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Structure and Mechanism of Vacuolar Na^+-Translocating ATPase From *Enterococcus hirae*

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V-type Na^+-ATPase from *Entercoccus hirae* consists of nine kinds of subunits (NtpA$_3$, B$_3$, C$_1$, D$_1$, E$_{1-3}$, F$_{1-3}$, G$_1$, I$_1$, and K$_{10}$) which are encoded by the *ntp* operon. The amino acid sequences of the major subunits, A, B, and K (proteolipid), were highly similar to those of A, B, and c subunits of eukaryotic V-ATPases, and those of β, α, and c subunits of F-ATPases. We modeled the A and B subunits by homology modeling using the structure of β and α subunits of F-ATPase, and obtained an atomic structure of NtpK ring by X-ray crystallography. Here we briefly summarize our current models of the whole structure and mechanism of the *E. hirae* V-ATPase.

KEY WORDS: Na^+-ATPase; vacuolar ATPase; *Enterococcus hirae*; membrane protein; crystal structure; Na^+ binding.

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- or V-ATPases (Stevens and Forgac, 1997), multisubunit complexes related to the F-ATPases (ATP synthetases) found in eubacteria, mitochondria, and chloroplasts. Both classes have globular catalytic domains, V_1 and F_1, where ATP is hydrolyzed (and synthesized in F-ATPases), and are attached by central and peripheral stalks to intrinsic membrane domains, V_0 and F_0, where ions are pumped across the membrane. ATP hydrolysis generates rotation of the central stalk and an attached membrane ring of hydrophobic subunits. Ions are pumped through a pathway in the interface between the rotating c ring and subunit a as a static membrane component, which is linked to the outside of the V_1 or F_1 domain by the peripheral stalk. In extensive studies of the structure of F-ATPase, atomic structures of most of the subunits except for subunit a have been so far obtained. However, the information about V-ATPase structure is limited; atomic structures of several minor subunits, e.g., C and H subunits from *S. cerevisiae* (Sagermann *et al*., 2001; Drory *et al*., 2004) and C subunit from *T. thermophilus* (Iwata *et al*., 2004) have been obtained.

V-ATPases are also found in prokaryotes. The enzyme in the nonrespiring bacterium *Enterococcus hirae* acts as a primary sodium extrusion system (Kakinuma *et al*., 1999). Its subunit composition is simpler than that of its eukaryotic counterparts, and its nine subunits are encoded in the *ntp* operon (Fig. 1(A)). We have established a purification and reconstitution system (Murata *et al*., 1997, 1999), and have been studying the molecular features of the enzyme. A current model for the structure and mechanism of the *E. hirae* V-ATPase is here briefly summarized.

Structure and Function of V_1 Domain

The V_1 domain is a 500-kDa peripheral complex responsible for ATP hydrolysis. V_1 is composed of Ntp

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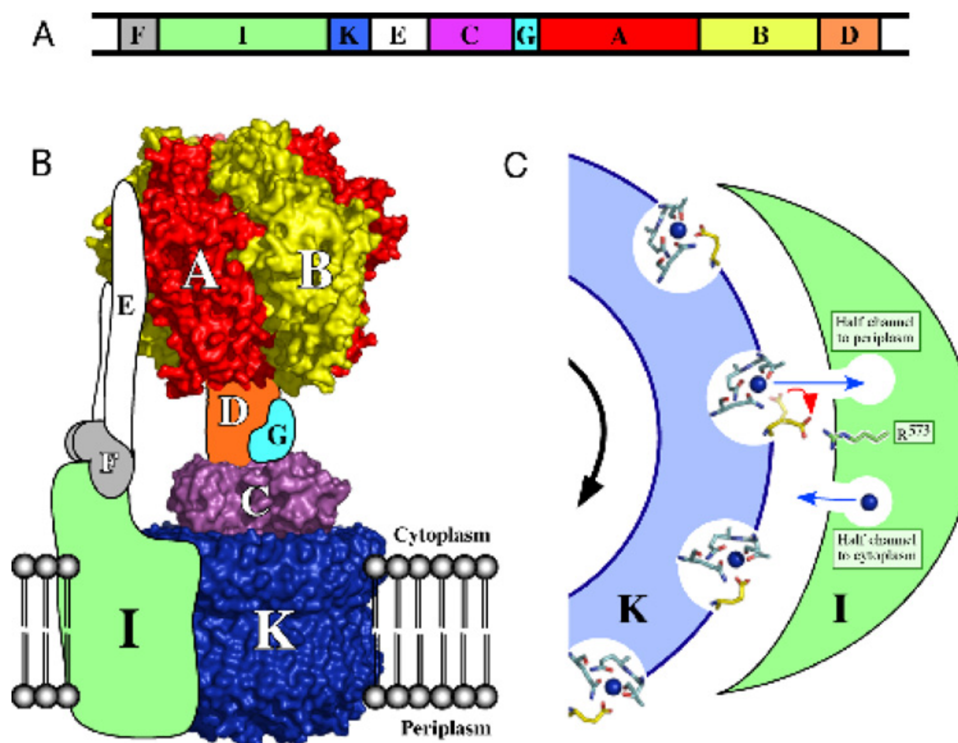

Fig. 1. Schematic structure and mechanism models of *E. hirae* V-type Na^+-ATPase. (A) Structure of the *ntp* operon. (B) Structure model. 3D-structures of NtpA, B and C were constructed by homology modeling based on the X-ray crystal structure of the β subunit, and α subunit of bovine mitochondrial F_1-ATPase (PDB code; 1BMF) and C subunit of *T. thermophilus* V-ATPase (PDB code; 1R5Z), respectively. NtpA, B, C and K were shown in space-filling representation, and the others whose structures are unknown were shown as hand-made cartoon. The color code for subunits is the same as that of the *ntp* operon. (C) ion transport mechanism. The model is based on the crystal structure of the K ring. The view is from the cytoplasm and shows the K ring-NtpI interface at the level of the Na^+-binding sites. Residues involved in Na^+ binding are shown in stick representation. Oxygen and nitrogen atoms are red and blue, respectively. Glu^{139} is shown in yellow, and other residues (Leu^{61}, Thr^{64}, Gln^{65}, and Gln^{110}) are in light blue. Sodium ions are shown as blue spheres. NtpI is in green and in close proximity to the K ring with the essential Arg^{573} in a half-channel connecting to the periplasm. Another half-channel links the ion-binding sites to the cytoplasm.

A, B, C, D, E, F, and G. The subunits A and B both participate in nucleotide binding with the catalytic site located on the A subunit, and are modeled based on the structure of the β and α subunits of the F-ATPases, respectively (Murata *et al*., 2002; Hosaka *et al*., 2004). Three copies of each subunit are arranged around the central stalk made of single copies of subunits D and G. The central stalk rotates by a conformational change of the A subunit caused by binding and hydrolyzing ATP. The precise locations of the F and E subunits are uncertain, but they are thought to make a subcomplex and to constitute the peripheral stalk. The C subunit has no counterpart in F-ATPases and may connect the foot of the central stalk to the membrane rotor ring of V_0 (Iwata *et al*., 2004).

Structure and Function of V_0 Domain

The V_0 domain is a 240-kDa integral complex that is responsible for Na^+ translocation across the membrane. V_0 contains the 16-kDa NtpK which forms a ring with 10-fold symmetry and a single copy of the I subunit. Recently we succeeded to solve the structure of the membrane rotor ring of the enzyme as the first high-resolution ring structure from either a V-type or an F-type enzyme, and proposed an ion transport mechanism based on the ring structure (Murata *et al*., 2005). NtpK has a binding site for Na^+ ion. Ten sodium ions are bound to the specific binding pockets each of which is composed of five oxygen atoms 2.2–2.3 Å distant, four of them in

the side chains of Thr^{64}, Gln^{65}, Gln^{110} and Glu^{139}, and the fifth in the main chain carbonyl of Leu^{61}. They are located on the external surface of the ring, and the hydrophobic surface arrangement places the Na^+-binding site close to the center of the bilayer. The ring structure supported a “two-half-channel” ion translocation model rather than “one-half-channel” model as proposed for F-ATPases (Dimroth *et al.*, 2000). The clockwise rotation of the K ring with D, G, and C subunits in V_1 by using ATP hydrolysis energy as shown in black arrow brings an occupied Na^+-binding site into the interface between the K-ring and I subunit (Fig. 1C). The proximity of the Na^+ site to NtpI-Arg^{573}, which is essential for ion transport (Kawano *et al.*, 2002), produces an electrostatic interaction between Arg^{573} and Glu^{139} as shown in red arrow, releasing the Na^+ ion into the periplasm via a half-channel in NtpI as shown in blue arrow. Further rotation, which may disrupt the Arg–Glu interaction, brings the site in contact with a second half-channel, resulting in the binding of a cytoplasmic Na^+ ion as shown in another blue arrow. Based on this model, we are now going to study the details of ion translocation mechanism of V-ATPase.

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