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Notes on the Mechanism of ATP Synthesis

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The most commonly quoted mechanism of the coupling between the electrochemical proton gradient and the formation of ATP from ADP and P_i assumes that all states of the F_1 portion of the ATP synthase have β subunits in "tight," " loose," and "open" conformations. Models based on this assumption are inconsistent with some of the available experimental evidence. A mechanism that includes an additional β subunit conformation, "closed," observed in the rat liver structure overcomes these difficulties.

KEY WORDS: F_1—ATPase; ATP synthesis; conformational changes.

In the last few years great progress has been made toward understanding the mechanism of ATP synthesis/hydrolysis by the F_oF_1 complex of mitochondria, chloroplast, and bacteria. This progress reflects, in part, the wealth of information provided by the determinations of the three-dimensional structure of the F_1 complex (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998) and, in part, the information provided by more traditional biochemical methods and by experiments that permitted a direct visualization of the movements of the γ subunit with respect to the major subunits of F_1 (Noji *et al.*, 1997; Yasuda *et al.*, 1998). In the interpretation of most of these experiments, it is assumed that ATP hydrolysis-driven proton translocation occurs by reversal of the ATP synthesis mechanism.

In the process of assimilating the new information into existing models, some inconsistencies and unlikely steps were incorporated into some of the proposed mechanisms. Since the proposals were made over a period of several years, it is difficult to know which ones are still considered viable and which ones are considered to have been superceded by newer proposals. The two mechanisms quoted by most investigators are those pioneered by the groups of Boyer (1997) and Walker (Abrahams *et al.*, 1994) (BW), and the modification of this mechanism proposed by Cross and co-workers (Duncan *et al.*, 1995) (DBZHC). A significant modification to these mechanisms was recently proposed by Amzel and co-workers (Bianchet *et al.*, 1998) (BHPA). All three mechanisms involve a "binding change": binding and release of reactants and products, as well as energy coupling, involve a concerted change of the catalytic sites of the three β subunits of the F_1 portion of the ATP synthase complex (Boyer, 1981). In addition, in all these mechanisms the energy that results from the dissipation of the electrochemical proton gradient is transmitted to the F_1 portion via movements of the γ subunit that include a 120° rotation (Abrahams *et al.*, 1994; Noji *et al.*, 1997). The major differences between these models reside either in the number of catalytic sites of the three β subunits that are occupied at any one time during catalysis (BW vs. DBZHC), or in the number of F_1 configurations necessary to fully describe the catalytic cycle (BW and DBZHC vs. BHPA), or both (BW vs. BHPA).

BW (Fig. 1) and DBZHC (Fig. 2) differ only in the number of sites in the β subunits that are occupied during catalysis. BW (Fig. 1) has two fundamental problems: (1) In BW, during the catalytic cycle, F_1 alternates between species that have either one or two of the nucleotide sites occupied. This characteristic is at variance with the results of recent experiments (Lobau *et al.*, 1998; Weber *et al.*, 1993; Dou *et al.*, 1998) that indicate that maximum activity is only achieved when *all three sites in β subunits are occupied.* (2) In the direction of ATP hydrolysis, the

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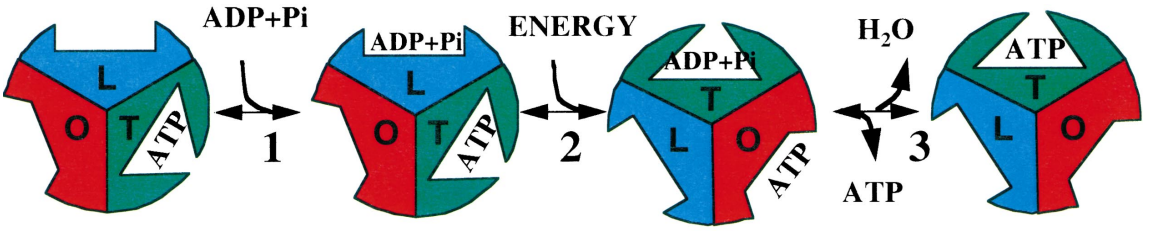

Fig. 1. BW Mechanism (Abrahams *et al.*, 1994; Boyer, 1981). The circles represent different conformations of the F_1 molecule. The three sectors correspond to the three $\alpha\beta$ pairs in one F_1-ATPase (see Fig. 4); green sectors represent the "tight" (T) conformation, blue sectors represent the "loose" (L) conformation, and red sectors represent the "open" (O) conformation.

reversal of step 3 (Fig. 1) involves binding of ATP to the O site while the L site is not occupied. This appears to be a thermodynamically extremely unfavorable step since the affinity of the L site for ATP is several orders of magnitude greater than that of the O site. DBZHC overcomes these difficulties by proposing a minimum modification to BW that contains species with either two or three sites occupied during the catalytic cycle (Duncan *et al.*, 1995). However, some other difficulties of the original BW mechanism are not resolved by this modification.

1. In the DBZHC mechanism (Fig. 2), for the enzyme to cycle while always having one site in the open (O) conformation, ADP and P_i have to bind to this site *and remain bound until the energy-coupled conformational change occurs.* Since the structure of the O site is the one for nucleotide release, *i.e.*, one that does not bind nucleotide, this condition presents contradictory requirements. This problem arises in the DBZHC mechanism because, in order to comply with the data on site occupancy, it invokes species with nucleotides bound to O sites. It appears that, for the reactants to remain bound while waiting for the major conformational change to occur, a local conformation change needs to occur that changes the β subunit in the O conformation to another conformation, C, that can retain the reactants until the proton translocation-coupled global conformational change takes place.
2. There is experimental evidence (reviewed in Boyer, 1997) indicating that in the tight (T) site, ADP and P_i are in equilibrium with ATP with an equilibrium constant $K_{eq} \approx 1$. This means that at any time half of the ATP synthase molecules have bound ATP and half the molecules have ADP + P_i. In the BW and DBZHC mechanisms, there is no provision to prevent that half the time the system will go through a full cycle, but, at the end, release ADP + P_i instead of ATP. For this kind of uncoupling to be prevented, the major energy-driven conformational change should only be allowed when the T site contains ATP. Thus, the T site must be in a slightly different conformation when it contains ATP (T) than when it contains ADP and P_i (T′).
3. One would expect that the mechanism of ATP hydrolysis-driven proton translocation is the exact reversal of the mechanism of ATP synthesis. If one attempts to use the BW or the DBZHC mechanism to run in the opposite direction, the step involving ATP hydrolysis occurs without consequences for proton translocation. Instead, binding of ATP to the O site—the site that is not

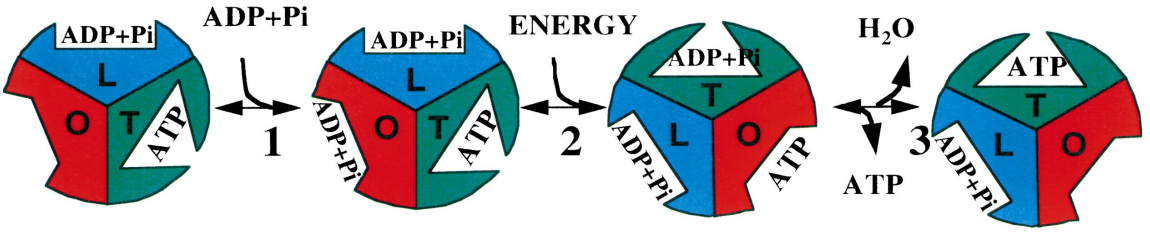

Fig. 2. DBZHC Mechanism (Duncan *et al.*, 1995). All symbols and colors are as in Fig. 1. The main differences with the BW mechanism are in the occupancy of the sites.

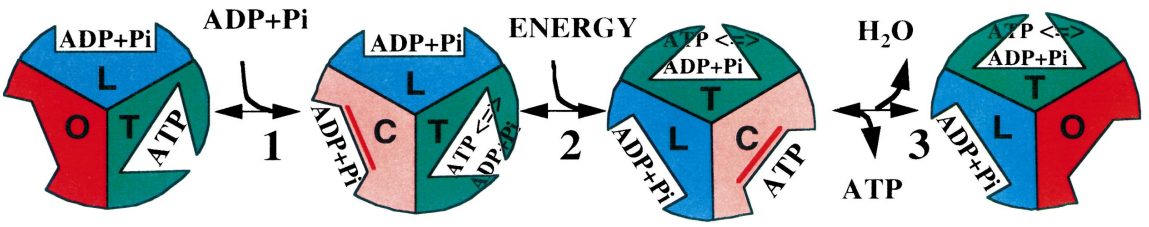

Fig. 3. BHPA Mechanism (Bianchet *et al.*, 1998). All symbols and colors are as in Fig. 1. A new conformation "closed" (C) is included. This conformation, observed in the rat liver structure, is much more similar to T and L than to O. In this mechanism, the major conformational change driven by the proton gradient (ENERGY in the figures) occurs with the $\alpha\beta$ pairs in the T, L, and C conformations. This major conformational change is triggered by a rotation of the subunit (not shown) driven by the proton gradient.

competent for ATP binding—is proposed to drive proton translocation. Furthermore, if the major conformational change can occur in the reverse direction with either ATP or ADP plus P_i bound to the T site, then half the time proton translocation against its concentration gradient will occur with release of ATP, that is, without ATP hydrolysis. Since this is not possible, the change from T to L, as part of the major conformational change when the enzyme is operating in the hydrolytic direction, must occur with ATP hydrolysis.

4. The BW (and the DBZHC) mechanism of ATP synthesis is described as one in which the energy of the electrochemical proton gradient, transmitted through the rotation of the γ subunit, is used for the release and not for the formation of ATP. Analyzing Figs. 1 and 2, it is clear that this statement does not correctly describe the events in the proposed mechanism. The energy of the electrochemical proton gradient drives a conformational change that has many effects. It not only produces a loosening of the tight site with ATP so that release of ATP becomes favorable, but also results in a tightening of the site that contains ADP and P_i so that formation of ATP is now favorable. Therefore, the energy in the gradient is used to change dissociation constant of ATP from the tight site from approximately 10^{-10} to 10^{-4} M (T -> O), and for changing the equilibrium constant for the formation of enzyme bound ATP ($ADP + P_i$ -> ATP) from 10^{-5} to approximately 1(L -> T).

Solutions to all these objections were incorporated into the mechanism proposed by Amzel and co-workers (Bianchet *et al.*, 1998) (BHPA, Fig. 3). Additional experimental information provided by the determination of the structure of the rat liver F_1-ATPase in the presence of physiological concentrations of nucleotides (Fig. 4), was incorporated into this mechanism.

Before the determination of the rat liver structure, Walker, Leslie, and co-workers (Abrahams *et al.*, 1994) had reported the structure of the F_1-ATPase from bovine heart.[4] This determination was carried out in the presence of AMP–PNP and Mg^{2+}, but with limiting amounts of ADP. Under these conditions, one of the β subunits is free of nucleotide (Lobau *et al.*, 1997) and has a conformation that is significantly different from the other two—the open (O) conformation. All mechanisms that were proposed based on this structure used it as the *only configuration of the F_1 present throughout the enzymic cycle*. The structure of the rat liver enzyme demonstrated that, in the presence of physiological concentrations of nucleotide, all three β subunits become occupied and adopt highly similar conformations (Bianchet *et al.*, 1998). Occupancy of all three catalytic sites is in full agreement with the data of Senior and co-workers (Lobau *et al.*, 1998; Weber *et al.*, 1993). As mentioned above, inclusion of the configuration with all catalytic sites occupied results in a mechanism (Fig. 3) that overcomes all the objections described above.

In this mechanism the F_1 molecule oscillates between two configurations that reflect the site occupancy of the β subunits: species with the three catalytic sites occupied adopt the configuration observed in the rat liver structure; species with two sites occupied adopt the configuration observed in the bovine heart structure. The two configurations differ mainly in the conformation of one of the β subunits. The three-nucleotide structure has β subunits in the T, L, and C conformations, all occupied with

[4] A structure of the *E. coli* F_1-ATPase was published recently Hausrath, A. C., Gruber, G., Matthews, B. W., and Capaldi, R. A. (1999). *Proc Natl Acad Sci USA* **96**, 13697–13702. It is a low-resolution determination (4.4 Å) of an inhibited form of the enzyme.

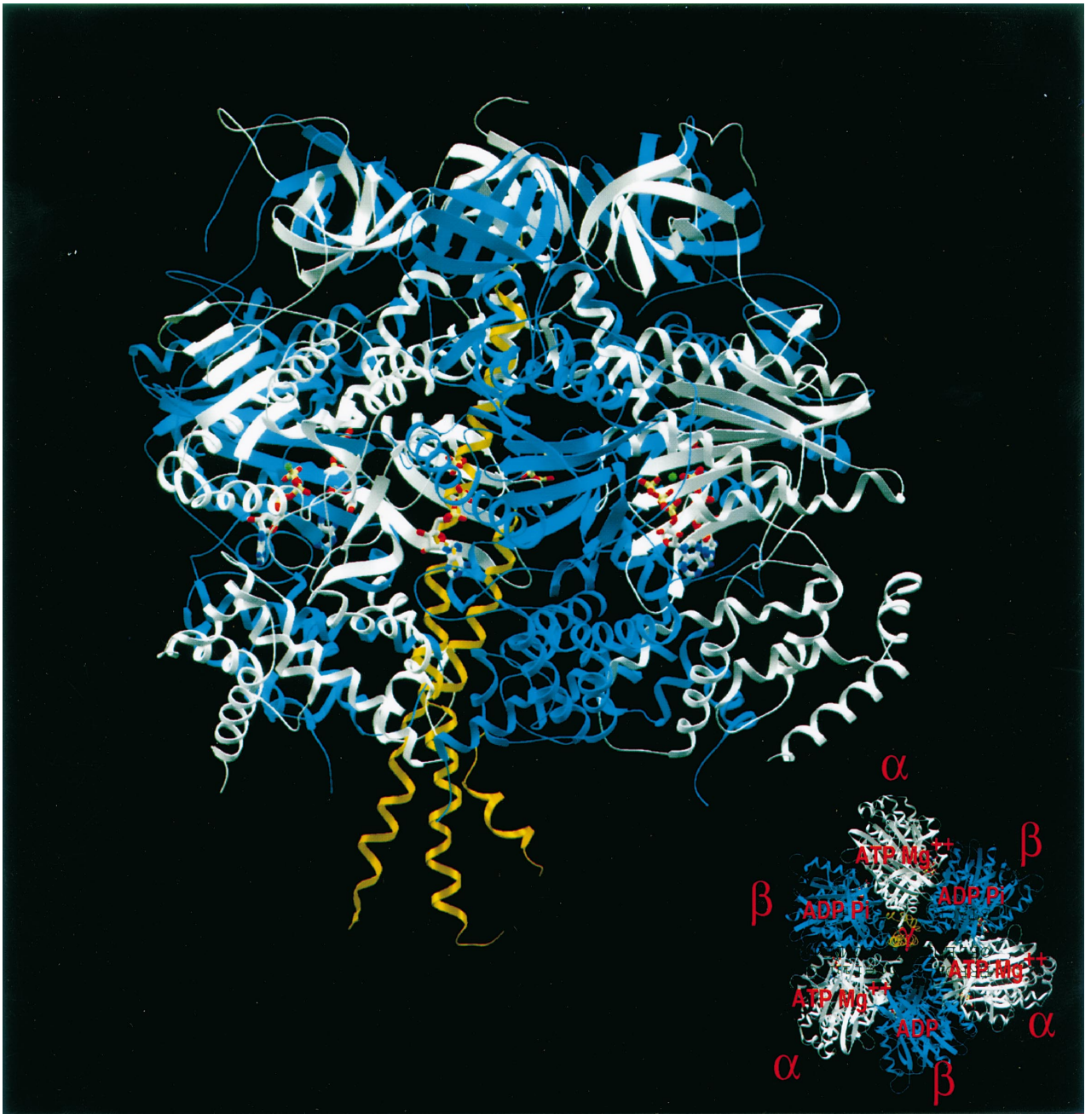

Fig. 4. Overall structure of the rat liver F_1 ATPase. α Subunits are in white, β subunits are in blue, and the γ subunit is in gold. Side view (large) and top view (small) are shown. The nucleotides bound to all β/α and α/β interfaces are shown. This conformation of F_1 has nucleotides in all three β subunits.

nucleotides; the two-nucleotide structure has β subunits in the T and L conformations occupied with nucleotide and one in the O conformation that contains no nucleotide. Nucleotide occupancy is not the only difference between the C and the O conformations. C has a well formed nucleotide-binding site similar to that of L; by contrast, in O, the carboxy-terminal domain has moved away from the nucleotide-binding domain, in effect, disrupting the nucleotide binding site.

The mechanism involves two types of conformational changes: local and global. Local conformational changes involve the changes in a single β subunit from O (open) to C (closed) as a consequence of nucleotide binding. Some other changes probably take place, especially

in the α/β interfaces, but the other two β subunits remain essentially unchanged. The global conformational change consists of a concerted transformation of all three β subunits. It can only occur in the three-nucleotide configuration and only when ATP is bound to the T site. Under these conditions the changes in the β subunits are: T to C, L to T, and C to L.

When all three catalytic sites are occupied they contain ADP (C site), ADP + P_i (L site), and ATP in equilibrium with ADP + P_i (T site). This configuration undergoes the proton-driven conformational change. In the direction of ATP synthesis, this global conformational change has the following characteristics:

1. The energy of the proton gradient is transmitted to the β subunits via a rotation of the γ subunit.
2. The change can only occur with ATP in the T site, not with ADP + P_i in this site.
3. The three β subunits undergo concerted conformational changes: T to C, L to T, and C to L.
4. After the conformational change, the overall configuration of the F_1 molecule is still the three nucleotide structure, but the sites now contain ATP (C site), ADP (L site), and ATP in equilibrium with ADP + P_i (T site).

In this mechanism the O site only occurs in the configurations with two nucleotides bound, after the release of ATP and before binding ADP.

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