Journal of Bioenergetics and Biomembranes, Vol. 24, No. 4, 1992

Structural Conservation and Functional Diversity of V-ATPases

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Received February 16, 1992; accepted March 16, 1992

The vacuolar system of eukaryotic cells contains a large number of organelles that are primary energized by an H^+-ATPase that was named V-ATPase. The structure and function of V-ATPases from various sources was extensively studied in the last few years. Several genes encoding subunits of the enzyme were cloned and sequenced. The sequence information revealed the relations between V-ATPases and F-ATPases that evolved from common ancestral genes. The two families of proton pumps share structural and functional similarity. They contain distinct peripheral catalytic sectors and hydrophobic membrane sectors. Genes encoding subunits of V-ATPase in yeast cells were interrupted to yield mutants that are devoid of the enzyme and are sensitive to pH and calcium concentrations in the medium. The mutants were used to study structure, function, molecular biology, and biogenesis of the V-ATPase. They also shed light on the functional assembly of the enzyme in the vacuolar system.

KEY WORDS: Proton pumps; ATPase; V-ATPase; vacuolar system; eukaryotes; chromaffin granules; yeast cells; mutants.

INTRODUCTION

Eukaryotic cells contain a wide variety of organelles that function in many different vital processes. The organelles maintain concentration gradients of solutes across their membranes, and consequently constant energy input is required to generate and maintain the gradients. V-ATPases provide the main energy source for these processes, and therefore they should function differently in the various organelles. The requirement of this versatility is in contrast with the conserved structure of the enzyme.

Proton pumping into the vacuolar system generates an electrochemical gradient of protons (protonmotive force–pmf) that is composed of a membrane potential and a proton gradient. The various organelles of the vacuolar system require different ratios of the two components of the protonmotive force. This bioenergetic requirement may have played a major role in the evolutionary selection of V-ATPases as the primary active transport protein of the vacuolar system of eukaryotic cells.

V-ATPases also function in the plasma membranes of specialized systems such as kidney cells and osteoclasts. Understanding the molecular biology and the bioenergetics of the various V-ATPases may advance our knowledge not only of the properties of one of the most fundamental enzymes in nature but also may advance our understanding of several basic biological processes and can help in the design of drugs for treatment of certain diseases.

STRUCTURE OF THE CHROMAFFIN GRANULE V-ATPase

Historically the chromaffin granules provided the first evidence for the existence of a proton ATPase in the vacuolar system of eukaryotic cells (Kirshner,

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0145-479X/92/0800-0407$06.50/0

1962; Carlsson *et al.*, 1962; Bashford *et al.*, 1975; Njus and Radda, 1978; Mellman *et al.*, 1986). It was demonstrated that catecholamine uptake is driven by protonmotive force generated by an ATP-dependent proton pump that was later named V-ATPase (Pollard *et al.*, 1976; Casey *et al.*, 1977; Holz, 1978, 1979; Schuldiner *et al.*, 1978; Johnson and Scarpa, 1979). Therefore it was established that an electrochemical gradient of protons generated by a H^+-ATPase is the driving force for catecholamine uptake, a process that is a fine example for the chemiosmotic theory (Mitchell, 1979). However, the identity of the H^+-ATPase from chromaffin granules remained elusive for some time (Buckland *et al.*, 1979; Apps and Glover, 1978; Apps and Schatz, 1979; Apps *et al.*, 1980; Cidon and Nelson, 1983, 1986; Percy and Apps, 1986; Moriyama and Nelson, 1987a,b, 1988a). The most conclusive information on the structure of V-ATPases from chromaffin granules came from studies on cold inactivation of the enzyme (Moriyama and Nelson, 1989a,b,c; Nelson, 1989). Incubation of membranes containing V-ATPases on ice in the presence of MgATP and salts such as 0.2 M NaCl results in inactivation of the ATPase and proton pumping activities of the enzyme and separation of the catalytic sector from the membrane. The cold inactivation of V-ATPases required both Mg^{+2} and ATP, and omission of one of them or inclusion of EDTA during the incubation on ice prevented the cold inactivation. Moreover, low NEM concentrations prevented the liberation of the catalytic sector from the membrane. Therefore, the detachment of the catalytic sector from the membrane is absolutely dependent on the proper binding of MgATP onto the catalytic site of the enzyme. It is likely that this binding results in a conformational change in the enzyme which renders the binding of the catalytic sector to the membrane sector hydrophobic in nature. Since hydrophobic interactions are weakened at low temperatures, the incubation on ice results in the dissociation of the catalytic sector from the membrane and loss of its enzymatic activities. Cold inactivation under these conditions is unique for V-ATPases and is a valuable tool for studying the structure of these enzymes. Cold inactivation of membranes from various sources containing V-ATPases resulted in a specific liberation of five polypeptides from the membranes with molecular weights of about 70, 60, 40, 35, and 30 kDa. These polypeptides were named subunits A to E of the catalytic sector (V_1) of the V-ATPases (Nelson, 1989). Four of the cold-release polypeptides (subunits A, B, C and E) were identified by antibodies and amino acid sequences identical to or related to gene products of cloned *c*DNAs. A fifth polypeptide was identified as subunit D only by immunological cross reactivity (Moriyama and Nelson, 1989a,b,c). So far the gene encoding this subunit has not been cloned. It is thought that the catalytic sector of the V-ATPase of chromaffin granules has a subunit stoichiometry similar to that established for the enzymes from clathrin-coated vesicles, namely 3A, 3B, 1C, 1D, and 1E (Arai *et al.*, 1988).

The purified and reconstituted V-ATPase from chromaffin granules contains at least four additional polypeptides. Two of them with apparent molecular weights of 16 and 20 kDa are very hydrophobic and may comprise the membrane sector (V_0) of the enzyme and function in proton conduction across the membrane (Nelson, 1989). The function of the other two is not clear and they were designated as accessory polypeptides. A polypeptide of apparent molecular weight of 39 kDa was isolated from the purified enzyme (Moriyama and Nelson, 1988a). The antibody that was raised against this subunit cross-reacted with all enzymes of mammalian sources that were tested (Moriyama and Nelson, 1989a,b,c). The gene encoding this polypeptide was cloned and sequenced and the deduced gene product was found to be hydrophilic in nature (Wang *et al.*, 1988). This polypeptide remains in the membrane following cold inactivation and it may be bound to one of the polypeptides of the membrane sector. One of the most notable polypeptides in preparations of V-ATPases from various sources is a polypeptide of about 115 kDa. This polypeptide is tightly bound to the membrane, sensitive to proteolytic digestion, and is the only subunit of V-ATPases that is glycosylated (Nelson, 1989; Adachi *et al.*, 1990). Recently a *c*DNA encoding this subunit in bovine brain was cloned and sequenced (Perin *et al.*, 1991). The predicted amino acid sequence contains six potential transmembrane segments. It was proposed that this subunit functions in proton conduction across the membrane. It is worth noting that one recently purified and reconstitutively active preparation of the plant V-ATPase did not contain the 115-kDa polypeptide (Ward *et al.*, 1992). This polypeptide was not detected in preparations of V-ATPase from the midgut of insects (Wieczorek *et al.*, 1991). If these observations are verified by other means, it would be unlikely that the 115-kDa polypeptide functions in proton transport in V-ATPases from other sources.

MOLECULAR BIOLOGY OF V-ATPases

The function of an individual subunit of a membrane protein complex can be better studied by cloning the gene encoding this subunit, interrupting the gene, and repairing the mutation by the original gene and forms of it modified by site-directed mutagenesis. The mammalian system falls short of providing ready access to all of these genetic manipulations. On the other hand, yeast cells are ideal for all the above genetic studies. Nevertheless, cloned mammalian genes provide valuable information including insights into the mechanism of action of the enzyme and its evolution. The antiquity of the H^+-ATPases and their presence in every known living cell makes them one of the best source of evolutionary dating (Nelson and Taiz, 1989). Indeed, the sequences of the various subunits of V-ATPases provide a fascinating evolutionary story that has been previously reviewed (Nelson and Taiz, 1989; Nelson, 1989). The *c*DNA encoding the A and B subunits were first cloned from plant and fungal sources (Zimniak *et al.*, 1988; Manolson *et al.*, 1988; E. J. Bowman *et al.*, 1988b; B. J. Bowman *et al.*, 1988; Nelson *et al.*, 1989). Recently a *c*DNA encoding the A subunit of the enzyme from bovine chromaffin granules and brain were cloned and found to be similar to the three known subunit A proteins from other sources (Pan *et al.*, 1991; Puopolo *et al.*, 1991). Partial amino acid sequences of subunits B and E from chromaffin granules were found to be very similar if not identical to the deduced sequences of these subunits from other mammalian sources (Hirsch *et al.*, 1988; Sudhof *et al.*, 1989; Nelson *et al.*, 1990 and unpublished observations). A *c*DNA encoding the C subunit of V-ATPase from bovine chromaffin granules was cloned and sequenced (Nelson *et al.*, 1990). It encodes a protein of 44 kDa with no homology to any of the F-ATPase subunits. It was proposed that the addition of the C and γ subunits to the respective V- and F-ATPases during evolution defined them as two separate families of proton pumps. Recently the gene encoding the C subunit in yeast cells was cloned and sequenced (Beltrán *et al.*, 1992). It encodes a protein of 42 kDa that is 37% identical to the C subunit of V-ATPase from bovine adrenal medulla. This relatively low identity is in contrast to the conservation of A and B subunits that are 70 to 80% identical among enzymes of fungal and mammalian sources (Nelson, 1989; Nelson *et al.*, 1989; Nelson and Nelson, 1989). Yeast cells in which the gene encoding subunit C was interrupted exhibited similar phenotype to cells in which the gene encoding subunits A, B, E, or c (proteolipid) were inactivated (Nelson and Nelson, 1990; Foury, 1990; Yamashiro *et al.*, 1990; Umemoto *et al.*, 1990; Ohya *et al.*, 1991; Noumi *et al.*, 1991; Beltrán *et al.*, 1992). Substitution of more than half of the coding region by a corresponding DNA fragment encoding the bovine subunit C resulted in a phenotype identical to wild-type (Beltrán *et al.*, 1992). This experiment suggests that the structure of the C subunit may be more important than its amino acid sequence for the activity of the enzyme. On the other hand, relatively small changes in the amino acids of the A subunit resulted in marked changes in the properties of the V-ATPase from yeast cells (Taiz, Taiz, Nelson, and Nelson, unpublished observations). Studies on the molecular biology of V-ATPases have advanced our knowledge of the structure, function, mechanism of action, and evolution of the enzyme. They have also provided important clues for the evolution of the vacuolar system in eukaryotic cells and the biogenesis of proteins that function in this system. A major tool in these studies was the mutated yeast cell.

The strategy of exploring a biochemical system in yeast is straightforward. First, clone as many genes encoding proteins involved in the biochemical process of interest. Second, interrupt the genes and look for a specific phenotype. Third, repair the mutants by transformation with a plasmid bearing the native gene or the gene modified by site-directed mutagenesis. Fourth, look for suppressor mutants that will give you valuable structural information. Immediately following the cloning of the first two genes encoding the B and c(proteolipid) subunits, we advanced to the second stage of interrupting the genes (Nelson *et al.*, 1989; Nelson and Nelson, 1989). We obtained mutants that were not able to grow at high pH (Nelson and Nelson, 1990; Yamashiro *et al.*, 1990; Umemoto *et al.*, 1990; Foury, 1990). The mutants are also sensitive to the Ca^{+2} concentration in the medium (Hirata *et al.*, 1990; Noumi *et al.*, 1991). This phenotype makes the yeast mutants amenable to genetic manipulations including site-directed mutagenesis in the various genes encoding subunits of the V-ATPase. The utilization of yeast mutants for the study of the catalytic sector of the enzyme will be reviewed elsewhere (Nelson, 1992, in preparation). The membrane sector was the focus of our interest in the last few years.

The first gene cloned in our laboratory was the gene encoding the proteolipid in bovine adrenal

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Bovine       MSEAKNGPEYASFFAVMGASAAMVFSALGAAYGTAKSGTGIAAMSVMRPEMIMKSIIPVVMAGIIAIYGLVVAVLIANSL
Mouse        MADI..N...S...G.....S......M.....................L.............................
Torpedo      MSTPGA....SA....I.............................L.............................
Drosophila MSSEFSSDN.I.GP.......A..II.........................L.........................GA.
Plant        M.SVFS.D.T.P..GFL..A..L...CM..........V.V.S.G.....LV....V......VLG....II..I.STGI
Yeast          MTELC.V..P..GAI.CAS.II.TS..........V..C.TC.L..DLLF.N.V..I...........S..VCY..


             NDGISLYRSFLQLGAGLSVGLSGLAAGFAIGIVGDAGVRGTAQQPRLFVGMILILIFAEVLGLYGLIVALILSTK
             T...T.....................................................................
             TED...FK..................................................................
           EEPSKY....G.IH.....A..F.....................................................IY.Y..
         NPKAKPYF.FDGYAH.SS..AC..A.....M..........AN....K.............A.A......GI.L.SRAGQSRAD
             GQKQA..PG.I..............................SS.........................L.NSRATQDVVC
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Fig. 1 Extensive similarity in amino acid sequences in the transmembrane helices of proteolipids from various sources. The sequences were published previously and aligned manually. Bovine: proteolipid of V-ATPase from chromaffin granules (Mandel *et al.*, 1988; Correction in Gene Bank); Mouse: mouse brain (Hanada *et al.*, 1991); Torpedo: *T. marmurata* electric organ (Birman *et al.*, 1990); Drosophila: *Drosophila mellanogaster* (Meagher *et al.*, 1990); plant: oat (Lai *et al.*, 1991); Yeast: *Saccharomyces cerevisiae* (Nelson and Nelson, 1989).

medulla (Mandel *et al.*, 1988). The sequence information revealed that the proteolipids of V-ATPases from eukaryotic cells evolved by gene duplication and fusion of a gene related to the proteolipid of F-ATPases. Subsequently we cloned the gene encoding the proteolipid of the V-ATPase in yeast cells and found it to be highly homologous to the bovine proteolipid (Nelson and Nelson, 1989). After a correction in the former sequence (see Hanada *et al.*, 1991 and correction in Gene Bank) it appeared as if the proteolipid of the V-ATPases was the most conserved hydrophobic protein sequenced so far. The gene encoding the yeast proteolipid was interrupted and the resulting mutant was identical, in its sensitivity to high pH and Ca^{+2} concentration, to all the other disruptant mutants in genes encoding V-ATPase subunits (Nelson and Nelson, 1990; Noumi *et al.*, 1991), confirming that the cloned gene encodes a V-ATPase subunit. An identical protein was reported to be present in preparations of Gap junctions (Dermietzel *et al.*, 1989; Leitch and Finbow, 1990). Moreover, a protein that mediates calcium-dependent acetylcholine release from synaptic vesicles in Torpedo electric organs was found to be highly homologous to the proteolipid of V-ATPases (Birman *et al.*, 1990). Figure 1 depicts the sequences of proteolipids from various sources. It is apparent that in all of these proteolipids the amino acid sequences of the last two transmembrane helices are nearly identical. There is no other example of a transmembrane helix that is identical in its amino acid sequence between yeast and mammalian brain. Site-directed mutagenesis in the yeast proteolipid revealed quite high sensitivity to amino acid replacements in the hydrophobic segments of the protein (Noumi *et al.*, 1991). But this observation cannot fully account for the unusual conservation of the protein. Moreover, it cannot explain why such a conserved protein serves in unrelated systems that are located in different kinds of membranes. A partial answer to these questions can be given by studying the biogenesis of V-ATPases in various cells.

Vacuolar acidification plays a role in protein sorting and receptor recycling (Mellman *et al.*, 1986). In yeast cells both the Golgi complex and vacuoles are likely to contain the V-ATPase and may operate under low pH inside the organelles. Neutralization of the internal pH in the yeast vacuolar system by the protonophore CCCP, or by acidotropic agents such as the weak base ammonium acetate, resulted in the partial missor-ing of vacuolar proteins (Banta *et al.*, 1988). These chemicals act in a nonspecific manner to disrupt the electrochemical gradient of protons in all intracellular compartments. Inhibition of the V-ATPase by the specific inhibitor bafilomycin A_1 (E. J. Bowman *et al.*, 1988a), caused an increase in the vacuolar pH and resulted in the missorting of soluble vacuolar proteins, but apparently not those of the vacuolar membrane (Benta *et al.*, 1988; Klionsky and Emr, 1989). The role of vacuolar acidification has been analyzed genetically, but it presented a problem in distinguishing the role of the V-ATPase in these mutants (Preston *et al.*, 1989; Rothman *et al.*, 1989). We used mutants in which genes encoding subunits of the V-ATPase were interrupted for the study of the biogenesis and assembly of the enzyme as well as the sorting of vacuolar proteins (Nelson and Nelson, 1990; Beltrán *et al.*, 1992,; Klionsky *et al.*, 1992). The mutants with chromosomal disruption of the genes

encoding the A, B, and c(proteolipid) subunits accumulated precursor forms of the soluble vacuolar hydrolase carboxypeptidase Y (CPY) and proteinase A (PrA). These Golgi-modified precursors are also secreted from the mutant cells. The data suggested that the missorting occurs in the Golgi complex or in the post-Golgi vesicles. Precursor forms of the membrane-associated alkaline phosphatase are also accumulated in the mutants but to a lesser extent, suggesting that sorting of vacuolar membrane proteins is less sensitive to changes in the luminal pH. It was concluded that acidification of the vacuolar system is important for efficient sorting of proteins to the vacuole (Klionsky *et al.*, 1992).

The mutant strains with chromosomal disruptions of the genes encoding subunits A, B, C, and c(proteolipid) were used for studying the assembly of the V-ATPase in yeast cells (Nelson and Nelson, 1990; Noumi *et al.*, 1991; Beltrán *et al.*, 1992; Kane *et al.*, 1992). All of these strains are unable to acidify their vacuoles, suggesting that the V-ATPase is the only protom pump operating in the yeast vacuolar system, and unlike plants it contains no H^+-pyrophosphatase. Each one of the disruptant mutants prevented the assembly of the catalytic sector of the enzyme. However, the disruptant mutants in genes encoding subunits A, B, and C of the catalytic sector had no effect on the synthesis and incorporation of subunit c(proteolipid) into the vacuolar membrane. It was concluded that the assembly of the catalytic sector is absolutely dependent on the proper assembly of the membrane sector of the enzyme. On the other hand, the assembly of the membrane sector is independent of the assembly of the catalytic sector. These data suggest that the sequence of events in the assembly of V-ATPases starts by incorporation and assembly of the membrane sector followed by assembly of the catalytic sector onto the membrane sector. The bioenergetic implication of the disruptant mutants and the assembly of the enzyme are discussed below.

BIOENERGETICS OF H^+-ATPases IN RELATION TO THEIR MOLECULAR AND CELLULAR BIOLOGY AS WELL AS EVOLUTION OF EUKARYOTIC CELLS

Proton electrochemical gradient (pmf) was elected by nature to serve as a universal intermediate between two distinct proton pumps (Mitchell, 1961). In one, pmf is generated by a downhill vectorial electron transport across membranes. The second system is a reversible ATP-dependent proton pump best represented by F- and V-ATPases. These two fundamental energy conversion systems are usually coupled to each other by the pmf, and due to the coupling they are mutually controlled. Because of their fundamental nature and their key role in biological processes, they evolved during the dawn of life on earth and they are highly conserved. pH homeostasis was of a prime concern to the primitive cells and proton-ATPases may have originally evolved to maintain proper pH inside the cells (Nelson and Taiz, 1989). Because the proton pumps driven by electron transport and the H^+-ATPases functioned in the same membrane generating pmf in the same direction, the coupling between them took place. The utilization of a proton gradient as a universal intermediate presented a problem on the extent of the gradient that could be formed. Overacidification could denature proteins, killing the very cell that is being energized. This obstacle was dealt with by three separate parameters. First, by increasing the membrane potential at the expense of the proton gradient, the pH differences inside the cell could be controlled. The second property is the stoichiometry of H^+ to ATP in the proton ATPases. In F-ATPases the ratio of $3H^+$ to ATP limits the pmf required for the formation of ATP to about 160 mV or less than ΔpH of 3 at $\Delta\Psi = 0$. However, in systems that have H^+ to ATP ratio smaller than 3, a higher ΔpH could be formed at thermodynamic equilibrium. Introduction of a proton slip or proton leak may also regulate acidification. However, if the slip is too large, the ATPase can no longer form ATP and it can function exclusively as an ATP-dependent proton pump. We suggested that V-ATPases of eukaryotic cells are controlled by a proton slip, preventing them from overacidification of the lumen of the vacuolar system (Moriyama and Nelson, 1988b; Nelson, 1991). Moreover, we proposed that this property governed the evolution of V-ATPases (Nelson, 1988, 1989).

Figure 2 depicts a schematic proposal of major events in the evolution of F- and V-ATPases. It was proposed that the catalytic sector of the primordial H^+-ATPase was related to the current catalytic sector of V-ATPases (Nelson, 1988, 1989; Nelson and Taiz, 1989). The increase in oxygen concentration in the atmosphere by oxygenic photosynthesis may have caused rapid changes in the amino acid composition and the size of the subunits during the evolution of the F-ATPases from the primordial enzyme. This evolutionary step was detrimental in the formation of

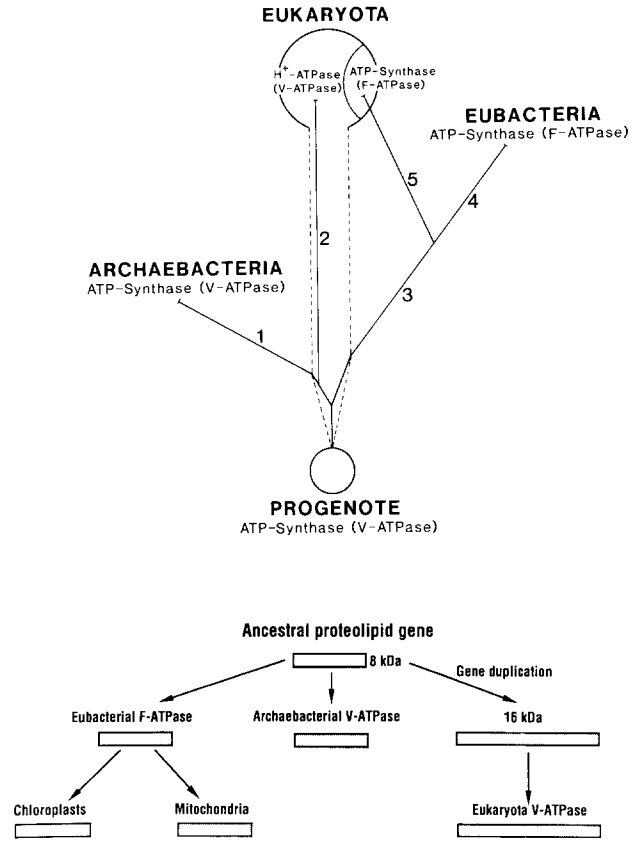

Fig. 2. Schematic proposal for events in the evolution of the catalytic and membrane sectors of F- and V-ATPases. The specific events are discussed in the text.

eubacteria as a separate kingdom from archaebacteria that maintained a catalytic sector of V-ATPase type (Nanba and Mukohata, 1987; Denda *et al.*, 1988a,b, 1989). However, the mechanism of ATP hydrolysis is probably very similar if not identical in the two families of F- and V-ATPases. Subunit A of the eukaryotic V-ATPase contains cysteine residues in the ATP-binding site as well as several tryptophan residues all over subunits A and B. This property may make the enzyme sensitive to oxygen and may play a role in its evolution. As proposed in Fig. 2 in step 1 the primordial enzyme evolved into the current H^+-ATPase in archaebacteria with relatively little change. Notably the increased oxygen tension in the atmosphere forced them to eliminate the cysteine residues, and therefore unlike the eukaryotic V-ATPases the archaebacterial V-ATPases are not sensitive to NEM (Lubben *et al.*, 1987; Schafer *et al.*, 1990). In step 3 major changes in the amino acid sequences and the sizes of the subunits took place to give rise to the F-ATPases that contain no cysteine in the ATP-binding site and are almost free of tryptophan residues. This helped to shape the new kingdom of eubacteria that by symbiosis with protoeukaryotes developed the chloroplasts and mitochondria which utilize F-ATPases exclusively. In step 2 the progenote's V-ATPase was introduced into the vacuolar system of eukaryotes. The main change in the property of the enzyme that took place during this evolutionary step was the introduction of a major slip in coupling between the ATPase and proton-pumping activity of the enzyme. Consequently the V-ATPase of the vacuolar system of eukaryotic cells can no longer generate ATP and operates exclusively in the ATP-dependent proton pumping mode of the enzyme. We proposed that the major structural change that led to this property was the gene duplication and fusion of the proteolipid to give a protein of about 16 kDa that spans the membrane four times. The correlation between the presence of the enlarged proteolipid with these phenomena is apparent. Therefore, it seems as if the most important differences between the two families of F- and V-ATPases resulted from the evolution of the membrane sector of the enzymes.

Recently we assayed the proton conduction of chromaffin granules and yeast vacuoles in which the catalytic sector was removed by cold inactivation or was not assembled due to a deletion mutant in one of the subunits of the catalytic sector (Beltrán and Nelson, 1992). We showed that in both cases the absence of the catalytic sector did not increase the proton permeability of the membranes. This is in contrast with the numerous studies with membranes containing F-ATPases from various sources (McCarty and Racker, 1966, 1967, 1968; Racker, 1976; Nelson, 1980). Upon removal of the catalytic sector of F-ATPase a marked increase in proton conductivity takes place. It is interesting that removal of the catalytic sector of the archaebacterial V-ATPase also generated a proton leak through the membranes (Bogomoini *et al.*, 1980). It is noteworthy that the archaebacterial enzyme contains the short version of proteolipid with only two transmembrane helices (Denda *et al.*, 1989). Therefore, there is a straightforward correlation between the capacity of an H^+-ATPase to operate as an ATP-synthase with the presence of the short proteolipid in the membrane sector and the generation of a proton leak by the removal of the catalytic sector from the membrane. Moreover, both the catalytic and the membrane sector of the V-ATPases are interdependent on the presence of each other for their partial activity. The membrane sector will conduct protons only in the presence of the

catalytic sector, and the catalytic sector of eukaryotic V-ATPases loses its ATPase activity upon removal from the membrane (Moriyama and Nelson, 1989a,b,c). In contrast, the ATPase activity of F-ATPases is enhanced by its removal from the membrane. We proposed that all of these properties of the eukaryotic V-ATPase evolved for gaining control against overacidification of the various organelles. The proton pumping is controlled by a slip and the proteolipid plays a major role in this control function.

The yeast mutants, in which genes encoding subunits of the V-ATPase were interrupted, provided additional support of these assumptions. It was proposed that the mutant yeast cells survive the absence of the V-ATPase because the acidification of the vacuolar system proceeded via endocytosis (Nelson and Nelson, 1990). It is quite likely the eukaryotic cells cannot live without acidification of the vacuolar system which is required for numerous functions including calcium homeostasis in plants and fungi. Inactivation of the gene encoding the proteolipid prevented the assembly of the catalytic sector of the enzyme (Noumi *et al.*, 1991). Inactivation of genes encoding subunits of the catalytic sector prevented the assembly of the other subunits of this sector, but the assembly of the membrane sector was not disrupted (Noumi *et al.*, 1991; Beltrán *et al.*, 1992). This observation supports the assumption that the removal of the catalytic sector does not cause a proton leak through the membrane sector. Were the vacuolar membranes to become permeable to protons, the cytoplasmic pH may drop to the external pH, and the mutants lacking the catalytic sector would not survive. Therefore, the phenotype of the yeast mutants added credence to the biochemical observations concerning the interdependence of the catalytic and membrane sectors in eukaryotic V-ATPases. Suppressor mutants are likely to provide a wealth of information on the structure and function of the V-ATPases.

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