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Review

Half channels mediating H^+ transport and the mechanism of gating in the F_o sector of *Escherichia coli* F_1F_o ATP synthase ☆

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ABSTRACT

H^+-transporting F_1F_o ATP synthase catalyzes the synthesis of ATP via coupled rotary motors within F_o and F_1. H^+ transport at the subunit *a*–*c* interface in trans-membranous F_o drives rotation of the *c*-ring within the membrane, with subunit *c* being bound in a complex with the γ and ε subunits extending from the membrane. Finally, the rotation of subunit γ within the $\alpha_3\beta_3$ sector of F_1 mechanically drives ATP synthesis within the catalytic sites. In this review, we propose and provide evidence supporting the route of proton transfer via half channels from one side of the membrane to the other, and the mechanism of gating H^+ binding to and release from Asp61 of subunit *c*, via conformational movements of Arg210 in subunit *a*. We propose that protons are gated from the inside of a four-helix bundle at the periplasmic side of subunit *a* to drive protonation of *c*Asp61, and that this gating movement is facilitated by the swiveling of trans-membrane helices (TMHs) 4 and 5 at the site of interaction with *c*Asp61 on the periphery of the *c*-ring. Proton release to the cytoplasmic half channel is facilitated by the movement of *a*Arg210 as a consequence of this proposed helical swiveling. Finally, release from the cytoplasmic half channel is mediated by residues in a complex of interacting extra-membraneous loops formed between TMHs of both subunits *a* and *c*. This article is part of a Special Issue entitled: 18th European Bioenergetic Conference.

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1. Introduction

The F_1F_o-ATP synthase of oxidative phosphorylation utilizes the energy of a trans-membrane electrochemical gradient of H^+ or Na^+ to mechanically drive the synthesis of ATP via two coupled rotary motors in the F_1 and F_o sectors of the enzyme (ref. [1] and Fig. 1). H^+ transport through the transmembrane F_o sector is coupled to ATP synthesis or hydrolysis in the F_1 sector at the surface of the membrane. Homologous ATP synthases are found in mitochondria, chloroplasts, and many bacteria. In *Escherichia coli* and other eubacteria, F_1 consists of five subunits in an $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry. F_o is composed of three subunits in a likely ratio of $a_1b_2c_{10}$ in *E. coli* and *Bacillus* PS3 [2,3] or $a_1b_2c_{11}$ in the Na^+ translocating *Ilyobacter tartaricus* ATP synthase [1,4] and may contain as many as 15 *c* subunits in other bacterial species [5].

Subunit *c* spans the membrane as a hairpin of two α-helices with the first TMH[1] on the inside and the second TMH on the outside of the *c* ring [1,4]. The binding of Na^+ or H^+ occurs at an essential, membrane embedded Glu or Asp on *c*TMH2. A high resolution X-ray structure of the *I. tartaricus* c_{11}-ring revealed a Na^+ binding Glu at the periphery of the ring with chelating groups to the bound Na^+ extending from two adjacent subunits in an extensive hydrogen bonding network [1,4,6]. A high resolution X-ray structure of the H^+-translocating c_{15}-ring of *Spirulina platensis* revealed a similar geometry for the H^+ binding site [5,7]. Several other neighboring residues form a hydrogen bonding network between TMHs 1 and 2 of one subunit and TMH2 from the adjacent subunit. In slight contrast, a recent structure of the c_{13} ring from *Bacillus pseudofirmus* OF4 [8] revealed a more hydrophobic H^+ binding site in which a water molecule is coordinated by the H^+-binding Glu and the backbone of the adjacent *c*TMH2. This H^+ binding mode is likely to also be found in *E. coli* subunit *c*, given the lack of hydrogen bonding side chains surrounding the Asp61 H^+-binding site. In the H^+-translocating *E. coli* enzyme, Asp-61 at the center of *c*TMH2 is thought to undergo protonation and deprotonation as each subunit of the *c* ring moves past the stationary subunit *a*. In the functioning enzyme, the rotation of the *c* ring is proposed to be driven by H^+ transport at the subunit *a*/*c* interface. Subunit γ physically binds to the cytoplasmic surface of the *c*-ring, which results in the coupling of *c*-ring rotation with rotation of subunit γ within the $\alpha_3\beta_3$ hexamer of F_1. The rotation of subunit γ then forces conformational changes in the catalytic sites at αβ subunit interfaces that lead to synthesis of ATP (reviewed in ref. [1]).

E. coli subunit *a* folds in the membrane with five TMHs and is thought to provide aqueous access channels to the H^+-binding Asp-61 residue in the *c*-ring [9,10]. Interaction of the conserved Arg-210 residue in *a*TMH4 with *c*TMH2 is thought to be critical during the deprotonation–protonation cycle of *c*Asp-61 [1,11,12]. Presently, only fragmentary

Abbreviations: N-side, the electrochemically negative side of the membrane; P-side, the electrochemically positive side of the membrane; TM, trans-membrane; TMH, trans-membrane helix; NEM, N-ethylmaleimide

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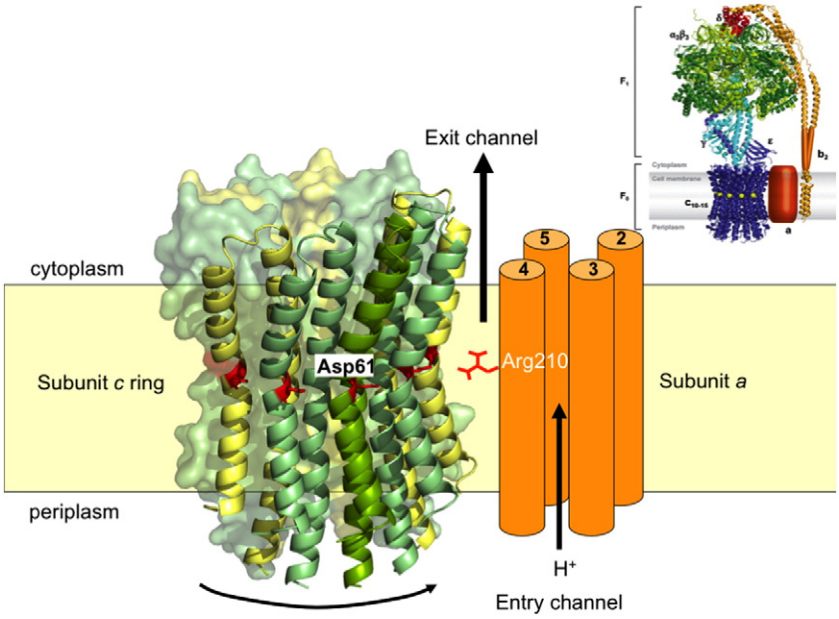

Fig. 1. H^+-transporting F_1F_o ATP synthase catalyzes the synthesis of ATP via coupled rotary motors within F_o and F_1. H^+ transport at the subunit *a*–*c* interface in F_o drives rotation of the *c*-ring-γ–ε complex, and rotation of subunit γ within the $\alpha_3\beta_3$ sector of F_1 then mechanically drives ATP synthesis within the catalytic sites. In this review, we propose the route of proton transfer via two separate half-channels extending from the periplasmic (P) or cytoplasmic (N) sides of the membrane to *c*Asp61 at the center of the membrane. The half channels are gated with the H^+ binding to and release from *c*Asp61 being coupled to conformational movements of Arg210 in subunit *a*. The structural model of F_1F_o shown is reproduced from ref. [1] with permission, and the structural model for the *E. coli* c_{10} ring is that predicted from the X-ray structure of the *I tartaricus* *c*-ring [4,26].

information is available on the three-dimensional arrangement of the TMHs in subunit *a*. TMHs 2–5 of subunit *a* pack in a four-helix bundle, which was initially defined by cross-linking ([13]; Fig. 2), but now such a bundle, packing at the periphery of the *c*-ring, has been viewed directly by high resolution cryo-electron microscopy in the *I. tartaricus* enzyme [14]. Previously published cross-linking experiments support the identification of *a*TMH4 and *a*TMH5 packing at the periphery of the *c*-ring and the identification of *a*THM2 and *a*TMH3 as the other components of the four helix bundle seen in these images [13,15,16]. More recently published cross-linking experiments identify the N-terminal α-helices of two b subunits, one of which packs at one surface of *a*TMH2 with close enough proximity to the *c*-ring to permit cross-linking [17]. The other subunit *b* N-terminal helix packs on the opposite peripheral surface of *a*TMH2 in a position where it can also be cross-linked to *a*TMH3 [17]. The N-terminal helix of subunit *b* (residues 1–34) appears to play an important structural role in formation of a functional F_0 [18]. The last helix density shown in Hakulinen *et al.* [14] packs at the periphery of the *c*-ring next to *a*TMH5 and is very likely to be *a*TMH1. If *a*TMH1 is packing at this position, the long cytoplasmic 1-2 loop must extend across *a*TMH5 before reaching *a*TMH2 (see Fig. 3).

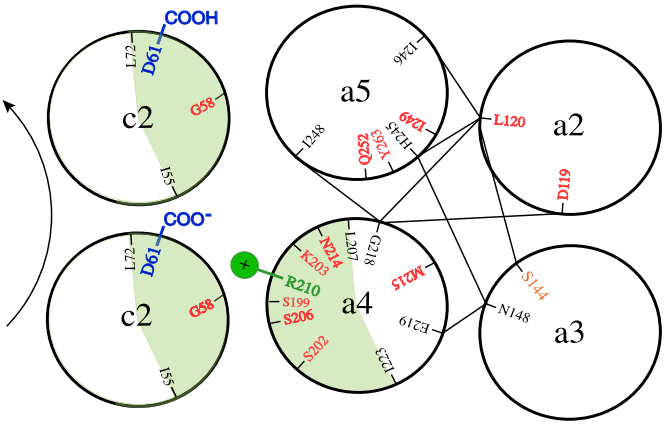

Fig. 2. The predicted cross-sectional packing of TMHs 2–4 of subunit *a* into a four helix bundle and the predicted faces of helix–helix interaction with the peripheral helices of the *c*-ring as viewed from the cytoplasm. The eight cross-links formed in high yield between pairs of Cys introduced into different TMHs of subunit *a* are indicated by the lines between the cross-linkable residues [13]. The predicted packing of TMH2 of subunit *c* with TMHs 4 and 5 of subunit *a* is also based upon Cys–Cys cross-linking [15,16]. Arrows indicate the direction of *c*-ring rotation during ATP synthesis. This figure is modified from that shown in ref. [32].

The aqueous accessibility of Cys residues introduced into the 5 TMHs of subunit *a* has been probed based upon their reactivity with and inhibitory effects of Ag^+ and other thiolate-reactive agents [22–24]. Two regions of aqueous access were found with distinctly different properties. One region in TMH4, extending from Asn-214 and Arg-210 at the center of the membrane to the cytoplasmic surface, contains Cys substitutions that are sensitive to inhibition by both NEM and Ag^+ ([22–24]; Fig. 3). These NEM and Ag^+ sensitive residues in TMH4 pack at or near the peripheral face and cytoplasmic side of the modeled four-helix bundle [11,13]. The route of aqueous access to the cytoplasmic side of subunit *c* packing at the *a*–*c* interface has also been mapped by the chemical probing of Cys substitutions and more recently by molecular dynamic simulations ([25–27]; Fig. 2).

A second set of Ag^+-sensitive substitutions in subunit *a* mapped to the opposite face and periplasmic side of *a*TMH4 [22,23], and Ag^+-sensitive substitutions were also found in TMHs 2, 3, and 5 where

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