Review

Structure, Function, and Evolution of Proton-ATPases

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NATHAN NELSON
Department of Biochemistry, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110

ABSTRACT

Proton-ATPases are among the most important primary ion pumps in nature. There are three classes of these enzymes which are distinguished by their structure, mechanism of action, and evolution. They function in ATP formation at the expense of a protonomotive force generated by oxidative and photosynthetic electron transports, maintaining a constant pH in the cytoplasm, and forming acidic spaces in special compartments inside and outside the cell. The three classes of proton-ATPases evolved in a way that prevents functional assembly in the wrong compartment. This was achieved by a triple genetic system located in the nucleus, mitochondria and chloroplast, as well as delicate control of the proton pumping activity of the enzymes.

The vital function of proton concentrations in homeostasis was the first to be recognized, and in the last two decades the crucial role of protonmotive force in bioenergetics was clearly demonstrated. Proton-ATPases are the enzymes responsible for controlling these processes. Their function is to balance the pH in the cell, to form protonmotive force, or to harness this potential energy for ATP formation. The ATP can be utilized by other primary ion pumps and the protonmotive force can drive many secondary processes such as ion and nutrient uptake. The central role of proton-ATPases is evident from the fact that they are vital for every living cell, and there is no known life without them. Proton-ATPases can be divided into three main classes: (a) Plasma membrane-type, operates via a phosphoenzyme intermediate; therefore it is part of the E\_1-E\_2 ion pumps, and it is inhibited by vanadate. The function of the plasma membrane proton-ATPase is to secrete protons, and by so doing to maintain ApH acidic outside the cell and membrane potential negative inside. (b) The eubacterial-type, which is present in eubacteria, mitochondria and chloroplasts. It operates without phosphoenzyme intermediate and its main function is to phosphorylate ADP at the expense of protonmotive force formed by oxidative or photosynthetic electron transport. In eubacteria it also functions in forming the protonomotive force for secondary uptake systems at the expense of ATP formed by substrate fermentation. (c) The vacuolar-type ATPase, which is present in the vacuolar system of eukaryotic cells and probably in archaea. It functions in a controlled pumping of protons into organelles of the vacuolar system via an unknown mechanism that presumably does not involve a phosphoenzyme intermediate. In archaea it may function in proton secretion and ATP formation.

THE PLASMA MEMBRANE-TYPE PROTON-ATPase

The gene coding for the enzyme of yeast and neurospora was cloned and sequenced (1, 3, 5, 21). The enzyme is composed of a single polypeptide of 100 kD that appears to cross the membrane 10 times with most of the hydrophilic parts facing the internal side of the membrane. While the function of the enzyme is probably restricted to the cell membrane, like most of the membrane proteins, the site of synthesis of it is on the endoplasmic reticulum. Following synthesis and posttranslational processing the enzyme is transported to the cell membrane via part of the secretory pathway (Fig. 1). Although it may serve as temporary proton pump on its way out, in most cases it should be inactive during its transport. This regulation may be achieved by a specific requirement for phospholipids that are more abundant in the plasma membrane or by a control imposed by phosphorylation of the enzyme by protein kinase.

Sequencing analysis of the plasma membrane proton-ATPases in comparison with other E\_1-E\_2 enzymes, revealed small stretches with quite extensive similarities especially in the suspected ATP

FIG. 1. Evolution of proton-ATPase and functional distribution in plant cell. Plasmamembrane proton-ATPase; ( ), eubacterial-type proton-ATPase; ( ), vacuolar proton-ATPase; CHROM, chromoplast; OX. PHOS, oxidative phosphorylation; OX. PHOTO, oxygenic photosynthesis; N, nucleus, M, mitochondria; CHL, chloroplast; V, vacuole; G, Golgi complex; ER, endoplasmic reticulum; E, endosome; CV, coated vesicle; SV, secretory vesicle; RV, recycling secretory vesicle, M-SV, membrane secretory vesicle for refurbishing the plasma membrane; o, ligand; Y, receptor.

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binding site (21). Among the enzymes that showed similarity were Na+/K+-ATPase, CA+-ATPase and K+-ATPase B from *Escherichia coli*. It was concluded that all of these enzymes may have evolved from a common ancestral gene (21). That the origin of this gene may reside in eubacteria is suggested by the presence of E1-E2 enzymes in *E. coli*. However, during its lifetime of nearly a billion years in the guts of animals, *E. coli* could fish for many genes, one of which may be Na+/K+-ATPase that later evolved into K+-ATPase and other related enzymes. As depicted in Figure 1, we favor the view that E1-E2 enzymes evolved from proteo-
karyons and spread to other organisms via natural gene transfer mediated by viruses or spontaneous transformation by DNA fragments. Of course, one cannot prove this hypothesis because we cannot go back in evolution even by simulation due to the lack of 'reverse translate' system.

**THE EUBACTERIAL-TYPE PROTON-ATPases**

The enzymes of this class consist of two distinct structures: a catalytic sector which is hydrophilic in nature and can catalyze the ATPase activity of the proton pump, and a membrane sector that is hydrophobic in nature and can conduct protons across the membrane. The entire enzyme is composed of 8 to 12 different subunits. The β subunit of the catalytic sector contains the active site and beside the β subunit only the α contains nucleotide binding sites (12, 16). Despite two decades of extensive studies, the mechanism of action of these enzymes was not resolved. However, a few features of the enzyme have been established. Mild treatments such as washing with EDTA or exposure to sonication can release the catalytic sector from the membrane (12). The resulting membranes are permeable to protons and this proton conductance can be blocked by the addition of the purified catalytic sector. The mechanism of proton conductance is not clear and it is not known whether all of the membrane sector is involved in proton conductance. The stoichiometry of proton conductance is most probably 3 H+/ATP. Therefore, this class of enzymes usually operates at the thermodynamic equilibrium, and in most cases the rates of ATP formation or hydrolysis are determined by the extent of the proton motive force across the membrane. Kinetic control of the enzyme is achieved by the presence of a specific polypeptide called ATPase inhibitor (16). This control is very important in chloroplasts which must prevent ATP hydrolysis in the dark.

There are three unique features of eukaryotic cells: separate nucleus, developed vacular system, and semiautonomous organelles. Even though most of the genetic information is stored in the nucleus, chloroplast and mitochondria retained control over the synthesis of several of their proteins via their own unique DNA and RNA molecules and protein-synthesis machinery (17). This has led to a widely accepted hypothesis that the origin of chloroplasts and mitochondria was endosymbiosis between a proteoekaryot (with a developed nucleus) and eubacteria possessing a fully developed machinery for carrying out oxygenic photosynthesis and oxidative phosphorylation (11, 19). These bacteria are now represented as chloroplast and mitochondria, and the special features of their DNA, RNA, and protein synthesis process are taken as solid evidence for the above evolutionary pathway. Regardless of how mitochondria and chloroplasts evolved, most of their oligomeric membrane protein complexes are now synthesized by both cytoplasmic and organelle ribosomes. The β subunit of ATPases present in chloroplasts and mitochondria is a eubacterial-type enzyme. In eukaryotic cells it is present exclusively in these organelles and there is no evidence for the existence of a functional enzyme of this type without the involvement of the organelle protein synthesis machinery. Even though the synthesis of their subunits is shared by cytoplasmic and organelar ribosomes, assembly takes place inside the organelles. Therefore, no transport of partially active unit is expected during the biogenesis of these enzymes. Reports on function of these enzymes outside mitochondria or chloroplasts proved to be wrong (16).

The β subunit of the catalytic sector contains the active site of the enzyme. This subunit is also the most conserved one, and this was evident from immunological cross-reactivity of proton-ATPases from *E. coli* through chloroplasts up to mammalian mitochondria (20). Recently this observation was extended to archaebacteria, suggesting that the eubacterial-type proton-ATPase evolved from an ancestral enzyme of archaebacteria (7). Sequence data revealed that the DNA and amino acid sequences of the β subunits from various sources are remarkably preserved during evolution from eubacteria up to the organelles of higher organisms. Moreover, the genes coding for the chloroplasts proton-ATPase exist in the same order in the chloroplast DNA as in the operon of *E. coli* (6). There are very few examples in which evolution left so many footprints in a single system. Therefore, it is likely that this class of proton ATPases evolved from a common ancestral gene that can be traced back to archaebacteria (Fig. 1).

**THE VACUOLAR-TYPE PROTON-ATPases**

As shown in Figure 1, the vacuolar type enzyme is the most diversified proton-ATPase in eukaryotic cells. It is present in lysosomes, plant and fungal vacuoles, the Golgi complex, clathrin-coated vesicles, synaptic vesicles, several secretory granules, and probably other organelles that are yet to be discovered (2, 4, 8, 9, 13, 14, 18, 23). The proton-ATPases function in a controlled acidification of the interior of the organelles and formation of a positive membrane potential inside. Since the stoichiometry of the proton pump is probably 2 H+/ATP, at equilibrium a ΔpH of over three units should be formed. Yet the most common ΔpH is about 1.5 units, and a kinetic control should be imposed on this class of proton-ATPases in order to prevent them from reaching thermodynamic equilibrium resulting in overacidification of the organelles (2, 14). This control may be achieved by ATPase inhibitors, specific requirement for phospholipids, and loosely coupled mechanism of proton pumping modulated by factors such as various anions. The proton pumping activity of the vacuolar ATPases is enhanced by Cl- ions and inhibited by nitrate. The ATPase activity of the same enzymes is less sensitive to these anions, a manifestation of the loose coupling between these two processes. Except for the inhibition by nitrate, vacuolar ATPases are inhibited by N-ethylmaleimide and unlike the plasma membrane enzymes they are not sensitive to vanadate. The vacuolar ATPases are composed of 3 to 8 different polypeptides tightly bound to each other and are not disrupted by treatment of EDTA. The site of synthesis of this class of enzymes is on the endoplasmic reticulum. It is not known precisely where each one of these enzymes is assembled, but they are likely to be assembled in the Golgi, and after sorting, to be transported to the target membranes. In some organelles such as the vacuole the enzyme may stay in the same compartment during the lifetime of the membrane, while in others such as secretory granules it may be temporarily disposed of on the plasma mem-

### Table 1. Subunit Structure of Vacuolar H+-ATPases

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<th>Source</th>
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<tr>
<td>Clathrin-coated vesicles</td>
<td>116</td>
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<tr>
<td>Chromaffin granules</td>
<td>115</td>
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<tr>
<td>Plant vacuoles</td>
<td>115</td>
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<td>Fungal vacuoles</td>
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<td>Archaeabacteria</td>
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<td>Source</td>
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brane during exocytosis and even recycled several times before it will turn over (13). During transport, exocytosis, and recycling, the activity of the enzyme must be controlled to prevent proton pumping into spaces that should not be acidified.

Figure 1 depicts proposed evolutionary pathways for the three classes of proton-ATPases. The vacuolar proton-ATPase is a landmark for the secretory pathway of eukaryotic cells. It was detected and partially purified from several organelles connected with this system (2–4, 8, 9, 13, 14, 23, 24). Table I depicts a probable subunit structure of the enzyme from various sources (see Refs. 3 and 13 for more detail). The enzyme from chromaffin granules and clathrin-coated vesicles contains a subunit of 115 kD that binds dicyclohexyl-carbodiimide (DCCD) and ATP, and may play an important role in the catalysis, assembly, or sorting out of these enzymes (14, 24). Another subunit of about 70 kD is present in the enzymes from all of the sources and it was implicated in the catalysis of ATPase and/or proton pumping activity of the enzyme (8, 14, 18). An immunological cross-reactivity among polypeptides of about 70 kD from various sources was reported (10, 18). The polypeptide of 115 kD was not detected so far in enzyme preparations from plant and fungal sources. If indeed it is not present there, it cannot be as important as the 70 kD polypeptide for the catalytic activity of the enzyme. However, the 115 kD subunit is easy to miss due to its sensitivity to proteolytic enzymes and its tendency to aggregate at elevated temperatures even in the presence of SDS. So far the common denominator of this class of proton-ATPases is the existence of a DCCD-binding polypeptide which may be analogous, but not homologous, to the eubacterial one (22). However, conclusions on its evolutionary origin can only be drawn when the sequences of these polypeptides become available.

An ATPase enzyme was detected and partially purified from the archaebacterium Halobacterium halobium (15). The polypeptides present in the preparation and the sensitivity to nitrate and N-ethylmaleimide suggested a relation to the vacuolar-type ATPase. Since the vacuolar type and the eubacterial type operate without phosphoenzyme intermediate they are likely to share some common features such as utilizing proteolipid and multnucleotide binding sites. These features cannot be taken as an indication for common evolutionary pathway. It is tempting to suggest that the vacuolar ATPases evolved together with the vacuolar system from archaebacteria and perhaps proteoekaryot (Fig. 1). It may be that the origin of the eubacterial type ATPase is also archaebacteria which may be evolutionarily very distant from the one that was bearing an ancestral gene for the vacuolar type enzyme. The vacuolar type ATPases may have segregated into a few subclasses quite early in evolution. Thus, the enzymes present in catabolic vesicles such as lysosomes and plant vacuoles may be somewhat different from those that function in receptor mediated endocytosis and those present in secretory granules. Therefore, it is of immense importance to reveal the relations among these enzymes via thorough study of their molecular biology.

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