ATP SYNTHASE — A MARVELLOUS ROTARY ENGINE OF THE CELL

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ATP synthase can be thought of as a complex of two motors — the ATP-driven F_1 motor and the proton-driven F_o motor — that rotate in opposite directions. The mechanisms by which rotation and catalysis are coupled in the working enzyme are now being unravelled on a molecular scale.

ELECTROCHEMICAL POTENTIAL GRADIENT
When two aqueous phases are separated by a membrane, the electrochemical potential difference of H^+ between the two phases is expressed as $\Delta\bar{\mu}_{H+} = F\Delta\psi - 2.3RT\Delta pH$, where F is the Faraday constant, $\Delta\psi$ is the electric potential difference between two phases, R is the gas constant, T is the absolute temperature and ΔpH is pH difference between two phases.

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"All enzymes are beautiful, but ATP synthase is one of the most beautiful as well as one of the most unusual and important" said Paul Boyer[1]. ATP synthase — also called the F_oF_1-ATP synthase or F_oF_1-ATPase — synthesizes cellular ATP from ADP and inorganic phosphate (P_i). The energy for ATP synthesis is provided from downhill H^+ (proton) transport along the gradient of ELECTROCHEMICAL POTENTIAL of protons across membranes[2] ($\Delta\bar{\mu}_{H+}$). This potential is built by the electron-transfer chains of respiration or photosynthesis, which pump protons against a gradient (FIG. 1).

Boyer's admiration for ATP synthase is justified for two reasons. First, ATP supports nearly all the cellular activities that require energy. ATP synthesis is the most prevalent chemical reaction in the biological world, and ATP synthase is one of the most ubiquitous, abundant proteins on Earth. From *Escherichia coli* to plants and mammals, this enzyme is one of the most conserved during evolution, with >60% of the amino-acid residues of the catalytic β-subunit being conserved[3–5]. Second, ATP synthase uses physical rotation of its own subunits as a step of catalysis — a novel mechanism, different from that of any other known enzyme. Rotation is not a favourite motion in living organisms; there is no animal with wheels, no bird with a propeller and no fish with a screw. On a molecular scale, apart from ATP synthase, only bacterial flagella are known as a rotary motor. The crystal structures of the main part of the ATP synthase show, in atomic detail, how the appearance of this tiny motor made from ~3,500 amino acids is remarkably reminiscent of man-made motors[6].

The story behind the study of the mechanism of ATP synthesis contains many dramas (BOX 1), and the tale is still being unravelled. In this review, we focus on the mechanism of rotary catalysis of ATP synthase — that is, how rotation and catalysis are coupled and regulated in the working enzyme.

F_1 and F_o are both rotary motors

ATP synthase is a large protein complex (~500 kDa) with a complicated structure. It is composed of a membrane-embedded portion, F_o (read as 'ef oh'), central and side stalks, and a large headpiece (FIG. 2). The central portion ($F_1\gamma\varepsilon$–$F_oc_{10–14?}$) rotates relative to the surrounding portion ($F_1\alpha_3\beta_3\delta$–F_oab_2) and, for convenience, we will call the former the 'rotor' and the latter the 'stator', although the rotor–stator relationship is relative. When the magnitude of $\Delta\bar{\mu}_{H+}$ is large, as in functional mitochondria, downhill proton flow through F_o causes rotation of the F_o rotor and, hence, rotation of the γε-subunits of F_1. The rotary motion of the γ alternates the structure of the β-subunit so that ATP is synthesized.

In the reverse reaction, ATP hydrolysis in F_1 induces the rotation of γ and, hence, of the F_o rotor in the reverse direction. This then drives proton pumping. In either case, the side stalk connecting the stator of F_o and that of F_1 prevents them being dragged by the central rotor. It is therefore possible to define the ATP synthase as a complex of two motors — an ATP-driven F_1 motor and a proton-driven F_o motor. They are connected by a common rotary shaft and their genuine directions of rotation are opposite. The motor motions have been visualized for F_1, as described below and in REF. 7, but this has yet to be done for F_o (REF. 8).

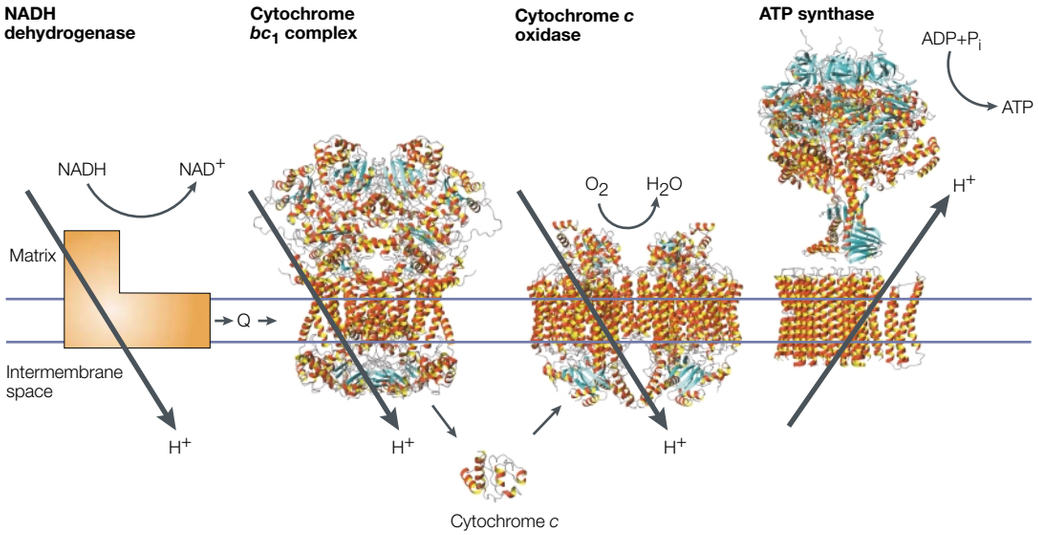


Figure 1 | **The respiratory chain and ATP synthase.** Electrons are transferred from NADH dehydrogenase to cytochrome *c* oxidase by coenzyme Q (Q), cytochrome bc_1 complex and cytochrome *c*. The established proton gradient across the inner mitochondrial membrane drives the proton flow in ATP synthase that accompanies ATP synthesis. Structures are taken from: cytochrome bc_1 complex[71]; cytochrome *c* oxidase[72]; the F_1 part of ATP synthase[52]; and the F_o part of ATP synthase[29].

Structure of F_1

In the initial crystal structure of the $(\alpha\beta)_3\gamma$-portion of native bovine mitochondrial F_1 (termed the 'native' structure)[6], three α-subunits and three β-subunits are arranged alternately, forming a cylinder of $(\alpha\beta)_3$ around the coiled-coil structure of the γ-subunit (FIG. 3a,b). The α- and β-subunits have a similar fold, as would be expected from their sequence similarity. All of the α-subunits are bound to the ATP analogue AMP–PNP, and the three subunits adopt very similar conformations. The three β-subunits, however, are in three nucleotide-bound states: the first, termed β_{TP}, has AMP–PNP in the catalytic site (FIG. 3c); the second (β_{DP}) has ADP; and the third (β_E) has no bound nucleotide (FIG. 3c–e). So, the native structure of F_1 looks like a snapshot of the working rotary engine, with three reaction chambers representing the moment just after exhaust and intake (β_E), ignition (β_{DP}) and compression (β_{TP}) (BOX 2). The lower part of the slightly bowing, asymmetric coiled-coil structure of the γ-subunit is displaced towards the β_E, forcing the carboxy-terminal domain of this β-subunit to swing ~30° downwards. Thus, the β_E adopts the 'open' (O) form, whereas β_{TP} and β_{DP} have the 'closed' (C) form.

A novel crystal structure of bovine F_1, with all three catalytic sites occupied by nucleotides, was recently reported[9]. Crystals were grown in Mg-ADP and aluminium fluoride (AlF_4^-; a dummy phosphate inhibitor that is expected to stabilize the conformations of a catalytic transition state). In this latest structure — termed $(ADP{\bullet}AlF_4^-)_2F_1$ — the two βs, which are identified as being equivalent to β_{TP} and β_{DP} in the native structure from their relative positions to the asymmetric γ-subunit, hold Mg-ADP•AlF_4 at their catalytic sites. Their structures are very similar to each other and to their equivalents in the native structure. The β-subunit equivalent to β_E takes a 'half-closed' (C′) conformation (FIG. 3d), and retains Mg-ADP and sulphate (a mimic of phosphate) at the catalytic site. In the C′-form β, the carboxy-terminal domain of the β_E swings ~23° upwards, and the distance between the β-phosphate of ADP and the sulphate would be too long to resynthesize ATP, even if sulphate were replaced by phosphate. The coiled-coil region of the γ-subunit twists ~20° in the region surrounded by the $(\alpha\beta)_3$ cylinder and ~10° in the region protruding from the $(\alpha\beta)_3$ cylinder.

Visualizing the rotation of F_1

ATP-driven rotation of the γ-subunit in the $(\alpha\beta)_3$ cylinder of F_1 has been visualized using the $(\alpha\beta)_3\gamma$-subcomplex from a thermophilic bacterium with three microprobes (FIG. 4). With reference to the crystal structure, the direction of the γ-rotation is such that one β undergoes a transition in the order β_{TP}, β_{DP} and β_E, consistent with ATP-hydrolysis-driven rotation. At very low concentrations of ATP, rotation occurs in discrete 120° steps, each driven by an ATP molecule that arrives at F_1. Distribution of the dwelling time (a period between one 120° step and the next) obeys an exponential decrease, confirming that one ATP is consumed per 120° step[10].

Long actin filaments (FIG. 4) rotate slowly and short ones rotate more rapidly, but the torque of rotary motion, calculated from the rotational velocity of an actin filament and the frictional resistance of water (viscous load), always reached ~40 pN nm^{-1}. The energy required to produce this magnitude of torque is ~8×10^{-20} J per 120° rotation; the free energy liberated from one molecule of ATP under the same conditions is ~9×10^{-20} J. So the efficiency of converting the energy of ATP hydrolysis into that of rotation seems to be very high —

SWITCH II REGION
The β-subunit of F_1 has a region that is topologically equivalent to the switch II region of guanine-nucleotide binding (G) proteins, which changes the conformation in response to the interconversion of GTP and GDP.

around 90% (REF. 10). Lower values (50–80%) have been reported on the basis of the rotation velocity of nickel bars attached to the γ-subunit[11]. Strictly speaking, a thermodynamically accurate efficiency of energy conversion requires the measurement of work taken out of the system (such as deflection of a laser trap), rather than rotation of a filament, which dissipates energy into the medium as heat[12].

120° = 90° (ATP on) + 30° (ADP/P_i off). When an actin filament is used as a rotation marker, the viscous load imposes an artificial limit on rotation (maximum 6–8 revolutions per second (rps)). A small gold bead (diameter 40 nm) obliquely attached to the γ-subunit, however, is not an impeding load, so is well suited for monitoring rapid rotation. Rotation is detected by light scattering of a laser beam and recorded by a video with 8,000 frames per second. Rapid monitoring of rotation thus attained has revealed insights into the mechanism of rotational catalysis by F_1 (REF. 13).

First, at high concentrations of ATP, a bead rotates at around 130 rps, consistent with a maximum rate of ATP hydrolysis (~300 s^{-1}; that is, about 100 rps). The velocities of rotation at ATP concentrations from 20 nM to 2 mM agree fairly well with those expected from the rates of ATP hydrolysis of free F_1 in the solution and obey simple Michaelis–Menten kinetics, with 15 μM being an ATP concentration that gives half-maximal rotation velocity. This indicates that it might not be necessary to assume a gear change mechanism as the ATP concentration varies in the range >20 nM.

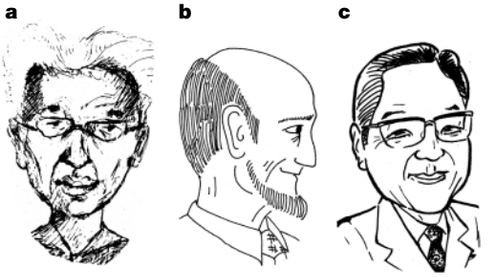
Second, the 120° step of rotation is further split into 90° and 30° sub-steps. Rotation velocities of the 90° and 30° sub-steps are very fast (<0.1 ms). Dwelling times between a 30° and a subsequent 90° sub-step become shorter as the concentration of ATP increases and finally disappear beyond the detection limit, indicating that the 90° rotation is triggered by binding of a single ATP to F_1 (but not by any subsequent catalytic event with a lifetime longer than 0.1 ms).

And last, dwelling times between a 90° and a subsequent 30° sub-step are always ~2 ms on average at all ATP concentrations. Analyses of dwelling-time distribution indicate that at least two events, each ~1 ms in length, occur sequentially in F_1. These events are most likely to be hydrolysis of a bound ATP (or the conformational transition necessary for hydrolysis of ATP), and release of the last remaining hydrolysis product, ADP or P_i. This second event triggers the 30° rotation, and F_1 is reset for the next round of catalysis. The physical events — the binding and release of substrate and product — are coupled with the power generation, just as Boyer predicted.

Open–closed motion of βs. The β-subunit in F_1 can take one of at least three forms — open (O), half-closed (C′) or closed (C) (FIG. 3c–e). Altering the contact with the asymmetric γ-subunit in F_1 will lead to a transition between these forms. Also, local changes of bound-nucleotide states at catalytic sites are amplified to the global open–closed transition of the βs. The isolated β-subunit takes the O form in the crystal structure (K. Miki and M.Y., unpublished observations), and it transforms to another conformation — most likely the C form — on binding nucleotide[14]. The O form β can readily accept ATP with 'zipping' of bonds between ATP and the catalytic sites, so that the open catalytic site is closed.

A comparison of the structures of the native structure (βs are in the CCO state) and $(ADP{\cdot}AlF_4^-)_2$ F_1 structure (CCC′ state) further suggests that when ATP is hydrolysed on the C-form β-subunit of F_1, the phosphate generated is dislocated 3.5–4 Å from the previous γ-phosphate position of ATP, and seems to push up the loop in the SWITCH II REGION. This, and other rearrangements of nearby residues, causes partial separation of

Box 1 | A brief history of research into ATP synthase



The molecular study of ATP synthase was initiated in 1960 when Efraim Racker (**a**) and his colleagues reported the isolation of a soluble factor from beef heart mitochondria. The factor, F_1, had ATP hydrolysis activity[61] and could restore ATP synthesis in membrane fractions that had lost this activity[62]. They also isolated a similar factor from chloroplasts, showing that the essential features of ATP synthesis in mitochondria and chloroplasts were the same. Since then, there have been at least two notable revolutions in the study of ATP synthase.

In 1961 (REF. 2), Peter Mitchell (a Nobel laureate of 1978 (REF. 63)) proposed the chemiosmotic hypothesis, in which a long-sought high-energy chemical intermediate that would connect the oxidation of respiratory fuel and ATP synthesis was declared to be an illusion. Instead, a high-energy state — the electrochemical potential of protons across a membrane $\Delta\bar{\mu}_{H+}$ — was postulated. Accordingly, the putative ATP synthase was predicted to be a proton-translocating ATP synthase/hydrolyase. This hypothesis was unfamiliar and very unpopular with biochemists of the time. But the situation was changed markedly in 1966 by Andre Jagendorf's (**b**) 'acid–base transition' experiment[64]. He imposed a pH gradient across chloroplast membranes and observed ATP synthesis in the absence of light. The chemiosmotic mechanism was finally established by experiments using the vesicle-reconstitution method initiated by Yasuo Kagawa (**c**) [65]. Driven by an artificially imposed $\Delta\bar{\mu}_{H+}$, vesicles containing purified ATP synthase catalysed ATP synthesis[66].

The next challenge was to discover how ATP synthase exchanges the energy of proton flow at F_o and ATP synthesis/hydrolysis at F_1. On the basis of the kinetics of enzyme-catalysed ^{18}O exchange between H_2O and P_i/ATP, Paul Boyer (a Nobel laureate of 1997 (REF. 67)) proposed the 'binding change' mechanism in 1977 (REFS 17,18). According to this mechanism, each of three catalytic β-subunits in ATP synthase alternates sequentially between states with different affinities to nucleotides. This binding affinity change — but not chemical conversion of ATP hydrolysis/synthesis — is coupled with energy input/output. Boyer further assumed physical rotation of the centrally located γ-subunit as a cause of sequential change[18].

Although the unusual cooperative kinetics of the enzyme (negative for ATP binding and positive for catalysis) supports the binding-change mechanism[68], the idea of the rotation was so novel that there were few serious attempts to test it until over a decade later, when John Walker (a Nobel laureate of 1997 (REF. 69)) and colleagues showed how the structure of bovine F_1 justified Boyer's prediction[6]. Once the rotation was considered to be plausible, it took only another three years to demonstrate it — ATP-dependent crosslinking exchange experiments came first[70], and then direct visualization of rotation of an actin filament attached to the γ-subunit swept away scepticism[7].

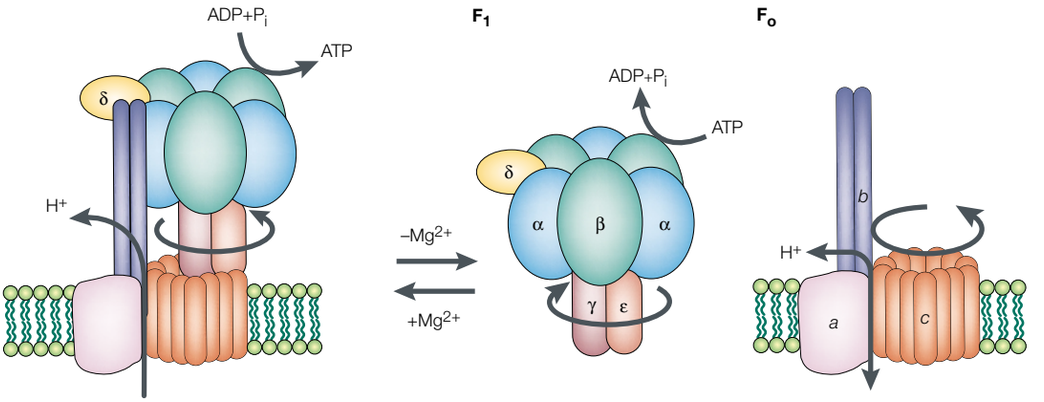


Figure 2 | **Structure of ATP synthase.** The bacterial ATP synthase is illustrated as the simplest version of ATP synthases. It is composed of a water-soluble protein complex of ~380 kDa, F_1, and a hydrophobic transmembrane portion, F_o. Removal of Mg^{2+} at low concentrations of salt allows the F_1 part to be extracted in water, leaving the F_o portion in the membrane. F_o and F_1 can, however, reassemble into the intact ATP synthase by adding back Mg^{2+} (REFS 61,73,74). This reversible separation of F_o and F_1 has benefited study in this field. F_o acts as a proton channel by itself, and isolated F_1 — often called the F_1-ATPase — catalyses ATP hydrolysis (which is considered a reverse reaction of ATP synthesis). The subunit structures of ATP synthases are well conserved during evolution, but there are some variations among sources. The simplest is bacterial ATP synthase, in which F_1 contains five kinds of subunit with a stoichiometry $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ and F_o contains three kinds of transmembrane subunit with a stoichiometry $a_1b_2c_{10-14?}$. The numbers of transmembrane helices are five (F_oa), one (F_ob) and two (F_oc). Chloroplast ATP synthase has the same subunit composition except that two kinds of F_ob homologue exist. Mitochondrial ATP synthase has at least six kinds of additional accessory subunit. Confusing subunit nomenclature remains for historical reasons (for example, mitochondrial δ corresponds to bacterial ε, and the mitochondrial subunit named OSCP corresponds to bacterial δ), but in this review we use the names of subunits according to the bacterial enzyme. The catalytic sites for ATP hydrolysis are located on the β-subunits of F_1, but residues of the α-subunits also contribute. The α-subunits contain a non-catalytic nucleotide-binding site, the function of which is not yet fully understood. The central stalk is made of the γ- and ε-subunits, and the side stalk from the $F_1\delta$- and F_ob_2-subunits.

the two β-strands (one before the P-LOOP and the other before the switch II loop), which leads to the outward (opening) movement of the whole carboxy-terminal domain of β. This movement produces the C′ form, and subsequent loss of ADP/P_i from the catalytic site sets the conformation of the β-subunit to the O form.

The open–closed state of each β in F_1 is thus decided by its bound-nucleotide state and by the orientation of γ. This means that the nucleotide states of βs in F_1 can determine the orientation of the γ-subunit and vice versa; the latter determines the former. The alternating, sequential ATP hydrolysis accompanies the coordinated open–closed motion of βs and thereby rotation of the γ-subunit. Conversely, if the γ-subunit is forced to rotate by the F_o motor, this can induce changes in the nucleotide-binding states of the β-subunits, resulting in the synthesis of ATP. Indeed, the dynamic occurrence of the CCO (or CCC′) state during catalytic turnover has been shown by the formation of a specific crosslink that can bridge only between two C-form β-subunits in an F_1 molecule[15], and the activity of F_1 is lost by fixing the β-subunits in the C form[16].

Models to account for catalysis and rotation

Boyer's classic model of rotary catalysis assumes that one or two catalytic sites are occupied by ATP (or ADP) at any moment of steady-state catalysis[17,18] (BOX 2). The $(ADP{\bullet}AlF_4^-)_2F_1$ structure, however, favours the models by which two or three catalytic sites are filled with ATP (or ADP), as previously indicated from the ATP-induced change of tryptophan fluorescence[19]. Walker and colleagues[9] have proposed a model by which concerted transitions — the closing of β_E driven by ATP binding and the opening of β_{DP} driven by ATP hydrolysis — induce the 120° rotation of the γ-subunit. A similar model was postulated previously by Ren and Allison[20].

The latest evidence of the 90°–30° sub-steps, as well as the $(ADP{\bullet}AlF_4^-)_2F_1$ structure, lead us to propose a model to explain the catalysis–rotation relationship (FIG. 5). Details can be found in the figure legend, but three main points are worth mentioning here. First, the γ-subunit in the $(ADP{\bullet}AlF_4^-)_2F_1$ structure has a ~20° clockwise twist (viewed from the membrane side) at the middle part compared with the native structure. So, the ADP/P_i release from the C′-form β (CCC′ → CCO) probably induces a relaxation of this torsion; that is, a ~20° anticlockwise rotation, which probably corresponds to the observed 30° sub-step.

Second, as pointed out by Walker and colleagues, when one β-subunit takes the C′ form, another β-subunit (located at the anticlockwise side, viewed from the membrane side) might adopt a catalytically active C form (denoted C_A), which mediates reversible cleavage of the γ-phosphate of ATP. Last, this model can explain why ATP synthase binds ADP and P_i preferentially in the presence of excess ATP in the ATP-synthesis reaction — proton flow through F_o drives the ~30° rotation of the γ-subunit, turning the empty O-form β to the empty C′-form β that can accept only ADP and P_i. Without ADP and P_i, the empty C′-form β is not filled and ATP synthesis would stop at this point. Consistent

P-LOOP
Various ATP-metabolizing proteins contain a consensus sequence Gly-X-X-Gly-X-Gly-Lys-Thr (X is variable). This sequence is found in a loop connecting a β-strand (adjacent to a β-strand of switch II region) and an α-helix. The lysine and threonine residues in the P-loop are recruited for binding the phosphate moiety of nucleotides.

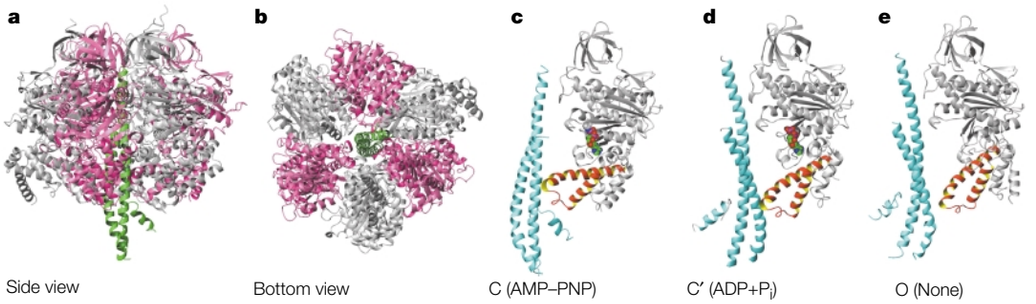


Figure 3 | **The crystal structure of mitochondrial F_1-ATPase.** Side view (**a**) and view from the bottom (**b**) of the $\alpha_3\beta_3\gamma$ part of bovine heart mitochondrial F_1 (REF. 3). A coiled-coil structure of the γ-subunit penetrates the $(\alpha\beta)_3$ cylinder. This structure apparently embodies Boyer's rotary catalysis hypothesis. **c**–**e** | Three conformations of β-subunits. The structure of the γ-subunit is also shown. **c** | Closed (C) form. A β-subunit with bound AMP–PNP (β_{TP}) is shown. β-subunit with bound ADP (β_{DP}) and the α-subunits are also in the closed form. **d** | Half-closed (C′) form. A β-subunit with bound ADP and sulphate (a mimic of phosphate) is shown. **e** | Open (O) form. A β-subunit with an empty catalytic site (β_E) is shown. The carboxy-terminal helix-rich domain of the C′ and O forms of βs swing ~23° and ~30° outwards, respectively, from the centre of the molecule as a rigid body. The helices of the domain are highlighted.

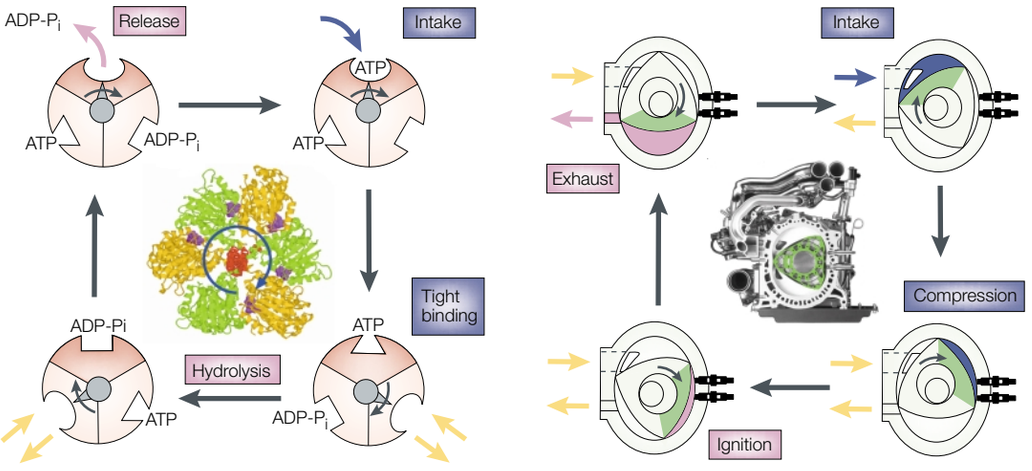
with this, $\Delta\bar{\mu}_{H+}$ alone is not enough to promote the release of product ATP from ATP synthase or to drive rotation of the γ-subunit; in both cases ADP and P_i are required[21–23].

The F_oc ring is a rotor

Although the whole structure of the F_o part of ATP synthase is not known, the crystal structure of $F_1F_oc_{10}$ of the yeast ATP synthase has shown that F_oc-subunits are arranged as a ring, and that the foot of the central shaft γε lands on — but does not penetrate into — the cytoplasmic surface of the ring[24]. It is generally thought that F_oa associates with the outside surface of the F_oc ring, and that F_ob_2 associates with F_oa and $F_1\delta$.

The F_oc ring is a rotor. Rotation of both the γ-subunit and the F_oc ring by ATP has been shown by an actin filament attached to the amino termini of F_oc on the immobilized ATP synthase[8,25,26]. However, rotation was even observed for an enzyme with inactivated F_o, which had lost the ability to transport protons, and it turned out that subunit associations in F_o were impaired by the detergents used[8]. In fact, all of the F_o-

Box 2 | **Rotary engines in the car and in the cell**



The F_1 motor reminds us of the rotary combustion engine, which was invented by Felix Wankel in 1957 and was first used in commercial cars by Mazda in 1967. The rotary engine is small, light, silent and simple because the engine can directly convert the fuel energy into rotation of the rotor. It can drive the intake of the fuel gas, compression, ignition and exhaust sequentially just by a simple rotation of the central rotor, which is quasi-triangular in shape (right panel). The events occurring on one side of the rotor (green) are annotated.

The F_1 also has a central rotor — the γ-subunit — and three reaction chambers (the catalytic β-subunits; left panels). The events occurring in one β-subunit (light red) are annotated according to Boyer's classic model. The basic principles behind the functioning of these rotors — three reaction sites in turn doing each of three cyclic steps in a 120° phase difference to cause rotary motion — are remarkably similar.

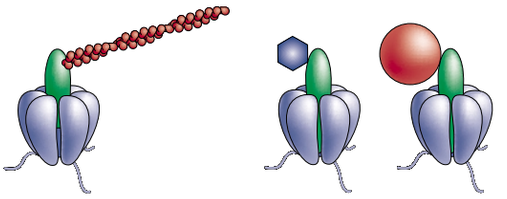


Figure 4 | **Microprobes to detect the rotation of a nanomotor.** The $(\alpha\beta)_3$ cylinder is fixed on the glass surface and one of three kinds of rotation marker is attached to the γ-subunit. **a** | A fluorescently labelled actin filament (1–4 μm)[7]. **b** | A single fluorescent dye (~2 nm)[75]. **c** | A bead (gold (40 nm) or polystyrene (0.5 μm))[13].

subunits, with the exception of $F_o c$, were lost from the crystals of yeast ATP synthase formed in dodecylmaltoside[24]. Evidence from biochemical studies showed that ATP synthase, in which γ, ε and $F_o c$ were crosslinked, retained activities of ATP synthesis and ATP-driven proton translocation[27]. Thus, γ, ε and the $F_o c$ ring must rotate together as one body without slack. Note that the $F_o c$ ring in ATP synthase is permanently asymmetric because the γε-subunits are connected to the fixed members of $F_o c$-subunits in the ring. ATP-dependent subunit rotation between the $F_o c$ ring and $F_o a$ was supported by $F_o c$–$F_o a$ crosslinking experiments[28].

Symmetry mismatch between F_1 and F_o. The assumption that ATP synthase contains 12 copies of $F_o c$-subunits used to be generally accepted. In this case, one proton moves the $F_o c$ ring by 30°, and four protons drive the 120° that matches one step of rotation of γ (proton:ATP ratio is 4). Models for the F_o motor have been proposed and discussed on this assumption[29], but several recent structural studies do not support it (FIG. 6). For example, the crystal structure of a partial complex of yeast ATP synthase contains ten copies of the $F_o c$-subunits[24]. Atomic-force and electron micrographs showed 14 copies of $F_o c$ in chloroplast ATP synthase[30], and 11 copies in *Ilyobacter tartaricus* ATP synthase[31]. The possibility that this is an artefact arising from the deteriorating effect of the strong detergents used in these experiments (sodium dodecylsulphate) has to be kept in mind, but a recent experiment in the absence of detergents using genetically fused $F_o c$ polymers in *E. coli* ATP synthase revised the previous stoichiometry of $F_o c$-subunits from 12 to 10 (REF. 32). So, the possibility is increasing that the number of copies of $F_o c$-subunits in the $F_o c$ ring differ, depending on the species or even on the growth conditions, as suggested by Schemidt and colleagues[33].

If this is the case, there are several serious — but interesting — consequences. The first is that the proton:ATP ratio can be non-integral and variable — analogous to the clutch plate of a car, which allows more slip than the tight toothing of two gears. Second, the rotation step of the γ-subunit (120°) cannot be a multiple of that of $F_o c$ (36° if 10 copies of $F_o c$ exist), despite the fact that the γε-subunits and the $F_o c$ ring rotate together as an ensemble without displacement (as shown by a γ–ε–$F_o c$ crosslink study[27]). Torque force is generated and transferred at the precisely aligned stator–rotor interfaces between the γ- and β-subunits and between the $F_o c$- and $F_o a$-subunits. However, after each step of rotation, misalignment inevitably happens to one of the contact sites and it must be adjusted to the right positions by some means. Both a side stalk, $F_o b_2$–$F_1\delta$ that has an extra flexibility[34], and the coiled-coil structure of the γ-subunit that allows some internal twisting, are good candidates for the 'absorber' of this transient structural torsion accompanied with the adjustment.

Electrostatic motor versus power stroke. The mechanism of the F_o motor remains more elusive than that of the F_1 motor. When a proton passes through $F_o c$, $F_o a$ exerts a sliding force on the $F_o c$ ring without breaking its association with the ring. Therefore, two kinds of interaction or contact site, a driving unit and a rail, are assumed between the $F_o c$ ring and $F_o a$. Two essential residues — proton-translocating carboxylate (aspartate or glutamate) in transmembrane helix 2 of $F_o c$ and the conserved arginine residue in transmembrane helix 4 of $F_o a$ — would lie closely and presumably form a proton channel. Some models propose that the change in electrostatic interactions between these two residues upon protonation/deprotonation is a key step of torque generation[35,36]. However, one constraint on this model is the varied arrangement of proton-translocating carboxylates seen in the V-ATPASE family, which is evolutionarily related to ATP synthase.

The proton motor of the V-ATPase is thought to have the same mechanism as that of the F_o motor. The

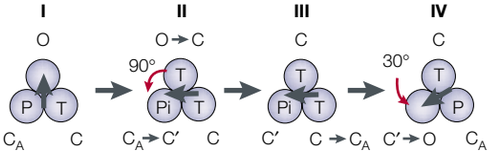


Figure 5 | **Model for the rotary catalysis of ATP synthase.** The change of states of the three β-subunits and the γ-subunit (arrow) during hydrolysis of one ATP (120° rotation) is illustrated. Starting from the CCO state (I), the sequence of events in ATP hydrolysis reaction is as follows. I→II, ATP binds to the O-form β, which undergoes an O→C transition. Simultaneously, the C_A form β undergoes a C_A→C′ transition and the γ-subunit rotates 90°. This process takes place within 0.1 ms. II→III, the C-form β undergoes a C→ C_A transition. This occurs at a rate constant of ~1 × 10^3 s^{-1} (lifetime ~1 ms). III→IV, ADP/P_i is released from the C′-form β which then undergoes a C′→O transition and the γ-subunit rotates 30°. This process takes place at a rate constant of ~1 × 10^3 s^{-1} (lifetime ~1 ms). Transitions II→III and III→IV are rate-limiting steps at saturating ATP concentrations. The C_A→C′ transition accompanies the separation of P_i from ADP on F_1. A single β-subunit undergoes a sequential change of states, C(ATP)→C_A(ADP•P_i)→C′(ADP+P_i)→O(empty)→C(ATP), during three catalytic turnovers of ATP hydrolysis. In ATP synthesis, the reversed order of events occurs. C, closed-form of β-subunit that accommodates ATP (denoted by T); C′, half-closed form of β-subunit that accommodates ADP and P_i (denoted by P_i); C_A, catalytically active closed form of β-subunit on which ATP and ADP•P_i are interconvertible (denoted by P); O, open form of β-subunit with an empty catalytic site.

V-ATPASE
V-ATPase is responsible for ATP synthesis in archaebacteria and a small number of eubacteria. In eukaryotic cells, it works as a proton-translocating machinery driven by ATP hydrolysis, and it is responsible for the acidification of lysosome lumens, chromaffin granules and vacuoles.

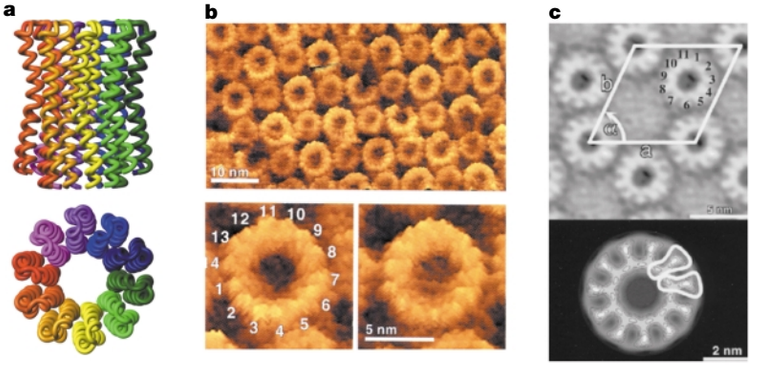


Figure 6 | **How many copies of the F_o*c*-subunit are in the ring?** Different numbers of F_o*c*-subunits in the F_o*c* ring have been reported. **a** | Ten copies. Crystal structure of the F_o*c* ring of yeast mitochondrial ATP synthase[24]. **b** | 14 copies. Atomic-force micrograph image of the spinach chloroplast F_o*c* ring[30]. **c** | 11 copies. Cryo-electron micrograph image of the F_o*c* ring from *Ilyobacter tartaricus* ATP synthase[31].

c-subunits of V-ATPases (V_0*c*) are mostly a fused dimer form of a prototype single-hairpin structure of F_o*c*, but the carboxylate (Glu or Asp) essential for proton translocation is found only in the second hairpin (helix 4) in V_0*c*. A fused trimer form of the V_0*c*-subunit is also known; here, essential carboxylates are conserved in the second and third hairpins but lost in the first[37]. ATP synthase containing fused dimer(s) of F_o*c*, with the essential carboxylate being only at helix 1, has also been reported[38]. The variable alignment of essential carboxylates in the ring does not favour the models that assume lateral proton diffusion among the carboxylates[39]. An NMR study of monomeric *E. coli* F_o*c*-subunits in a water-saturated organic solvent showed that deprotonation of an essential carboxylate induced rotation of helix 2 as a unit about its axis by 140° (REF. 29). If this takes place in F_o, local rotations within the F_o*c*-subunit at the contact surface with F_o*a* would push the F_o*c* ring. This mechanism is more like a power stroke than an electrostatic motor. However, F_o*c* retains considerable activity when the essential carboxylate is moved from helix 2 to an opposite position of helix 1 of the hairpin structure of F_o*c* (D61G/A24D)[40]. It is not easy — but provocative — to think of a motor mechanism that allows, or even favours, variable copies of F_o*c* and variable arrangement of essential carboxylates in the *c* ring.

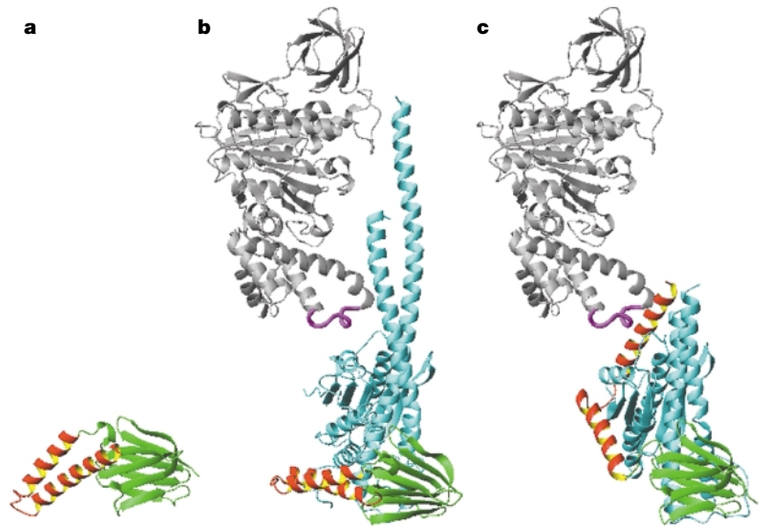


Figure 7 | **Conformational transition of the ε-subunit. a** | Structure of the isolated ε-subunit of *Escherichia coli* ATP synthase[76]. This is a 'down' conformation. **b** | Structure of bovine F_1 (REF. 52). The β_{TP}-subunit is shown, but other α- and β-subunits are omitted for simplicity. The structure of mitochondrial δ-subunit (equivalent to the bacterial ε-subunit) is similar to, if not the same as, the down conformation. **c** | Structure of the ε-subunit co-crystallized with the truncated γ-subunit of *E. coli* ATP synthase[51]. Using the structure of the γ-subunit as a reference, the β_{TP}-subunit has been put into the figure. Whereas the amino-terminal β-barrel domain of the ε-subunit (green) remains largely unchanged, the two carboxy-terminal helices (red) stand up along the γ-subunit and can reach the DELSEED region (violet) of the β_{TP}-subunit.

Control of organellar ATP synthases

In living cells, the demand for ATP and the supply of respiration fuel (and, for photosynthetic organisms, sunshine) for the synthesis of ATP varies from one moment to the next. ATP synthase starts hydrolysing ATP as the magnitude of $\Delta\bar{\mu}_{H+}$ becomes small, and, to prevent the wasteful consumption of ATP, the activity of ATP synthase must be suppressed.

Chloroplast ATP synthase is regulated by the formation or cleavage of a disulphide bond between two cysteine residues in a chloroplast-specific extra sequence in the γ-subunit. When exposed to the light, chloroplasts reduce this disulphide bond through thioredoxin, and ATP synthase is activated to synthesize ATP (REF. 41). In the dark, sulphydryls are oxidized to form a disulphide bond, and ATP hydrolysis activity is suppressed. The peptide segment containing two cysteines can work as a transferable 'micro-switch' cassette, and its introduction into F_1s from cyanobacteria[42] and thermophilic bacteria[43] makes their ATPase activities redox-sensitive. Rotation of F_1 pauses frequently when the γ-subunit is oxidized (D. Bald and T.H., unpublished observations).

The ATP hydrolytic activity of mitochondrial ATP synthase is inhibited by binding of a 9-kDa basic protein[44]. Binding depends on the presence of ATP-Mg and acidic pH; unsuitable conditions for ATP synthesis. Recent structural studies indicate that, as pH is lowered, a non-inhibitory tetramer of the protein dissociates into an inhibitory dimer, which connects two ATP synthases through their F_1 portions[45,46].

Kinetic and mechanistic regulation. In all ATP synthases from mitochondria, chloroplasts and bacteria, kinetic and mechanistic regulation is known. The catalytic turnover of ATP hydrolysis by ATP synthase and F_1 is interrupted by occasional trapping of ADP-Mg at the catalytic site(s). The binding of ATP to non-catalytic nucleotide-binding sites on the α-subunits facilitates the release of ADP-Mg from the affected catalytic sites, thereby recovering the ATP-hydrolysis activity[47,48]. At the single-molecule level, the ADP-Mg inhibition is recognized as long (~30 s) pauses of rotation (Y. Hirono-Hara and M.Y., unpublished observations). ATP synthase during steady-state ATP hydrolysis is, therefore, a dynamic mixture of the inhibited and uninhibited molecules. So, spontaneous switching of the enzyme between active

and inactive states, with a timescale much slower than the catalytic turnover, contributes to regulation. Interestingly, ATP synthesis is free from this type of inhibition[49,50].

The ε-subunit is an endogenous inhibitor of ATP synthase. It undergoes a drastic conformational change, with a non-inhibitory 'down' form and an inhibitory 'up' form. The carboxy-terminal α-helix of the ε-subunit lies on the F_o*c* ring in the down-form, or it is lifted up to reach the bottom of an $(\alpha\beta)_3$-cylinder in the up-form[51–53] (FIG. 7). Electrostatic interactions between basic residues in the α-helix of the ε-subunit and acidic residues of the conserved 'DELSEED' region of the β-subunit seem to stabilize the association of the ε- and β-subunits, and the rotation is blocked[54]. The occupation and absence of AT(D)P at the second catalytic site facilitates up-to-down and down-to-up transitions, respectively[55]. The ratchet-like function of the ε-subunit is intriguing; when the ε-subunit is fixed in the up-form by covalent crosslinking to the γ-subunit, ATP-synthesis activity is maintained whereas ATP hydrolysis is blocked[56].

Unidirectional inhibition of the motor. In nature, all enzymes catalyse both forward and reverse reactions, and it is impossible to block the reverse reaction without affecting the forward one. Therefore, the apparently unidirectional inhibition of ATP hydrolysis described above can be explained only by transformations of ATP synthase. ATP synthase somehow senses conditions favourable for ATP synthesis and transforms itself into a high-catalytic-activity form. One signal could be a large $\Delta\bar{\mu}_{H+}$, and indeed, activation by $\Delta\bar{\mu}_{H+}$ was reported for ATP synthases from chloroplasts[57], mitochondria[58] and bacteria[59].

$\Delta\bar{\mu}_{H+}$ is composed of ΔpH and $\Delta\psi$ (the electric potential difference across membranes), and an exclusive role for $\Delta\psi$ as a signal has been proposed[60]. The transformation could involve the following: release of the inhibitory ADP-Mg; transition of the ε-subunit to the non-inhibitory form; cleavage of a disulphide bond (chloroplast ATP synthase); and release of an inhibitor protein (mitochondrial ATP synthase). This high-activity form of the enzyme has the potential to catalyse both hydrolysis and synthesis at high efficiency, but the thermodynamic balances allow only the net synthesis of ATP. Under conditions that are favourable for ATP hydrolysis, but not for ATP synthesis, ATP synthase drops into a low-activity form (both for ATP synthesis and hydrolysis), and ATP hydrolysis is suppressed. How the thermodynamic conditions define ATP synthase activity is yet to be clarified.

Perspectives

ATP synthase is a splendid enzyme. But why is it so? For the purpose of proton-flow-driven ATP synthesis alone, a much simpler enzyme could have been adopted. The most challenging question is why ATP synthase needs to rotate if it is not a machine of movement. Probably related to this, the reversible separation of F_1 from F_o must have a functional basis that we do not know.

The study of how ATP synthase is regulated is just beginning to come into our molecular scope and two research directions — the actual role in living cells under various conditions and the mechanisms that allow the dynamic behaviours of enzyme molecules — need to be explored.

As a motor protein, ATP synthase offers a rare research opportunity. Structures of the F_1 motor, both rotor and stator in the same assembly, are known in atomic detail for the first time, and rotation can be analysed at sub-millisecond time resolution. And the atomic structure of the F_o motor is expected to be determined in the near future. An emerging possibility of step-size mismatch between the F_1 and F_o motors provides an opportunity to find a novel coupling mechanism of the two motors that will explain why the mismatch is good for the enzyme. Finally, one can even dream of using this, the world's tiniest motor, as an engine part in the fabrication of nano-machines. The marvel of ATP synthase will continue.

Links

FURTHER INFORMATION Yoshida lab | Hisabori lab

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