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The Coupling of the Relative Movement of the *a* and *c* Subunits of the F_0 to the Conformational Changes in the F_1-ATPase

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F_0F_1-ATPase structural information gained from X-ray crystallography and electron microscopy has activated interest in a rotational mechanism for the F_0F_1-ATPase. Because of the subunit stoichiometry and the involvement of both the *a*- and *c*-subunits in the mechanism of proton movement, it is argued that relative movement must occur between the subunits. Various options for the arrangement and structure of the subunits involved are discussed and a mechanism proposed.

KEY WORDS: F_0F_1-ATPase; rotation; *a*-, *b*-, and, *c*-subunits.

The elucidation of the crystal structure of a major part of mitochondrial F_1-ATPase (Abrahams *et al.*, 1994; Bianchet *et al.*, 1991, 1994; Pedersen *et al.*, 1995) has led to renewed interest in a rotational mechanism for the synthesis of ATP by the F_0F_1-ATPase (Boyer and Kohlbrenner, 1981; Cross *et al.*, 1984; Mitchell, 1985; Cox *et al.*, 1984, 1986; Schneider and Altendorf, 1987; Rottenberg, 1990; Vik and Antonio, 1994; Duncan *et al.*, 1995). Cryoelectron microscopy of the F_1-ATPase of *Escherichia coli* (see Gogol, 1994) has also indicated a movement of the smaller subunits (γ, δ, and ε) with respect to the α and β subunits and also with respect to each other. However, it is in the "engine room" of the F_0 where a requirement for movement became obvious. Critical residues for proton translocation in the *E. coli* F_0F_1-ATPase have been identified on both the *a*-subunit (R210, E219, H245) and *c*-subunit (D61) of the F_0 (see Cox *et al.*, 1992). With a single copy of the *a*-subunit and 9 (or 12) copies of the *c*-subunit plus a requirement for interaction of the *a*-subunit with all of the D61 residues on the *c*-subunits (Hermolin and Fillingame, 1989), relative movement of these two subunits during proton translocation is obviously essential. What is not obvious is the arrangement of the *c*-subunits, the position of the *a*-subunit relative to the *c*-subunits, the location, role, and structure of the *b*-subunit and whether there are any stable interactions between F_1 subunits and F_0 subunits other than those involving *b*-subunits. Definitive solutions to these problems will require structural elucidation by diffractive methods although sufficient information from indirect methods has allowed a number of plausible models to be proposed (Cox *et al.*, 1984, 1986; Vik and Antonio, 1994; Duncan *et al.*, 1995).

THE NATURE OF THE *c*-SUBUNIT OLIGOMERS AND THE RELATIVE POSITION OF THE *a*-SUBUNIT

The structure of the *E. coli c*-subunit monomer in organic solvents has been examined by NMR (Girvin *et al.*, 1989, 1994; Norwood *et al.*, 1992) and the pre-

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dicted largely α-helical hairpin structure has been confirmed. This structure is consistent with mutational analysis and in particular those double mutants in which the key residues D61 and P64 have been transferred to positions 24 and 21 respectively with retention of activity (Miller *et al.*, 1990; Fimmel *et al.*, 1983). However, Deckers-Hebestreit *et al.* (1986) prepared antibodies against undenatured subunit *c* that interacted with the subunit *c* incorporated into liposomes but not with subunit *c* prepared by chloroform/methanol extraction and incorporated into liposomes. This antibody may be detecting differences in the bend region of the *c*-subunit hairpin that result from organic solvent extraction. Since all of the D61 residues of the oligomeric *c*-subunits are required to interact with the *a*-subunit the most likely arrangement of the *c*-subunits is in the form of a ring. Furthermore the efficient assembly of an oligomeric complex would require a single preferred interacting face between subunits. The CF_0 III (equivalent to *E. coli* *c*-subunit) oligomer has been isolated from chloroplasts and the dimensions of this structure are consistent with a ring of subunits with a diameter of about 60 Å (Fromme *et al.*, 1987). Such a ring structure in *E. coli* with equivalent access to all of the aspartate residues at position 61 and also position 24 since aspartate in this position is functional, would necessarily have a large central hole. A similar ring formation has been demonstrated for the oligomeric light harvesting complex (McDermott *et al.*, 1995).

The *a*-subunit is larger than the *c*-subunit and its folded structure remains controversial with models ranging from five to eight transmembrane helices (Cox *et al.*, 1986; Lewis *et al.*, 1990; Bjørbæk *et al.*, 1990; Vik and Dao, 1992). Our bias is of course towards a five transmembrane helix model (Hatch *et al.*, 1995) which has been developed as a result of extensive mutational analysis, particularly double mutants (see Fig. 1) and which conforms with structural principles derived from the crystal structure of the photosynthetic reaction center (Deisenhofer *et al.*, 1985) and cytochrome oxidase (Iwata *et al.*, 1995) and the two-dimensional crystal structure of bacteriorhodopsin (Henderson *et al.*, 1990). One of the important double mutants affecting the *a*-subunit involved the transfer of the key residue R210 to position 252 with retention of partial activity (Hatch *et al.*, 1995). The ability to transfer key residues in both the *a*-subunit and *c*-subunits (see above) to adjacent helices, but in the same plane of the membrane, and retain activity, is possible only because these two

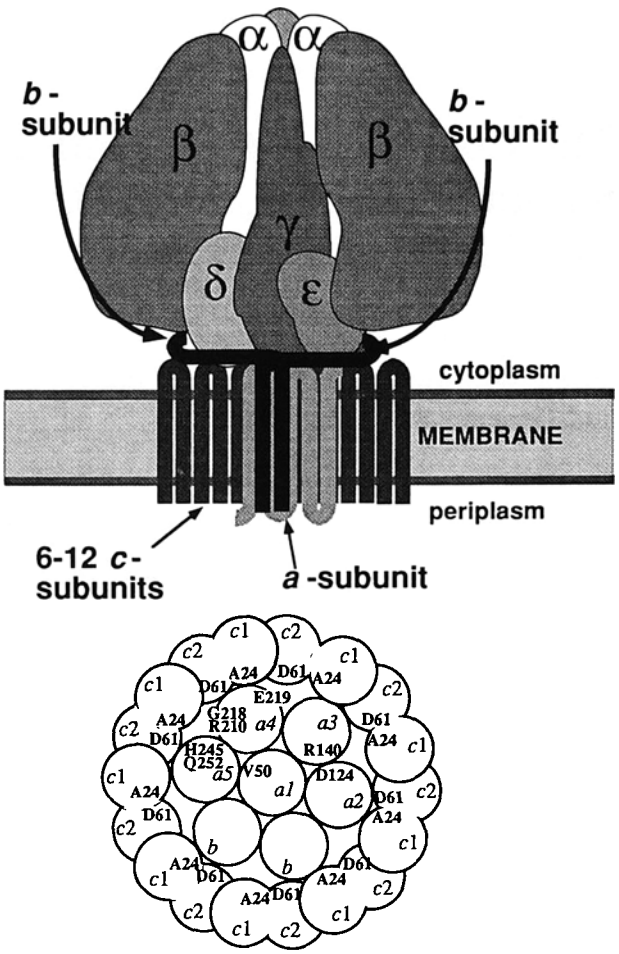

Fig. 1. A model of the subunit arrangement in *E. coli* F_0F_1-ATPase including a cross section of the F_0 in the plane of the membrane. Interacting amino acid residues (summarized in Hatch *et al.*, 1995) are shown.

subunits move rotationally relative to each other on an axis vertical to the plane of the membrane. The five transmembrane helix *a*-subunit would also readily fit inside a ring of 9 (or 12) *c*-subunits (Fig. 1). Models proposed by Schneider and Altendorf (1987), Vik and Antonio (1994), and Duncan *et al.* (1995) have placed the *a*-subunit on the outside of a ring of *c*-subunits and, while this may be correct, we favor an internal location of the *a*-subunit. Steffens *et al.* (1988) labelled *c*-subunits incorporated into liposomes with the lipophilic photo-activatable compound 3-(trifluoromethyl)-3-(*m*-iodophenyl diazirine) with the most significant labelling occurring on methionine residues 11, 16, 17, 57, and 65. If an *ac* complex was incorporated into liposomes, then residues 16 and 17 were no longer labelled and the labelling of residue 57 was much reduced. Because there is only one copy

of the *a*-subunit and 9 (or 12) copies of the *c*-subunit, an external location of the *a*-subunit would be unable to block the labelling of all the *c*-subunits simultaneously.

If the *a*-subunit is inside the ring of subunits then the D61 residues of the ring of *c*-subunits must be accessible from inside the ring. The D61 residue reacts covalently with the lipophilic inhibitor N,N′-dicyclohexylcarbodiimide (DCCD) which presumably gains access from the lipid bilayer. However, the reactivity of DCCD with D61 in normal ATPase is restricted since, in a P64L mutant, the reactivity of D61 with DCCD increases 6–7-fold (see Cox *et al.*, 1992). The *c*-subunit must therefore be arranged such that D61 has only limited access to the membrane bilayer. As referred to above, the CF_0III oligomeric complex has a diameter of about 60 Å. The F_0 portion of the F_0F_1-ATPase, when examined by cryoelectron microscopy, has also been estimated to have a diameter of about 60 Å (Gogol, 1994). However, a wide range of diameters has been observed for the F_0 (for an excellent discussion concerning the variation obtained, see Gogol, 1994).

Apart from experimental data, the location of the *a*-subunit outside the ring of *c*-subunits would present a design problem in that the two subunits required to move relative to one other are both exposed to the bilayer and as they are required to rotate at about 100 times/sec represent a distinct hazard to adjacent F_1F_0 assemblies or any other proteins embedded in the bilayer.

THE LOCATION, ROLE, AND STRUCTURE OF THE *b*-SUBUNIT

The crystal structure of the F_1-ATPase (Abrahams *et al.*, 1994) clearly indicated that the central hole formed by the ring of α and β subunits is filled by two helices from the γ-subunit in a coiled-coil conformation. If the *E. coli* structure is similar, then there is no room for the *b*-subunits to extend through the middle of the F_1 as previously proposed (Cox *et al.*, 1984, 1986). A more recent model (Duncan *et al.*, 1995) has the *b*-subunits functioning as a "stator" forming a rigid connection between the αβ subunits and the *a*-subunit outside the ring of *c*-subunits.

Kumamoto and Simoni (1986, 1987) have described mutations in the *a* and *c* subunits of *E. coli* which suppress the deleterious effect of a G9D mutation in the *b*-subunit. The *a*-subunit suppressor mutations replace proline at position 240 with either alanine or leucine. The *c*-subunit suppressor mutation replaced alanine at position 62 with serine. The *b*-subunit mutation and the *a* and *c*-subunit suppressors all affect amino acid residues in the phospholipid bilayer. The *b*-subunit would therefore be expected to be in close contact in the bilayer with both the *a*- and *c*-subunits. This close proximity is supported by the cross-linking studies of Aris and Simoni (1983) in which the following cross-linked products were observed: c_2, b_2, *c–a*, *a–b*, *b–c*, and $a–b_2$. Since we favor the location of the *a*-subunit inside the ring of *c*-subunits (see above), then we also favor the location of the *b*-subunit inside the ring of *c*-subunits. Friedl *et al.* (1984) also demonstrated effects of mutations in the *a*- and *c*-subunits of *E. coli* on the extractability from the membrane of the *b*-subunit by a combination of the detergents cholate and desoxycholate. In the wild type, subunit *b* was very tightly bound to the membranes in the presence of the detergents. Mutations in subunit *a* or subunit *c* (D61G or D61N) greatly increased the amount of extractable subunit *b*.

In the model proposed by Duncan *et al.* (1995) the *b*-subunit extramembranous domain extends from the membrane and interacts with the external surface of the α and/or β subunits. Hermolin *et al.* (1983) investigated the effect of trypsin on the *b*-subunit in native membranes and in membranes that had been treated to remove the F_1-ATPase. The *b*-subunit was rapidly degraded in the stripped membranes but was protected from degradation when F_1 was bound to the membrane. An additional problem with the *b*-subunit functioning as a "stator" by interacting with the α and β subunits might be the introduction of asymmetry in the α and β subunits since there are two *b*-subunits and three α and β subunits. In the binding change model proposed by Cross (1981) each of the three active sites proceeded through an identical sequence of conformational changes. This requirement may be difficult to achieve if the *b*-subunits form a tight interaction with one or two of the αβ subunits.

Dunn (1992) expressed the extramembranous domain of the *b*-subunit in *E. coli* and demonstrated that the truncated *b*-subunit formed a highly elongated dimer with a high α-helical content. The soluble dimer bound to the F_1-ATPase and inhibited the binding of the F_1 to F_1-stripped membranes. The F_1-ATPase/*b* dimer complex was examined by cryoelectron microscopy (Wilkens *et al.*, 1994). The *b* dimer appeared not to be located in the central region, partly occupied by the N-terminal domain of the γ-subunit, but interacted

on the periphery where the α, β, δ, and ε subunits and the *C*-terminal domain of the γ-subunit are located, although the data was not convincing.

Since we favor the location of the N-terminal region of the *b*-subunit to be within the ring of *c*-subunits and therefore part of the rotor, we suggest that the extramembranous domain might be interacting with the γ, δ, and ε subunits. Howitt *et al.* (1996) have shown that a single mutation A128D prevents dimerization of the soluble extramembranous *b*-subunit domain. Such a result suggests that the wild type dimer is not formed by interactions along the long arms of the *b*-subunit. Preliminary sedimentation velocity experiments (A. J. W. Rodgers and P. D. Jeffrey, unpublished observation) comparing the wild type dimer with the mutant monomer suggest that the dimer is formed by end-to-end interaction of the monomers. With the N-terminal regions of the *b*-subunits associated with the *a*-subunit the extramembranous regions of the pair of *b*-subunits may extend away from each other essentially parallel to the surface of the membrane and forming a divalent-cation-bridged interaction with the γ, δ, and ε subunits (see Fig. 1).

THE NATURE OF THE "STATOR"

In a model proposed previously (Cox *et al.*, 1986) the "stator" was postulated to comprise the α-, β-, and *c*-subunits. This was, and is, an attractive proposition because these three subunits may conform to a three-fold symmetry whereas the remaining subunits which do not conform to a three-fold symmetry form the "rotor." This arrangement would overcome the dilemma of a functionally symmetrical but structurally asymmetrical enzyme complex. There is, however, little evidence from experiments in *E. coli* that directly support the proposed "stator" structure. In spinach chloroplast ATP synthase there is evidence to suggest that subunit III (equivalent to the *E. coli c*-subunit) may be involved in the binding of CF_1 to CF_0 (Feng and McCarty 1990a; Wetzel and McCarty 1993a, b). CF_0F_1 could be purified and depleted of subunits IV (equivalent to the *E. coli a*-subunit), I and II (homologs of the *E. coli b*-subunit), leaving a complex consisting of subunit III and CF_1 (Feng and McCarty, 1990a; Wetzel and McCarty, 1993a). The subunit III-CF_1 complex could be incorporated into liposomes and its properties were more similar to those of CF_0F_1 than those of CF_1. CF_1 could be removed from the CF_0F_1 complex by treatment with EDTA and the liposomes containing subunit III were competent to bind CF_1, suggesting a direct interaction between subunit III and CF_1. CF_1 from which ε had been removed was also able to bind to subunit III, although cross-linking studies indicate that the ε subunit is close to subunit III (Süss, 1986).

The δ and ε subunits are not required for the binding of CF_1 to CF_0 (Andreo *et al.*, 1982; Patrie and McCarty, 1984; Xiao and McCarty, 1989; Feng and McCarty, 1990b). It has also been shown that the β- and γ-subunits can be selectively removed from *Rhodospirillum rubrum* chromatophores and functional reconstitution can be achieved with purified β and γ (Khananshvili and Gromet-Elhanan, 1982). The α-subunit is therefore a prime candidate for binding to F_0. Support for this idea comes from proteolysis studies showing that the α-subunit is susceptible to proteolysis in the soluble CF_1 but is protected when bound to thylakoids (Moroney and McCarty, 1982a, b). However, the α subunit was not protected in the CF_1–subunit III complex (Wetzel and McCarty, 1993b), indicating that other CF_0 subunits are required for protection. From these studies it was suggested that the binding of CF_0III to CF_1 influenced the tightness of the binding of the ε subunit and therefore the ATPase activity. It was not determined if the interaction between CF_0III and the ε subunit was direct or indirect.

Recent experiments with the *E. coli* F_0F_1-ATPase (Zhang *et al.*, 1994; Zhang and Fillingame, 1995) indicate that the ε- and *c*-subunits are in close proximity. A mutation in which Q42 of the *c*-subunit was replaced by glutamate resulted in an uncoupling of proton transport and ATP hydrolysis/synthesis. This could be overcome by the mutations E31G, E31V, and E31K in the ε subunit (Zhang *et al.*, 1994). The proximity of the ε- and *c*-subunits was confirmed by the finding that disulfide bridges were formed between cysteine residues introduced into the polar loop of the *c*-subunit and position 31 of the ε subunit (Zhang and Fillingame, 1995). Complexes in which disulfide bridges formed showed an inhibition of enzyme function. Since movements of the ε subunit may be involved in energy coupling (Capaldi, 1994), it has been suggested that an interaction between the *c*- and ε-subunits is involved in the transmission of the conformational change between the F_0 and the F_1 (Zhang and Fillingame, 1994). However, the inhibition seen in cross-linked F_0F_1-ATPase complexes suggests that movement of the ε subunit with respect to the *c*-subunit may be required for function.

CONCLUDING REMARKS

While it is clear that the *a*- and *c*-subunits move with respect to each other, the mechanism by which this movement causes the conformational change in the F_1-ATPase remains unknown. The mechanism by which the proton movement through the F_0 drives the relative movement of the *a*- and *c*-subunits is also unknown and will only be understood when the structure of the *a*-subunit, the *c*-subunit oligomer, and their relative locations are known. In the model proposed here we suggest that the key interaction is a salt-bridge formed between the R210 of the *a*-subunit and a D61 residue of the *c*-subunit oligomer. This salt-bridge is broken by protons arriving via the H245 (and maybe E219) of the *a*-subunit and forcing a separation of R210 and D61. The proton would be released from R210 (probably by interaction with H_2O) on the F_1 side of the membrane and the R210 would then reform a salt-bridge with the D61 on the next *c*-subunit in the ring. The driving force for the reverse reaction would be the large conformational changes in the F_1-ATPase driving the asymmetric rotor in the opposite direction. The complete understanding of this fascinating enzyme will require elucidation of the crystal structure of the entire F_0F_1-ATPase complex.

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