**RESEARCH ARTICLES**

**Structure of the Rotor of the V-Type Na\(^{+}\)-ATPase from Enterococcus hirae**

Takeshi Murata, Ichiro Yamato, Yoshimi Kakinuma, Andrew G. W. Leslie, John E. Walker

The membrane rotor ring from the vacuolar-type (V-type) sodium ion–pumping adenosine triphosphatase (Na\(^{+}\)-ATPase) from Enterococcus hirae consists of 10 NtpK subunits, which are homologs of the 16-kilodalton and 8-kilodalton proteolipids found in other V-ATPases and in F\(_{1}\)F\(_{0}\) or F-ATPases, respectively. Each NtpK subunit has four transmembrane \(\alpha\) helices, with a sodium ion bound between helices 2 and 4 at a site buried deep in the membrane that includes the essential residue glutamate-139. This site is probably connected to the membrane surface by two half-channels in subunit NtpI, against which the ring rotates. Symmetry mismatch between the rotor and catalytic domains appears to be an intrinsic feature of both V- and F-ATPases.

In eukaryotic cells, vital processes such as protein trafficking, endocytosis, neurotransmitter release, and intracellular pH regulation depend on the movement of ions across membranes by vacuolar adenosine triphosphatase (V-ATPases) (1), which are multisubunit complexes related to the F\(_{1}\)F\(_{0}\) or F-ATPases found in eubacteria, mitochondria, and chloroplasts (2). Both classes have globular catalytic domains, \(V_{1}\) and \(V_{0}\), where ATP is hydrolyzed (and synthesized in F-ATPases), which are attached by central and peripheral stalks to the intrinsic membrane domains \(V_{p}\) and \(V_{o}\), where ions are pumped across the membrane. ATP hydrolysis generates rotation of both the central stalk and an attached membrane ring of hydrophobic subunits. Ions are pumped through a pathway in the interface between the rotating ring and a static membrane component, which is linked to the outside of the \(V_{1}\) or \(V_{0}\) domain by the peripheral stalk.

Eukaryotic V-ATPases contain 13 different polypeptides (I). Subunits A to H form the \(V_{1}\) domain. Subunits A and B are the counterparts of subunits \(\beta\) and \(\alpha\), respectively, in the F-ATPases; three copies of each subunit are arranged around the central stalk, which is made of single copies of subunits D and F.

The precise locations of the remaining subunits (C, E, G, and H) are uncertain, but they are thought to contribute to the peripheral stalk. The remaining subunits—a, c, \(c'\), \(c''\), and \(d\)—form \(V_{o}\). The closely related \(c\), \(c'\), and \(c''\) subunits are each composed of tandem homologous repeats of the sequences of F-type \(c\) subunits (I). The F-type subunits are folded into two transmembrane \(\alpha\) helices that are linked by a loop oriented to the same side of the membrane as the \(F_{1}\) domain (3, 4). Their C-terminal \(\alpha\) helices contain a conserved carboxylate side chain that is essential for ion transport (5). A carboxylate is also conserved in the equivalent position of helix 4 of the V-type subunits \(c\) and \(c'\) (in the second repeat) but not in helix 2 (the first repeat). Subunit \(c''\) has a fifth nonessential transmembrane \(\alpha\) helix at its N terminus, and its essential glutamate is in helix 3 (6, 7). In the V-ATPase from Saccharomyces cerevisiae, all three homologs are assembled into \(V_{o}\) and are essential for a functional enzyme (7); and, like the \(c\) subunits in \(F_{1}\), they probably form the \(V_{o}\) rotary ring. V-ATPases also occur in prokaryotes; in Enterococcus hirae, a V-ATPase acts as a sodium ion extrusion pump (8). Its nine subunits are encoded in the Na\(^{+}\)-dependent triphosphatase (ntp) operon. Subunits A, B, D, and G make up the \(V_{1}\) domain and are the homologs of subunits \(\beta\), \(\alpha\), \(\gamma\), and \(\delta\), respectively, in mitochondrial F-ATPases. Subunit C of V-ATPase has no counterpart in F-ATPase and may connect the foot of the central stalk to \(V_{o}\), which is made of subunits K and I, the homologs of the F-ATPase subunits \(c\) and \(a\), respectively. The \(K\) subunit (NtpK) is also the homolog of the eukaryotic V-type \(c\) subunits (Fig. 1A). Like the \(c\) subunits in eukaryotic V-ATPases, the sequence of NtpK contains tandem repeats in residues 1 to 79 and 80 to 156 with 22% identity and 44% similarity (Fig. 1B).

A surprising feature of F-ATPases is that the number of \(c\) protomers in the ring structures appears to vary between species. A complex of the \(F_{1}\) domain with the \(c\) ring from the hydrogen ion–pumping H\(^{+}\)-F-ATPase from S. cerevisiae contains 10 c protomers (4). Isolated \(c\) rings from spinach chloroplasts consist of 14 c protomers (9), and rings from the sodium ion–pumping Na\(^{+}\)-F-ATPases from Hyobacter tartaricus are made of 11 protomers (10). Biochemical and genetic evidence suggests that the H\(^{+}\)-ATPases in Escherichia coli and Bacillus PS3 have 10 protomers per \(c\) ring (11, 12).

Here we describe the structure at 2.1 Å resolution of the \(K\) ring from E. hirae, which is the first high-resolution ring structure from either a V-type or an F-type enzyme.

**Structure of the \(K\) ring.** The structural data are summarized in Table 1. The 10 protomers of the \(K\) ring have very similar structures (Fig. 2); the root mean square deviation (RMSD) between \(c\) carbons is less than 0.06 and 0.08 Å for all atoms, except for crystal contact sites, where greater deviations occur. Each protomer is folded into five \(\alpha\) helices, H0 to H4. H1 to H4 are transmembrane segments, and as expected from sequence homology, H1 and H2 resemble H3 and H4. The RMSD between all main-chain atoms in H1 and H2 (residues 11 to 46 and 51 to 79, respectively) compared with H3 and H4 (residues 87 to 122 and 127 to 155, respectively) is 1.0 Å. The 40 transmembrane \(\alpha\) helices are packed in two concentric rings. H1 and H3 alternate in the inner ring, and H2 and H4 alternate in the outer ring. H1 and H3 (36 and 40 amino acids, respectively) are considerably longer than H2 and H4 (29 and 33 residues, respectively). H1 is linked to H2 and H3 to H4 by loop regions (residues 47 to 50 and 125 to 126, respectively), which, by analogy with F-ATPases, would lie proximal to the central-stalk region in the intact enzyme on the cytoplasmic side of the membrane. Another short loop (residues 80 to 84) links H2 and H3 on the periplasmic side of \(V_{o}\) in the intact enzyme. H0 (residues 1 to 8) also lies distal from \(V_{o}\). It points inward and slightly upward at 61° to the axis of \(H_{1}\) toward the center of the ring. H2 is kinked around Pro\(^{62}\) and Gly\(^{63}\), and H4 bends in the region of Met\(^{137}\) and Val\(^{138}\). The lower parts of H2 and H4 bend outward from the axis of the ring to approximately the same extent, as do the upper parts (mean values 11° and 26°, respectively); the bends in H1 and H3 are less pronounced. The effect of these curvatures is

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to widen the upper and lower parts of the ring relative to the central region, especially on the cytoplasmic side (external and internal diameters of 83 and 54 Å, respectively). On the opposite side of the membrane, the inward-pointing H0 regions narrow the internal diameter of the ring to 29 Å.

The height of the K ring (68 Å) is similar to that of the c rings from the F-ATPases from *S. cerevisiae* (58 Å) (4) and *I. tartaricus* (70 Å) (10). These similarities arise from the relationships between the sequences of the tandem repeats in subunit K with the sequence of the c subunit (Fig. 1B). For example, the first K repeat and the *S. cerevisiae* c subunit are 45% similar and 17% identical, respectively, and the second K repeat and c subunit are 52% similar and 25% identical, respectively. However, these similarities in sequence and structure of the V- and F-type protomers notwithstanding, their ring diameters differ greatly, and the V-type ring is much larger than the F-type ring (external and internal diameters at the top of the F-type ring in *S. cerevisiae* are 55 and 27 Å, respectively, and in *I. tartaricus*, the values are 50 and 17 Å; the corresponding values in the K ring are 83 and 54 Å) (Fig. S1). These differences in diameter probably reflect significant differences in the association of the ring with the foot of the central stalk. In F-ATPases, the interaction between the ring and the central stalk subunits (γ, δ, and ε in the mitochondrial enzyme) is direct (4). In bacterial V-type enzymes, the C subunit is thought to be interposed between the equivalent central stalk subunits and the ring (13) (in eukaryotic V-ATPases, the homolog of bacterial subunit C is subunit d and not subunit C).

**The Na⁺-binding sites.** In each NtpK subunit, a strong peak of density between H2 and H4 adjacent to the γ carbonyl of Glu139 was interpreted as a sodium ion. Other metal cations were also considered (Ca, Mg, Mn, Fe, Cu, Zn, and K), but Na⁺ was the only metal cation in the crystallization medium, and it fit the coordination geometry best (14). Sodium ions bind to the *E. hirae* V-ATPase with high affinity (15). Each sodium ion is surrounded by five oxygen atoms 2.2 to 2.3 Å distant, four of them in the side chains of Thr64, Gln65, Glu110, and Gln139 (Fig. 3A), and the fifth in the main-chain carbonyl of Leu61.

Thus, residues in H2, H3, and H4 all contribute to the Na⁺-binding pocket (Fig. 3). The bound sodium ions are occluded by the side chain of Glu139, and the position of its γ carbonyl is stabilized by hydrogen bonds with the side chains of Gln110, Tyr68, and Thr64 (Fig. 3A). Access to the bound ion to allow its exchange with the bulk phase (via a half-channel in subunit I, see below) can be achieved by changing the torsion angles of the side chain of Glu139 without causing steric hindrance (Fig. 3C), although additional movements of adjacent side chains may also be necessary. Residues above and below the Na⁺-binding site are all hydrophobic, and there is no plausible intrinsic channel within the K ring itself to provide access to the binding site from either the cytoplasm or the periplasm.

**Bound phospholipid, detergent, and water molecules.** After the 10 NtpK protomers had been fitted into the electron density, many peaks of positive density around the ring remained uninterpreted. Twenty of them lying close to the inside surface are bifurcated, which suggests that they arise from the acyl side chains of phospholipids. They are disordered.

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**Table 1.** Data collection and crystallographic analysis. Values in parentheses indicate the highest resolution shell. The overall figure of merit (after density modification) is 0.247 (0.458).

<table>
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<th>Data set</th>
<th>Native 1</th>
<th>Native 2</th>
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<th>K2PtCl6</th>
<th>KAuCl4</th>
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<td>1.0397</td>
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<td>Resolution range (Å)</td>
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<td>87.7–2.50</td>
<td>67.4–2.30</td>
<td>67.4–2.90</td>
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<tr>
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<td>9.1 (54.3)</td>
<td>7.1 (52.5)</td>
<td>10.0 (59.8)</td>
<td>17.0 (70.6)</td>
<td>9.1 (42.8)</td>
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<tr>
<td>Completeness (%)</td>
<td>99.9 (97.5)</td>
<td>88.4 (64.0)</td>
<td>99.9 (99.8)</td>
<td>98.8 (93.3)</td>
<td>97.9 (86.8)</td>
</tr>
<tr>
<td>Unique observations</td>
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<td>124,148</td>
<td>78,994</td>
<td>48,674</td>
<td>69,975</td>
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<td>Redundancy</td>
<td>3.7 (2.9)</td>
<td>3.8 (3.1)</td>
<td>13.7 (8.8)</td>
<td>4.0 (4.0)</td>
<td>4.4 (3.8)</td>
</tr>
<tr>
<td>%A</td>
<td>9.1 (1.8)</td>
<td>12.1 (2.3)</td>
<td>17.5 (2.9)</td>
<td>7.1 (2.1)</td>
<td>12.2 (2.7)</td>
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<td>Wilson B factor (Å²)</td>
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<td>44.9</td>
<td>72.1</td>
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</table>

**Phasing statistics**

- **Number of heavy atom sites**: 20, 10, 19
- **Rcalc [centric]**: 0.653, 0.907, 0.899
- **Phasing power** (iso/ano): 1.40/1.120, 0.70, 0.59

* * *

**Fig. 1.** Sequences of proteolipids from *V*- and F-ATPases (32). They were aligned with ClustalW (33). Dashes denote insertions. (A) The 16-KD proteolipids from V-ATPases from *E. hirae* (NtpK), the yeast vacuole (Yv-c), and human lysosomes (Hs-16k), and from gap junctions of *Nephrops norvegicus* (Nn-16k). The secondary structure, the positions of helices H0 to H4, and residue numbers above are shown. Red and blue residues are exposed inside and outside the K ring, respectively. Violet and light blue vertical boxes denote identical and similar residues, respectively.

(B) The tandem repeats of NtpK aligned with c subunits of F-ATPases from *E. coli* (Ec-c), yeast mitochondrial (Ym-c), *P. modestum* (Pm-c), and *I. tartaricus* (It-c). The N- and C-terminal residues are numbered on the left and right, respectively. Residues with conserved essential side chains are shaded red. Amino acids forming the Na⁺-binding site are boxed in (A) and (B).
distributed equally between the upper and lower regions of the model, with the openings of the bifurcation oriented downward in the upper layer and upward in the lower region, as expected in a phospholipid bilayer. The upper bifurcated moieties, but not the lower ones where the density is more limited, extend into regions of density that have been interpreted as phosphatidylglycerol (PG) head groups, because PG is the most abundant (57.1%) phospholipid head group in E. hirae (16). Because the most common fatty acyl chain in E. hirae phospholipids is C₁₆ (cause the most common fatty acyl chain in glycerol O₉ of DPG in the lower layer forms the side chain of Phe₁₀₁ in the adjacent protomer (to the left) and to Leu₁⁰⁷ of the protomer two to the left. The other acyl chain binds to Met¹ and Met² in H₀; Leu¹⁷ in H₁; and Leu⁰⁹, Leu⁹³, and Leu⁹⁷ in the adjacent protomer to the left. The thickness of the internal hydrophobic region of the lipid bilayer (the separation between the lipid glycerol backbone region on the two sides of the membrane) is about 25 Å. In a lipid bilayer of di-(C₁₆:0), this thickness is estimated to be about 26 Å (18).

Within the annulus of the bound phospholipid bilayer, additional regions of electron density are associated with the upper phospholipid leaflet but not the lower one. Thirty regions of elongated density were interpreted as acyl side chains (C₅ to C₁₀) in a second shell of phospholipids, although the possibility that they derive from detergent molecules cannot be excluded (Fig. 4, A and C). There was no significant electron density in the central part of the ring, which suggests that phospholipids in this region are mobile and disordered.

It is evident that all external bound phospholipids were lost during the isolation of K rings, because no bifurcated regions of density were associated with the outside of the ring. During crystallization, both dodecyl- and undecylmaltosides were present, and a number of features next to the external regions toward the top of H₂ and H₄ have been interpreted as detergent molecules. The maltose ring is resolved in two molecules at crystal contact sites (Fig. 4A), and they appear to be undecyl-rather than dodecylmaltosides, but it is not possible to be certain. Twenty additional elongated features at approximately the same distance from the top of the ring have been interpreted as undecyl moieties, but the maltose moieties are disordered (Fig. 4, A to D).

The model also contains 348 water molecules (Fig. 4) (16).

**Coupling of the K ring and the central stalk.** In the H⁺V-ATPase from Thermus thermophilus, subunit C is thought to be at the foot of the central stalk (13). It is a pointed molecule made of 12 α helices surrounding a core of six additional α helices, all lying approximately parallel to a threefold axis of pseudosymmetry. It has been proposed that the tapered end of this bundle (diameter, 28 Å) penetrates 12 Å into the membrane ring (13). In T. thermophilus, the c protomers (subunit L) have two transmembrane α helices, similar to the F-type c subunits, and the ring was modeled as 12 protomers of the c monomer.
from E. coli F-ATPase determined by solution nuclear magnetic resonance (NMR) (3), giving a pore diameter of 30 Å, to act as a closely fitting socket for the 28 Å taper of subunit C. The sequence of NtpC is 20% identical and 40% similar to that of the C subunit of T. thermophilus, and it is likely to have a similar structure. The T. thermophilus C subunit structure was docked with the K ring, and, with the taper of subunit C penetrating into the central cavity almost to the narrowest region at the midpoint of the ring, it occupies most of the cavity, although some structural changes would be needed to achieve an extensive interaction (Fig. 4E). This model of the K ring with the C subunit differs substantially from the proposal (13) for the interaction of the C subunit with the c ring in T. thermophilus, where the smaller diameter of a ring assumed to be composed of 12 F-type c protomers restricts the penetration to the tip of the taper in subunit C.

**Sodium ion translocation.** Three key questions about the mechanism for transmembrane Na\(^+\) translocation are as follows: (i) How does the outer surface of the ring interact with the phospholipid bilayer. (ii) What is the nature of the interactions of the ring with subunit I? (iii) To what extent is the Na\(^+\)-binding site accessible to the external milieu? Although no phospholipids are bound to the outer surface, their likely regions of interaction with the K ring can be inferred from features such as the distribution of charged and hydrophobic side chains and the locations of bound water molecules. In known structures of membrane proteins, tyrosine, asparagine, and lysine residues (and also arginine, histidine, tryptophan, and serine) are often found near phospholipid head groups, acting as primary ligands for phosphodiester groups, and additional stabilization sometimes comes from threonine and glutamine residues (18, 19). The outside of the K ring has a hydrophilic belt 39 Å wide (Fig. 4D), a carbon distance between Lys\(^{130}\) and Asn\(^{155}\) corresponding to the head-group separation in a lipid bilayer (18). This position for the lipid bilayer allows interactions between phosphodiester groups in the upper leaflet and the side chains of Gln\(^{54}\), Thr\(^{129}\), and Lys\(^{130}\), and in the lower leaflet with Asn\(^{79}\) and Asn\(^{155}\). The distribution of bound water and detergent molecules supports this interpretation. This arrangement places the Na\(^+\)-binding site close to the center of the bilayer, as in the c ring in I. tartaricus (10). Although several polar residues involved in binding the Na\(^+\) ion are exposed on the outer surface of the K ring, the network of hydrogen bonds surrounding the Na\(^+\) site satisfies all potential hydrogen-bonding groups (Fig. 3A). With a bound Na\(^+\), there is no energy penalty for these residues being near the center of the bilayer.

The position of the Na\(^+\)-binding site close to the middle of the lipid bilayer, and the absence of any intrinsic K-ring channel leading to the site, support a “two-half-channel” ion translocation model (Fig. 5) as proposed for F-ATPases (20). The clockwise rotation of the K ring, as viewed from the cytoplasm, brings an occupied Na\(^+\)-binding site into the K-ring–subunit-I interface. The proximity of the Na\(^+\) site to NtpI-Arg\(^{573}\), which is essential for ion transport (21), produces an electrostatic interaction between Arg\(^{573}\) and Glu\(^{39}\), disrupting the hydrogen-bond network and releasing the Na\(^+\) ion into the periplasm via a half-channel in NtpI (the ion release may possibly be assisted by electrostatic repulsion by R\(^{573}\)). Further rotation, which may disrupt the Arg-Glu interaction, brings the site into contact with a second half-channel, resulting in the binding of a cytoplasmic Na\(^+\) ion. With the site occupied, the energy barrier to further rotation is lowered, bringing the site beyond the I–K-ring interface. This model differs from an earlier one for the V-ATPase based on Na\(^+\)-exchange experiments in detergent (8, 15) where a single channel connected the ion-binding site to the periplasm at the I–K-ring interface, giving free cytoplasmic access to most Na\(^+\)-binding sites. The earlier model is incompatible with the structure, and the kinetics of exchange (fast- and slow-exchange sites >1.7 and 0.16 min\(^{-1}\), respectively) are much slower than physiological ion-pumping rates.

A “single-channel” model of Na\(^+\) translocation has also been proposed for the F-ATPase from Propioniogenium modestum (22), where the Na\(^+\)-binding site is placed close to the membrane center on the basis of biochemical evidence (23, 24), but Na\(^+\)-exchange experiments have been interpreted as suggesting free cytoplasmic access to the sites (25). The observations were reconciled by proposing an intrinsic c-ring channel connecting the ion-binding sites to the cytoplasm (10, 23, 2).

In a three-dimensional model of the c ring of the Na\(^+\)-F-ATPase from I. tartaricus based

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**Fig. 4.** Localization of lipid, detergent, and water molecules and interaction of the K ring with subunit C. The solvent-accessible surface of the K ring is colored according to the electrostatic potential (red, negative; blue, positive; gray, neutral). Lipids and detergents are shown in space-filling representation (C and O atoms are green and red, respectively). The C atoms of incomplete acyl chains from either lipid or detergent are orange. Yellow spheres are water molecules. (A) View from the cytoplasm. Three circles show crystal contacts. (B) Cut-away view showing the interior surface of one half of the ring and one pair of lipids in the bilayer inside the ring. Dashed lines show the location of the hydrophobic core of the lipid bilayer inside the ring, which is defined as the region between the lipid glycerol backbone moieties. (C and D) Side views, respectively, of the surface of the K monomer with lipids and detergent tails and of the outer surface of the whole ring with all resolved detergent and water molecules. In (D), the extent of the hydrophobic belt, 39 Å, is indicated by a vertical line. (E) Coupling of the K ring and the central stalk. The T. thermophilus subunit C (colored red, Protein Data Bank code 1R5x) (13) was docked manually into the K ring: one half of the K ring (blue) is shown with interior lipids (in space-filling representation; C and O atoms are green and red, respectively). The marked distances, 21 and 24.5 Å, are, respectively, the depth of penetration of subunit C into the K ring and the extent to which subunit C protrudes from the ring and thus its contribution to the height of the central stalk.
on electron cryogenic microscopy (10), rods of density for the N- and C-terminal helices in an 11-fold ring and weaker connecting densities were observed, but side chains could not be identified. The model was constructed with a series of conserved glycines (residues 27, 29, 31, and 33) facing neighboring subunits in the inner ring of helices, with Gln52 (involved in Na+ binding) facing the C-terminal helix to achieve the tight packing of the inner helices suggested by conservation of the glycines. There was less information to constrain the orientation of the C-terminal helix, but because both Glu65 and Ser66 are implicated in Na+ binding (20), the face of the helix containing these residues was placed toward the N-terminal helix, allowing a chain of polar residues (Gln61, Thr56, Ser55, Asp52, and Lys40) to connect the Na+ binding site to the cytoplasm, providing free access to the Na+ binding site.

To explore this proposal, a model of the I. tartaricus c ring based on the NtpK structure has been constructed (16). From this model, it appears that there is no intrinsic ion channel in the I. tartaricus ring. Thus, it is likely that Na+ translocation in the F-ATPases from I. tartaricus and P. modestum also relies on two half-channels (in subunit a) rather than on a single periplasmic channel, and this feature is common to ion transport mechanisms in both F- and V-ATPases.

The overall fold of helices H3 and H4 of NtpK is very similar to the low-pH NMR structure of an E. coli c monomer (3). However, the position of the essential carboxylate (Glu139 in NtpK and Asp61 in E. coli subunit c) differs by almost half an α-helical turn, corresponding to a rotation around the axis of the helix of approximately 150°. In view of the sequence similarity and because Asp61 is assumed to be protonated in the NMR structure so that the ion binding site is occupied in both proteins, this result is surprising. A more detailed comparison of the NtpK and c-ring models will be described elsewhere.

**Symmetry of rotor rings in V- and F-ATPases.** By single-particle averaging of the same preparation of K rings as used for crystallography, a sevenfold symmetry was observed (27), which is incompatible with the structure of the K ring. A proper explanation of this discrepancy has not yet been found, but the x-ray structure demonstrates the 10-fold symmetry unequivocally, and it is likely that this is the symmetry of the ring in the fully assembled V-ATPase. Also, given the close sequence relationship between K subunits and c subunits in other V-ATPases, the 10-fold symmetry of the K rings is likely to have wider significance for V-ATPases. On the basis of various strands of evidence, including chemical analysis (28) and electron microscopy (29), it has become accepted that the symmetry of V-type rings is sixfold. This situation is reminiscent of the history of c-ring stoichiometries in F-ATPases, where, on the basis of similar kinds of experimentation, it was thought that the c rings had 12-fold symmetry, until higher resolution structural information demonstrated clearly the 10-fold symmetry in the F1-c10 complex from F-ATPase from S. cerevisiae (4) and 11-fold and 14-fold symmetries in rings isolated from the Na+-ATPases from I. tartaricus (10) and the H+-ATPase from spinach chloroplasts (9), respectively. Thus, the earlier sixfold symmetries of c rings from V-ATPases will require reevaluation by high-resolution structural methods.

**Fig. 5.** A model for ion translocation by the V-ATPase of E. hirae. The model is based on the structure of the K ring, shown in space-filling representation with Glu139 in yellow with red oxygen atoms. Subunit I (structure unknown) is green and in close proximity to the K ring in space-filling representation with the essential residue Arg523 (21) in a half-channel connecting to the periplasm. Another half-channel links the ion-binding site to the cytoplasm (Na+ ions are blue). Clockwise rotation of the ring (arrow) driven by ATP hydrolysis in V1 transfers bound Na+ ions, which have reached the ion-binding site via a half-channel connecting to the cytoplasm, to a position where they transfer to a second half-channel leading to the periplasm.

The fundamental importance of the observed ring stoichiometries is twofold. First, the range of values suggests that not all species pay the same energetic price either to make an ATP molecule in the F-ATPases or to pump a proton or a sodium ion in V-ATPases and bacterial F-ATPases. According to extant models for ATP synthesis, each 360° rotation of the central stalk and the attached c ring generates three ATP molecules in the F1 domain, and the generation of a 360° rotation requires each of the c subunits in the ring to translocate an ion. Thus, the number of protons (or sodium ions) required to make an ATP molecule in enzymes with c ring symmetries of 10, 14, and 11 will be 3.3, 4.7, and 3.7, respectively. Similarly, hydrolysis of an ATP molecule in the V-ATPase from E. hirae will be coupled to the outward translocation of 3.3 sodium ions. Second, the mismatch of symmetries between catalytic and membrane domains observed now in both F- and V-ATPases could be an important intrinsic feature of both enzyme types, helping to reduce potential-energy barriers to rotation that might otherwise exist in enzymes with matching symmetries (4). Symmetry mismatch is also a feature of the machinery for packaging DNA into bacteriophage heads (30) and of the bacterial flagellar motor (31). Like the F- and V-ATPases, both machines have a rotary action.

**References and Notes**

16. Materials and methods are available as supporting material on Science Online.
Structure of the Rotor Ring of F-Type Na+-ATPase from Ilyobacter tartaricus

Thomas Meier,1 Patrick Polzer,2 Kay Diederichs,2* Wolfram Welte,2 Peter Dimroth1*

In the crystal structure of the membrane-embedded rotor ring of the sodium ion–translocating adenosine 5’-triphosphate (ATP) synthase of Ilyobacter tartaricus at 2.4 angstrom resolution, 11 c subunits are assembled into an hourglass-shaped cylinder with 11-fold symmetry. Sodium ions are bound in a locked conformation close to the outer surface of the cylinder near the middle of the membrane. The structure supports an ion-translocation mechanism in the intact ATP synthase in which the binding site converts from the locked conformation into one that opens toward subunit a as the rotor ring moves through the subunit a/c interface.

In the F1F0 ATP synthase, the cytoplasmic F1 catalytic domain (subunits αβγδε) is linked by means of a central and a peripheral stalk (subunits γε and b2γε, respectively) to the intrinsic membrane domain called F0 (subunits ab2c10-14). Each of these domains functions as a reversible rotary motor and exchanges energy with the opposite motor by mechanical rotation of the central stalk. During ATP synthesis, energy stored in an electrochemical gradient of protons or Na+ ions fuels the F0 motor, which causes the stalk to rotate with the inherently asymmetric γε subunit acting as a camshaft to continuously change the conformation of each catalytic β subunit. These sequential interconversions, which result in ATP synthesis, endow the binding sites with different nucleotide affinities [for reviews see (1–3)]. The rotational model, which explains a wealth of biochemical and kinetic data, is impressively supported by the crystal structure of F0 (4) and was experimentally verified by biochemical, spectroscopic, and microscopic techniques (5).

The F0 motor consists of an oligomeric ring of c subunits that is abutted laterally by the a and b2 subunits (6). The c ring, together with γε subunits, forms the rotor assembly, which spins against the stator components ab2δα1β3.

In the crystal structure of the membrane-embedded rotor ring of the Ilyobacter tartaricus ATP synthase, the cytoplasmic F1 catalytic domain (subunits αβγδε) is linked by means of a central and a peripheral stalk (subunits γε and b2γε, respectively) to the intrinsic membrane domain called F0 (subunits ab2c10-14). Each of these domains functions as a reversible rotary motor and exchanges energy with the opposite motor by mechanical rotation of the central stalk. During ATP synthesis, energy stored in an electrochemical gradient of protons or Na+ ions fuels the F0 motor, which causes the stalk to rotate with the inherently asymmetric γε subunit acting as a camshaft to continuously change the conformation of each catalytic β subunit. These sequential interconversions, which result in ATP synthesis, endow the binding sites with different nucleotide affinities [for reviews see (1–3)]. The rotational model, which explains a wealth of biochemical and kinetic data, is impressively supported by the crystal structure of F0 (4) and was experimentally verified by biochemical, spectroscopic, and microscopic techniques (5).

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