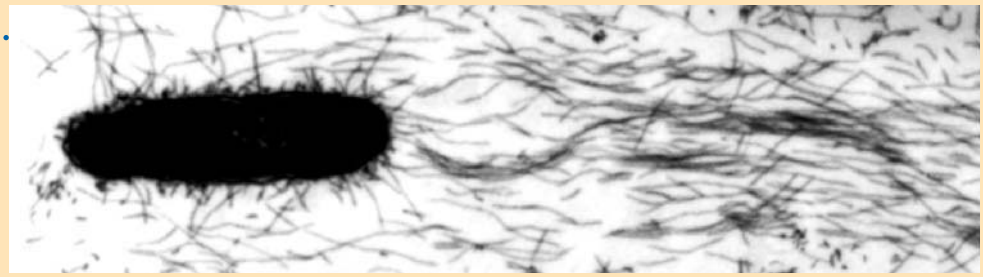
Cell biology

Pathogen propulsion



Bacterial pathogens have a cunning way of moving about inside the cells they infect — they harness components of the host's cytoskeletal machinery, in particular, the protein actin.

One such pathogen, *Rickettsia conorii*, is transmitted to humans through tick bites, and causes Mediterranean spotted fever. This bacterium assembles an elaborate 'tail' made of actin filaments (pictured) to propel itself through the cytoplasm of the infected host cell and to invade neighbouring cells. *Rickettsia* tails consist of parallel, unbranched filaments that closely resemble those present in thread-like cellular projections called filopodia.

Elsewhere in this issue, Pascale Cossart and colleagues shed light on the molecular mechanism that generates *Rickettsia* tails (E. Gouin *et al. Nature* **427,** 457–461; 2004). The authors had previously identified a *Rickettsia* protein, RickA, that is structurally similar to members of the WASP family found in higher organisms. WASPs are potent activators of the Arp2/3 complex, which nucleates actin filaments.

In the latest development, Cossart's group has found that RickA occurs on the bacterial surface, where actin filaments are generated. Using an *in vitro* actin polymerization assay, they go on to show that although RickA on its own cannot form actin filaments, it can activate the Arp2/3 complex — thereby stimulating actin polymerization. They extend these studies to show that *Rickettsia* uses the Arp2/3 complex *in vivo* to form actin tails, and that RickA can induce the formation of filopodium-like projections when it is targeted to the plasma membrane. So future work on *Rickettsia* motility might help to clarify the poorly understood process of filopodium formation.

Rickettsia actin tails are very different to those created by another pathogen, *Listeria monocytogenes,* which consist of shorter, highly branched arrays of actin filaments. Nevertheless, the *Listeria* tails are also formed by the Arp2/3 complex, which the bacterium recruits from the host cell using the surface protein ActA. So pathogens may have evolved different ways to induce actin polymerization using the same effector, the Arp2/3 complex.

Deepa Nath

Ashassi-Sorkhabi and colleagues' work got me wondering whether there were any other natural compounds that act as inhibitors. A quick bibliographic search revealed a long history of research on the anti-corrosion properties of naturally occurring materials: extracts of prickly pear[2], henna[3], rosemary[4] and honey[5] have all been cited as providing a greater than 50% reduction in the corrosion rate of carbon steels, aluminium alloys and copper alloys, in chloride media of up to one-molar concentrations. And historically, tannins[6] were used in the steam age to reduce boiler corrosion.

Particularly fascinating, however, is recent work using organisms (bacillae or fungi) that have been genetically modified to secrete corrosion-inhibiting species, such as polyphosphate[7]. Normally, biofilms tend to accelerate corrosion, but inoculation with these 'smart' organisms can, in appropriate environments, substantially reduce it. Such technology potentially offers immense benefits for environmentally friendly corrosion control. I look forward to the day when we might all be advised to add yoghurt to our central heating systems! Corrosion might then cease to be the hidden enemy no one wants to discuss. ■

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Molecular motors

Turning the ATP motor

Richard L. Cross

A long-standing question regarding ATP synthase — a cellular energy-generator — has been which direction it spins in when generating ATP. Some elegant experiments have revealed the answer.

The ATP synthase not only lays claim to being nature's smallest rotary motor, but also has an extremely important role in providing most of the chemical energy that aerobic and photosynthetic organisms need to stay alive. On page 465 of this issue, Itoh and colleagues[1] describe how they used electromagnets to force this motor to rotate and generate chemical energy (adenosine trisphosphate, ATP). In the process, they determined the direction of rotation during ATP synthesis.

ATP synthase is composed of two linked multi-subunit complexes, called F_0 and F_1. F_0 is embedded in cellular membranes and conducts protons, whereas F_1 is a peripheral complex and contains the catalytic sites. Together they couple the flow of protons down an electrochemical gradient to the synthesis of ATP from ADP (adenosine diphosphate) and inorganic phosphate.

The major — and initially controversial — features of this process were first recognized by Paul Boyer, who developed the concept of the 'rotary binding-change' mechanism. The first surprise came from his discovery that the energy derived from proton transport is not used to promote the synthesis of ATP at the catalytic sites on F_1. Instead, ATP forms spontaneously from tightly bound ADP and phosphate (Fig. 1a, step 2, overleaf); the energy instead drives the subsequent release of ATP from that site[2]. Also associated with this dissociation step (Fig. 1a, step 1) is an increase in the affinity of adjacent catalytic sites for ADP and phosphate[3]. A second surprise was the recognition that the coupling process probably involves subunit rotation[4].

In the binding-change model, then, F_0 and F_1 function as a pair of rotary motors linked by a central rotor and a peripheral stator (Fig. 1b). Rotation of the ring of *c*-subunits in F_0 is proposed to allow protons to be carried between two channels, formed by the *a*-subunit, that connect to opposite sides of the membrane. Because of the existence of the stator[5,6], and because the γ- and ϵ-subunits of the central rotor are firmly attached to the top of the *c*-ring[7], rotation of the *c*-ring forces the γ-subunit to rotate in the centre of F_1. This in turn drives net ATP synthesis by inducing cyclical conformational changes in the surrounding subunits, thereby altering binding affinities at the catalytic sites (hence 'binding-change' model; Fig. 1a, step 1). Strikingly, under

conditions in which more energy is stored in the ATP pool than in the electrochemical proton gradient, the engine can carry out the opposite reaction, consuming (hydrolysing) ATP to rotate F_0 and pump protons up a concentration gradient.

The rotary aspect of this theory remained a popular but speculative idea for years, until compelling evidence was provided by structural[8], biochemical[9] and spectral[10] studies. Then, in a dramatic visual demonstration, a fluorescent actin filament attached to one end of the γ-subunit of immobilized F_1 was seen by fluorescence microscopy to undergo multiple unidirectional rotations during ATP hydrolysis[11]. The direction of rotation, as viewed from the position of F_0, was anticlockwise. Subsequent biochemical experiments[12] established that energy-driven rotation of the γ-subunit occurs during ATP synthesis as well. But in this case the direction of rotation could not be determined by the approach used. So the question of whether or not the direction of rotation reverses when the direction of catalysis reverses has, until now, remained unanswered.

In this regard, it is interesting that the only other well-characterized biological rotary motor — the bacterial flagellar motor — has a transmission that, in response to regulatory signals, can shift the direction of rotation between forward and reverse without changing the direction of proton movement through the membrane. In contrast, the ATP synthase is smaller and simpler, and because of this it has long been thought that the direction of the coupled chemical events (ATP synthesis or hydrolysis) and proton transport (down or up a gradient) would indeed be reversed upon reversing the direction of subunit rotation. In the experiments presented by Itoh *et al.*[1], this prediction is at last confirmed.

The authors accomplished this feat by attaching magnetic beads to the γ-subunits on isolated F_1 complexes, which were immobilized by fixing their tops to a glass surface. The authors then used electromagnets to rotate the beads (substituting for proton transport), which in turn forced the γ-subunits to rotate. ADP and inorganic phosphate were included in the experiment, as well as an assay for monitoring the concentration of ATP. Itoh *et al.* found that rotating the γ-subunit in the anticlockwise direction — previously shown to accompany ATP hydrolysis — caused a decrease in ATP levels. But rotating the γ-subunit in a clockwise direction caused an increase in ATP. The results establish a hard-wired reversibility in the F_0F_1 rotary motor.

An interesting feature of Itoh and colleagues' experiment is that it works only at low rotational speeds, that is, roughly 5% of the maximal ATP-driven rate. As the speed increases, bead rotation becomes uncoupled from the magnet. This is not surprising because the rotary torque is applied at a constant rate in the experiments, whereas under physiological conditions the γ-subunit can stop rotating for significant periods during substrate binding, product release, and catalysis[13,14]. So it might have been predicted that, with increasing speed, a point would be reached at which the pauses limit bead rotation such that it falls out of synchronization with the rotating magnetic field.

As often happens with significant advances, another laboratory has independently reached the same conclusion about the mechanical properties of ATP synthase. Diez and colleagues[15] report the use of fluorescence resonance energy transfer to measure the distance between a pair of probes attached to the *b*- (stator) and γ- (rotor) subunits. Three distinct distances are clearly evident, and correspond to the orientation of the γ-subunit when paused at 120° intervals (see Fig. 1a). As expected, the distance between the probes changes during catalytic turnover, in repeat sequences that progress at a rate equal to that of catalysis. Furthermore, the order of the sequence reverses in going from ATP hydrolysis (close to intermediate to distant) to ATP synthesis (distant to intermediate to close), again establishing a correspondence between the direction of catalysis and the direction of rotation. These studies[1,15] not only further our understanding of an essential energy-transducing complex but also enhance the prospects for exploiting this rotary motor to develop nanodevices. ■

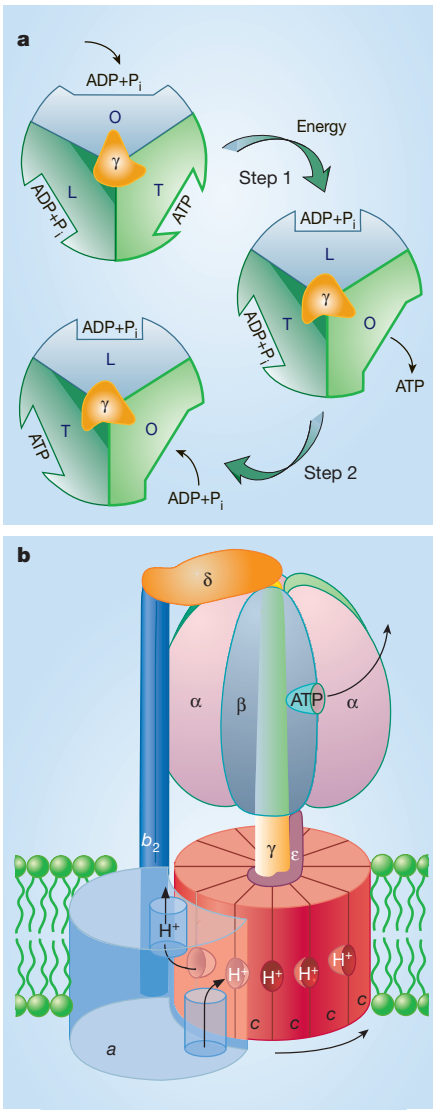

Figure 1 **The binding-change model for F_0F_1 ATP synthase.** a, **Looking up at F_1 from the membrane. Each blue or green area represents a pair of α- and β-subunits, in which the catalytic sites are interfacial but mostly on the β-subunit. In step 1, the γ-subunit rotates through 120°, driving conformational changes in the three surrounding catalytic sites that alter their affinities (O, L or T, for open, loose or tight) for substrates and product. In step 2, ATP forms spontaneously from tightly bound ADP and inorganic phosphate (P_i).** b, **View from the side of F_0F_1. The *a*-subunit contains two partial channels. For a proton to traverse the membrane, it must move through one channel to the centre, bind to one of the *c*-subunits and then be carried to the other partial channel by rotation of the *c*-ring. The *c*-subunits are anchored to the γ-subunit (part of the rotor), whereas the *a*-subunit is anchored through $b_2\delta$ (the stator) to the $\alpha_3\beta_3$ hexamer. Hence, rotation of the *c*-ring relative to the *a*-subunit in F_0 will drive the rotation of the γ-subunit relative to the $\alpha_3\beta_3$ hexamer in F_1. New results[1,15] show that the γ-subunit rotates in a clockwise direction when the engine generates ATP.**

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