Energy coupling mechanism of F_0 in a rotary ATP synthase: a model update

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INTRODUCTION

In nearly all living cells, rotary ATP synthases of the F_1F_0 family are essential to inter-converting of energy between the transmembrane electrochemical potential and the ATP form (Boyer 1997). During ATP synthesis, the F_0 sector of the ATP synthase machinery converts the electrochemical energy of either protons or sodium ions into mechanical energy of a rotor; in turn, this energy is converted into chemical energy of ATP by the F_1 sector (Gruber *et al.* 2014; Nakamoto *et al.* 2008). A fundamental question here is as to why these driving substances are all electrically charged. The answer to this conundrum is likely to be related to the electric nature of the transmembrane potential. For a membrane protein whose functional cycle is driven by proton motive force (Δp), be it an F_0 complex (Boyer 1988) or a secondary transporter (Zhang *et al.* 2015b, 2017), the driving force includes two parts, namely the electrostatic membrane potential ($\Delta\Psi_M$) and ΔpH . The corresponding energy terms are expressed as $F\Delta\Psi_M$ and $-2.3RT\Delta pH$, respectively. Whereas $\Delta\Psi_M$ is influenced by the capacitance of the lipid bilayer, ΔpH depends on buffer strength on either side of the membrane. Furthermore, the ΔpH -associated energy term is equivalent to the Nernst voltage of protons (Benedek and Villars 2000), *i.e.*, $V_N = -2.3(RT/F)\Delta pH$. Therefore, Δp itself can be represented by an overall, effective membrane potential $\Delta\Psi_p = \Delta\Psi_M + V_N$.

Thus, the biophysics of the transmembrane proton wire in F_0 appears to be similar to that of selective ion channels (Zhang *et al.* 2018). In particular, the movement of protons along its transmembrane path is

dictated by electrostatic forces. For instance, the repulsion forces between neighboring protons possibly serve as a medium to transduce concentration (*i.e.*, pH) information between the source, path, and sink of protons.

Based on moderate-resolution structures of F_1F_0 ATP synthases, we previously proposed a model to illuminate the energy coupling mechanism of F_0 (Zhang *et al.* 2015a), which is in agreement with an earlier hypothesis put forward by Dimroth and coworkers (Dimroth *et al.* 2003). In both models, $\Delta\Psi_M$ plays a major role in driving the F_0 machinery during ATP synthesis. To enable the energy coupling process to proceed effectively, a large drop in the electrostatic potential of the driving substance (*e.g.*, proton) at the interface between the stator (*i.e.*, a-subunit) and the rotor (*i.e.*, c-ring, the symmetric homo-oligomer formed by c-subunits) is essential and must be associated with unidirectional rotation of the rotor. Equivalently, the proton trajectory viewed in the rotor reference system (R-trajectory) must lag behind that viewed in the stator reference system (S-trajectory) so that catching up of the R-trajectory with the S-trajectory coincides with the unidirectional rotation of the c-ring. For instance, if $\Delta\Psi_M$ were uniformly distributed across the membrane in the interface region between the a-subunit and c-ring (a-c interface) where energy conversion occurs, the R-trajectory and S-trajectory of the protons might assume shapes of a straight line and a spiral curve, respectively (Zhang *et al.* 2015a). Although the actual shape of the trajectory depends on structural details of the a-c interface, the “ideal” spiral-shaped trajectory may qualitatively represent the energy landscape for the proton movement.

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NEW STRUCTURAL OBSERVATIONS

Recently, a number of studies reported high-resolution, cryo-electron microscopy structures of F_1F_0 ATP synthases (Guo *et al.* 2017; Hahn *et al.* 2018; Srivastava *et al.* 2018). In agreement with the reversibility of the functional cycle of ATP synthase, the F_0 sector, especially the a-c interface, shows a pseudo twofold symmetry with the axis parallel to the membrane plane. Two pieces of proton wire, which half-way penetrate into the lipid bilayer from opposite sides of the membrane, are well defined by polar and/or charged residues (Fig. 1) and are symmetrically arranged relative to the pseudo-axis. According to the current mainstream “double half-channel” hypothesis (Junge *et al.* 1997), these two proton wire segments are referred to as half-channels. It is reasonable to speculate that additional water molecules infiltrate into these half-channels, where they become coordinated by the polar groups from the stator, and remain titratable by protons moving from sites of lower pK_a along the proton wire. The two half-channels comprise the static components of a complete proton wire, along which protons translocate across the membrane in the energy-descending direction. Intriguingly, these half-channels (especially the entrance one) are located between transmembrane helices of the a-subunit in such a manner that the corresponding segments of the proton wire are mostly

buried inside the a-subunit, but are not in extensive contact with polar groups from the rotor. This structural feature dictates that proton movement inside these half-channels is not directly involved in the energy conversion. Furthermore, at the middle level of the lipid bilayer, the two half-channels appear to be connected to the two ends of a dynamic proton wire formed by a set of titratable acidic residues of the c-ring. This latter imaginary proton wire may assume its role in carrying protons only when the c-ring rotates continuously. Despite support from newly reported structures, the current “double half-channel” hypothesis remains to be extended to formally take $\Delta\Psi_p$ into account.

FOCUSED ELECTRIC FIELDS

Because of the presence of the two half-channels, the dielectric constant of the F_0 complex cannot be treated as uniform. Thus, the above-mentioned “ideal” picture of the uniformly distributed electric field is severely distorted. In particular, the electric field around the a-c interface becomes focused (Fig. 2). More specifically, the electric field between the inner surface of a half-channel and its closest protein surface on the opposite side of the membrane is reinforced, as it is associated with patches of higher charge-densities compared to their surrounding areas. In addition, because of the much

Fig. 1 Structure of F_0 . The peptide backbone traces of the F_0 complex from yeast mitochondria (PDB ID: 6CP7) are shown in tube representations. The c-ring is indicated in yellow; the a-subunit in wheat color; and other subunits of the stator are presented in gray. Positions of selected C_α atoms are marked with spheres: D/E sites in the c-ring, red; Arg-finger in the a-subunit, blue; residues contributing sidechain or maintain polar groups to the entrance half-channel, cyan; and residues of the exit half-channel, rose color

Fig. 2 Schematic diagram of the energy coupling mechanism of F_0 . The positive side of the membrane potential is shown in cyan, and the negative side in pink. For simplicity, we assume that the electric potential inside each half-channel equals to that of the surface of the same-side membrane leaflet. Selected electric lines of forces are represented as black arrows. For instance, in the lipid bilayer region away from the a-c interface, the membrane potential is uniformly distributed. In contrast, near the two half-channels, the membrane potential becomes focused. The dynamic proton wire formed by the rotating c-ring is presented as a green curve, and the direction of the proton movement (i.e., rotation of the c-ring) during ATP synthesis is indicated by the yellow arrow. Energy coupling between Δp and the rotation of the c-ring occurs at both the V_+-V_0 and V_0-V_- junctions. The conserved Arg-finger is represented with a blue sphere

higher dielectric constant inside the half-channels relative to their surrounding nonpolar structure, the electric fields inside the half-channels are significantly weaker than that inside the remaining F_0 . Consequently, at the inner end of each half-channel, the electrostatic potential is roughly the same as that on the surface of the same leaflet of the lipid bilayer, depicted as V_+ or V_- (see Fig. 2). As first approximation, one may consider a scenario in which the effective membrane potential, $\Delta\psi_p$ (i.e., $V_- - V_+$), is directly applied between the tips of the two half-channels (Dimroth *et al.* 2003). A benefit of such a focused electric field is that proton movements inside the two stator-buried half-channels consume negligibly amount of energy, thus ensuring that ATP synthesis remains a highly efficient energy-generating process (Silverstein 2014). A conserved Arg-finger is located between the half-channels and on the pseudo twofold axis of F_0 (Lightowlers *et al.* 1987; Zhang *et al.* 2015a). This Arg-finger generates a formidable energy barrier for protons. As a result, futile discharge of protons directly across the ends of the two half-channels is prevented. Therefore, the dynamic proton wire created by the rotating c-ring becomes necessary and effective for discharging the energy potential acutely accumulated between the two half-channels.

The dynamic part of the proton wire in F_0 is comprised of a series of titratable acidic residues, referred to as D/E sites, around the circumference of the c-ring. In order to maintain both high speed and high efficiency of energy conversion in ATP synthesis, this proton wire

should present low or no kinetic barriers. On the one hand, the multiple protonated D/E sites (positioned away from the a-c interface) experience a constant voltage, V_0 , which assumes a value between V_- and V_+ . Thus, a given D/E site senses a flat energy landscape when it moves from one position to the next during rotation of the c-ring. On the other hand, during the process of continuing ATP synthesis, the overall movement of protons does proceed from higher voltage sites to lower sites. Therefore, two drastic voltage drops likely exist at the a-c interface: one from the entrance half-channel to the c-ring (V_+-V_0 junction) and the other drop from the c-ring to the exit half-channel (V_0-V_- junction). These jumps of protons across the two voltage gaps convert the electrostatic energy into the unidirectional rotation of the c-ring, similar to a ratchet-like movement. From a mechanistic point of view, a large energy drop in a small angular range obligatorily generates a strong torque, favoring a rotation along the energy-descending direction. Furthermore, by properly arranging the two half-channels relative to the proton loading/releasing subunits in the c-ring, the most likely scenario is that the pulses of energy conversion occur sequentially to generate smooth rotation of the c-ring. For instance, the proton release at the V_0-V_- junction may slightly proceed of the proton loading at the V_+-V_0 junction (Srivastava *et al.* 2018).

THE PATH FOR PROTON TRANSLOCATION

In agreement with the monotonically descending energy landscape of the dynamic proton wire, the titratable D/E sites assume distinct pK_a values in different phases of the function cycle of the c-ring. More specifically, the pK_a value of a given D/E site (e.g., Glu^{c59} in the yeast mitochondrial F_0) transiently decreases when approaching the Arg-finger (Arg^{a176}), as a result of which the proton is released. Since it is accompanied by a large energy drop, the proton release at the V_0-V_- junction is a high probability event; so is the unidirectional rotation of the c-ring, because both events are in the same chain of Bayesian probabilities. Therefore, one can conclude that, analogous to a dropping chain at a table edge, the proton release drags the corresponding c-subunit to move toward the Arg-finger. Concurrently, the rigid-body rotation of the c-ring moves the neighboring c-subunit on the proton loading side away from the Arg-finger, and the pK_a of the D/E site in this c-subunit starts returning to “normal,” permitting proton loading. Thus, the proton loading at the V_+-V_0 junction requires and drives the corresponding

c-subunit to move away from the Arg-finger. Being buried in the lipid bilayer environment, the “normal” pK_a of the D/E site seems to be sufficiently high to attract protons from the entrance side of the membrane. At this point, it is important to clarify a common, yet false belief about the role of the Arg-finger. It is often assumed that on the proton-releasing side of the Arg-finger, the electrostatic attraction between the Arg-finger of the stator and incoming deprotonated D/E site of the c-ring contributes to the overall driving force for the c-ring rotation. However, a second, similar attraction on the proton loading side would energetically disfavor the unidirectional rotation, thus canceling the first attraction. Unfortunately, this second attraction is usually selectively ignored. In agreement with the pseudo twofold symmetry of F_o as well as the reversibility of the F_1F_o type of ATP synthase machinery, our arguments presented here are likely suitable for both the Δp -driven ATP synthesis and the ATP hydrolysis-driven proton pumping process.

In summary, we argue here that $\Delta\Psi_p$ is the sole driving force in ATP synthesis, maintaining the rotation of the c-ring via accelerating proton movements across the two junction points between the stator and the rotor. Any future attempt of a more complete understanding of the F_oF_1 ATP synthase mechanism must take $\Delta\Psi_p$ into account; otherwise, the high efficiency of this elegant machinery would be left unexplained.

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Compliance with Ethical Standards

Conflict of interest XC Zhang and M Liu declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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References

- Benedek GB, Villars F (2000) Physics, with illustrative examples from medicine and biology (electricity and magnetism), 2nd edn. AIP Press, New York
- Boyer PD (1988) Bioenergetic coupling to protonmotive force: should we be considering hydronium ion coordination and not group protonation? *Trends Biochem Sci* 13:5–7
- Boyer PD (1997) The ATP synthase—a splendid molecular machine. *Annu Rev Biochem* 66:717–749
- Dimroth P, von Ballmoos C, Meier T, Kaim G (2003) Electrical power fuels rotary ATP synthase. *Structure* 11:1469–1473
- Gruber G, Manimekalai MS, Mayer F, Muller V (2014) ATP synthases from archaea: the beauty of a molecular motor. *Biochem Biophys Acta* 1837:940–952
- Guo H, Bueler SA, Rubinstein JL (2017) Atomic model for the dimeric F_o region of mitochondrial ATP synthase. *Science* 358:936–940
- Hahn A, Vonck J, Mills DJ, Meier T, Kuhlbrandt W (2018) Structure, mechanism, and regulation of the chloroplast ATP synthase. *Science* 360:eaat4318
- Junge W, Lill H, Engelbrecht S (1997) ATP synthase: an electrochemical transducer with rotatory mechanics. *Trends Biochem Sci* 22:420–423
- Lightowers RN, Howitt SM, Hatch L, Gibson F, Cox GB (1987) The proton pore in the Escherichia coli F_oF_1 -ATPase: a requirement for arginine at position 210 of the a-subunit. *Biochem Biophys Acta* 894:399–406
- Nakamoto RK, Baylis Scanlon JA, Al-Shawi MK (2008) The rotary mechanism of the ATP synthase. *Arch Biochem Biophys* 476:43–50
- Silverstein TP (2014) An exploration of how the thermodynamic efficiency of bioenergetic membrane systems varies with c-subunit stoichiometry of $F(1)F(0)$ ATP synthases. *J Bioenerg Biomembr* 46:229–241
- Srivastava AP, Luo M, Zhou W, Symersky J, Bai D, Chambers MG, Faraldo-Gomez JD, Liao M, Mueller DM (2018) High-resolution cryo-EM analysis of the yeast ATP synthase in a lipid membrane. *Science* 360:eaas9699
- Zhang XC, Liu M, Zhao Y (2015a) How does transmembrane electrochemical potential drive the rotation of F_o motor in an ATP synthase? *Protein Cell* 6:784–791
- Zhang XC, Zhao Y, Heng J, Jiang D (2015b) Energy coupling mechanisms of MFS transporters. *Protein Sci* 24:1560–1579
- Zhang XC, Liu M, Lu G, Heng J (2017) Thermodynamic secrets of multidrug resistance: a new take on transport mechanisms of secondary active antiporters. *Protein Sci* 27:595–613
- Zhang X, Yang H, Liu Z, Sun F (2018) Thermodynamics of voltage-gated ion channels. *Biophys Rep*. <https://doi.org/10.1007/s41048-018-0074-y>