1 and their function are summarized in Table 1. We have dissected ATP synthesis into its fundamental elements; each catalytic site conformation carries out a specific elementary chemical process, as shown in Table 1. Looked at in this way, the efficiency and economy of nature's design of this molecular machine is revealed.

The molecular mechanism of ATP synthesis proposed here is supported by a number of *independent* experimental evidences. There exists strong structural, biochemical and spectroscopic evidence for intersubunit rotation in both hydrolysis and synthesis modes[1–9]; moreover, rotation in F_1ATPase has been observed directly in recent works[10,11]. The interactions of the γ and ε subunits with the β subunits are consistent with the crystal

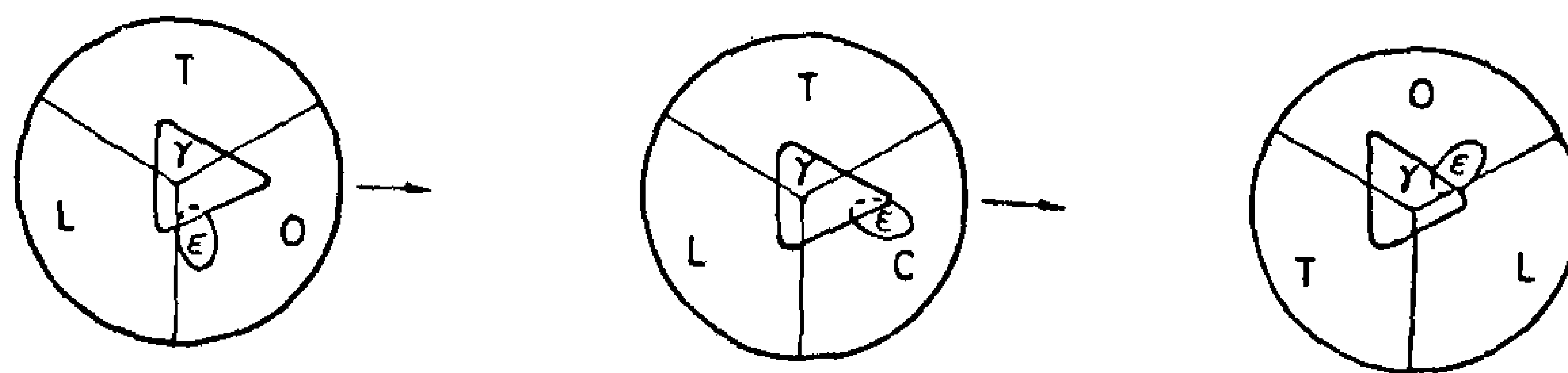

Figure 3. One-third of the catalytic cycle according to our proposed torsional mechanism of ATP synthesis by ATP synthase (viewed from the F_1 side). The conformations of the F_1 catalytic sites in the diagram on the left are: open, O (β_E); tight, T (β_{DP}); and loose, L (β_{TP}). The rotating ε subunit has caused β_E to open (see text), thereby releasing the bound MgATP from the previous one-third of the enzyme cycle. MgADP diffuses into this open conformation, but in the absence of a binding site it cannot bind to it. Thus, the open conformation (β_E) contains no *bound* nucleotide. The middle diagram is drawn for the case when the ionmotive force has caused the ε subunit to rotate 60° counterclockwise (*by two 30° steps*, each 30° step corresponding to the translocation of one ion through F_0)[12,16]; the top of the γ subunit remains stationary in this interval of time because of constraints arising from its interactions with the catalytic sites[16]. Due to the removal of ε–β_E interactions, the substrate binds and the open catalytic site changes to a closed conformation (β_C). β_C therefore contains bound MgADP. The tight and the loose catalytic sites contain bound MgATP and bound (MgADP + P_i) respectively. With the passage of four ions through F_0, sufficient *torsional energy* is accumulated in the γ subunit to enable the constraints at the top to be broken, and the top of the γ subunit rotates *120° counterclockwise in a single step*[16] (i.e. while the bottom of the γ subunit, together with ε, rotates from 90° to 120°, the top of the γ subunit rotates from 0° to 120°), leading to the enzyme conformation depicted in the diagram on the right. This rotation of the top of the γ subunit converts the L site (β_{TP}) to the T site (β_{DP}), and the C site (β_C) to the L site (β_{TP}). Interaction of the ε subunit converts the T site (β_{DP}) to the open site (β_E), and an enzyme conformation similar to the diagram on the left appears, but shifted counterclockwise by 120°, and the entire cycle repeats.

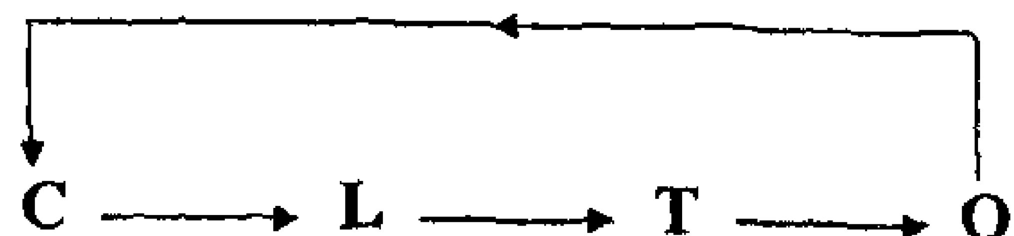

Figure 2. *The sequence of conformations that a particular β subunit cycles through during ATP synthesis, as proposed by our molecular mechanism.* C represents the closed conformation (β_C), L the loose conformation (β_{TP}), i.e. site 2, T the tight conformation, i.e. site 1 or the highest-affinity catalytic site (β_{DP}), and O the open conformation, i.e. the least-affinity catalytic site (β_E) (site 3).

Table 1. Conformations of the F_1 catalytic sites and the *elementary chemical process taking place inside them* during ATP synthesis

Conformation	Elementary chemical process during ATP synthesis
O (β_E, open)	MgATP leaves; MgADP enters
C (β_C, closed)	MgADP binds
L (β_{TP}, loose)	P_i enters
T (β_{DP}, tight)	MgATP synthesized during L → T transition remains bound

structures of F_1ATPase reported in the literature[1,18]. Recent crosslinking studies lend additional support[19,20]. Moreover, our labeling of the β subunits is also in accord with the recent finding that the catalytic site containing bound MgADP in the two-nucleotide structure is of higher affinity than the site containing bound MgAMP–PNP[21,22]. Finally, definitive evidence for our mechanism comes from recent kinetic experiments[23] which emphasize that (i) P_i cannot simply bind spontaneously, (ii) an enzyme species with all three sites occupied is the only catalytically competent species, and (iii) release of product and binding of substrate cannot be simultaneous, rather product release must precede substrate binding.

A few remarks based on the referee comments are in order. It is true that in a recent crystal structure of the rat liver F_1-ATPase enzyme[24], a more symmetrical structure of the α and β subunits was obtained. We do not believe that the data adequately support their structural interpretations. Unlike the bovine heart F_1-ATPase enzyme[1], the rat liver enzyme was crystallized in the absence of Mg^{2+}. Mg^{2+} is critical for the structure and mechanism, and, as shown and emphasized in this work, Mg^{2+} has a crucial role in catalysis; we therefore cannot conceive how a closed catalytic site conformation can occur in the absence of Mg^{2+}. It is also true that our assignment of sites contradicts the assignment made by Grüber and Capaldi[25]. However, it is interesting to note that recently[26], the Capaldi group published a new assignment that contradicts their earlier work[25], reflecting uncertainty in their own interpretations. In our view, both these assignments[25,26] are incorrect, and looking at ATP synthase as a cyclic molecular machine, the assignment given in this work is the correct one. In our view, b_2 and δ form part of the stator connecting $\alpha_3\beta_3$ and a. Thus, any change in length of b_2 should necessarily result in bending of the $\alpha_3\beta_3$ complex or movement of the a subunit. Therefore, we see absolutely no merit in the proposal that the b subunit 'serves as an elastic element which transiently stores free energy[27,28]' and disagree completely with a recent assertion that the 'interaction between the b_2 dimer and the δ subunit is designed to be broken[29]', i.e. the peripheral stalk transiently dissolves and reforms during ATP synthesis. Further, *these proposals[27–29] are not in* accordance with recent crosslinking experiments which demonstrate that cross-linking of a b subunit to an α subunit does not affect multisite ATPase activity[30]. What is perhaps most important, in the final outcome, is to recognize and understand the imperative need to consider novel ideas with an open mind, and to overcome the limitations imposed on our own thinking by currently accepted mechanisms[3,5,24,31], by paradigms that are no longer applicable.

We finally comment briefly on the major differences of our molecular mechanism from those proposed by other workers. Previous workers have only addressed parts of the complete mechanism of ATP synthesis: thus, mechanisms *of torque generation in* F_0 *(refs 13–15,* 27, 28) and catalytic mechanisms in F_1 (refs 3–5, 31) have been proposed. Others have proposed mechanisms of ATP hydrolysis[4,32], which, in our view, is a non-physiological mode of operation of the synthase. The molecular mechanism proposed by us is the only *unified* mechanism of ATP synthesis to date, addressing as it does the issues of ionmotive torque generation in F_0 (ref. 12), torque transmission from F_0 to F_1 (refs 12, 16), energy storage in the enzyme[16], conformational changes in F_1 (this work), and the catalytic cycle of ATP synthesis (this work). This, in our opinion, is a great leap in our understanding. Several models of torque generation employ thermal fluctuations for performing useful molecular work[13,27,28]; however, we visualize the ATP synthase as an *enthalpy-driven* machine (and *not* as an entropic machine), and we conceive of the free energy transfer process in a very different way: as a precise, ordered, quantized, nonthermal and non-stochastic process. According to our molecular mechanism, there is no site–site cooperativity in ATP synthase; in fact there is no need for cooperativity! It is interesting to note that a recent work advocated the absence of cooperativity[33], though, in our view, based on incorrect interpretations; it was claimed that throughout the range from unisite to multisite reaction conditions, the rate constant for ATP hydrolysis remains relatively constant! In other words, slow unisite catalysis was not observed[33]. A possible reason for this anomaly could be that they used a luciferase-based chemiluminescent assay that measured binding, but not necessarily hydrolysis, of ATP. In our view, there does exist a rate enhancement on transition from unisite to multisite catalysis in ATP synthase; however, *this rate enhancement is caused, not* by positive cooperativity among catalytic sites, but is a consequence of the mode of functioning of the enzyme itself, as clearly illustrated by our molecular mechanism (text and Figure 3). For instance, in unisite catalysis, the MgADP bound to the T site is released during the T → O transition (text and Figure 3), with essentially no (or only background) ATP synthesis; in fact, according to our mechanism, physiological rates of catalysis are achieved only when all three catalytic sites are occupied by bound nucleotide. In current *catalytic mechanisms of the working of* F_1 (refs 3,5) (unlike in our mechanism), the binding of P_i is taken to be spontaneous, which, in our conception, is a very serious defect. Further, the implications of the binding change mechanism mean that ATP release at a catalytic site precedes ATP synthesis at another catalytic site[5], and that substrate binding enhances product release from a different catalytic site, which in turn promotes ATP synthesis[34]. In other versions, substrate binding to one site and product release from another site occur simultaneously[35]. Such mechanisms are incorrect, because product must first be released to reveal an open catalytic site, into

which a substrate molecule can enter from the medium and subsequently bind (Figure 3). In other words, product release must precede substrate binding.

Several other differences may be highlighted. We consider it highly unlikely that nucleotide can bind to the catalytic site in the O conformation, because of the absence of a proper binding site. This difficulty does not exist in our mechanism. In our mechanism, ATP synthesis is associated with the L → T conformational transition of a catalytic site; this contradicts the proposal that reversible formation of tightly bound ATP occurs at a single catalytic site[36]. In a recent work, it was postulated that liberation of ATP provides the driving force for releasing elastic strain in the enzyme[37], while according to our mechanism, the torsional strain in the enzyme drives ATP release. Further, P_i binding does not drive ATP release (as proposed in a recent work)[31] from site 1, or, for that matter, from any other site, according to our mechanism. Finally, it is not correct to say that the energy from oxidation is not used to make the ATP molecule, but to release the tightly bound ATP[36]; in fact, our mechanism shows that P_i binding, ATP synthesis, and ATP release all require energy, and we predict the energy requirement to follow the order, ATP release > ATP synthesis > P_i binding. Space limitations preclude further discussion; however, we can say with certainty that our molecular mechanism of ATP synthesis differs completely from all other (partial) mechanisms that have been proposed in the literature to date.

In summary, we have explained the functioning of ATP synthase as a molecular machine; we have shown how the ion-motive torque generated in the F_0 portion of ATP synthase[12] is transmitted from F_0 to F_1, a biologically long distance of approximately 10 nm, and how energy is stored in this mechanoelectrochemical enzyme[16]. We have proposed how the transmitted torque causes conformational changes in F_1, and what the catalytic cycle of ATP synthase looks like. By elucidating the cycle of events at the catalytic sites, we have provided the first motion picture of the ATP synthase at work, and the dynamics of its interacting parts. We have dissected ATP synthesis into its fundamental elements, and the specific elementary chemical process taking place in each catalytic site conformation has been illustrated. The crucial role of Mg^{2+} in catalysis has been accentuated. The proposed molecular mechanism of ATP synthesis has been shown to be consistent with a number of independent experimental observations. The radical differences of our unified molecular mechanism with all other (partial) mechanisms in the literature have been summarized. Finally, it has not escaped our notice that the (self) regulation of ATP synthesis follows naturally, and is in fact self-evident from our proposed molecular mechanism. Taken together, our work presents the molecular mechanism of coupling ion translocation through F_0 to ATP synthesis in the F_1 domain of ATP synthase, the smallest known molecular machine, and a remarkable mechano-electrochemical transducer.

1. Abrahams, J. P., Leslie, A. G. W., Lutter, R. and Walker, J. E., *Nature*, 1994, **370**, 621–628.
2. Shirakihara, Y., Leslie, A. G. W., Abrahams, J. P., Walker, J. E., Ueda, T., Sekimoto, Y., Kambara, M., Saika, K., Kagawa, Y. and Yoshida, M., *Structure*, 1997, **5**, 825–836.
3. Boyer, P. D., *Biochim. Biophys. Acta*, 1993, **1140**, 215–250.
4. Weber, J. and Senior, A. E., *Biochim. Biophys. Acta*, 1997, **1319**, 19–57.
5. Zhou, Y., Duncan, T. M. and Cross, R. L., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10583–10587.
6. Wilkens, S. and Capaldi, R. A., *Nature*, 1998, **393**, 29.
7. Ogilvie, I., Aggeler, R. and Capaldi, R. A., *J. Biol. Chem.*, 1997, **272**, 16652–16656.
8. Sabbert, D., Engelbrecht, S. and Junge, W., *Nature*, 1996, **381**, 623–625.
9. Sabbert, D. and Junge, W., *Proc. Natl. Acad. Sci. USA*, 1997, 94, 2312–2317.
10. Noji, H., Yasuda, R., Yoshida, M. and Kinosita, K., *Nature*, 1997, **386**, 299–302.
11. Yasuda, R., Noji, H., Kinosita, K. and Yoshida, M., *Cell*, 1998, **93**, 1117–1124.
12. Rohatgi, H., Saha, A. and Nath, S., *Curr. Sci.*, 1998, **75**, 716–718.
13. Elston, T., Wang, H. and Oster, G., *Nature*, 1998, **391**, 510–513.
14. Vik, S. B. and Antonio, B. J., *J. Biol. Chem.*, 1994, **269**, 30364–30369.
15. Cox, G. B., Fimmel, A. L., Gibson, F. and Hatch, L., *Biochim. Biophys. Acta*, 1986, **849**, 62–69.
16. Nath, S., Rohatgi, H. and Saha, A., *Curr. Sci.*, 1999, **77**, 167–169.
17. Nath, S., *Pure Appl. Chem.*, 1998, **70**, 639–644.
18. Uhlin, U., Cox, G. B. and Guss, J. M., *Structure*, 1997, **5**, 1219–1230.
19. Aggeler, R., Haughton, M. A. and Capaldi, R. A., *J. Biol. Chem.*, 1995, **270**, 9185–9191.
20. Aggeler, R. and Capaldi, R. A., *J. Biol. Chem.*, 1996, **271**, 13888–13891.
21. Löbau, S., Weber, J., Wilke-Mounts, S. and Senior, A. E., *J. Biol. Chem.*, 1997, **272**, 3648–3656.
22. Weber, J., Hammond, S. T., Wilke-Mounts, S. and Senior, A. E., *Biochemistry*, 1998, **37**, 608–614.
23. Löbau, S., Weber, J. and Senior, A. E., *Biochemistry*, 1998, **37**, 10846–10853.
24. Bianchet, M. A., Hullihen, J., Pedersen, P. L. and Amzel, L. M., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 11065–11070.
25. Grüber, G. and Capaldi, R. A., *Biochemistry*, 1996, **35**, 3875–3879.
26. Wilkens, S. and Capaldi, R. A., *J. Biol. Chem.*, 1998, **273**, 26645–26651.
27. Junge, W., Lill, H. and Engelbrecht, S., *Trends Biochem. Sci.*, 1997, **22**, 420–423.
28. Engelbrecht, S. and Junge, W., *FEBS Lett.*, 1997, **414**, 485–491.
29. Sorgen, P. L., Caviston, T. L., Perry, R. C. and Cain, B. D., *J. Biol. Chem.*, 1998, **273**, 27873–27878.
30. Rodgers, A. J. W. and Capaldi, R. A., *J. Biol. Chem.*, 1998, **273**, 29406–29410.
31. Al-Shawi, M. K., Ketchum, C. J. and Nakamoto, R. K., *Biochemistry*, 1997, **36**, 12961–12969.
32. Wang, H. and Oster, G., *Nature*, 1998, **396**, 279–282.
33. Reynafarje, B. D. and Pedersen, P. L., *J. Biol. Chem.*, 1996, **271**, 32546–32550.
34. Souid, A.-K. and Penefsky, H. S., *J. Biol. Chem.*, 1995, **270**, 9074–9082.
35. Cross, R. L., in *Molecular Mechanisms in Bioenergetics* (ed. Ernster, L.), Elsevier, Amsterdam, 1992, pp. 317–330.
36. Boyer, P. D., *Angew. Chem. Int. Edn. Engl.*, 1998, **37**, 2296–2307.
37. Cherepanov, D. A., Mulkidjanian, A. Y. and Junge, W., *FEBS Lett.*, 1999, **449**, 1–6.

ACKNOWLEDGEMENTS. S.N. thanks DST and the All-India Council for Technical Education, for research grants obtained.

The authors are at the Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Hauz Khas, New Delhi 110 016, India.