Mitochondrial adenosine triphosphate (ATP) synthase produces the majority of ATP in eukaryotic cells, and its dimerization is necessary to create the inner membrane folds, or cristae, characteristic of mitochondria. Proton translocation through the membrane-embedded F$_0$ region turns the rotor that drives ATP synthesis in the soluble F$_1$ region. Although crystal structures of the F$_1$ region have illustrated how this rotation leads to ATP synthesis, understanding how proton translocation produces the rotation has been impeded by the lack of an experimental atomic model for the F$_0$ region. Using cryo-electron microscopy, we determined the structure of the dimeric FO complex from Saccharomyces cerevisiae at a resolution of 3.6 angstroms. The structure clarifies how the protons travel through the complex, how the complex dimerizes, and how the dimers bend the membrane to produce cristae.

 Atomic model for the dimeric F$_0$ region of mitochondrial ATP synthase

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Mitochondrial adenosine triphosphatase (ATPase) stop in different rotational states when extracted from membranes and phosphate (ADP) and phosphate (P). Proton translocation is thought to occur through two offset half-channels, one opening to the IMS and the other to the mitochondrial matrix (3, 4). Protons from the IMS half-channel neutralize conserved Glu residues in the c$_{50}$ ring (Glu$^{50}$ in S. cerevisiae), travel through the lipid bilayer as the ring rotates, and are delivered to the matrix half-channel. The $\alpha$-helical complex is prevented from rotating along with the central rotor by the peripheral stalk subcomplex, consisting of subunits OSCT (oligomycin sensitivity conferring protein), d, and the soluble region of subunit b. Mammals lack subunits i/j, k, and l but possess the additional subunits DAPI and 6SPL (5).

Mitochondrial ATP synthase complexes assemble into long ribbons of dimers (6, 7) that bend the mitochondrial inner membrane as the result of the presence of a domain containing subunits e and g in the F$_0$ region (7–11). These bends are needed to form cristae, highly folded membrane structures that maximize the surface area available for respiration, giving mitochondria their characteristic appearance (6, 7). X-ray crystallography has revealed the atomic structures of most of the soluble subunits of mitochondrial and bacterial ATP synthase (2). However, most of the membrane-embedded region of the complex (subunits a, b, e, f, g, i/j, k, l, and 18) has resisted high-resolution structural characterization. Despite recent advances, the resolution in cryo-electron microscopy (cryo-EM) maps of intact ATP synthases have been insufficient to allow construction of atomic models (9, 12–15).

Cryo-EM has shown that rotary adenosine triphosphatases (ATPases) stop in different rotational states when extracted from membranes (9, 13, 15, 16). We hypothesized that this conformational heterogeneity limits cryo-EM resolution and that analysis of dissociated F$_0$$_1$ regions would reduce heterogeneity, improve resolution, and enable construction of an experimental atomic model. A similar approach was successful with the related V$_0$ complex from the S. cerevisiae V$_1$-type ATPase (17). However, whereas yeast V$_1$-ATPases dissociate in response to glucose depletion (18), ATP synthases do not offer a physiological route to separating the F$_1$ region from the F$_0$ region. Therefore, we used sodium bromide to dissociate the F$_0$$_1$ regions from inside-out submitochondrial particles (19). Similar to the natural product digitonin (9), we found that the recently described synthetic detergent glycodiesogen (GDN) (20) extracts the dimeric form of ATP synthase from yeast mitochondrial membranes for both the intact ATP synthase and the F$_0$ complex (Fig. 1B). Cryo-EM of the F$_0$ complex allowed for determination of the structure to 3.6 Å resolution (Fig. 1, C to E, and fig. S1). Side-chain detail could be seen for subunits a, f, i/j, and 18; the transmembrane $\alpha$ helix of k; the c-subunits; the transmembrane $\alpha$ helices of subunit b; and part of subunit d. This resolution allowed construction of atomic models for these subunits (fig. S2 and table S1). Density for subunits e and g allowed construction of polyalanine models for these proteins. The recently identified subunit l, which has no known function and a sequence similar to that of subunit k (17), could not be identified in the cryo-EM map. Together with existing atomic models of ATP synthase subcomplexes, the F$_0$ complex structure determined here allows for construction of a nearly complete “mosaic model” (2) of mitochondrial ATP synthase (fig. S3).

The core subunits of the F$_0$ complex, found in both mitochondria and bacteria, are subunits a, b, and c. The fold observed for subunit a (Fig. 2, A and B, green) matches the fold deduced from a low-resolution map of the bovine enzyme combined with evolutionary covariance analysis (13). The protein contains five transmembrane $\alpha$ helices and an amphipathic $\alpha$ helix ($\alpha$ helix 2) that lies along the matrix surface of the detergent micelle; $\alpha$ helices 3 and 4 form a transmembrane hairpin, as do $\alpha$ helices 5 and 6, with the latter being the long and tilted hairpin that is characteristic of rotary ATPases (12, 13, 15, 16, 21). The simpler bacterial ATP synthase contains two copies of subunit b, each with a single N-terminal transmembrane $\alpha$ helix and an elongated C-terminal region that constitutes most of the peripheral stalk (15). In contrast, the mitochondrial enzyme has a single subunit b with two N-terminal transmembrane $\alpha$ helices. The two transmembrane $\alpha$ helices of the mitochondrial subunit do not pack against each other, as suggested previously (9, 13, 14).

Instead, subunit b begins with a short, presumably amphipathic $\alpha$ helix and a transmembrane $\alpha$ helix that together form a domain with subunits e and g (Fig. 2A, dashed line). A short loop connects this domain to the second transmembrane $\alpha$ helix of subunit b, which packs against subunit a. The soluble C-terminal portion of subunit b enters the mitochondrial matrix as part of the peripheral stalk (22, 23) and is mostly disordered in the isolated F$_0$$_1$ complex.

Subunits f and 8, which are essential for mitochondrial respiration, previously had no clear function in the ATP synthase. Subunit f (Fig. 2, A and C, yellow) consists of a soluble N-terminal sequence of 50 residues that binds to the helix terminus of the peripheral stalk, followed by a single transmembrane $\alpha$ helix. The transmembrane $\alpha$ helix of subunit f was mistaken for the first transmembrane $\alpha$ helix of subunit b in lower-resolution cryo-EM studies of the bovine (13) and yeast (9, 14) enzymes. Subunit 8 (Fig. 2, A and C, pink), known as A6L in mammals, is almost entirely $\alpha$-helical, with the N-terminal part of the helix embedded in the membrane in contact with the first $\alpha$ helix of subunit a and the C-terminal 14 residues contributing to the base of the peripheral stalk. Subunits f and 8 can therefore be assigned as components of the peripheral stalk. The C-terminal 56 residues of

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subunit d (Fig. 2, A and C, brown), which were absent from earlier crystal structures (22, 23), wrap around subunit 8 and subunit b as they leave the detergent micelle to clamp together the base of the peripheral stalk. The structure shows that the base of the peripheral stalk in the mitochondrial ATP synthase has a more complicated arrangement of subunits than predicted (22, 23), but found in the bacterial enzyme (15).

Unlike the dimeric ATP synthase from *Polytomella* sp., which is held together by subunits specific to algae (12), the yeast dimer is held together by subunits a, i/j, k, and e (Fig. 3, A to C). The interface formed by subunit i/j, which was mistaken for subunit f in a lower-resolution structure (9), occurs through two short stretches of ~10 residues that pack together in the dimer (Fig. 3, A and B, magenta). The lack of homologs for subunit i/j in mammals could explain why yeast ATP synthase dimers are more stable than mammalian dimers (9). Unexpectedly, subunit a also forms a dimer interface, with each monomer contributing two strands of a four-stranded planar structure (Fig. 3, A and B, green) with one hydrophobic surface and one hydrophilic surface. This motif superficially resembles an antiparallel β sheet, with amino acid side chains pointing away from the plane of the motif. The two dimerization motifs are stacked together, with the subunit i/j motif in the IMS and the subunit a motif on the IMS surface of the detergent micelle. In an alignment of subunit a sequences from different species (9), the dimerization motif begins just four residues N-terminal of the first residue in the bovine and human subunit a, which have shorter N-terminal

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**Fig. 1. Overall structure of the F₀ complex.** (A) Cartoon of the ATP synthase dimer, with subunits found in the F₀ region outlined in black. (B) The detergent GDN extracts dimeric *S. cerevisiae* ATP synthase from mitochondrial membranes intact (left) or as the F₀ complex after sodium bromide treatment of the membranes (right). Scale bar, 25 Å. (C) Example of map density that allowed construction of an atomic model (subunit a, residues Ala168 to Phe196 and Pro212 to Leu242). Carbon, oxygen, nitrogen, and sulfur atoms are colored green, red, blue, and yellow, respectively. Scale bar, 5 Å. (D and E) Top and side views, respectively, of the F₀ dimer, revealing the arrangement of subunits. One monomer is outlined with a dashed line in (D). Scale bar, 25 Å.
Fig. 2. Subunit folds for individual subunits. (A) Matrix view of the F$_{0}$ complex monomer structure. A dashed line encloses the domain formed by subunits e and g and the N-terminal $\alpha$ helices of subunit b. (B) Fold of subunit a. (C) Side view of the F$_{0}$ monomer showing that subunits 8, f, and b contribute to the base of the peripheral stalk, with subunit d acting as a clip. Scale bar, 25 Å.

Fig. 3. Dimerization and membrane bending. (A) Subunits a and i/j contribute dimerization motifs. (B) Enlargement of (A). (C) The unsharpened map shows that subunit k extends into the IMS to contact subunit e. (D and E) Top and side views of the F$_{0}$ dimer show that subunits b, e, g, and k create the structure that bends the lipid bilayer by almost 90°. The dashed orange line indicates the full length of subunit k. Scale bars, 25 Å.
regions than the yeast subunit a. Consequently, the mammalian subunit a dimerization motif likely begins immediately before the subunit’s first transmembrane α helix. Subunits k and e contribute the final monomer-monomer interaction. Subunit k possesses an N-terminal transmembrane α helix in the map that is well ordered (Fig. 2, orange). Inspection of an unsharpened version of the map, which allows visualization of lower-resolution features, shows additional density from subunit k (Fig. 3C, orange). This density continues into the IMS to contact subunit e, which extends into the IMS as an α helix (Fig. 2A and Fig. 3C, blue). The lack of this interaction in the bovine ATP synthase monomer may explain why the extended IMS α helix of subunit e is in different orientations in the monomeric bovine ATP synthase and intact dimeric yeast ATP synthase (9, 13). It has been proposed that dissociation of ATP synthase dimers triggers opening of the mitochondrial permeability transition pore, which can initiate regulated cell death (24). Consequently, the dimer contacts described above warrant further investigation for their role in this phenomenon.

The unusually shaped domain containing subunits e, g, and the N-terminal ~50 residues of subunit b (Fig. 3, D and E) is responsible for bending the mitochondrial inner membrane (11, 25). Subunit e (Fig. 3, D and E, blue) begins with an N-terminal transmembrane α helix that continues ~40 Å into the IMS. Although this feature is conserved from yeast to mammals, it does not appear to have a role in proton translocation or rotary catalysis. Subunit g has two N-terminal α helices that lie on the matrix surface of the detergent micelle and a single transmembrane α helix that interacts with subunit e, probably via the conserved Gly-X-X-Gly motifs of the two proteins (26, 27). The curved structure of the domain formed by subunits e, g, and b offers an explanation for how subunits e and g bend the lipid bilayer and why deletion of the genes for these subunits in yeast leads to defects in cristae formation (8). The combination of membrane-surface and membrane-embedded α helices from these subunits, with further support from subunit k, creates a curved structure that enforces curvature of the lipid bilayer itself. The membrane surface α helices of subunits g and b are reminiscent of the structure of BAR domains, which also induce curvature in lipid bilayers through amphipathic α helices (28).

Proton translocation in ATP synthase involves subunit a and the c-ring. Remarkably, the c10-ring has a well-defined orientation relative to subunit a (Fig. 4A), at least after the rapid cooling that occurs during cryo-EM specimen preparation. This constrained orientation shows that the highly tilted α helices of subunit a maintain a tight interaction with the ring, although the rotor must still be able to turn during ATP synthesis. Two pores are visible in the F0 complex that correspond to the two half-channels required for proton translocation (3, 4). A cavity on the IMS side of the detergent micelle behind the tilted α helices 5 and 6 forms the IMS half-channel (Fig. 4A, left, circled in red) while an opening on the matrix side of the detergent micelle between subunit a and the c-ring forms the matrix half-channel (Fig. 4A, right, circled in red) (12, 13, 17). The positions of these half-channels are consistent with the proton translocation path proposed from lower-resolution studies of rotary ATPases (9, 12, 13, 16, 17, 21). Arg276 from subunit a, the only absolutely required residue of the subunit for enzyme activity (29, 30), extends toward the c-ring (Fig. 4B). X-ray crystallography and simulations of isolated c-rings have shown that under deprotonating conditions, the proton-carrying Glu residues adopt an extended (deprotonated) conformation. The remaining Glu residues are in proton-bound conformations (31). Under protonating conditions, these residues fold toward the center of the c-ring (31). Under protonating conditions, these residues fold toward the center of the c-ring (31). Under protonating conditions, these residues fold toward the center of the c-ring (31). Under protonating conditions, these residues fold toward the center of the c-ring (31). 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Glu162 residues closest to the c-ring are readily visible in the map (Fig. 4B). From the cryo-EM map (Fig. 4B), the conserved Glu162 residues of the two c-subunits nearest to Arg176 appear to be in deprotonated (negatively charged) conformations (31) but too far from Arg176 for either Glu to form a direct salt bridge with Arg176. The remaining c-subunits appear to be in the proton-bound (neutral) conformation (Fig. 4B). Consequently, the resting conformation of the c-ring in the F0 structure differs subtly from the V-type enzyme, where the Glu residue from one of the c-subunits was close enough to interact with the conserved Arg176 (17).

The surface of subunit a that contacts the c-ring is mostly hydrophobic, with a patch of positive charge from Arg176 (Fig. 4C, blue) and two patches of negative charge (red) at the expected positions of the half-channels. Near the matrix surface, these negative patches are due to Glu223 and Asp244, whereas near the IMS surface the negative charge is due to Glu233; all of these residues are conserved (9) and likely pass protons between the half-channels and the Glu residues of the c-ring. Numerous other conserved and functionally characterized residues of subunit a surround the locations of the two half-channels (Fig. S4). The positions of the half-channels and the orientations of the Arg and Glu residues support an emerging model for proton translocation: Donation of a proton from the aqueous IMS half-channel via Glu233 neutralizes the Glu162 residue of the subunit c adjacent to the channel. Neutralization of the Glu residue allows the c-ring to rotate as a result of Brownian motion (3, 4), counterclockwise when viewed from F1 toward F0 (Fig. 4D), thereby placing the residue in the hydrophobic environment of the lipid bilayer. Rotation of the c-ring brings a neutral Glu162 residue from a different c-subunit into the matrix half-channel, where it is stripped of its proton by Arg176 of subunit a. This proton is accepted by Glu162 or Asp244, which in turn lose the proton to the mitochondrial matrix. Loss of the proton from the Glu162 residue in the matrix half-channel resets the motor for the next proton. The directionality and force of rotation in this model is governed by the difference in probability of a c-subunit binding a proton from either half-channel, which depends on the ΔG of proton translocation established by the proton motive force.

REFERENCES AND NOTES
Atomic model for the dimeric $F_O$ region of mitochondrial ATP synthase
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**How protons power rotation**

Synthesis of adenosine triphosphate (ATP) in mitochondria is accomplished by a large molecular machine, the $F_1 \times F_O$ ATP synthase. Proton translocation across the $F_O$ region that spans the mitochondrial inner membrane drives ATP synthesis in the $F_1$ region through a rotational mechanism. Guo *et al.* present a high-resolution structure of the dimeric $F_O$ complex from *Saccharomyces cerevisiae*, determined by electron microscopy. The structure gives insights into how proton translocation powers rotation and suggests how $F_O$ dimers bend the membrane to give mitochondria their characteristic cristae.

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