Vacuolar ATPase (V-ATPase) is a multi-subunit complex found in all eukaryotic cells that is responsible for the acidification of intracellular compartments. These compartments include endosomes, lysosomes, Golgi membranes, clathrin-coated vesicles, several types of secretory granules, and the central vacuoles of plants and yeast (48, 53, 62). Each of these intracellular compartments has a specific requirement for the internal pH that is generated by the V-ATPase function (23, 52). In addition to pumping protons into the various organelles and membranes of eukaryotic cells, the pmf generated by V-ATPases is utilized as a driving force for numerous secondary transport processes. V- and F-ATPases have similar structure and mechanism of action, and several of their subunits evolved from common ancestors.

Electron microscopy studies of V-ATPase revealed its general structure at low resolution. Recently, several structures of V-ATPase subunits, solved by X-ray crystallography with atomic resolution, were published. This, together with electron microscopy low-resolution maps of the whole complex, and biochemistry cross-linking experiments, allows construction of a structural model for a part of the complex that may be used as a working hypothesis for future research.

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against the concentration gradient. The two sectors are held together by a stator composed of the membrane-anchored subunit b in F-ATPase and subunits E and G in V-ATPase (Table 1, **FIGURE 1**).

Although the function of the F-ATPase homologous subunits could be easily suggested, V-ATPase has several unique subunits: subunits d, C, and H. These nonhomologous subunits may be responsible for the unique V-ATPase properties. Subunit d (Vma6p) is a unique V-ATPase subunit that was found to be peripherally attached to the cytoplasmic face of the membrane sector (9, 48, 62, 69). Both subunits H and C are present only in eukaryotic V-ATPases. Subunit H (Vma13p) is required for the activity of the enzyme, but the other subunits could be assembled in its absence, suggesting a regulatory function (59). Subunit C (Vma5p) is required for proper assembly of the V-ATPase (9, 14, 34). It has been shown that this subunit has actin- and nucleotide-binding properties (6, 14, 68). Furthermore, it is the only subunit that reversibly leaves the enzyme in glucose deprivation, causing the catalytic subcomplex to detach from the membrane sector (14). This suggests that it acts as part of the stator.

During the last few years, structural information was accumulated on V-ATPase and prokaryotic V-ATPase homologs, which, with additional biochemical, genetic, and electron microscopy data, opened the opportunity to construct a working model for the complete V-ATPase complex.

### Table 1. Subunit composition of F-ATP synthase and V-ATPases from different species

<table>
<thead>
<tr>
<th>Subcomplex</th>
<th>Prokaryotic F-ATPase (E. Coli)</th>
<th>Eukaryotic V-ATPase (S. Cerevisiae)</th>
<th>Bacterial V-ATPases (E. Hirae)</th>
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<tr>
<td>Soluble F1 or V1</td>
<td>Name</td>
<td>Mass</td>
<td>Gene</td>
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<tr>
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<td>50*</td>
<td>VMA2</td>
<td>B</td>
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<td>55*</td>
<td>VMA1</td>
<td>A</td>
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<td>19*</td>
<td>VMA8</td>
<td>D</td>
</tr>
<tr>
<td>γ*</td>
<td>31*</td>
<td>VMA4</td>
<td>E</td>
</tr>
<tr>
<td>ε*</td>
<td>15*</td>
<td>VMA7</td>
<td>F</td>
</tr>
<tr>
<td>Membrane F0 or V0</td>
<td>Name</td>
<td>Mass</td>
<td>Gene</td>
</tr>
<tr>
<td>a</td>
<td>30</td>
<td>VPH/STV1</td>
<td>a</td>
</tr>
<tr>
<td>b</td>
<td>19</td>
<td>VMA10</td>
<td>G</td>
</tr>
<tr>
<td>c</td>
<td>8</td>
<td>VMA3</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VMA11</td>
<td>c’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VMA16</td>
<td>c&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VMA6</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VMA9</td>
<td>e</td>
</tr>
</tbody>
</table>

*Subunits with known structure.
catalytic activity. Furthermore, it has been shown that subunit H interacts with several other proteins: HIV-Nef (21, 40) and ecto-apyrase (74). It was the first eukaryotic V-ATPase subunit to be crystallized, and its structure was determined at 2.95-Å resolution (60). Subunit H is an all alpha helical structure composed of two distinct domains: the NH2-terminal domain (amino acids 2–352) and a smaller COOH-terminal domain (amino acids 353–478). The NH2-terminal domain has five HEAT or armadillo repeat fold, which is the hallmark of the importin family. The importin family import proteins that contain NLS (nuclear localization signal) to the nucleolus. The groove that binds the NLS in importins is occupied in subunit H by its own 10 amino acid starch in the NH2-terminal. The COOH-terminal domain is connected by a short, probably flexible loop, has eight alpha helices, and a similar arrangement and fold as the NH2 terminus but is less ordered (FIGURE 2B). Expression of the NH2-terminal domain, but not the COOH-terminal domain, in yeast vma13 null mutants partially complements the growth defects of the mutant (38). Expression of both domains gave better complementation than either fragment alone.

The structure of subunit H suggests interaction with nuclear components. This is one of several features that distinguish V-ATPase from F-ATPase, which is confined to the interior of the semiautonomous organelle chloroplasts or mitochondria and is precluded from interaction with cytoplasmic components.

Yeast V-ATPase Subunit C

The cDNA encoding subunit C was first cloned from bovine’s adrenal medulla (47, 51). Subsequently, the yeast gene (VMA5) was cloned, and a null mutant, with a typical V-ATPase conditional lethal phenotype, was constructed (9). In this mutant, both V1 and V0 subcomplexes were assembled without binding together to form the full active complex (15, 26). The yeast subunit C is the only subunit that reversibly leaves the enzyme in glucose deprivation, causing the catalytic subcomplex to detach from the membrane sector (14). This suggests that it is part of the peripheral stator of V-ATPase. In addition, it was shown to have actin-binding activity (68) and may undergo structural changes by ADP binding (6, 19). The crystal structure of the yeast V-ATPase subunit C (Vma5p) was recently solved at 1.75-Å resolution (18). It was showed to be composed of three distinct domains: “head” and “foot” that are connected by a flexible elongated “neck” (FIGURE 2C). The foot domain is composed of four anti-parallel beta strands and two short alpha helices in a globular fold formed by the NH2 and COOH terminals. The head domain (amino acids 166–263) has the same structural fold as the foot domain of four anti-parallel beta strands and two short alpha helices, even though there was no obvious sequence homology. The elongated neck domain (amino acids 49–165 and 264–323) is composed of two 80-Å-long alpha helices and a shorter 48-Å-long helix in an alpha helical bundle fold. Vma5p was crystallized in two crystal forms (18): a tetragonal crystal, which diffracted to 1.75 Å, and trigonal crystals, which diffracted to 2.9 Å. The two crystal structures share the same foot and lower neck domain orientations, but the head and the upper neck domains had different conformations. This elastic movement of the head domain was suggested to have a functional role in the smooth coupling of torque energy between the V1 and V0 subcomplexes as part of its stator function.

Crystal Structure of F-ATPase F1 Subcomplex

A major breakthrough in the understanding of F-ATPase function was achieved with the elucidation of the structure of bovine’s heart mitochondria F-ATPase F1 sector in 1994 (1). This crystal structure, at 2.8-Å resolution, of the AMP-PNP-inhibited state of the F1 sector revealed a molecule consisting of alternating α and β subunits in a 3α/3β complex that creates a ring structure. The NH2 terminus of the elongated α helical subunit γ protrudes to the shaft created by the ring structure, whereas its COOH terminus stretches 45 Å away from the 3α/3β complex. The structure and location of subunit γ were found in following structural studies. The subunits ε and γ are bounded, forming a foot-like structure that connects to the c ring of the F0 part (Ref. 63; FIGURE 2A). Three catalytic sites were observed in the 3α/3β complex that varies depending on the nucleotide occupancy: empty, with ADP, or with AMP-PNP (or “open,” “loose,” and “tight” sites, respectively). Those structural features of 3α/3β complex supported the rotational catalysis mechanism.
and were in agreement with Boyer’s hypothesis of the binding change mechanism (12, 13).

The F-ATPase $F_1$ subcomplex known structure was utilized as a homolog of V-ATPase $V_1$ subcomplex for the model (FIGURE 4).

**Crystal Structure of Prokaryotic V-ATPase Subunits**

The ATP-synthase of archaea (A-ATPase) is essentially a chimeric complex, where the catalytic subunits A and B are closely related to the corresponding V-ATPase subunits, and the proteolipids are similar to the F-ATPase c subunits in their size, assembly, and function (45, 46). Even though ATP-synthases of the F- and A-type are predicted to have similar if not identical mechanism of ATP-dependent proton uptake, major differences were recently discovered (29). In A-ATPase (V-type) of *Thermus thermophilus*, at low ATP concentrations, the D subunit rotated stepwise, pausing every 120 degrees, and it consumes one ATP per step. It is in contrast to F-ATPase, which cleaves one ATP at 80 degrees posterior to the binding of ATP. Although A-ATPase generates a torque of 35 pN•nm, F-ATPase generates a torque of 46 pN•nm. This finding suggests that the mechanism of ATP-dependent proton uptake by eukaryotic V-ATPase will be quite different from F-ATPase and even its relative A-ATPase.

**Structure of Subunit B of the Archaea Methanosarcina Mazei**

Subunits A of A- and V-ATPases contain sequences of the canonical ATP-binding site just as the $\beta$ subunit of

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**FIGURE 2. Crystal structure of available yeast V-ATPase subunits and homolog subunits A- and F-ATPases**

A: the highest resolution crystal structure of bovine mitochondrial $F_1$-ATPase that we used for modeling V-ATPase, with all three catalytic sites occupied (PDB 1HBE). $\alpha$ subunits are in red, $\beta$ subunits are in yellow, $\gamma$ subunit is in blue, $\delta$ subunit is in green, and $\varepsilon$ subunit is in cyan. B: crystal structure of yeast V-ATPase subunit H (PDB 1HO8): the different domains are shown. C: crystal structure of yeast V-ATPase subunit C (PDB 1U7L): the different domains are highlighted. D: crystal structure of the NtpK subunits Ring (as homolog of subunits C, C’, and C” of yeast V-ATPase) from Na$^+$-translocating V-ATPase (PDB 2BL2): each NtpK subunit is colored differently. The conserved glutamate residue is colored by CPK and highlighted in red circle. E: crystal structure of *Thermus thermophilus* A-ATPase subunit C (PDB 1RSZ, suggested homolog of yeast V-ATPase subunit D).
F-ATPase. Subunit B of A- and V-ATPases do not have the consensus GxGKxGKT/S that is the phosphate binding P-loop present in F-ATPase catalytic subunits. Thus the function of ATP binding demonstrated for B subunits is not clear. Recently, subunit B of the archaea *M. mazei* was expressed, crystallized, and solved at 1.5-Å resolution (61). Superposition of the B subunit of A-ATPase and the α subunit of F-ATPase provided new insights into the similarities and differences between these two subunits. The overall structure of subunit B is similar to that of the related noncatalytic α subunit of F-ATPase (1, 61). Remarkably, even though their sequence identity is only 25%, both subunits superimpose with root mean square deviation of 1.85 Å. Subunit B of *M. mazei* is about 60% identical to the corresponding subunit B in V-ATPases, and it is likely that it will closely represent the subunit structure. Yet the published structure reveals no further information on the possible rotary mechanism of ATP-dependent proton uptake by eukaryotic V-ATPases.

**The Structure of NtpK K Subunit that is Homologous to Subunit c, c’, and c” of Eukaryotic V-ATPase**

Subunit c (proteolipid) of V-ATPase was first identified and cloned from bovine’s adrenal medullas (41). The yeast gene (VMA3) was subsequently cloned, and a null mutant was constructed. It showed a typical V-ATPase conditional lethal phenotype (49, 50, 67). The V-type ATPase c subunit (16 kDa) is twice as large as the equivalent F-type ATPase proteolipid subunit (8 kDa) and has twice as much transmembrane helices. It is thought to be evolved from gene duplication and fusion events from an ancestral 8-kDa proteolipid (45, 46, 49). Subsequently, two other homologous proteins were found in the yeast genome (VMA11 and VMA16) and their gene products were denoted as c’ and c” (25). Similar genes were found in *C. elegans*, mammals, and plants (7, 55). The yeast deletion mutants in these subunits share the same V-ATPase null-mutant phenotype. This observation was supported by substitution of the critical glutamate residue at the proton binding pocket, which resulted in properly assembled but not active complex (25, 54). It was proposed that the three different proteolipid subunits in eukaryotic V-ATPase were evolved by the need to change the coupling efficiency of V-ATPases by a "slip" mechanism (52).

The eubacteria *Enterococcus hirae* has a V-type sodium ATPase (NtpK) that exhibits a significant homology to the eukaryotic V-ATPase in most of its subunits (43). Recently, its rotor ring that is composed of 10 c subunits, each of four transmembrane helices, was crystallized and solved to 2.1-Å resolution (44). Simultaneously, the crystal structure of F-type sodium ATPase from *Ilyobacter tartaricus* was solved to 2.4-Å resolution (42). Remarkably, despite the fact that it is half the size of the NtpK ring (the subunits are composed of only two transmembrane helices), its rotor ring is composed of 10 proteolipids as well. Thus the rotor ring of NtpK has a significantly larger diameter, but since each c subunit binds one sodium ion, the stoichiometry of ATP/Na is identical in the two enzymes (33). The sodium binding sites of the NtpK rotor ring are located in a pocket deeply embedded in the membrane, formed between helix H2 and H4 (FIGURE 2D). This pocket includes the conserved and essential amino acids that are present in all V-type ATPases. The high resolution that was obtained pro-
vided the possibility to fit bound lipids and detergent molecules, which are situated in both the middle hollow part of the ring and outside facing the membrane.

**T. Thermophilus Subunit C Structure**

The A-ATPase of *T. thermophilus* contains a subunit that unfortunately is denoted as subunit C but is not homologous, analogous, or structurally related to the C subunit of eukaryotic V-ATPase (18, 73). Recently, the structure of this protein was solved to 1.95-Å resolution (31). The crystal structure revealed an all alpha helical, threefold symmetry, funnel-shape fold with dimensions of $45 \times 50 \times 50$ Å (FIGURE 2E). This subunit co-purified with the membrane sector and was shown to cross-link to the proteolipid ring subunit L, which is the *T. thermophilus* homolog of eukaryotic V-ATPase subunit c proteolipid (73). This bacterial subunit C has very low (if any) sequence homology to the eukaryotic V-ATPase subunit d; however, it was suggested to function similarly (31). Subunit d was initially cloned from bovine’s adrenal medulla (69). The yeast gene encoding this subunit (VMA6) was cloned, and a null mutant was constructed (8). The vma6 null mutants exhibited identical properties compared with the other V-ATPase null mutants, and V1 failed to associate with V0. These findings suggested a possible function in linking the two parts of the enzyme. Subunit d is part of the V0 sector, but since it is not an integral membrane protein it is peripherally associated with the V0 sector on the cytosolic side of the membrane.

The structure of the *T. thermophilus* enzyme has a polar cavity open to one side and a polar surface with no apparent hydrophobic anchor to the membrane sector. The structure consists of three distinct domains related by a nonperfect threefold symmetry, suggesting gene triplication (even though there is no obvious sequence homology). Each domain is composed of two central 50-Å-long and four 25-Å-long peripheral

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**FIGURE 4. A proposed model of yeast V-ATPase**

The different subunits are colored as in Figure 1, assembled together using the data summarized in Table 1 and Figure 2. Subunits with no structural data are shown as spheres with arbitrary size. The model was built by manually placing the different structures with DeepView program (http://www.expasy.org/spdbv/).

<table>
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<tr>
<th>Experimental Method</th>
<th>Species</th>
<th>V0→V0 Interactions</th>
<th>V0→V1 Interactions</th>
<th>V1→V1 Interactions</th>
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<td>a→c'</td>
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<td></td>
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<td></td>
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<td></td>
<td>T. therm</td>
<td></td>
<td></td>
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</table>

helices. The central helices create the central cavity, which has a remarkable fitting to the cavity at the cytoplasmic face of the L (or c) ring. This observation was supported by a site-directed mutagenesis and cysteine-mediated chemical cross-linking (31).

The question as to whether *T. thermophilus* subunit C is structurally, and functionally, related to V-ATPase subunit d remains open, but, as frequently happens, highly resolved structures easily capture our imagination. Therefore, for the time being, the notion that *T. thermophilus* subunit C is homologous to subunit D of V-ATPase and fitted its structure is adopted for the model presented in this review.

**Subunit Interactions in V-ATPase**

The connection between subunits in the V-ATPase complex was studied by a variety of methods: binding assays, yeast two hybrid system, and chemical cross-linking. A summary of the published observations is given in Table 2. Knowledge of the overall structure of V-ATPase and especially high-resolution structure of individual subunits allowed the construction of sensible mutations. Insertions of cysteine residues in structurally defined sites and performance of cysteine-mediated cross-linking have recently helped to locate those areas in subunit C that interact with subunits E and G (30). This data was used for the construction of a working model depicted in FIGURE 3. Each of the methods used for the study of subunit interactions might give erroneous results. Although there is no replacement for straight-forward structural data, while awaiting the crystal structure of V-ATPase, one must resort to other methods that provide relatively low-resolution structural data.

**Electron Microscopy Studies**

Electron microscopy (EM) was successfully used for obtaining low-resolution structures of macromolecules and protein complexes. Resolution better than 10 Å may be obtained by single-molecule cryo-electron microscopy, especially if high symmetry objects are studied (10). The first electron microscopy images of the V-ATPase revealed a two-sector shape that closely resembled the overall structure of the well-described F-ATPase (11). An excellent review on the structure of V-ATPases revealed by EM was published recently (71). The EM structures from bovine brain (70), insects (59), yeast (75), and plants (37) have been presented, and all share the same structural motifs. The EM maps (16, 31, 70) were used to dock the high-resolution structures of V-ATPase and its homologs, which adds more restraints to the cross-linking experiments and other protein-protein interaction assays when one attempts to construct a structural model. Furthermore, by a differential imaging of a complex with and without subunit h, its precise location in the complex was determined (70). The model suggested in this review utilized the published EM structure of yeast V-ATPase. Direct docking of the suggested model to EM electron density maps is hard to accomplish due to the absence of EM electron density map data files.

**Conclusions and Future Directions**

A model of V-ATPase structure, based on known X-ray structures of V-ATPase subunits and homologs, and information on subunit interactions observed by different biochemical methods was presented. The model illustrates the overall dimensions of this complex and the subunit interactions within it. Only two subunits (C and H) have been solved so far from the yeast V-ATPase. Interestingly, both subunits assemble at the last stage of the complex formation, since one can purify large assemblies of V-ATPase subcomplex in the absence of those subunits. This fact suggests that both subunits should be stable monomers in vivo, which facilitate their ability to crystallize. All other subunits, except subunit C of thermophilus (homologs to subunit d of eukaryotic V-ATPase), were solved as a complex with other subunits, and some of them seemed to be unstable as monomers. In contrast to the F-ATPases, which can be purified in abundance from mitochondria, chloroplast, or bacteria, there is no good source for V-ATPase complex. To overcome this problem, single V-ATPase subunits were expressed in *E. coli*. This approach yielded the first two eukaryotic V-ATPase structures. Not all the yeast V-ATPase subunits could be readily expressed or, even if large amounts of highly purified protein were obtained, crystallized. Two approaches are accessible: first, co-expression and co-crystallization of V-ATPase subunits in *E. coli*, which are known to form stable intermediate subcomplexes (E and G, A and B, D and F, A+B+D+E, and more combinations). The second approach is purification of the native or the separate sectors of V-ATPase from the available sources. This is not an easy path to follow, but this road could hopefully yield a complete structural model for this important enzyme.

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**References**


