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review

Catalytic and mechanical cycles in F-ATP synthases

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Cycles have a profound role in cellular life at all levels of organization. Well-known cycles in cell metabolism include the tricarboxylic acid and the urea cycle, in which a specific carrier substrate undergoes a sequence of chemical transformations and is regenerated at the end. Other examples include the interconversions of cofactors, such as NADH or ATP, which are present in the cell in limiting amounts and have to be recycled effectively for metabolism to continue. Every living cell performs a rapid turnover of ATP to ADP to fulfil various energetic demands and effectively regenerates the ATP from ADP in an energy-consuming process. The turnover of the ATP cycle is impressive; a human uses about its body weight in ATP per day. Enzymes perform catalytic reaction cycles in which they undergo several chemical and physical transformations before they are converted back to their original states. The ubiquitous F_1F_o ATP synthase is of particular interest not only because of its biological importance, but also owing to its unique rotational mechanism. Here, we give an overview of the membrane-embedded F_o sector, particularly with respect to the recent crystal structure of the c ring from *Ilyobacter tartaricus*, and summarize current hypotheses for the mechanism by which rotation of the c ring is generated.

Keywords: ATP cycle; catalytic reaction cycle; F-ATP synthase; ion translocation model; mechanical rotation; c ring structure; Na^+ binding site

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Introduction

The F-ATP synthase is a nano-size rotary engine that manufactures most of the ATP required to drive the many energy-consuming reactions in living cells. The overall construction of this enzyme complex—which is conserved from bacteria to humans—is an assembly of two entities, F_1 and F_o (Fig 1; Boyer, 1997; Capaldi & Aggeler, 2002; Dimroth *et al*, 2003). Either unit can operate as a reversible rotary motor and exchanges energy with its partner motor by mechanical rotation of the central stalk. F_1 is a water-soluble protein complex (subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$) and F_o (subunit composition ab_2c_{10-15}) is embedded in the membrane. In synthesis mode, the F_o motor converts the electrochemical gradient of protons or Na^+ ions into torque to force the F_1 motor to act as an ATP generator. In hydrolysis mode, F_1 converts the chemical energy of ATP hydrolysis into torque, causing the membrane-embedded F_o motor to act as an ion pump. The F_1 motor consists of a hexameric assembly of alternating α- and β-subunits around a central coiled-coil γ-subunit. The F_1 complex is intrinsically asymmetric, owing to different interactions of the central γ-subunit with each of the catalytic β-subunits, and provides them with different conformations and nucleotide affinities at their catalytic sites. On rotation of the γ-subunit, the conformations of the three β-subunits change sequentially such that each β-subunit successively adopts the same conformations of varying affinity during one rotational cycle. As a result, three molecules of ATP are synthesized. This model, known as the binding change mechanism (Boyer, 1993), explains a wealth of biochemical and kinetic data. The rotation of the γ-subunit is consistent with the crystal structure of F_1 (Abrahams *et al*, 1994) and has been verified by various methods, most convincingly by direct observation in a video microscope (Noji *et al*, 1997).

A prominent part of the F_o motor consists of an oligomeric c-subunit assembly in the shape of a ring, the stoichiometry of which varies depending on the species. For example, rings consisting of 10 monomers have been found in yeast mitochondria (Stock *et al*, 1999), the thermophilic *Bacillus* PS3 (Mitome *et al*, 2004), and possibly in *Escherichia coli* (Jiang *et al*, 2001), whereas the rings found in the bacterium *Ilyobacter tartaricus* (Stahlberg *et al*, 2001) and *Propionigenium modestum* (Meier *et al*, 2003) have 11 monomers. Furthermore, ring stoichiometries of 14 and 15 monomers have been found in chloroplasts (Seelert *et al*, 2000) and the cyanobacterium *Spirulina platensis* (Pogoryelov *et al*, 2005), respectively. The number of subunits in each ring also indicate the number of ions transported across the membrane during each cycle of the ATP synthase. Consequently, as the F_1 motor contains three catalytic sites and synthesizes three molecules of ATP per cycle, a variation in the number of c-subunits and ion binding sites automatically leads to different H^+ (Na^+) to ATP ratios. ATP synthases with large c rings have a high H^+ (Na^+) to ATP ratio, which would be advantageous for ATP synthesis at low ion motive force. Conversely, ATP synthases with small c rings might prevail in organisms with constantly high ion motive force: the low H^+ (Na^+) to ATP ratio of these enzymes results

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in a more efficient use of energy. A mismatch to the threefold symmetry of the F_1 motor created by F_o motors with 10, 11, 13 or 14 c-subunits has been regarded as functionally important (Murata *et al*, 2005; Stock *et al*, 1999). However, recent data from the c_{15} ring of the *S. platensis* F-ATP synthase show that symmetry mismatch is not mandatory for function.

In the F_o complex, the c ring is flanked by the a- and b_2-subunits (Mellwig & Böttcher, 2003; Rubinstein *et al*, 2003). The c ring interacts with the γ/ε pair in the intact ATP synthase, forming the rotor assembly that spins against the stator components $ab_2\alpha_3\beta_3\delta$ (Fig 1; Capaldi & Aggeler, 2002; Tsunoda *et al*, 2001). Ion translocation at the a/c subunit interface, driven by the ion motive force, is thought to generate the torque applied to the rotor subunits—which is then used to promote the conformational changes required for ATP synthesis at the F_1 catalytic sites.

Proton or sodium ion-cycles energize ATP synthesis

The ATP synthase recruits energy for the formation of the terminal phospho-anhydride bond of ATP from an electrochemical gradient of either protons or Na^+ ions across the membrane. In the process of ATP synthesis, these ions are translocated by the ATP synthase through the membrane along the electrochemical gradient. Conversely, enzymes coupling an exergonic reaction to ion pumping against their electrochemical gradient have to be part of the ATP-synthesizing system to close the cycle and re-energize the membrane (Fig 2). For example, mitochondria and aerobic bacteria perform the electron transfer from NADH to O_2 to form H_2O through a series of respiratory chain complexes. Some of the complexes couple electron transfer to proton translocation to store the free energy of the oxidation as an electrochemical proton gradient. The gradient drives ATP synthesis by the H^+-translocating F_1F_o ATP synthase, thereby recycling the protons to their original compartment. A similar, but Na^+ ion-cycle-based ATP synthesis mechanism, is common in fermenting bacteria (Fig 2; Dimroth, 1997; Dimroth & Cook, 2004). The model is *P. modestum*, which grows by the fermentation of succinate to propionate and CO_2. During metabolism, succinate is converted to methylmalonyl-CoA, which is decarboxylated to propionyl-CoA by a membrane-bound decarboxylase acting as a Na^+ pump. Consequently, the free energy of the decarboxylation reaction is converted into an electrochemical gradient of Na^+ ions, which are the only source of free energy available for ATP synthesis (Hilpert *et al*, 1984). The bioenergetic parameters deserve special attention in this ATP-synthesis mechanism. The free energy of methylmalonyl-CoA decarboxylation is approximately –20 kJ/mol and the free energy required for ATP synthesis in a growing bacterium is estimated to be about 60 kJ/mol. As a result, approximately three consecutive Na^+ export cycles, driven by decarboxylation events, are required to accumulate enough energy for the synthesis of one ATP in the Na^+ import cycle. In the export cycle, one Na^+ is pumped electrogenically per decarboxylation event across the membrane. In the import cycle, the ATP synthase—with its 11 c-subunits—translocates 11 Na^+ ions backwards into the cytoplasm and thereby synthesizes three molecules of ATP. Therefore, to turn the c ring one step, the free energy of one Na^+ traversing the membrane must equal 0.27 molecules of ATP, corresponding to 16.2 kJ/mol, which is below the free energy of one decarboxylation event (–20 kJ/mol). These calculations show that the stoichiometries of Na^+ translocation by the decarboxylase and the ATP synthase are properly adjusted to account for the synthesis of one ATP by three decarboxylation events (Dimroth & Cook, 2004).

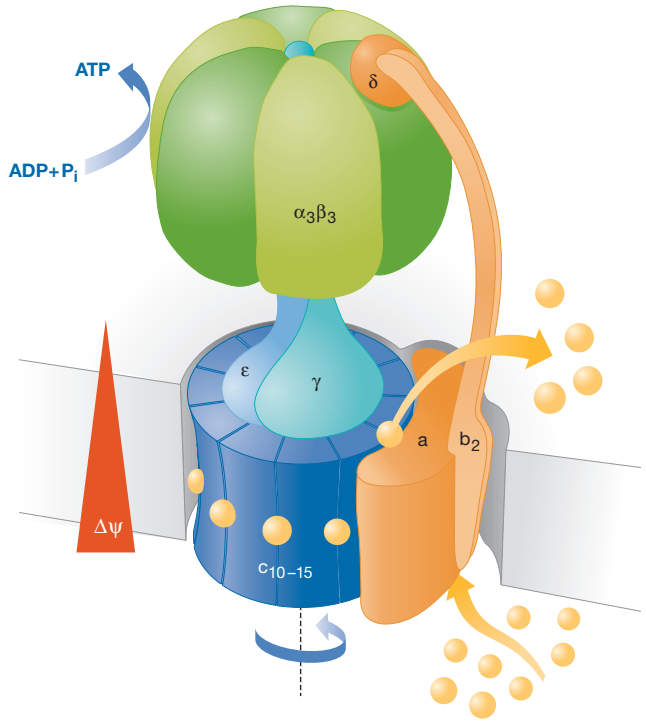

Fig 1 | Structure and function of a bacterial ATP synthase in a biological membrane. F_1 (subunits $\alpha_3\beta_3\gamma\delta\varepsilon$) and F_o (subunits ab_2c_{10-15}) are two motors that exchange energy by rotational coupling. The rotary subunits ($\gamma\varepsilon c_{10-15}$) are shown in blue and the membrane-anchored and cytoplasmic stator subunits ($ab_2\alpha_3\beta_3\delta$) are shown in orange and green. During ATP synthesis, coupling ions (orange) pass through the F_o motor from the periplasm to the cytoplasm inducing rotation and enabling the F_1 motor to synthesize ATP.

Ion-cycling across membranes: the a/c subunit interface

In the membrane-embedded F_o complex of the ATP synthase, the c ring rotates against the laterally orientated subunits a and b_2 with up to 150 Hz, thereby conducting protons or Na^+ ions across the membrane. The mechanism of ion translocation involves sophisticated interactions between subunit a and the rotating c ring. Basically, subunit a acts as a mediator for ion transport from the periplasm to the middle of the membrane, where the incoming ion is passed to the adjacent binding site in the c ring. The ion remains bound for an almost complete rotation and is released to the cytoplasm when it reaches the a/c interface again.

Recently, the structure of the c_{11} oligomer from the Na^+-translocating F-ATP synthase of *I. tartaricus* was solved, first at medium and then at high resolution (Meier *et al*, 2005; Vonck *et al*, 2002). This was complemented with the structure of the k ring from the Na^+-translocating V-type ATPase from *Enterococcus hirae* (Murata *et al*, 2005). In the *I. tartaricus* structure (Fig 3A), each ring is composed of 11 c-subunits. Of these, each monomer is folded as a helical hairpin with the loop at the cytoplasmic side and the termini at the periplasmic side, as was predicted earlier by independent methods (Girvin *et al*, 1998; Hoppe *et al*, 1984). The structure shows a cylindrical, hourglass-shaped protein complex with a hydrophobic cavity in the centre of the ring. In the natural membrane environment, the cavity is filled with lipids (Oberfeld *et al*, 2006). The 11 N-terminal helices

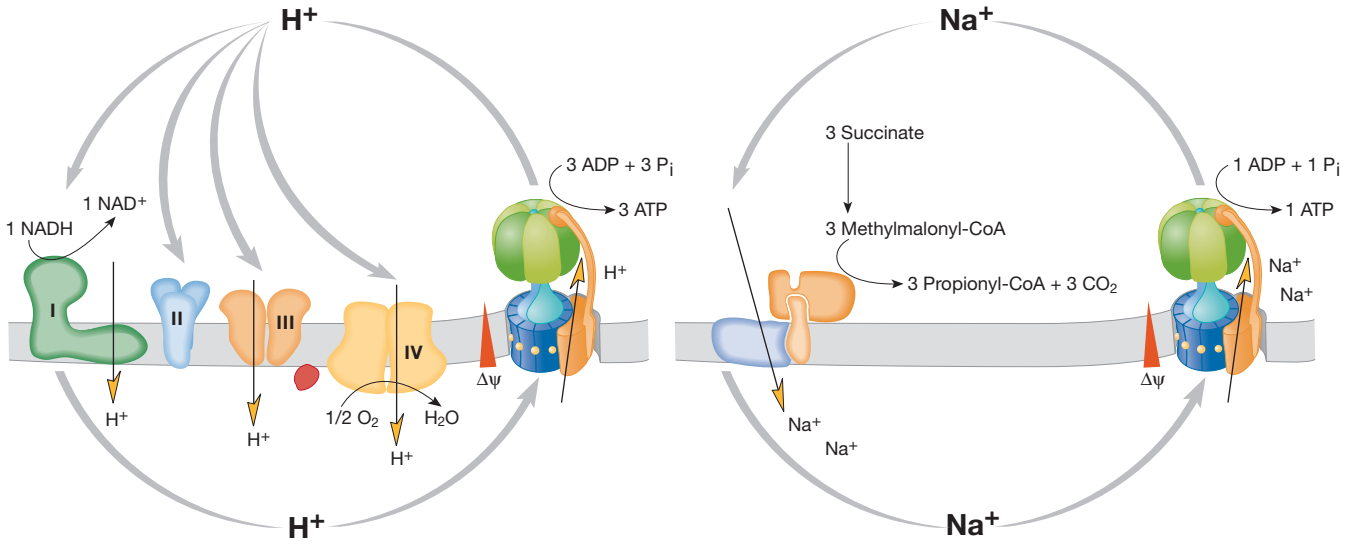

Fig 2 | Energy conversions by ion-cycling across membranes. (**A**) Proton cycle coupling respiration to ATP synthesis in mitochondria or aerobic bacteria. In the respiratory chain from NADH to O_2, the number of protons pumped across the membrane by the chain complexes I, III and IV is sufficient for the synthesis of approximately three molecules of ATP by the ATP synthase. (**B**) Na^+ ion-cycle coupling ATP synthesis to the decarboxylation of methylmalonyl-CoA during succinate fermentation in *Propionigenium modestum*.

form a tightly packed inner ring, whereas the 11 C-terminal helices of the outer ring pack into the grooves of the inner helices. Eleven Na^+ ions are bound at their binding sites, at the middle of the membrane bilayer facing towards the outer surface of the c ring, which confirms previous crosslinking data (von Ballmoos *et al*, 2002a). The arrangement of the binding site residues and the coordination of the coupling ion is probably the most instructive feature of the c ring structure. As shown in Fig 3B, each of the 11 Na^+ ions is bound at the interface of three helices: an N-terminal helix and two C-terminal helices. The coordination sphere is formed by side-chain oxygen molecules of Q32 and E65 of one subunit and the hydroxyl oxygen molecule of S66, with the backbone carbonyl oxygen molecule of V63 of the neighbouring subunit. A fifth coordination site was found in the structure of the k ring but could not be identified clearly in the structure of the c ring. The structure thus confirms previous mutational studies in which Q32, E65 and S66 were identified as Na^+-binding ligands (Kaim *et al*, 1997). An intriguing observation is that E65 acts not only as one of the four Na^+-binding ligands but also as the recipient of three hydrogen bonds. This arrangement generates a stable, locked conformation of the binding site, from which horizontal transfer of the Na^+ ion to subunit a is prevented. This implies that the present locked conformation of the c ring has to be converted to an open one in the a/c interface.

Subunit a is an extremely hydrophobic protein of ~32 kDa containing 5–6 transmembrane α-helices (Zhang & Vik, 2003), which is difficult to handle experimentally. In particular, the interface with the c ring must be ingeniously designed to grant the stability of the a/c complex and at the same time allow an almost frictionless rotation of the c ring against subunit a, as revealed by single-molecule spectroscopy (Ueno *et al*, 2005). These peculiarities have impeded structural determinations of subunit a. So far, biochemical and mutational studies suggest that the universally conserved aR227 residue (*P. modestum* numbering), which is localized approximately at the same level in the membrane as the cE65, is important in ion translocation. Whereas the residue seems to be irreplaceable in the H^+-translocating enzymes (Valiyaveetil & Fillingame, 1997), some variability is possible in the Na^+-dependent ATP synthase of *P. modestum* (Wehrle *et al*, 2002). Experiments with site-specific mutants of aR227 indicate that this positively charged amino acid is essential for dislodging the bound Na^+ ion from its binding site. Electrostatic interactions between aR227 and cE65 are therefore thought to be crucial for the ion translocation mechanism.

The c ring structure supports a model (Fig 4) in which aR227 acts as a switch to open and close the gate between the binding site and subunit a. In the direction of synthesis, the positively charged arginine repels the Na^+ from the approaching binding site and simultaneously exerts a pull on the negatively charged side chain of cE65, keeping it in its original conformation. Therefore, the Na^+ is thought to escape vertically from the binding site into the cytoplasm. On passing the aR227 residue, the electrostatic attraction to cE65 is retained, thus opening the gate between subunit a and the binding site to allow reloading of the site through the periplasmic entrance channel in subunit a. This model is consistent not only with the structure but also with various biochemical data showing direct access of Na^+ ions to the binding site in the isolated c ring—without the participation of subunit a (Meier *et al*, 2003; von Ballmoos *et al*, 2002b).

However, in alternative models that have been proposed for the *E. coli* ATP synthase, both the entrance and the exit pathway of the ion to and from the binding site were placed in subunit a (Aksimentiev *et al*, 2004; Feniouk *et al*, 2004; Junge *et al*, 1997; Vik & Antonio, 1994). One of these models suggests a slight, but crucial difference in the function of the stator arginine. The interaction between aR210 and the cD61 (*E. coli* numbering) is proposed to regulate the access of two vertically orientated proton donor and acceptor sites, which are spatially and electrostatically separated by aR210. Large conformational changes were observed in nuclear magnetic resonance studies of

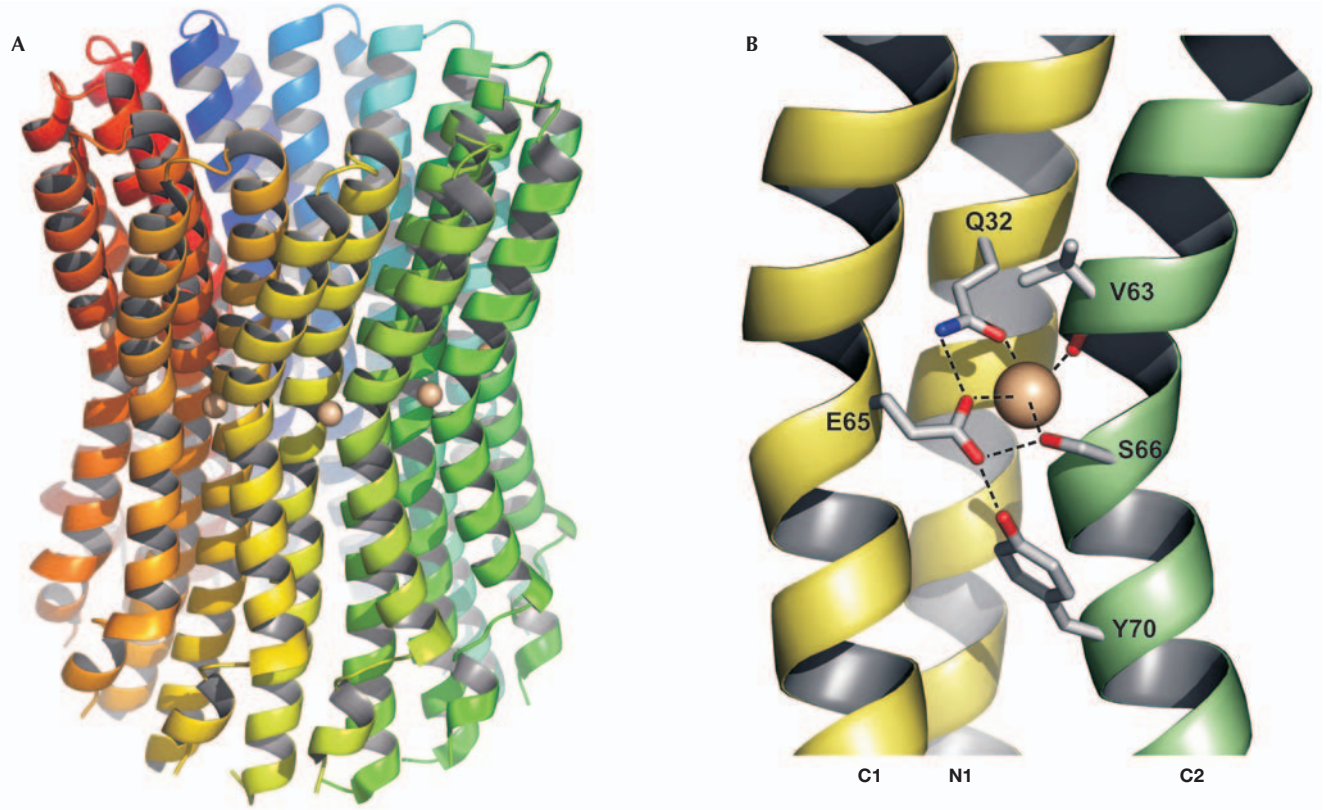

Fig 3 | Structure of the *I. tartaricus* c_{11} ring in ribbon form. (**A**) Individual subunits are shown in different colors. The grey spheres indicate the bound Na^+ ions. The structure shows a cylindrical, hourglass-shaped protein complex with an outer diameter of ~45 Å in the middle of the membrane and ~50 Å at the top and bottom. The ring has a height of ~70 Å and therefore protrudes out of the membrane on both sides. A hydrophobic cavity with a diameter of ~17 Å at its narrowest part is located in the centre of the ring. (**B**) Close-up of the Na^+ binding site formed by the inner (N1) and outer helix (C1) of one c-subunit and the outer helix (C2) of the neighbouring c-subunit. Na^+ coordination and selected hydrogen bonds are indicated with dashed lines. This structure shows the locked conformation. During opening, the side chain of Y70 might relocate into a cavity underneath the binding site, thus destabilizing the hydrogen bonding network and allowing unloading and loading of the binding site to and from subunit a.

monomeric subunit c in organic solvent mixtures at pH 5 and 8 (Girvin *et al*, 1998; Rastogi & Girvin, 1999). To account for these observations, the model suggests a 180° rotation of subunit c by the C-terminal versus the N-terminal helix in the subunit a/c interface (Aksimentiev *et al*, 2004). However, this model gains no support from the present c ring structure (Meier *et al*, 2005).

Torque generation by the F_o motor of the ATP synthase

Torque generation in the F_o is intimately joined with the transport of coupling ions across the membrane. Therefore, experiments concerning the driving forces for ATP synthesis and the ion pathway have to be interpreted in parallel. A key feature of the ATP synthesis mechanism is the finding that the two components of the proton (sodium) motive force, the H^+ (Na^+) concentration gradient and the membrane potential, which are thermodynamically equivalent, are not kinetically equivalent. Experiments with purified and *in vitro* reconstituted ATP synthases from *E. coli*, spinach chloroplasts or *P. modestum* showed that ATP synthesis requires a threshold membrane potential between 20 and 40 mV. This minimal potential is essential and cannot be replaced by large proton or sodium ion concentration gradients (Kaim & Dimroth, 1999). These results indicate that a potential-dependent reaction step has a fundamental role in the generation of torque by the F_o motor (Dimroth *et al*, 1999).

Because the motor can rotate in both directions (Diez *et al*, 2004), it is not surprising that, in the absence of an external energy source—ion motive force or ATP—the enzyme is in a relaxed state. In this idling mode, the ATP synthase from *P. modestum* performs Na^+ ion exchange between the two reservoirs by Brownian back-and-forth rotations of the rotor versus the stator in a narrow sector (Dimroth *et al*, 2003; Kaim & Dimroth, 1998). External forces are required to switch the enzyme out of this state into unidirectional rotation, which is the prerequisite for performing work. It is important to note that for the switch from this relaxed state to rotation, which terminates Na^+ exchange, the membrane potential is compulsory and cannot be substituted by a large Na^+ concentration gradient (Kaim & Dimroth, 1998).

Despite a common general mechanism, there is an ongoing debate about the molecular details of how torque is generated in F_o. The problem has been addressed by biochemical, biophysical, structural and even computational approaches, and an impressive amount of data has accumulated during the past two decades. The so-called 'two-channel model', which is the most popular for H^+-translocating enzymes, relies on a ratchet-type mechanism,

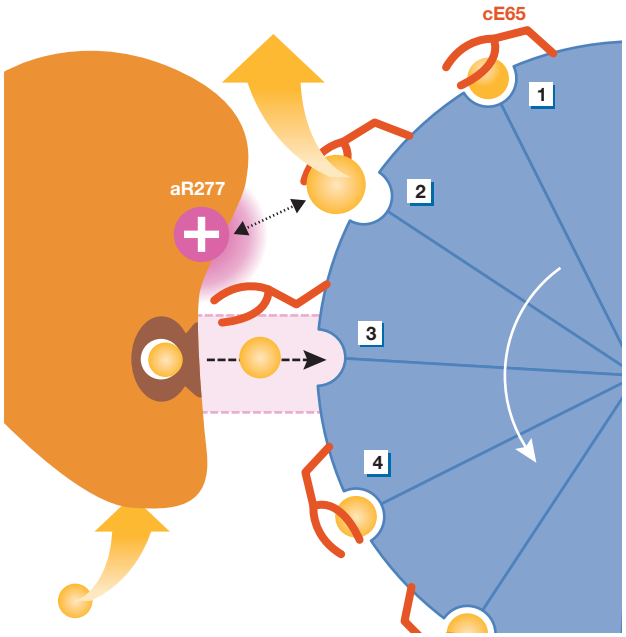

Fig 4 | Model for ion translocation in the a/c subunit interface. The c ring (blue) rotates counterclockwise against subunit a (orange) during ATP synthesis. (1) The Na^+ ion (orange) is coordinated by the E65, which is on the outer helix distal to subunit a, closing the horizontal gate towards subunit a. (2) On reaching the aR227 in the a/c subunit interface, electrostatic interactions stabilize the closed conformation of the glutamate and simultaneously repel the Na^+ ion vertically out of its site. (3) After passing aR227, the electrostatic attraction between cE65 and aR227 is retained. This opens the gate and allows a Na^+ ion to enter the site from subunit a. (4) On coordination of the incoming Na^+ ion, the E65 switches back into the closed conformation and the cycle can start again.

predicting that only a protonated site can move out of the a/c interface into the lipid phase (Junge *et al*, 1997; Vik & Antonio, 1994). This initial model was broadened to include the essential stator charge arginine to couple proton transport effectively to rotation and to prevent proton leakage in the a/c subunit interface (Elston *et al*, 1998). Possible molecular events of the operation of the motor have been calculated (Aksimentiev *et al*, 2004; Elston *et al*, 1998). The model predicts that inlet and outlet channels are located in a non-coaxial manner in subunit a (Fig 5A). The ion is proposed to enter the site through the inlet channel from the low pH reservoir and to exit through the outlet channel into the high pH reservoir, after performing an almost complete rotation. The unprotonated site is located between the two channels, where its negative charge is compens ated by the stator arginine. Without an external driving force, the motor is in idling mode, and the site shuttles between these channels with equal probability to either side. In the presence of a ΔpH, the proton concentration in each of the separate aqueous access channels is unequal, and the site will be protonated more frequently at the position of the channel with higher proton concentration. The protonated site is now able to move out of the a/c subunit interface and into the lipid phase. Simultaneously, a new site enters the interface, where its ion is displaced by the stator arginine. The negatively charged empty site acts as a ratchet, due to the large energy penalty of its backwards movement into the lipid phase. In this model, the pH gradient determines the direction of rotation and simultaneously acts as the main driving force for the generation of torque. H^+-transport experiments with the isolated F_o have shown that ions are transported across the membrane with a pH gradient only. The obligatory role of the membrane potential is thereby attributed to the interaction of F_1 and F_o during ATP synthesis, but cannot be specified in molecular terms (Feniouk *et al*, 2004).

In the model for torque generation by the Na^+-translocating F_o motor, a mechanism was created, on the basis of a variety of experimental data (Xing *et al*, 2004). This interpretation can be shown by empirical energy profiles of the transport processes in the a/c interface, which were used to build the mathematical model. The binding sites involved have to interact in a coordinated manner with the coupling ion, the positive stator charge (aR227) and the membrane potential, as depicted schematically in Fig 5B. In the depicted model, the F_o motor rotates in the direction of ATP synthesis. A rotor site passing through the interface with subunit a experiences the following events: as the occupied site (site 1) enters the a/c interface, it is in the vicinity of the positive stator charge, which forces the bound Na^+ ion to strip off. The negative charge of site 1 is compensated by the positive stator charge. In the idling mode, site 1 can move by thermal fluctuations to either side if no external energy source is applied. However, in the presence of a membrane potential (cytoplasmic negative), the movement becomes directional (see below), bringing the empty site 1 into the periplasmic input channel in subunit a. The hydration of site 1 in the aqueous channel results in a drop of the free energy, which traps it at this position until a Na^+ ion has been bound. This directed movement has simultaneously 'pulled' the next occupied rotor site (site 2) on the c ring into the a/c interface. After displacement of the Na^+ ion, site 2 is pulled into juxtaposition with the stator charge, which 'pushes' the occupied site 1 from the a/c interface into the lipid phase. Therefore, the two rotor sites in the interface with the stator operate together in a 'push-and-pull' mechanism to achieve rotation of the rotor versus the stator.

How does the potential make the rotation unidirectional? It can be assumed that only part of the membrane potential drops vertically between the periplasmic channel entrance and its terminus in the middle of the membrane. However, the two-ion access routes from the periplasm and towards the cytoplasm also allow for a drop in horizontal potential in the middle of the membrane. This horizontal potential causes the stator charge to orientate towards the incoming rotor sites and the rotor charge to become attracted to the periplasmic entrance channel (Fig 5B). In this model, the membrane potential acts as the main kinetic driving force of unidirectional rotation and ATP synthesis.

In the ATP hydrolysis mode of the enzyme, the F_1 motor drives F_o in reverse to pump Na^+ ions from the cytoplasm into the periplasm. According to the model, a Na^+-loaded rotor site rotates to the left into the a/c interface. In the absence of a membrane potential, the stator arginine is close to the periplasmic channel and dislodges the Na^+ ion into it on ATP-driven rotation. The now empty site passes the stator charge and becomes occupied again through the cytoplasmic inlet pathway before the site leaves the interface to perform a new cycle.

The two models described above and in Fig 5 are distinct in their use of the ion release pathway, which is located in subunit a in the proton motor, whereas evidence now suggests the presence of an ion release pathway in the c ring of the Na^+-motor. The other main difference is in the energy source that drives each F_o motor out of the relaxed, idling mode and into unidirectional rotation. Apart from

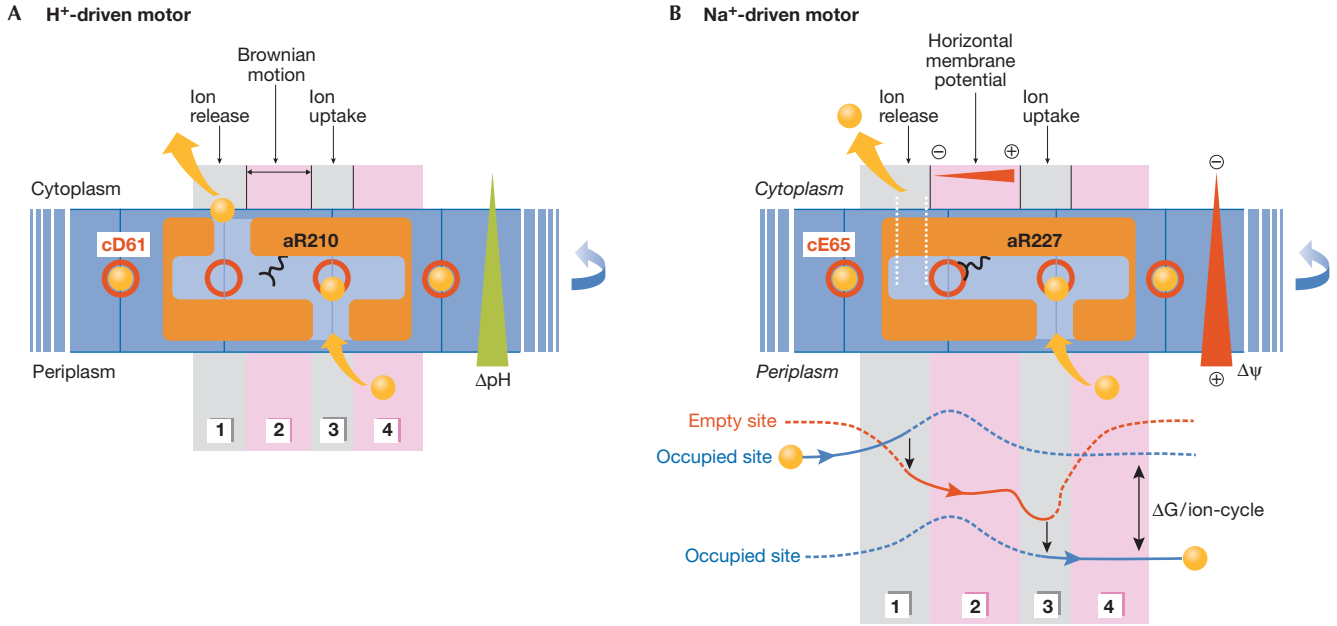

Fig 5 | Model for torque generation in the H^+- and Na^+-translocating F_o motor. (**A**) Two-channel model with a ratchet-type mechanism for H^+-dependent enzymes. The crucial events during ion translocation in ATP synthesis direction in the a/c interface can be divided into four different zones. (1) The occupied rotor site enters the interface and releases its coupling ion through the outlet channel into the cytoplasm with high pH. The deprotonation of the binding site prevents backwards rotation into the lipid phase and acts as a molecular ratchet. (2) The negative charge of the binding site is compensated by the stator arginine. In this functionally symmetric state, Brownian back-and-forth motions towards either channel are possible. (3) As the inlet channel, which is in contact with the periplasm where there is a low pH, contains more protons than the outlet channel, which is in contact with the cytoplasm, the binding site is more frequently protonated from the periplasm. Therefore, the ΔpH determines the direction of rotation. (4) The loaded binding site can now move out of the interface into the lipid bilayer, whereby the next binding site enters the interface and experiences the events described in (1). (**B**) Push-and-pull model for Na^+-dependent enzymes. The upper part shows the events taking place in the a/c subunit interface and the lower part shows the calculated free energy (ΔG) profiles of an empty or occupied site during ion translocation. Arrows indicate where a Na^+ ion is released or taken up, respectively. (1) In ATP synthesis direction, an occupied rotor site enters the interface from the left and releases its bound Na^+ ion towards the cytoplasm. This process is aided by the stator arginine. (2) The stator arginine compensates for the now negatively charged empty binding site. The horizontal component of the membrane potential, however, pulls the arginine to the left and pushes the glutamate to the right. Therefore, the electrical component of the ion motive force determines the direction of rotation from left to right. (3) The hydration of the binding site within the inlet channel stabilizes this conformation and allows loading of the binding site from the periplasm. Movement of the binding site from zone 2 to 3 pulls the next rotor site into the a/c interface as described in (1). (4) The binding site that has been occupied from the periplasm is allowed to rotate out of the interface into the lipid phase. This event is aided by a push mechanism during the events described in (2).

these differences, both models are similar. Each motor operating in the direction of ATP synthesis performs a molecular cycle, in which a specific binding site on the c ring captures a coupling ion from the side with high electrochemical potential and releases it to the side with low electrochemical potential. As loading and unloading of the binding site can only be accomplished at defined positions at the a/c subunit interface, ion translocation is closely connected to the rotation of the c ring. Therefore, a cyclic mechanical movement is linked to the ion translocation events in the F_o motor components and is transmitted through the camshaft-like rotating central stalk into the ATP synthesizing F_1 motor. These features of the ATP synthase impressively show that a combination of mechanical and catalytic cycles is the fundamental principle for the function of this sophisticated enzyme machinery.

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