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Frontiers in ATP Synthase Research: Understanding the Relationship Between Subunit Movements and ATP Synthesis

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How biological systems make ATP has intrigued many scientists for well over half the 20th century, and because of the importance and complexity of the problem it seems likely to continue to be a source of fascination to both senior and younger investigators well into the 21st century. Scientific battles fought to unravel the vast secrets by which ATP synthases work have been fierce, and great victories have been short-lived, tempered with the realization that more structures are needed, additional subunits remain to be conquered, and that during ATP synthesis, not one, but several subunits may undergo either significant conformational changes, repositioning, or perhaps even physical "rotation" similar to bacterial flagella[1,2]. In this introductory article, the author briefly summarizes our current knowledge about the complex substructure of ATP synthases, what we have learned from X-ray crystallography of the F_1 unit, and current evidence for subunit movements.

KEY WORDS: ATP synthesis; ATP synthase; F_1-ATPase; oxidative phosphorylation; molecular motors; subunit movements; subunit rotation; mitochondrial function.

INTRODUCTION

The purpose of this minireview series is to summarize current views about subunit movements in ATP synthases and their possible relationship to function, namely ATP synthesis and ATP hydrolysis (for recent reviews see Refs. 3–9). This minireview series has not been programmed to arrive at any consensus, but to emphasize that our current understanding of the proposed dynamic features of ATP synthases, although enlightening, is at a very early stage. Certainly, much more work is essential to arrive at any definitive conclusions, similar to those reached for bacterial flagella where rotational movements are clear.

In this brief introductory article the author's purpose is threefold: (1) to summarize our current knowledge about the complex substructure of ATP synthases, (2) to summarize what we have learned from X-ray crystallography about the F_1 unit and the possible relationship of this knowledge to subunit changes/movements during ATP synthesis, and (3) to summarize the types of "small subunit" movements that are believed to occur during ATP synthase function, and some of the supporting evidence.

ATP SYNTHASE SUBSTRUCTURE

Figure 1A depicts a conservative attempt to illustrate overall structural features of ATP synthases from bacteria (*E. coli*) and animals (rats and cows).[3,10–22] In the electron microscope, ATP synthases from both sources exhibit a tripartite structure consisting of a headpiece, basepiece (membrane sector), and a connecting stalk.[10,11] However, these enzymes resolve most readily into only two units, a water-soluble unit called F_1 and a detergent-soluble unit called F_0. Although the F_1 unit is predominantly "headpiece" and

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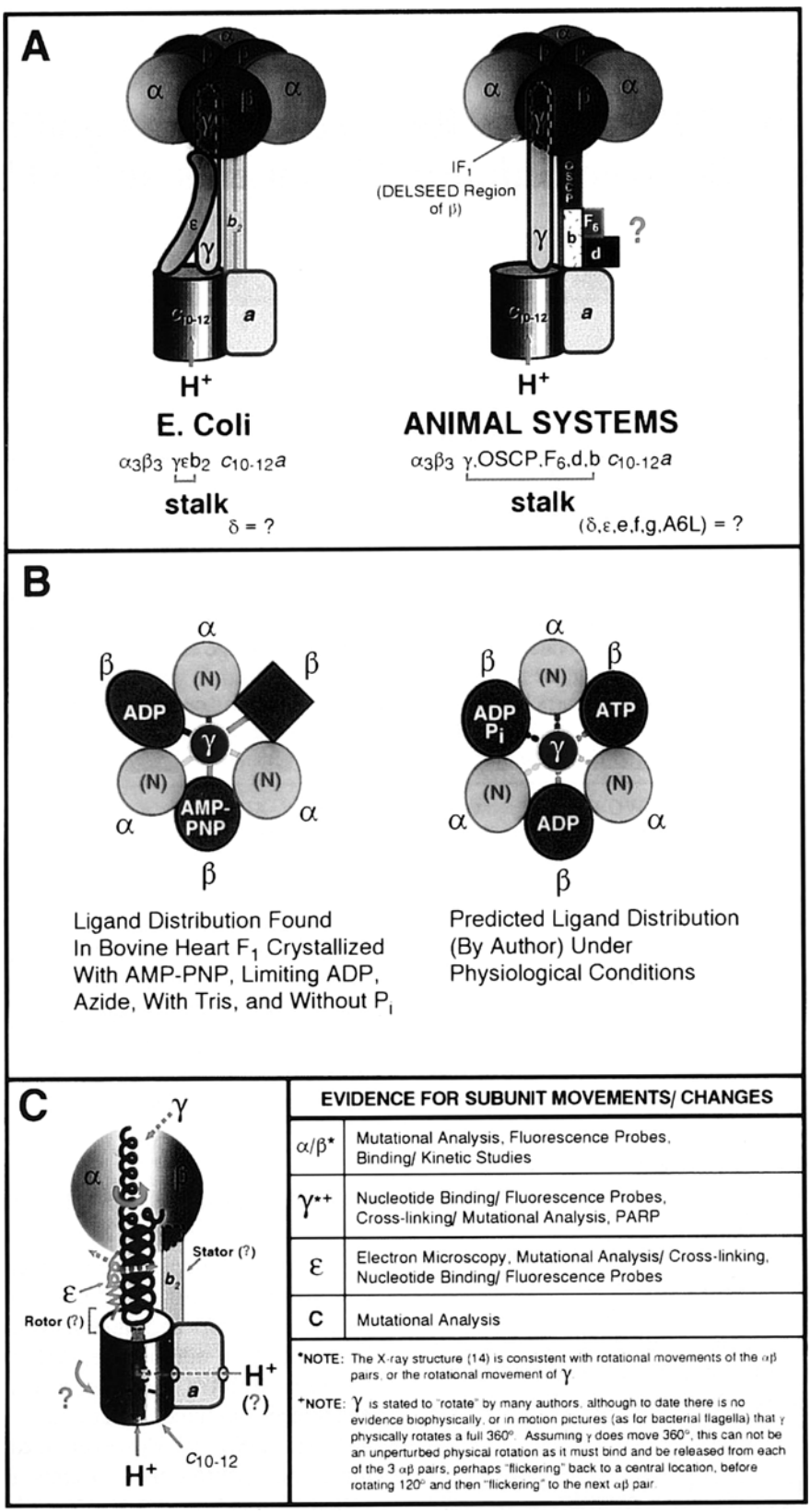
A
α
β
γ
ε
b_2
a
c_{10-12}
H^+
E. Coli
$\alpha_3\beta_3$ $\gamma\varepsilon b_2$ $c_{10-12}a$
stalk
δ = ?
IF_1
(DELSEED Region of β)
F_6
b
d
?
ANIMAL SYSTEMS
$\alpha_3\beta_3$ γ.OSCP.F_6.d.b $c_{10-12}a$
stalk
(δ,ε,e,f,g,A6L) = ?
B
(N)
ADP
AMP-PNP
ATP
P_i
Ligand Distribution Found In Bovine Heart F_1 Crystallized With AMP-PNP, Limiting ADP, Azide, With Tris, and Without P_i
Predicted Ligand Distribution (By Author) Under Physiological Conditions
C
Stator (?)
Rotor (?)
EVIDENCE FOR SUBUNIT MOVEMENTS/ CHANGES
α/β*
Mutational Analysis, Fluorescence Probes, Binding/ Kinetic Studies
γ*+
Nucleotide Binding/ Fluorescence Probes, Cross-linking/ Mutational Analysis, PARP
ε
Electron Microscopy, Mutational Analysis/ Cross-linking, Nucleotide Binding/ Fluorescence Probes
C
Mutational Analysis
*NOTE: The X-ray structure (14) is consistent with rotational movements of the αβ pairs, or the rotational movement of γ
+NOTE: γ is stated to "rotate" by many authors, although to date there is no evidence biophysically, or in motion pictures (as for bacterial flagella) that γ physically rotates a full 360°. Assuming γ does move 360°, this can not be an unperturbed physical rotation as it must bind and be released from each of the 3 αβ pairs, perhaps "flickering" back to a central location, before rotating 120° and then "flickering" to the next αβ pair.

the F_0 unit predominantly "basepiece," some components of both F_1 and F_0 contribute to the "stalk" region.[3,19–22]

The F_1 unit of both the *E. coli* and animal ATP synthases is comprised of five subunit types in the stoichiometric ratio $\alpha_3\beta_3\gamma\delta\varepsilon$ (~371 kDa). Also, the F_0 unit of both enzymes contains homologous subunits called a, b, and c, which in the *E. coli* enzyme are believed to be in the stoichiometric ratio ab_2c_{10-12}. However, the F_0 complex of the animal enzyme is much more complex, consisting of additional subunits called OSCP, F_6, d, e, f, g, and A6L.[16–21] In both enzymes the F_1 hexagonal subunit array $\alpha_3\beta_3$ comprises the bulk of the headpiece, and the F_0 subunits $c_{10-12}a$ comprise the bulk of the basepiece. However, the "stalk" differs considerably between the two enzymes. For example, in the *E. coli* enzyme there is evidence that both the γ and ε subunits of the F_1 unit and the b subunit of the F_0 unit contribute significantly to the stalk (reviewed in Ref. 3). In the animal enzymes, there is evidence that the γ and b subunits also contribute to the stalk,[14,19,21] but with likely contributions from several other F_0 subunits including OSCP, F_6, and d. The locations of *E. coli* subunit δ and animal subunits δ, ε, f, g and A6L are not clearly apparent at this time.

In summary, the *E. coli* ATP synthase can be defined "substructurally" at this time as follows:

$$\alpha_3\beta_3\underline{\gamma,\ \varepsilon,\ b_2}c_{10-12}a \qquad (\delta = ?)$$

Subunits known to contribute to the stalk region are underlined.

In an analogous manner, the animal ATP synthases can be defined "substructurally" at this time only partially as follows:

$$\alpha_3\beta_3\underline{\gamma,\ \text{OSCP},\ F_6,\ \text{d},\ \text{b}},\ c_{10-12}a$$

$$(\delta,\ \varepsilon,\ \text{e},\ \text{f},\ \text{g and A6L} = ?)$$

Subunits believed to contribute to the stalk region are underlined.

Finally, there are some subtleties regarding the subunits of ATP synthases that should be noted (reviewed in Ref. 5). Thus, the ε subunit of *E. coli* F_1 is not related to the ε subunit of F_1 from animals. In fact, there is no counterpart for the animal ε subunit in *E. coli*. Rather, it is frequently assumed that the *E. coli* ε subunit corresponds to the animal δ subunit. However, this assumption is based on a low sequence homology and to date there is no evidence that *E. coli* ε and animal δ subunits play similar roles. The *E. coli* ε subunit is readily removed from its F_1 unit and can

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Fig. 1. (A) Structures of *E. coli* and animal ATP synthases. Both structures exhibit a tripartite arrangement consisting of a headpiece, basepiece, and connecting stalk. The height of the basepiece, stalk, and headpiece range respectively in literature reports from 50–68 Å, 37–45 Å, and 85–100 Å. The stalk in the illustration is drawn out of proportion for illustrative purposes. Animal ATP synthases are more complex than the *E. coli* enzyme, with such complexity due to additional subunits types within the stalk and basepiece regions. Experimentally both enzymes can be resolved into two units, "F_1" consisting of five subunit types in the stoichiometric ratio $\alpha_3\beta_3\gamma\delta\varepsilon$, and "$F_0$" consisting of all other subunit types. The F_1 unit of both rat liver and bovine heart have been crystallized and X-ray structures solved at 3.6 Å and 2.8 Å, respectively. These structures show that α and β subunits are arranged as indicated in an alternating manner. The higher resolution of the bovine heart enzyme reveals nucleotide binding sites centrally located within α and β subunits at opposite interfaces, and shows that the γ subunit in the center of F_1 extends from near the top of the headpiece to well below its bottom. The position of the stalk subunits relative to the headpiece and basepiece are not known with certainty. Although most authors now believe on the basis of electron microscopic evidence[22] that subunits a and b reside outside the central core of c subunits, this view is not shared by all investigators.[45] Animal ATP synthases contain an extra readily dissociable subunit called "IF_1" which is an inhibitor of ATPase activity. IF_1 is believed to bind near or to a β subunit sequence DELSEED, characterized by a preponderance of acid residues. (B) Subunit distribution of nucleotides in the F_1 moiety of ATP synthase. The model on the left depicts the subunit distribution observed in the 3-dimensional structure of bovine heart F_1. Here "N" refers to AMP-PNP. The model on the right is the author's prediction for the subunit distribution of nucleotides under physiological conditions for one conformational state of F_1. Refinement of the 2.8 Å map of rat liver F_1 which was crystallized[24] in the presence of P_i, ATP, and ADP resulting from ATP hydrolysis, should help confirm or negate this prediction. Here "N" stands for ATP or ADP. (C) Summary of subunit movements in ATP synthases for which there is some evidence. As indicated in the figure and the text, there is evidence from a variety of approaches that significant functionally related movements/changes take place in $\alpha\beta$ pairs (particularly β subunits), in the γ subunit, and in the ϵ subunit (*E. coli*). Evidence that the oligomer of subunit c (c_{10-12}) "rotates" as implicated by some authors is derived primarily from genetic engineering effects on function, with no accompanying biophysical measurements. Current views by some of the more speculative contributors to this review series envision the γ subunit, together with the c_{10-12} oligomeric ring, as comprising the major elements of a "rotor," and that subunit b in *E. coli*, and perhaps OSCP and/or b in eukaryotes, may comprise a "stator," a stationary part in a machine in or about which a rotor revolves.[46] Thus, one could argue that the stator is necessary to stabilize the $\alpha_3\beta_3$ core while the γ subunit repositions ("rotates") among the 3 $\alpha\beta$ pairs. Finally, it remains possible also that the central γ subunit, and perhaps other small subunits, undergo up and down movements during catalysis.

act as an ATPase inhibitor, whereas animal δ subunits remain tightly associated with their F_1 units, and a subunit distinct from the five F_1 subunits called IF_1 serves as an ATPase inhibitor.[23] It is assumed also that the *E. coli* δ subunit may correspond, at least in part, to the OSCP subunit of animal ATP synthases, again based on some sequence homology. Whether these two subunits in fact play similar or different roles remains to be established.

In the above discussion no mention was made of either the chloroplast or yeast enzymes, both of which have been extensively studied. Suffice it to say here, that in terms of substructure, the chloroplast enzyme corresponds very closely but not identically to the *E. coli* enzyme, and the yeast enzyme corresponds very closely but not identically to the higher eukaryotic enzymes. The *Rhodospirillum rubrum* enzyme, also a well-studied ATP synthase, corresponds most closely in its substructure to the chloroplast enzyme.

ATP SYNTHASE F_1 UNIT: X-RAY STRUCTURES AND MECHANISTIC IMPLICATIONS

Both the rat liver and bovine heart F_1 units (catalytic moieties) have been studied by X-ray crystallography.[13,14] The rat liver enzyme was crystallized successfully in a buffer containing ATP and P_i[24] conditions which maintain the F_1 unit in an active form. Redissolved crystals, even after a year, exhibit ATPase activity comparable to uncrystallized F_1.[25] The X-ray structure derived at 3.6 Å from diffraction quality crystals shows that the large subunits α and β are arranged in an alternating manner about a 3-fold axis.[13] The small subunits γ, δ, and ε, present in SDS PAGE gels of redissolved crystals, are not observed in the X-ray structure.[13] The γ subunit is interpreted to reside, at least in part, within the center of the molecule, very much in line with the 3-fold axis.[13,26] The δ and ε subunits and a part of the γ subunit may not be ordered and conform to the crystallographic symmetry.[13,26] Chain tracing identified a nucleotide binding fold on α-subunits near α/β interfaces.[13,26] An atomic resolution structure at 2.8 Å has now been obtained and is currently being refined to clearly identify the precise locations of ADP and ATP.

In contrast to rat liver F_1, bovine heart F_1 was crystallized in a buffer containing ADP, AMP-PNP (a nonhydrolyzable ATP analog), Mg^{+2}, and the ATPase inhibitor sodium azide.[27] Thus, the enzyme within the crystals is clearly in an inhibited state. Similar to the rat liver X-ray structure,[13] the structure of bovine heart F_1[14] does not reveal the small subunits δ and ε. It also reveals only part of the γ subunit: However, the high resolution (2.8 Å) of the bovine heart structure, a monumental achievement in X-ray crystallography, has allowed the identification of 2,983 of the 3,444 amino acids including most of the α and β subunits and 127 of the 272 amino acids within the γ subunits. Five nucleotide molecules are bound at centrally located sites on the α and β subunits near opposite interfaces separated by 27 Å. Each α subunit contains an AMP-PNP molecule whereas one β subunit contains an AMP-PNP, a second β contains an ADP, and the third β is open (Fig. 1B). Moreover, each β subunit is in a different conformational state presumably induced by the centrally located γ subunit which contacts each β subunit differently. Primarily because of differences in conformational states of β subunits, the overall structure of bovine heart F_1, crystallized under inhibited conditions deviates, from perfect 3-fold symmetry. Consistent with the "binding change" mechanism,[4,9,28] the structure is viewed as a conformational state in which the empty β subunit has just released bound ATP in response to an electrochemical proton gradient, a second β subunit has just synthesized ATP from bound ADP and P_i, and the third β subunit has bound ADP but awaits the entry of P_i.[14]

It remains possible that the bovine heart F_1 conformation revealed by X-ray crystallography is not on the main path for ATP synthesis. Thus, the conditions[27] used to crystallize the enzyme include not only the ATPase inhibitor azide, but other nonphysiological components, e.g., TrisCl and deuterium oxide, and fail to include one of the critical substrates of oxidative phosphorylation, namely P_i. Also, the crystallization conditions[27] were adjusted so that no more than one ADP molecule could bind to F_1. Therefore, the finding that an "open" β-subunit exists, (Fig. 1B) a key factor in relating the conformation obtained to the "binding change" mechanism, may be of questionable physiological significance. This does not mean that many of the features of the final structure are not relevant to mechanism, but those related to subunit distribution of nucleotides and subunit movements/changes *per se*, e.g., β subunit conformational differences, will remain in question until a structure is obtained where nonphysiological components, in particular azide, are eliminated. It should be noted that azide via a slow transition is known to convert F_1 to an inhibited state, either by forming an azide-F_1-Mg^{++}[29] or an azide-F_1-ADP[30]

complex. Such inhibited states may not be on the main pathway for ATP synthesis. The completion of the rat liver F_1 structure may provide a more realistic view of the physiological subunit distribution of nucleotides as rat liver F_1 was crystallized in the presence of P_i and ATP, some of which is hydrolyzed to give ADP. Therefore, rat liver F_1, while crystallizing "sees" a surrounding environment with the same substrate and product "ingredients" as present in the mitochondrial matrix. The author's predicted view of the physiological distribution of nucleotides on F_1 at one point in the reaction cycle is shown in Fig. 1B.

Concerning the presence of different forms of F_1, several other points are pertinent. First, it is well known that during steady state kinetic measurements in the presence of certain anions like bicarbonate some investigators have clearly demonstrated that F_1 behaves as a Michaelis-Menten enzyme[31,32] in which all catalytic sites may be kinetically equivalent. Bicarbonate, as phosphate, is a normal physiological component of mitochondria. In fact, phosphate and bicarbonate appear to compete for the same locus on F_1.[33] A second important point relates to the most commonly quoted evidence for nonequivalency of catalytic sites during ATP synthase function.[34] These are experiments in which bovine F_1-ATPase activity is compared in a "two point" experiment, i.e., under "unisite conditions" where the ATP/F_1 ratio is 1 or less, and "multisite conditions" where this ratio is very high (i.e., saturating) in which all catalytic sites are believed to be occupied. The multisite/unisite ratio calculated in the original study was $\sim 10^6$ fold, suggestive of significant positive cooperativity among catalytic sites. Unfortunately, the unisite measurement was not based on the "rate" of ATP hydrolysis *per se* under unisite conditions but on the rate of dissociation of ADP remaining bound to ADP after catalysis had taken place.[34] Significantly, if the unisite measurements had been based on the initial ATP hydrolytic rate actually observed in the 2 sec prior incubation period, the multisite to unisite reaction rate ratio would have been $\sim 10^4$, consistent with Michaelis-Menten behavior. Recently, using the bovine heart enzyme, but monitoring the reaction immediately after addition of ATP, experiments conducted in the author's laboratory demonstrate that bovine heart F_1 obeys perfect Michaelis-Menten kinetic behavior from unisite to multisite conditions.[35] A third important point related to kinetic studies with F_1-ATPase preparations concerns product inhibition. If for any reason ADP is trapped within a catalytic site prior to initiating the reaction with ATP, deviations from Michaelis-Menten behavior will be obtained, which almost invariably is interpreted incorrectly as reflecting "cooperativity". Erroneous kinetic interpretations relating to ADP inhibition may arise for any one of the following reasons: (a) prior incubation of the enzyme with ATP before recording what is called the initial rate, (b) recording what is called the "initial rate" too slowly after addition of ATP, or (c) working with an "undefined" F_1 or F_0F_1 (membrane) preparation in which entrapment of inhibitory ADP at a catalytic site has already occurred.

From this author's viewpoint, the mechanism by which ATP is synthesized and hydrolyzed in biological systems needs much further investigation. It remains possible that what some investigators have loosely interpreted as "cooperativity" in kinetic studies may only reflect returning an inhibited intermediate to the main reaction pathway by adding excess ATP, thus reversing/relieving an inhibited state, most likely an ADP inhibited state. As the currently depicted "binding change" mechanism for ATP synthesis[4,9,28] is based primarily on the nonequivalency of catalytic sites, it may need some serious surgery as newer structural and kinetic data is published.

SMALL SUBUNIT MOVEMENTS DURING ATP SYNTHESIS (See Fig. 1C)

All subunits within ATP synthases except the α and β subunits (>50 kDa) are considered to be "small" subunits. Among these, the γ subunit is the largest (30–38 kDa), extending centrally from an interaction with the "c" subunit within the basepiece of the ATP synthase molecule, through the stalk region, and on to the top of the F_1 unit.[14,36] Evidence that the γ subunit undergoes significant movements during ATP synthase function is compelling. Data derived from the following types of experiments support this view: crosslinking studies,[37] nucleotide binding-fluorescence studies,[38,39] and most recently PARP studies (polarized absorption relaxation after photobleaching).[40] X-ray crystallographic studies[14] are consistent with γ subunit movements, perhaps rotation, but do not rule out preferential repositioning or rotation of α and β subunits.

PARP experiments provide some biophysical indication of the extent of γ subunit movement with an angular span near 200° being reported.[40] This falls significantly short of the 360° movement expected for rotation, and still leaves open the possibility that γ

only "moves aside" while αβ pairs reposition. Accepting the possible limitations of the PARP experiments, and assuming that γ does eventually move through a 360° angle, it is important to note that this movement cannot represent an unperturbed physical rotation as in the case of bacterial flagella. Rather γ must bind and be released from each αβ pair before it can move to the next αβ pair. Although in this case, a "net" 360° rotation of γ would be achieved in completing the catalytic cycle involving 3 αβ pairs, γ would be predicted to "flicker" every 120°. That is, γ would move back and forth 3 times between a more centrally located position and each αβ pair during the catalytic cycle.

In addition to γ, there is also compelling evidence for movements associated with the *E. coli* ε subunit during ATP synthase function.[41,42] This movement involves repositioning of a region of the ε subunit between F_1 α and β subunits.[42] Also genetic engineering of the *E. coli* c subunit has been interpreted to indicate that its oligomeric form (c_{10-12}) may undergo rotation during ATP synthesis.[43]

Finally, as the central cavity of the F_1 molecule, together with its occupancy by the γ subunit represent a cylinder/piston like relationship, up and down like movements during catalysis are also possible.

FUTURE DIRECTIONS

Although our knowledge of subunit movements within ATP synthase molecules is lacking in many important details, it seems clear from recent work in a number of different laboratories that such movements involving multiple subunits do occur, and that these are related to function (see Fig. 1C and subsequent articles in this series). One major challenge in ATP synthase research in the future is to define more clearly, both biochemically and biophysically, the functions of the stalk subunits, particularly in the more complex animal systems. As higher eukaryotes are much more subject to signal transduction pathways as a mode of regulation than are bacteria, it is possible that this type of control in animals is directed at the stalk subunits. It is, therefore, of considerable interest that recent studies with neuronal cells, stimulated with platelet-derived growth factor (PDGF), provide evidence for covalent phosphorylation of the F_1-δ subunit.[44] A second major challenge of the future will be to obtain more X-ray crystallographic "snapshots" of F_1, of F_1 together with "stalk" subunits, and of F_0F_1 (the complete ATP synthase) during the catalytic cycle. Finally, a third major challenge will be to develop methodologies that will allow subunit movements to be monitored as a function of time and captured directly on film, as in the case of bacterial flagella.[1,2]

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REFERENCES

1. Berg, H. C. (1975). *Nature* **254**, 389–392.
2. Macnab, R. and Aizawa, S. I. (1994). *Ann. Rev. Biophys. Bioengineering* **13**, 51–83.
3. Capaldi, R. A., Aggeler, R., Turina, P., and Wilkins, S. (1994). *Trends Biochem. Sci.* **12**, 186–189.
4. Boyer, P. D. (1993). *Biochem. Biophys. Acta.* **1140**, 215–250.
5. Pedersen, P. L. and Amzel, L. M. (1993). *J. Biol. Chem.* **268**, 9937–9940.
6. Penefsky, H. S. and Cross, R. L. (1991). *Adv. Enzymol. Relat. Areas Mol. Biol.* **64**, 173–214.
7. Senior, A. E. (1990). *Ann. Rev. Biophys. Biophys Chem.* **19**, 7–41.
8. Fillingame, R. H. (1990). In *The Bacteria*, Academic Press, New York, pp. 345–391.
9. Boyer, P. D. (1989). *FASEB J.* **3**, 2164–2178.
10. Soper, J. W., Decker, G. L., and Pedersen, P. L. (1979). *J. Biol. Chem.* **254**, 11170–11176.
11. Gogol, E. P., Lucken, U., and Capaldi, R. A. (1987). *FEBS Lett.* **219**, 274.
12. McCarty, R. E. and Hammes, G. G. (1987). *Trends Biochem. Sci.* **12**, 234–237.
13. Bianchet, M., Ysern, X., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1991). *J. Biol. Chem.* **266**, 21197–21201.
14. Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). *Nature*, **370**, 621–628.
15. McEnery, M. W., Hullihen, J., and Pedersen, P. L. (1989). *J. Biol. Chem.* **264**, 12029–12036.
16. Walker, J. E., Gay, N. J., Powell, S. J., Kostina, M., and Dyer, M. R. (1987). *Biochemistry* **26**, 8613–8619.
17. Walker, J. E., Runswick, M. J., and Poulter, L. (1987). *J. Mol. Biol.* **197**, 89–100.
18. Yosihare, Y., Nagase, H., Yamani, T., Oka, H., Tani, I., and Higuti, T. (1991). *Biochem.* **30**, 6854–6860.
19. Hekman, C. and Hatefi, Y. (1991). *Arch. Biochem. Biophys.* **284**, 90–97.
20. Belogrudov, G., Tomich, J. M., and Hatefi, Y. (1995). *J. Biol. Chem.* **270**, 2053–2060.
21. Collinson, I. R., van Raaij, M. J. Runswick, M. J., Fearnley, I. M., Skehel, J. M., Orriss, G. L., Miroux, B., and Walker, J. E. (1994). *J. Mol. Biol.* **242**, 408–421.
22. Birkenhäger, R., Hoppert, M., Deckers-Hebestreit, Mayer, F., and Altendorf, K. (1995). *Eur. J. Biochem.* **230**, 58–67.
23. Pullman, M. E. and Monroy, G. C. (1963). *J. Biol. Chem.* **238**, 3762–3769.

24. Amzel, L. M. and Pedersen, P. L. (1978). *J. Biol. Chem.* **253**, 2067–2069.
25. Pedersen, P. L., Hullihen, J. Bianchet, M., Amzel, L. M., and Lebowitz, M. S. (1995). *J. Biol. Chem.* **270**, 1775–1784.
26. Amzel, L. M., Bianchet, M., and Pedersen, P. L. (1992). *J. Bioenerg. Biomemb.* **24**, 429–433.
27. Lutter, R., Abrahams, J. P., van Raaij, M. J., Todd, R. J., Lundquist, T., Buchanan, S. K., Leslie, A. G. W., and Walker, J. E. (1993). *J. Mol. Biol.* **229**, 787–790.
28. Cross, R. L. (1981). *Ann. Rev. Biochem.* **50**, 681–714.
29. Daggett, S. G., Tomaszek, T. A., Jr., and Shuster, S. M. (1985). *Arch. Biochem. Biophys.* **236**, 815–824.
30. Vasilyeva, E. A., Minkov, I. A., Fitin, A. F., and Vinogradov, A. D. (1982). *Biochem. J.* **202**, 15–23.
31. Ebel, R. E., and Lardy, H. A. (1975). *J. Biol Chem.* **250**, 191–196.
32. Pedersen, P. L. (1976). *J. Biol. Chem.* **254**, 934–940.
33. Catterall, W. A. and Pedersen, P. L. (1974). *Biochem. Soc. Spec. Publ.* **4**, 63–88.
34. Cross, R. L., Grubmeyer, C., and Penefsky, H. S. (1982). *J. Biol. Chem.*, 12101–12105.
35. Reynafarje, B. D. and Pedersen, P. L. (1996). *J. Biol. Chem.*, in press.
36. Watts, S. D., Zhang, Y., Fillingame, R. H., and Capaldi, R. A. (1995) *FEBS Lett.* **368**, 235–238.
37. Duncan, T. M., Bulygin, V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995). *Proc. Natl. Acad. Sci. (USA)* **92**, 10964–10968.
38. Turina, P. and Capaldi, R. A. (1994). *Biochem.* **33**, 14275–14280.
39. Turina, P. and Capaldi, R. A. (1994) *J. Biol. Chem.* **269**, 13465–13471.
40. Sabbert, D., Engelbrecht, S., and Junge, W. (1996). *Nature,* 623–625.
41. Gogol, E. P., Johnston, E. Aggeler, R., and Capaldi, R. A. (1990). *Proc. Natl. Acad. Sci. (USA)* **87**, 9585–9589.
42. Capaldi, R. A., Aggeler, R., Wilkens, S., and Grüber, G. (1996). *J. Bioenerg. Biomembr.* **28**, 397–401.
43. Vik, S. B. and Antonio, B. J. (1994). *J. Biol. Chem.* **269**, 30364–30369.
44. Zhang, F. X., Pan, W., and Hutchins, J. B. (1995). *J. Neurochem.* **65**, 2812–2815.
45. Howitt, S. M., Rodgers, A. J. W., Hatch, L. P., Gibson F., and Cox, G. B. (1996). *J. Bioenerg. Biomemb.* **28**, 415–420.
46. *Webster's New Collegiate Dictionary* (1974). G. & C. Merriam Co., Springfield, Ma, p. 1137.