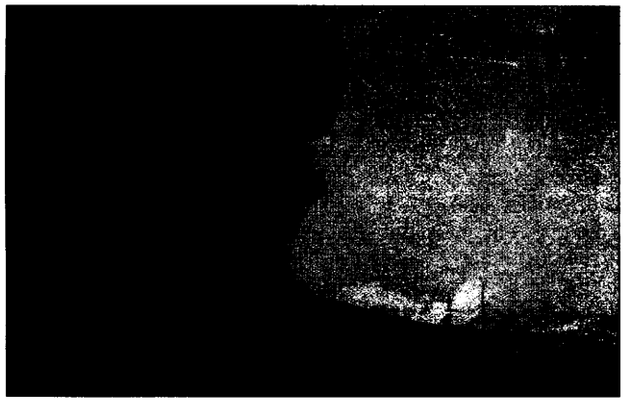

Past Vesuvian activity. This painting of the 1794 eruption as viewed from Naples is typical of Strombolian activity between 1631 and 1944.

termine which, we must know if there is magma within the crust below Vesuvius.

Multiple geophysical surveys by 12 groups from the European Community culminated in the recent TOMOVES (Vesuvius tomography experiment) (*5–7*). These surveys included seismic surveys across the Campanian Plain (see the figure on the previous page) that were linked to seismic surveys in the Bay of Naples. The surveys verified an interface at a depth of 2 km between the limestone "basement" and a sequence of overlying volcanic rocks and marine sediments. Knowledge of this interface is important for identifying the depths of potential aquifers where magma/water mixing occurred and generated highly explosive activity. A high seismic velocity zone directly below the summit has been interpreted as a solid dike network left by past eruptions. A low-velocity zone, possibly a partial melt, has been tentatively identified at a depth of 10 km. Teleseismic events suggest the presence of low-velocity zones below Vesuvius at depths of 15 and 300 km. These data have helped geophysicists to understand the overall structure of the volcano but are insufficient to identify specific locations of magma bodies.

Prediction of volcanic activity is thus not possible yet. But multiple monitoring networks are in place should Vesuvius resume its volcanic activity. The OV's integrated geophysical network covers the volcano and surrounding plain and would provide the first indication of rising magma. Any ground deformation caused by rising magma would be detected by leveling, tiltmeter, Global Positioning System (GPS), and tide-gauge networks. Gas compositions at high-temperature fumaroles are continuously measured.

Over the past decade, no signs of volcanic activity at Vesuvius have been detected (*8*). But people living on or near the volcano cannot be indifferent. Around the globe, many supposedly dormant or extinct volcanoes have erupted in the past 20 years. Where local populations were well-informed about volcanic eruptions and their effects, timely evacuations saved lives. People living near Vesuvius need ongoing educational programs to accompany state-of-the-art monitoring and detailed research on the volcano's past activity.

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8. Beginning on 9 October 1999, Vesuvius has experienced a series of earthquakes with hypocenters at about 3-km depth. The focal mechanisms were tectonic (strike-slip), and there was no accompanying ground deformation or change in gas compositions to indicate that the earthquakes were volcanic.

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PERSPECTIVES: PROTEIN STRUCTURE

Molecular Rotary Motors

Robert H. Fillingame

The enzymes that synthesize adenosine triphosphate (ATP) in the mitochondria and chloroplasts of animal and plant cells, and in bacteria, are the world's smallest rotary motors. These enzymes, the F_1F_0 ATP synthases, provide the cell with its energy currency (ATP) by catalyzing the addition of inorganic phosphate (P_i) to adenosine diphosphate (ADP). The synthesis of ATP is driven by a proton electrochemical potential (the proton-motive force) generated across the bacterial plasma membrane (or mitochondrial membrane) by a chain of electron transport proteins. The ATP synthase is composed of an F_1 catalytic domain that projects into the cytoplasm of a bacterial cell (or into the mitochondrial matrix) and an F_0 domain that traverses the membrane (see the figure). This enzyme is able to reversibly couple the rotation of several of its subunits (mechanically driven by the proton-motive force) to the generation of the chemical bond between ADP and P_i. On page 1722 of this issue, Sambongi *et al.* define the molecular components of the rotary motor of bacterial ATP synthase (*1*), and on page 1700 Stock *et al.* present the general structure of the rotary unit in yeast mitochondrial ATP synthase, explaining how it interacts with the other components of the enzyme (*2*). Together these two reports provide a coherent structural explanation for the rotary mechanism of ATP synthesis.

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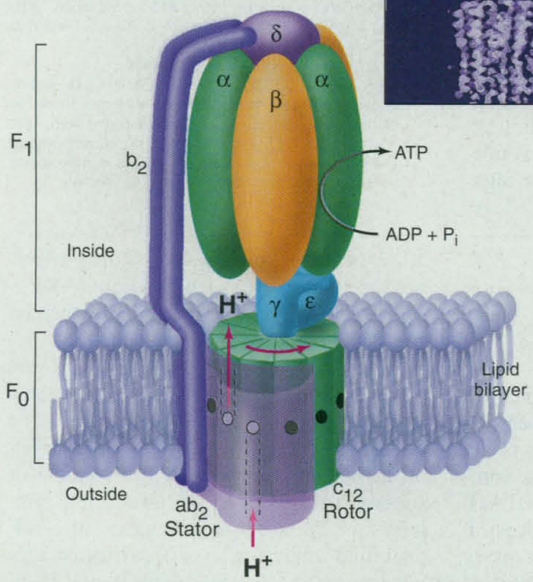
The F_1 domain of the *Escherichia coli* ATP synthase consists of five subunits (α, β, γ, δ, ε) in an $\alpha_3\beta_3(\gamma\delta\varepsilon)_1$ arrangement. The F_0 inner membrane sector is composed of three subunits (a, b, c) in an $a_1b_2c_{12}$ complex (see the figure). ATP synthesis is driven by rotation of the single γ subunit within a hexamer of alternating α and β subunits that contains three catalytic sites. As the γ subunit moves from site to site, it alternately promotes tight (ADP + P_i) substrate binding at one site and ATP product release at the next. The changes in binding affinities are cooperative, such that tight (ADP + P_i) binding at one site occurs simultaneously with ATP release at the next site.

This "binding change" mechanism was originally proposed by Boyer (*3*). An atomic-resolution x-ray diffraction structure of a bovine mitochondrial $\alpha_3\beta_3\gamma'$ subcomplex of the enzyme (where γ' is the segment of subunit γ resolved in the structure), published by Walker and colleagues in 1994, provided structural verification of this mechanism (*4*). In 1997, Noji *et al.* (*5*) directly demonstrated rotation of the γ subunit in the $\alpha_3\beta_3\gamma$ subcomplex. This water-soluble subcomplex hydrolyzes ATP in a reaction that is the reverse of ATP synthesis. Noji *et al.* immobilized the $\alpha_3\beta_3\gamma$ subcomplex on a microscope slide and decorated the protruding end of the γ subunit with a fluorescent actin filament. Upon addition of ATP to the immobilized enzyme, rotation of the fluorescent filament was observed directly with a microscope. Following this demonstration of rotary catalysis, the major question became the mechanism by which the proton-motive force drives rotation of the γ subunit. The favored hypothesis is that the proton-motive force drives rotation of an oligomeric ring of c

subunits in the transmembrane F_0 sector of the enzyme. Because subunit γ is permanently fixed to the surface of the oligomeric c ring, it moves as the ring rotates (*6, 7*).

Sambongi *et al.* (*1*) now provide the first evidence that the c subunit ring rotates during ATP hydrolysis. They immobilized the F_1F_0 complex from *E. coli* on a microscope slide with a polyhistidine tag attached to the α subunit at the top of the F_1 assembly (see the figure). Then, they attached fluorescent actin filaments to genetically engineered cysteine residues on the ends of the c subunits farthest away from F_1. Upon addition of ATP, the attached actin filaments started to spin. Their frequency of rotation was similar to that of actin filaments attached directly to subunit γ. Control experiments clarified that filament rotation was driven directly by turning of the c ring rather than by the possible adventitious attachment of the filament to subunit γ.

In a companion paper, Stock *et al.* (*2*) report the 3.9 Å resolution x-ray diffraction structure of yeast mitochondrial F_1, crystallized in association with the c ring oligomer. They were able to resolve substantial regions of the γ subunit that were not clearly resolved in the earlier structure of bovine $\alpha_3\beta_3\gamma$. In addition, the structure of a fourth subunit, yeast mitochondrial subunit δ (equivalent to bacterial subunit ε), also became clear. The investigators report that the γ and δ subunits form a footlike structure at the bottom of the F_1 molecule that sits on the surface of a ring of 10 c subunits. The multiple surface contacts between subunits γ and ε and 6 or 7 of the c subunits in the ring provide a structural explanation for how the subunits remain fixed to each other during rotation. The crystals were grown from a solution of a complete F_1F_0 complex and some subunits were lost, perhaps through being sheared from the side of the enzyme during crystallization. The missing subunits include those making up the stator component of the assembly (a, b, and δ in the bacterial enzyme).



Molecular carousel. Rotary model of how the *E. coli* F_1F_0 ATP synthase catalyzes the synthesis of ATP (**left**). The proton-motive force drives rotation of a ring composed of 12 c subunits (10 in the yeast). Protons enter the assembly through a periplasmic inlet channel and bind to the Asp^{61} carboxylate (open circle) of the c subunits. The protonated binding site (filled circle) then moves from the a_1b_2 stator component to the lipid phase of the membrane. After 12 steps the protons reach an outlet channel on the F_1-binding (cytoplasmic) side of the membrane. The γ and ε subunits remain fixed to the top of a set of c subunits so that rotation of the c subunit oligomer also drives rotation of subunit γ within the $\alpha_3\beta_3$ hexamer of F_1. The b_2 and δ subunits of the stator hold the $\alpha_3\beta_3$ subunits in a fixed position as the γ subunit turns inside them to drive ATP synthesis. Electron density map of the yeast mitochondrial F_1-c_{10} subcomplex (*2*) (**right**) with the positions of different subunits indicated in the inset. Yeast mitochondrial subunit δ is equivalent to *E. coli* subunit ε.

The new x-ray diffraction F_1-c_{10} structure correlates remarkably well with previous models of the structure of the c subunit oligomeric ring and the packing interactions between subunits c, ε, and γ (*8–10*). Nuclear magnetic resonance (NMR) and x-ray diffraction show that isolated subunit ε is folded into two domains with a 10-stranded β barrel at its amino terminus and a helix-loop-helix motif at its carboxyl terminus (*11, 12*). The packing interactions between the surfaces of the β barrel and subunits γ, ε, and c were predicted from cross-linking studies (*9, 10*) and now have been borne out by the yeast F_1-c_{10} structure. However, there is one discrepancy between the new structure and previous data. The helix-loop-helix domain of ε is positioned near the surface of the c subunit oligomer in the crystal structure but has been placed near the bottom of the $\alpha_3\beta_3$ hexamer by cross-linking experiments (*13*). In a chloroform-methanol-water solvent mixture, monomeric *E. coli* subunit c folds into a hairpin composed of two extended α helices, and this structure has been solved by NMR (*14*). The α-helical hairpin seen in the F_1-c_{10} structure closely matches the structure predicted by NMR. Furthermore, the c_{10} ring closely resembles models derived from the NMR structure and distance constraints revealed by cross-linking studies (*8*).

Perhaps the biggest surprise in the new structure is the finding that there are 10 c subunits in the oligomeric ring, not 12 (*6–8*). In *E. coli*, radiolabeling experiments indicate a stoichiometry of 9 to 12 c subunits per F_0 complex, and recent studies show that the correct stoichiometry must be a multiple of 2×3 (*15*). Dimeric and trimeric versions of c subunits are active in *E. coli* (*15*) and also occur in other species (*16*). There are several possible explanations for these differences: Subunits may have been lost during the yeast F_1-c_{10} crystallization; the *E. coli* enzyme composed of trimeric subunits may be functioning suboptimally because of an abnormally expanded c ring; the number of subunits may vary among different organelles and different species. This paradox will have to be solved by further experiments. The answer is important because the number of c subunits dictates how many protons will be transported per ATP molecule synthesized (H^+/ATP ratio). A nonintegral H^+/ATP ratio, as suggested by the Stock *et al.* (*2*) structure, would require elasticity in the F_1-F_0 connection.

The new reports provide a visual picture of how the proton-motive force drives ATP synthesis. A crucial unanswered question is the mechanical means by which proton transport drives the stepwise rotation of the c subunit ring relative to the a_1b_2 stator component. The structure of the a subunit and the half channels within it that transport protons have yet to be resolved. Finally, the mechanism by which energy is stored in the enzyme during the stepwise transport of three to four protons across the membrane (which takes place between each step of ATP release) needs further investigation.

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