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ATP Synthase - The Structure of the Stator Stalk

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Abstract

ATP synthase synthesizes ATP from ADP and inorganic phosphate by a unique rotary mechanism where two subcomplexes move relative to each other, powered by a proton or sodium gradient. The non-rotating parts of the machinery are held together by the “stator stalk”. Significant progress towards a structural model for the holoenzyme was made recently, when the structure of a major portion of the stator stalk of mitochondrial ATP synthase was resolved.

ATP synthase - an overview

The final step in oxidative phosphorylation and photophosphorylation, the synthesis of ATP from ADP and inorganic phosphate, is catalyzed by F_1F_0 -ATP synthase. The driving force for ATP synthesis is a transmembrane proton (or, in some organisms, sodium ion) gradient. Coupling between proton flow and ATP synthesis is achieved by a unique rotary mechanism (for recent reviews, see [1-3]; for a historical perspective, see [4]). ATP synthase consists of two connected rotary motors. Flow of protons, down the gradient, through the F_0 motor drives rotation of the ring of c subunits of the rotor (Fig. 1). The c ring connects to subunit γ . Rotation of γ causes conformational changes in the catalytic nucleotide binding sites on the three β subunits of the F_1 motor, resulting in synthesis and release of ATP. However, this can only happen if the non-rotating, static portions of both motors are affixed to each other, to prevent co-rotation of the $\alpha_3\beta_3$ ring with the γ subunit. This attachment is achieved via the “stator stalk” (Fig. 1). Despite the progress in the structural analysis of other parts of ATP synthase, a high-resolution structural model of the stator stalk has been lacking so far, with available data limited to a few fragments of the stalk [5-9]. Now the situation has markedly improved with the publication of the x-ray structure of a major portion of the stator stalk of mitochondrial ATP synthase by Kane Dickson *et al.* [10].

The structure of the stator stalk of mitochondrial ATP synthase

In the mitochondrial enzyme the stator stalk consists of one copy each of subunits b, d, F_6 and OSCP (the “oligomycin-sensitivity conferring protein”, homologous to bacterial subunit δ in Fig. 1). The crystallized subcomplex of the stator stalk [10] contains about half of subunit b, three quarters of subunit d and all of subunit F_6 (Figure 2). What immediately catches the eye is the curved shape of the stator stalk, with the long (105 residues), continuous helix formed by the portion of b contained in the crystallized subcomplex providing the “backbone” of the structure. The portion of subunit d included in the subcomplex contains 5 helices; the three longer helices run alongside the middle region of the subunit b helix, either parallel or antiparallel to it. As with the isolated subunit [5], F_6 contains two helices but they are arranged in a more open way in the subcomplex. These two helices interact with the C-terminal quarter

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of the b helix, and are followed by an extended region that stretches near to the middle of b (Fig. 2).

Kane Dickson *et al.* [10] demonstrate that, together with the structures of the F_1c_{10} complex [11] and of the N-terminal domain of OSCP [6], the structure of the b/d/ F_6 subcomplex fits very well into a 32 Å resolution structure of mitochondrial ATP synthase obtained by cryo-electron microscopy [12]. The N-terminus of the portion of the b subunit included in the crystallized b/d/ F_6 subcomplex is located very close to the transmembrane region of the enzyme. The curvature of the stator stalk follows the outer surface of the $\alpha_3\beta_3$ ring, and the end with the C-terminus of the long b helix and the N-terminus of F_6 comes close to the “top” of F_1 , where OSCP (or, in the bacterial or chloroplast enzyme, δ) is located, bound to the N-terminus of an α subunit [6,13]. Comparison of the x-ray structure of the stator stalk with the cryo-electron microscopy structure even suggests where the “missing pieces” (C-terminal helix of b, C-terminus of d, C-terminal domain of OSCP) might be localized [10].

The stator stalk in ATP synthase from bacteria and chloroplasts

Whereas most essential functional elements of ATP synthase are remarkably conserved during evolution, the architecture of the mitochondrial stator stalk differs significantly from that of bacterial or chloroplast ATP synthase. Instead of a single long b helix that is surrounded by shorter helical and extended portions of d and F_6 , the bacterial and chloroplast enzymes have two b-type subunits, but do not contain homologues of mitochondrial subunits d and F_6 . Most bacteria carry two identical copies of subunit b. In photosynthetic bacteria and in chloroplasts, the two b subunits are replaced by non-identical, but (rather weakly) homologous subunits b and b'. According to secondary structure predictions, in all b-type subunits an N-terminal transmembrane helix (two helices in the case of the mitochondrial enzyme) is followed by a long, probably continuous, helix of 100-110 residues (corresponding to the b helix contained in the structure of the mitochondrial b/d/ F_6 subcomplex [10]). For the *Escherichia coli* enzyme, it has been shown that the central portion of these two long b helices serves to provide the binding energy for dimerization of b (for a recent review, see [14]). In b, but not in b' subunits, there is, after a short break, another predicted helix of 15-20 residues. It had been proposed that this C-terminal helix is bent back in hairpin-like fashion [14,15]; the new stator stalk structure could accommodate such an arrangement [10].

The function of the stator stalk

Despite their structural differences, the mitochondrial and the bacterial/chloroplast stator stalk have to perform the same function. In addition to attaching subunit a to the $\alpha_3\beta_3$ ring, another possible function is to provide the elasticity necessary to efficiently couple two motors with different stepping angles [16]. The F_o motor has a stepping angle of $360^\circ/n$ (where n is the number of c subunits which can differ from 10 to 15 depending on the organism), associated with the translocation of a proton. The F_1 motor has a stepping angle of 120° , associated with the synthesis (or hydrolysis) of one molecule of ATP. The 120° step is divided into two substeps of 80° and 40° [17,18]; if the elastic strain can be relieved between the substeps, this will reduce the degree of elasticity required, but not eliminate the need for it. Elasticity can be provided by the rotor, the stator, or both. The new structure of the b/d/ F_6 subcomplex and comparison with its bacterial counterpart indicates how the stator stalk might perform this function.

For the *E. coli* enzyme it was shown that the b subunits could be shortened [19], lengthened [20], or even that b subunits of different length could be incorporated [21], with preservation of enzyme function. The conclusion that the stator stalk “seems much more likely to be a flexible rope” [20] is obviously not supported by the new structure of the mitochondrial b/d/ F_6 subcomplex [10]. On the other hand, the location of the deletions and insertions in the *E. coli* enzyme, i.e. in the portion of the stator stalk between the membrane part and the $\alpha_3\beta_3$ ring

[19–21], corresponds approximately to the region where the mitochondrial stator stalk is reduced to a single helix [10]. Thus, this portion of the stator stalk might have some flexibility, allowing bending motions which could serve as elastic element in the coupling machinery. In the case of the *E. coli* enzyme, a bend was detected at the C-terminus of the transmembrane helix [8], which could facilitate variations of the angle between the transmembrane helix and the long, cytoplasmic helix [14].

Another architectural motif that both types of stator stalk appear to have in common, despite their differences in overall structure, was recently pointed out by Del Rizzo *et al.* [22]. In *E. coli*, the two b subunits were found to form a right-handed coiled coil with an offset between the helices. In the mitochondrial stator stalk, a number of interactions between subunit b and helical or extended portions of subunits d and F₆ also seem to have a right-handed twist. These right-handed structural elements are tightened upon rotation, which might enable them to contribute to the storage of elastic energy [22].

Outlook

Beyond investigating the possible role of the stator stalk as elastic element in rotational catalysis, the new structure [10] opens a lot of opportunities for future experimentation. One important aim would be a better definition of the interaction between the stator stalk and the $\alpha_3\beta_3$ ring, including the localization of the stator stalk and the affinity of the interaction. Of course, at the top of the wish list of everybody working in the field remains a high-resolution structure of the entire ATP synthase.

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References

1. Wilkens S. Rotary molecular motors. *Adv. Protein Chem* 2005;71:345–382. [PubMed: 16230116]
2. Weber J, Senior AE. ATP synthesis driven by proton transport in F₁F₀-ATP synthase. *FEBS Lett* 2003;545:61–70. [PubMed: 12788493]
3. Capaldi RA, Aggeler R. Mechanism of the F₁F₀-type ATP synthase, a biological rotary motor. *Trends Biochem. Sci* 2002;27:154–160. [PubMed: 11893513]
4. Boyer PD. A research journey with ATP synthase. *J. Biol. Chem* 2002;277:39045–39061. [PubMed: 12181328]
5. Carbajo RJ, et al. Solution structure of subunit F₆ from the peripheral stalk region of ATP synthase from bovine heart mitochondria. *J. Mol. Biol* 2004;342:593–603. [PubMed: 15327958]
6. Carbajo RJ, et al. Structure of the F₁-binding domain of the stator of bovine F₁F₀-ATPase and how it binds an α subunit. *J. Mol. Biol* 2005;351:824–838. [PubMed: 16045926]
7. Wilkens S, et al. Solution structure of the N-terminal domain of the δ subunit of the *E. coli* ATP synthase. *Nat. Struct. Biol* 1997;4:198–201. [PubMed: 9164460]
8. Dmitriev O, et al. Structure of the membrane domain of subunit b of the *Escherichia coli* F₀F₁ ATP synthase. *J. Biol. Chem* 1999;274:15598–15604. [PubMed: 10336456]
9. Del Rizzo PA, et al. The “second stalk” of *Escherichia coli* ATP synthase: structure of the isolated dimerization domain. *Biochemistry* 2002;41:6875–6884. [PubMed: 12022893]
10. Kane Dickson V, et al. On the structure of the stator of mitochondrial ATP synthase. *EMBO J* 2006;25:2911–2918. [PubMed: 16791136]
11. Stock D, et al. Molecular architecture of the rotary motor in ATP synthase. *Science* 1999;286:1700–1705. [PubMed: 10576729]
12. Rubinstein JL, et al. Structure of the mitochondrial ATP synthase by electron cryomicroscopy. *EMBO J* 2003;22:6182–6192. [PubMed: 14633978]

13. Wilkens S, et al. Structural characterization of the interaction of the δ and α subunits of the *Escherichia coli* F_1F_0 -ATP synthase by NMR spectroscopy. *Biochemistry* 2005;44:11786–11794. [PubMed: 16128580]
14. Weber J. ATP synthase: subunit-subunit interactions in the stator stalk. *Biochim. Biophys. Acta* 2006;1757:1162–1170. [PubMed: 16730323]
15. Revington M, et al. Folding and stability of the b subunit of the F_1F_0 ATP synthase. *Protein Sci* 2002;11:1227–1238. [PubMed: 11967379]
16. Junge W, et al. Inter-subunit rotation and elastic power transmission in F_0F_1 -ATPase. *FEBS Lett* 2001;504:152–160. [PubMed: 11532447]
17. Yasuda R, et al. Resolution of distinct rotational substeps by submillisecond kinetic analysis of F_1 -ATPase. *Nature* 2001;410:898–904. [PubMed: 11309608]
18. Shimabukuro K, et al. Catalysis and rotation of F_1 motor: Cleavage of ATP at the catalytic site occurs in 1 ms before 40° substep rotation. *Proc. Natl. Acad. Sci. U. S. A* 2003;100:14731–14736. [PubMed: 14657340]
19. Sorgen PL, et al. Deletions in the second stalk of F_1F_0 -ATP synthase in *Escherichia coli*. *J. Biol. Chem* 1998;273:27873–27878. [PubMed: 9774398]
20. Sorgen PL, et al. Lengthening the second stalk of F_1F_0 ATP synthase in *Escherichia coli*. *J. Biol. Chem* 1999;274:36261–36266. [PubMed: 10593914]
21. Grabar TB, Cain BD. Integration of b subunits of unequal lengths into F_1F_0 -ATP synthase. *J. Biol. Chem* 2003;278:34751–34756. [PubMed: 12842903]
22. Del Rizzo PA, et al. ATP Synthase b subunit dimerization domain: A right-handed coiled coil with offset helices. *J. Biol. Mol* 2006;364:735–746.
23. Gibbons C, et al. The structure of the central stalk in bovine F_1 -ATPase at 2.4 Å resolution. *Nat. Struct. Biol* 2000;7:1055–1061. [PubMed: 11062563]
24. Rastogi VK, Girvin ME. Structural changes linked to proton translocation by subunit c of the ATP synthase. *Nature* 1999;402:263–268. [PubMed: 10580496]

1.

The rotary mechanism of ATP synthase. A model of the *E. coli* enzyme is shown (taken from Ref. [14], with permission). The portions of the enzyme for which a high-resolution structural model was available are shown in surface representation. The individual structural models ($\alpha_3\beta_3\gamma\epsilon$ [23], N-terminal domain of δ bound to N-terminus of α [13], $a c_n$ [24], membrane portion of b [8], “dimerization domain” of b [9]) were docked by eye. Structural data for parts of a and b as well as the C-terminal domain of δ were lacking. ATP synthase can be separated into a membrane-standing subcomplex, F_0 , which, in *E. coli*, consists of subunits $a b_2 c_n$ (brown, pink, and alternating cyan/light green, respectively) and a soluble subcomplex, F_1 , consisting of subunits $\alpha_3\beta_3\gamma\delta\epsilon$ (red, yellow, blue, orange, and green, respectively). A newer, more mechanically-based division differentiates between the “rotor” (in *E. coli*, subunits $\gamma\epsilon c_n$) and the “stator” (subunits $\alpha_3\beta_3\delta a b_2$). The dashed blue line indicates the part of γ hidden from view in the center of the $\alpha_3\beta_3$ ring. Flow of protons, down a gradient, through channels in the a subunit at the interface to the c_n ring drives the rotor, whose movement results in synthesis of ATP from ADP and P_i in the catalytic nucleotide binding sites on the β subunits and release of product ATP from these sites. The enzyme can also run in reverse, hydrolyzing ATP to generate a proton gradient.

In the mitochondrial enzyme, the b dimer is replaced by a single b subunit plus subunits d and F_6 . The $b/d/F_6$ subcomplex whose structure was resolved by Kane Dickson *et al.* [10]

corresponds to the hatched area. The mitochondrial counterparts of *E. coli* subunits δ and ϵ are termed OSCP (“oligomycin-sensitivity conferring protein”) and δ , respectively. Figures 1 and 2A were created with PyMOL (pymol.sourceforge.net).

2.

Structure and localization of the stator stalk of mitochondrial ATP synthase. (A) Structure of the b/d/F₆ subcomplex [10]. Subunit b is depicted in red, subunit d in green, subunit F₆ in blue. For each subunit, the N-terminus of the portion included in the crystallized subcomplex is marked. Black arrows indicate where some of the subunit fragments not included in the crystallized subcomplex might be accommodated. b_C, C-terminus of subunit b; d_C, C-terminus of subunit d; OSCP_C, C-terminus of subunit OSCP (OSCP corresponds to the bacterial subunit δ in Fig. 1). (B) Localization of the stator stalk. The b/d/F₆ subcomplex [10] (red/green/blue) was docked by eye to the F₁C₁₀ subcomplex [11] (grey), using RasMol (www.bernstein-plus-sons.com), and both were modeled into a model of mitochondrial ATP synthase derived by cryo-electron microscopy (orange; after [12], with permission). The approximate location of subunit a, the N-terminus of b ("b_N"), and the N-terminus of OSCP ("OSCP_N") is indicated. Mitochondrial ATP synthase contains a number of supernumerary proteins in the transmembrane region (marked with an asterisk) that are absent in the bacterial and chloroplast enzymes.