J. exp. Biol. 172, 19-27 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

THE VACUOLAR H^+-ATPase – ONE OF THE MOST FUNDAMENTAL ION PUMPS IN NATURE

BY NATHAN NELSON

Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA

Summary

An electrochemical gradient of protons (PMF) is a universal high-energy intermediate in biological systems. Two related families of proton pumps, denoted F- and V-ATPases, are among the principal generators of a PMF from ATP and can form ATP at the expense of a PMF. The enzymes of these two families share a similar structure and subunit composition; some subunits in the two families evolved from common ancestors. Other subunits having no common ancestry were added independently to the various enzymes and defined the two separate families. The general mechanism for the proton pumping activity is similar in the two families. However, whereas F-ATPases can act in both proton pumping and ATP formation, the V-ATPases of eukaryotes function exclusively as ATP-dependent proton pumps. The catalytic and membrane sectors of F-ATPases and archaebacterial V-ATPases can separately catalyze their specific partial activities of ATPase and proton conduction. The catalytic and membrane sectors of the eukaryotic V-ATPases cannot act separately. This property is correlated with the presence of a large proteolipid that traverses the membrane four times. The gene duplication of the smaller proteolipid in the formation of the large proteolipid was one of the most important events in the evolution of the V-ATPases of eukaryotic cells.

Introduction

H^+-ATPases function in biological energy conversion in every known living cell. They can generate a proton-motive force (PMF) by hydrolyzing ATP and may utilize the energy of the PMF for ATP formation (Mitchell, 1968). They also function in homeostasis, maintaining an appropriate cytoplasmic pH and acidifying the interior of several organelles of eukaryotic cells. These activities are among the most fundamental processes in nature.

There are two mechanistically distinct groups of ATP-dependent ion pumps. One group, the P-ATPases, operates with a phospho-enzyme intermediate and its members are usually sensitive to low concentrations of vanadate. Na^+/K^+-ATPase and the gastric H^+-ATPase are notable members of the P-ATPase family. The other group, the families of F- and V-ATPases, functions without a phosphorylated intermediate (Nelson and Taiz, 1989). They are not sensitive to low vanadate concentrations and are sensitive to the specific inhibitor bafilomycin A (Bowman *et al.* 1988*b*). The family of F-ATPases functions in eubacteria, chloroplasts and mitochondria, and the family of V-ATPases is

Key words: F-ATPase, V-ATPase, structure, proton pumping, evolution.

present in archaebacteria and the vacuolar system of eukaryotic cells. Eukaryotic F-ATPases are present exclusively in chloroplasts and mitochondria because they require organellar gene products for their functional assembly (Nelson, 1989). In contrast, V-ATPases are composed only of nuclear gene products and are present in organelles of the vacuolar system and in the plasma membrane of specialized cells.

The most fundamental difference between F- and V-ATPases is a consequence of their function and origin. Although the main function of F-ATPases is to synthesize ATP at the expense of a PMF generated by electron transport chains, the main function of V-ATPases in eukaryotic cells is to generate a PMF at the expense of ATP and to cause limited acidification of the internal space of several organelles of the vacuolar system. The origin of F-ATPases is rooted in the eubacteria that evolved into chloroplasts and mitochondria, whereas the origin of V-ATPases is related to archaebacteria. Therefore, the evolution of the two families of proton pumps is highly relevant to their structure, function and mechanism of action.

Structure of F- and V-ATPases

Both F- and V-ATPases are multisubunit protein complexes built of distinct catalytic and membrane sectors. F-ATPases from *Escherichia coli* and chloroplasts contain eight and nine subunits, respectively, and we are not certain about the precise number of subunits in the mitochondrial enzyme (Nelson, 1989; Futai *et al.* 1989). However, the catalytic sector of all of these enzymes is composed of five subunits denoted α, β, γ, δ and ϵ. The membrane sector contains a minimum of three subunits denoted in *E. coli* as *a*, *b* and *c* (proteolipid), and more than six subunits in mitochondria from various sources. In *E. coli,* the stoichiometry of the various subunits is 3α, 3β, 1γ, 1δ, 1ϵ, $1a$, $2b$ and 6–$12c$. This stoichiometry is largely maintained for the corresponding subunits in the different F-ATPases from various sources (Penefsky and Cross, 1991).

The subunit structure of V-ATPases from various sources has been studied in several laboratories during the last few years (Xie and Stone, 1986; Cidon and Nelson, 1986; Arai *et al.* 1988; Moriyama and Nelson, 1989*a,b,c*; Bowman *et al.* 1989; Lai *et al.* 1988; Kane *et al.* 1992; Umemoto *et al.* 1991). The study underwent the usual evolution of biological observations, from conflicting data to general agreement into reasonable doubts. Recent studies utilizing chaotropic agents and cold-inactivation in the presence of MgATP have revealed that the catalytic sector of V-ATPases may be composed of five different subunits denoted as subunits *A* to *E* in order of decreasing relative molecular mass from 70×10^3 to 26×10^3, respectively (Nelson, 1989). The genes encoding four of these subunits (*A*, *B*, *C* and *E*) in mammalian, plant and fungal cells have been cloned and sequenced (Hirsch *et al.* 1988; Zimniak *et al.* 1988; Bowman *et al.* 1988*a,c*; Nelson *et al.* 1989, 1990; Südhof *et al.* 1989; Beltrán *et al.* 1992; Hirata *et al.* 1990; Puopolo *et al.* 1991, 1992*a*). Disruption of these genes in yeast cells gave similar phenotypes, leaving little doubt that they are indeed subunits of the V-ATPase (Nelson and Nelson, 1990; Hirata *et al.* 1990; Noumi *et al.* 1991). Subunit *D* has been identified by immunological cross reactivity in preparations of mammalian V-ATPases and in the supernatants of cold-inactivated enzymes (Moriyama and Nelson 1989*a,b,c*). Cloning of the gene

encoding this polypeptide in yeast may verify its integrity as a subunit of the enzyme. A stoichiometry of 3*A*, 3*B*, 1*C* and 1*E* has been reported for the catalytic sector of V-ATPases from clathrin-coated vesicles and chromaffin granules (Arai *et al.* 1988; Nelson, 1989). Two recent studies provided some doubts concerning the number of subunits in V_1. It was demonstrated that the lack of a $40\times10^3 M_r$ polypeptide (subunit *C*?) did not prevent reconstitution of the solubilized catalytic sector onto depleted membranes (Puopolo *et al.* 1992*b*). Recently, a yeast gene encoding a protein of about $54\times10^3 M_r$ was cloned and shown to be necessary for the assembly of the enzyme (Anraku *et al.* 1992). Moreover, the observation that this protein was present in the assembled enzyme suggests additional subunits for the V_1 sector from yeast vacuoles. Additional polypeptides of about $50\times10^3 M_r$ have been reported in preparations of mammalian V-ATPases (Moriyama and Nelson, 1989*b*,*c*). Further studies on the structure of V-ATPases and the crystallization of this enzyme may help to settle the arguments regarding its structure.

Owing to its hydrophobic nature, the study of the structure of the membrane sector of V-ATPases is lagging behind. A pioneering work of over ten years ago revealed the presence of a $16\times10^3 M_r$ proteolipid in membranes containing V-ATPases (Sutton and Apps, 1981). This observation has been verified in every enzyme of eukaryotic cells studied so far (Nelson, 1989). The gene encoding this proteolipid has been cloned from a large variety of sources and its disruption in yeast cells has given a phenotype similar to that of mutants in which other genes encoding subunits have been interrupted (Nelson and Nelson, 1990). Therefore, there is no doubt that the proteolipid is an integral part of the membrane sector of V-ATPases. A stoichiometry of six proteolipids per enzyme has been reported for V-ATPases from clathrin-coated vesicles and plant vacuoles (Arai *et al.* 1988; Lai *et al.* 1991). An analogy to the membrane sector of F-ATPases suggests that additional subunits should function in the membrane sector of V-ATPases. Recently it was proposed that the $115\times10^3 M_r$ subunit of V-ATPases functions as the counterpart of the proteolipid in proton conduction across the membrane (Perin *et al.* 1991). This subunit is present in most if not all V-ATPases of eukaryotic cells (Kane *et al.* 1992). Its presence in all V-ATPases is a prerequisite for assigning it as an integral functional subunit in the membrane sector. An equivalent subunit could not be detected in archaebacteria, and a highly purified and active enzyme from plant vacuoles has been shown to lack the $115\times10^3 M_r$ polypeptide (Ward and Sze, 1992). Therefore, the admission of the $115\times10^3 M_r$ polypeptide to the club of the integral V-ATPase membrane sector is contingent upon its presence in all enzymes, including those of plant sources. However, the detection of this subunit in the enzyme from yeast cells suggests an important role for the $115\times10^3 M_r$ polypeptide in the correct assembly and proper functioning of the enzyme. Another polypeptide of $39\times10^3 M_r$ has been detected in the membrane sector of V-ATPase from several mammalian sources (Cidon and Nelson, 1986; Arai *et al.* 1988). Cloning of the gene encoding this polypeptide revealed no potential transmembrane helices and, therefore, it may be associated with the membrane by binding onto another membrane protein (Wang *et al.* 1989). A highly hydrophobic protein of about $20\times10^3 M_r$ has been detected in a few preparations of mammalian V-ATPases (Moriyama and Nelson, 1989*b*,*c*). Cloning of the gene encoding this

polypeptide should clarify whether this protein is analogous to subunit *a* of F-ATPases. When the dust has settled, we anticipate a complicated membrane sector for V-ATPases with more subunit and accessory polypeptides.

Molecular biology and evolution of V-ATPases

The sequences of cDNAs and genes encoding subunits *A* and *B* of V-ATPases have shed light on the relationship between F- and V-ATPases and the evolution of these two families of proton pumps (Bowman *et al.* 1988*a,c*; Zimniak *et al.* 1988; Manolson *et al.* 1988). The *A* subunit of V-ATPases has been shown to be homologous to the β subunit of F-ATPases. The alignment of the two amino acid sequences created a gap of about 150 amino acids in the middle of the sequence of the β subunit. The origin and function of the non-homologous sequence in the middle of the *A* subunit are not known; it might have been removed during the evolution of the β subunit. The *B* subunit of V-ATPases has been shown to be homologous to the α subunit of F-ATPases. Several stretches of amino acids in the α and β subunits implicated in ATP and ligand binding were conserved in the corresponding *B* and *A* subunits of V-ATPases (Zimniak *et al.* 1988; Bowman *et al.* 1988*a,c*). It is assumed that the *A* subunit has a similar function to the β subunits and the *B* subunit has a similar function to the α subunit in the catalytic and regulatory activities, respectively, of these proton pumps. The amino acid sequence homologies suggest that the α, β, *A* and *B* subunits evolved from a common ancestral gene. The property that separates the F- and V-ATPases into two distinct families of proton pumps is the lack of any sequence homology among the *C*, *E*, γ, δ and ϵ subunits (Nelson *et al.* 1991). The possible presence of extra subunits in the catalytic sector of V-ATPases may further separate two families (Anraku *et al.* 1992).

The most likely time for the early evolution of the catalytic sector of F-ATPase from that of the V-ATPase was when oxygen rapidly accumulated in the atmosphere as a result of the proliferation of organisms capable of water photolysis (Nelson and Nelson, 1989). The amino acid compositions of V-ATPases suggest higher sensitivity to oxygen than their related F-ATPases. There are several tryptophan and cysteine residues positioned in catalytically sensitive places in V-ATPases. Most notable is a cysteine residue right inside the P-loop structure of the *A* subunit implicated in the binding of ATP (Zimniak *et al.* 1988). This cysteine is sensitive to oxidation and its modification by ligands such as *N*-ethylmaleimide (NEM) inactivates the enzyme. This cysteine is missing in the V_1 sector of archaebacteria, indicating the necessity of its removal for operating in an oxygenic environment (Denda *et al.* 1988). In eukaryotic cells, V_1 is present on the cytoplasmic side of the membranes that maintain a rather reduced environment.

The membrane sector of F- and V-ATPases followed a separate evolutionary pathway. So far we have sequence information only for the proteolipids, which bind dicyclohexylcarbodiimide (DCCD), of the two families of proton pumps (Mandel *et al.* 1988; Nelson and Nelson, 1989; Umemoto *et al.* 1990). It is likely that the ancestral proteolipid was of the short version ($8\times10^3 M_r$) present in all F-ATPases and archaebacterial V-ATPases (Nelson, 1989). The proteolipid of eukaryotic V-ATPases evolved by gene duplication and fusion into a double-size proteolipid. The

archaebacterial proteolipid may have diverged from the F-ATPase proteolipid prior to the gene duplication event that led to the present shape of the eukaryotic V-ATPases. The mechanistic implication of this event of gene duplication was far reaching; several specific properties of the eukaryotic V-ATPases may be correlated with the presence of a $16\times10^3 M_r$ proteolipid in these enzymes.

Structure–function relations among various F- and V-ATPases

Both families of F- and V-ATPases share several structural and mechanistic properties. They are composed of several subunits assembled into distinct catalytic and membrane sectors. The underlying mechanism of ATPase and proton pumping activities is quite similar for the members of the two families (Nelson, 1989). F-ATPases operate close to thermodynamic equilibrium and, depending on the level of the PMF, may operate in ATP synthesis or ATP-dependent proton pumping. In contrast, V-ATPases of eukaryotic cells operate exclusively as ATP-dependent proton pumps. Apparently these enzymes operate below thermodynamic equilibrium and therefore can no longer synthesize ATP at the expense of a PMF (Moriyama and Nelson, 1988). It was proposed that a proton slip (a voltage-dependent change in ATP/H^+ stoichiometry) in eukaryotic V-ATPases rendered them incapable of ATP synthesis while gaining better control over the extent of the PMF in the various organelles. Can we find structural features that may be responsible for these properties of the different proton pumps? It has been proposed that the size of the proteolipid may be a major factor in transforming eukaryotic V-ATPases into exclusive proton pumps (Nelson, 1989). It has been demonstrated that all F-and V-ATPases capable of ATP synthesis contain a short proteolipid of about $8\times10^3 M_r$ (Futai *et al.* 1989; Denda *et al.* 1989). We proposed that concomitantly with the introduction of V-ATPases to eukaryotic cells the gene encoding the proteolipid was duplicated and fused to give a protein of about $16\times10^3 M_r$ that spans the membrane four times. This event completed the shaping of the eukaryotic V-ATPase and may have generated the proton slip. Recently, we observed that removal of the catalytic sector of eukaryotic V-ATPases from the membrane failed to generate increased proton conduction across the depleted membranes (Beltrán and Nelson, 1992). This observation contrasts with F_1-depleted membranes and archaebacterial V_1-depleted membranes, which exhibited a marked increase in proton conduction (Racker, 1976; Nelson, 1980). Fig. 1 illustrates the results of removing F_1 and V_1 from membranes of chloroplasts and chromaffin granules, respectively. Upon removal of V_1 from the chromaffin granule membrane, no increase in proton conduction could be observed for proton uptake in the presence of valinomycin in K^+-loaded granules or for a proton leak in NH_4Cl-loaded vesicles (Beltrán and Nelson, 1992). In contrast, chloroplasts depleted of F_1 by NaBr treatment exhibited proton conduction of over 10^6 protons per second for each F_o in the depleted membranes (Nelson, 1980). Therefore, the capacity of an H^+-ATPase to operate as an ATP synthase is positively correlated with the presence of a short proteolipid and the generation of a proton leak by the removal of the catalytic sector from the membrane. Moreover, both the catalytic and membrane sectors of eukaryotic V-ATPases are interdependent in their

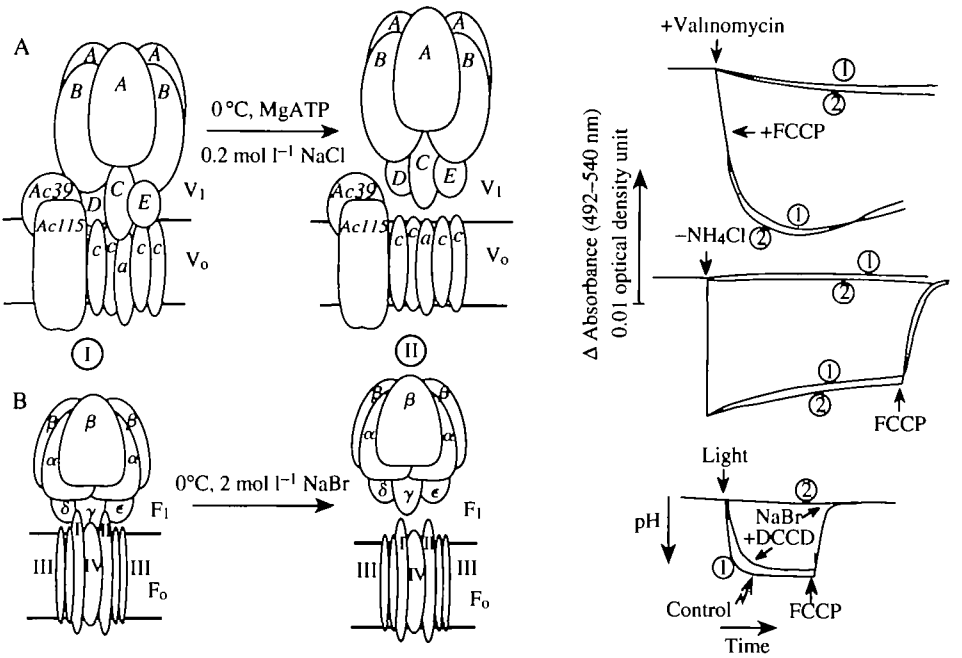

Fig. 1. Schematic presentation of the effect of removal of the F_1 or V_1 sector from the membrane on the proton conductivity across the depleted membranes. (A) The effect of cold-inactivation of membranes, such as chromaffin granules, containing V-ATPase. Incubation on ice in the presence of 0.2 mol l^{-1} NaCl and 5 mmol l^{-1} MgATP results in the dissociation of V_1 from the membranes. The free V_1 is no longer active in ATPase hydrolysis. The depleted membranes can be loaded with KCl by a prolonged incubation with the salt. Dilution of the loaded membranes into a potassium-free medium containing valinomycin results in a rapid outward movement of K^+, generating a negative membrane potential inside the vesicles. As shown in the upper right-hand corner, regardless of the presence (1) or absence (2) of V_1, no significant proton uptake could be detected for a period of over 10 min. If 0.01 μmol l^{-1} of the uncoupler FCCP is present in the medium, a rapid decrease in the absorption of Acridine Orange is detected, indicating rapid proton uptake mediated by the FCCP. The middle trace in the right-hand side of the figure shows the effect of cold-inactivation on proton conduction in the opposite direction. The treated and untreated chromaffin granules are loaded with NH_4Cl. Upon dilution into a medium without NH_4Cl, NH_3 diffuses out of the vesicles, resulting in a rapid decrease in the internal pH (indicated by drop in the absorbancy of Acridine Orange). Extremely slow proton release is observed both in control (1) and V_1-depleted membranes (2). Addition of FCCP causes a rapid proton secretion. The upper two traces are from a similar experiment except that valinomycin was present in the medium. Upon dilution of the membranes in the presence of valinomycin the NH_4^+ is conducted through the antibiotic and no pH gradient could be established. (B) A similar experiment with chloroplasts. In chloroplasts incubated for 1 h in the presence of 2 mol l^{-1} NaBr more than 95% of the F_1 is dissociated from the membrane. Light-dependent proton uptake is assayed by following the external pH in the medium in the presence of phenazine methosulfate (PMS). As shown in the lower right-hand side of the figure, illumination of control chloroplasts (1) causes a rapid proton uptake into the chloroplasts as measured by increase in the external pH. The depleted membranes (2) show no net proton uptake in the light. The addition of DCCD, which specifically blocks proton conduction through F_0 results in the restoration of proton accumulation. Therefore, in contrast to the V_1-depleted membranes, F_1-depleted membranes conduct protons at a rapid rate and proton conduction proceeds through F_0.

partial activity; the separated V_1 sector cannot catalyze the ATPase reaction (Moriyama and Nelson, 1989*a*). In contrast, the ATPase activities of F_1 and archaebacterial V_1 are enhanced when the sectors are removed from the membranes (Racker, 1976; Lübben *et al.* 1987). I have proposed that all of these differential properties evolved concomitantly with the duplication of the proteolipid in eukaryotic V-ATPases (Nelson, 1989, 1992).

Studies of the yeast V-ATPase provided additional insight into the specific properties of the eukaryotic enzyme. Mutant strains with chromosomal disruptions of the genes encoding subunits *A*, *B*, *C* and *E* of V_1 and the proteolipid of V_0 exhibit a similar phenotype that cannot grow at a pH above 7 and is sensitive to low and high Ca^{2+} concentrations in the medium (Nelson and Nelson, 1990; Foury, 1990; Noumi *et al.* 1991; Ohya *et al.* 1991). It was proposed that these mutants survive the inactivation of V-ATPase because the acidification of the vacuolar system proceeds *via* fluid-phase endocytosis (Nelson and Nelson, 1990). Inactivation of the gene encoding the proteolipid prevented the assembly of the catalytic sector (Noumi *et al.* 1991). Inactivation of genes encoding subunits of the catalytic sector prevented the assembly of the other subunit of this sector, but the assembly of the membrane sector was not disrupted (Noumi *et al.* 1991; Beltrán *et al.* 1992; Kane *et al.* 1992). This observation supports the hypothesis that the removal of the catalytic sector does not cause a proton leak through the membrane sector. If the vacuolar membranes were to become permeable to protons, the cytoplasmic pH would drop to the external pH, killing the mutants. Therefore, the chromosomal disruptant yeast mutants add credence to the biochemical observations that the V_1 and V_0 sectors are interdependent in their partial activities.

The vacuolar H^+-ATPase (V-ATPase) is one of the most fundamental enzymes in nature. Very little attention was given to this enzyme by the most prestigious scientific journals. Now that the enzyme has been implicated in human diseases this attitude will change drastically.

References

ANRAKU, Y., HIRATA, R., WADA, Y. AND OHYA, Y. (1992). Molecular genetics of the yeast vacuolar H^+-ATPase. *J. exp. Biol.* **172,** 67–81.

ARAI, H., TERRES, G., PINK, S. AND FORGAC, M. (1988). Topography and subunit stoichiometry of the coated vesicle proton pump. *J. biol. Chem.* **263**, 8796–8802.

BELTRÁN, C., KOPECKY, J., PAN, Y.-C. E., NELSON, H. AND NELSON, N. (1992). Cloning and mutational analysis of the gene encoding subunit C of yeast V-ATPase. *J. biol. Chem.* **267**, 774–779.

BELTRÁN, C. AND NELSON, N. (1992). The membrane sector of vacuolar H^+-ATPase by itself is impermeable to protons. *Acta. physiol. scand.* (in press).

BOWMAN, B.J., ALLEN, R., WECHSER, M. A. AND BOWMAN, E. J. (1988*a*). Isolation of genes encoding the *Neurospora* vacuolar ATPase. *J. biol. Chem.* **263**, 14002–14007.

BOWMAN, B. J., DSCHIDA, W. J., HARRIS, T. AND BOWMAN, E. J. (1989). The vacuolar ATPase of *Neurospora crassa* contains an F_1-like structure. *J. biol. Chem.* **264**, 15606–15612.

BOWMAN, E. J., SIEBERS, A. AND ALTENDORF, K. (1988*b*). Bafilomycins: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. *Proc. natn. Acad. Sci. U.S.A.* **85**, 7972–7976.

BOWMAN, E. J., TENNEY, K. AND BOWMAN, B. J. (1988*c*). Isolation of genes encoding the *Neurospora* vacuolar ATPase. *J. biol. Chem.* **263**, 13994–14001.

CIDON, S. AND NELSON, N. (1983). A novel ATPase in the chromaffin granule membrane. *J. biol. Chem.* **258**, 2892–2898.

DENDA, K., KONISHI, J., OSHIMA, T., DATE, T. AND YOSHIDA, M. (1988). The membrane-associated ATPase from *Sulfolobus acidocaldarius* is distantly related to F_1-ATPase as assessed from the primary structure of its α-subunit. *J. biol. Chem.* **263**, 6012–6015.

DENDA, K., KONISHI, J., OSHIMA, T., DATE, T. AND YOSHIDA, M. (1989). A gene encoding the proteolipid subunit of *Sulfolobus acidocaldarius* ATPase complex. *J. biol. Chem.* **264**, 7119–7121.

FOURY, F. (1990). The 31 kDa polypeptide is an essential subunit of the vacuolar ATPase in *Saccharomyces cerevisiae*. *J. biol. Chem.* **265**, 18554–18560.

FUTAI, M., NOUMI, T. AND MAEDA, M. (1989). ATP synthase (H^+-ATPase): Results by combined biochemical and molecular biological approaches. *A. Rev. Biochem.* **58**, 111–136.

HIRATA, R., OHSUMI, Y., NAKANO, A., KAWASAKI, H., SUZUKI, K. AND ANRAKU, Y. (1990). Molecular structure of a gene, *VMA1*, encoding the catalytic subunit of H^+-translocating adenosine triphosphatase from vacuolar membranes of *Saccharomyces cerevisiae*. *J. biol. Chem.* **265**, 6726–6733.

HIRSCH, S., STRAUSS, A., MASOOD, K., LEE, S., SUKHATME, V. AND GLUCK, S. (1988). Isolation and sequence of a cDNA clone encoding the 31-kDa subunit of bovine kidney vacuolar H^+-ATPase. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3004–3008.

KANE, P. M., KUEHN, M. C., HOWALD-STEVENSON, I. AND STEVENS, T. H. (1992). Assembly and targeting of peripheral and integral membrane subunits of the yeast vacuolar H^+-ATPase. *J. biol. Chem.* **267**, 447–454.

LAI, S., RANDALL, S. K. AND SZE, H. (1988). Peripheral and integral subunits of the tonoplast H^+-ATPase from oat roots. *J. biol. Chem.* **263**, 16731–16737.

LAI, S., WATSON, J. C., HANSEN, J. N. AND SZE, H. (1991). Molecular cloning and sequencing of cDNAs encoding the proteolipid subunit of the vacuolar H^+-ATPase from a higher plant. *J. biol. Chem.* **266**, 16078–16084.

LÜBBEN, M., LÜNSDORF, H. AND SCHÄFER, G. (1987). The plasma membrane associated ATPase of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*. Purification and immunological relationships to F_1-ATPases. *Eur. J. Biochem.* **167**, 211–219.

MANDEL, M., MORIYAMA, Y., HULMES, J. D., PAN, Y.-C. E., NELSON, H. AND NELSON, N. (1988). Cloning of cDNA sequence encoding the 16-kDa proteolipid of chromaffin granules implies gene duplication in the evolution of H^+-ATPases. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5521–5524.

MANOLSON, M. F., OUELLETTE, B. F. F., FILION, M. AND POOLE, R. J. (1988). cDNA sequence and homologies of the '57-kDa' nucleotide-binding subunit of the vacuolar ATPase from *Arabidopsis*. *J. biol. Chem.* **263**, 17987–17994.

MITCHELL, P. (1968). *Chemiosmotic Coupling and Energy Transduction*. Bodmin.

MORIYAMA, Y. AND NELSON, N. (1988). The vacuolar H^+-ATPase, a proton pump controlled by a slip. In *The Ion Pumps, Structure, Function and Regulation* (ed. W. D.Stein), pp. 387–394. New York: Alan R. Liss, Inc.

MORIYAMA, Y. AND NELSON, N. (1989*a*). Cold inactivation of vacuolar H^+-ATPases. *J. biol. Chem.* **264**, 3577–3582.

MORIYAMA, Y. AND NELSON, N. (1989*b*). H^+-translocating ATPase in Golgi apparatus: Characterization as vacuolar H^+-ATPase and its subunit structures. *J. biol. Chem.* **264**, 18445–18450.

MORIYAMA, Y. AND NELSON, N. (1989*c*). Lysosomal H^+-translocating ATPase has a similar subunit structure to chromaffin grauune H^+-ATPase complex. *Biochim. biophys. Acta* **980**, 241–247.

NELSON, H., MANDIYAN, S. AND NELSON, N. (1989). A conserved gene encoding the 57 kDa subunit of the yeast vacuolar H^+-ATPase. *J. biol. Chem.* **264**, 1775–1778.

NELSON, H., MANDIYAN, S., NOUMI, T., MORIYAMA, Y., MIEDEL, M. C. AND NELSON, N. (1990). Molecular cloning of cDNA encoding the C subunit of H^+-ATPase from bovine chromaffin granules. *J. biol. Chem.* **265**, 20390–20393.

NELSON, H. AND NELSON, N. (1989). The progenitor of ATP synthases was closely related to the current vacuolar H^+-ATPase. *FEBS Lett.* **247**, 147–153.

NELSON, H. AND NELSON, N. (1990). Disruption of genes encoding subunits of yeast vacuolar H^+-ATPase causes conditional lethality. *Proc. natn. Acad. Sci. U.S.A.* **87**, 3503–3507.

NELSON, N. (1980). Proton channels in chloroplast membranes. *Ann. N.Y. Acad. Sci.* **358**, 25–36.

NELSON, N. (1989). Structure, molecular genetics and evolution of vacuolar H^+-ATPases. *J. Bioenerg. Biomembr.* **21**, 553–571.

NELSON, N. (1991). Structure and pharmacology of the proton-ATPases. *Trends pharmac. Sci.* **12**, 71–75.

NELSON, N. (1992). Organellar proton-ATPases. *Curr. Opinions Cell Biol.* (in press).

NELSON, N. AND TAIZ, L. (1989). The evolution of H^+-ATPases. *Trends biol. Sci.* **14**, 113–116.

NOUMI, T., BELTRÁN, C., NELSON, H. AND NELSON, N. (1991). Mutational analysis of yeast vacuolar H^+-ATPase. *Proc. natn. Acad. Sci. U.S.A.* **88**, 1938–1942.

OHYA, Y., UMEMOTO, N., TANIDA, I., OHTA, A., IIDA, H. AND ANRAKU, Y. (1991). Calcium-sensitive *cls* mutants of *Saccharomyces cerevisiae* showing a pet^- phenotype are ascribable to defects of vacuolar membrane H^+-ATPase activity. *J. biol. Chem.* **266**, 13791–13977.

PENEFSKY, H. S. AND CROSS, R. L. (1991). Structure and mechanism of F_oF_1-type ATP synthases and ATPases. In *Advances in Enzymology and Related Areas of Molecular Biology* (ed. A. Meister), vol. 64, pp. 173–214. New York: John Wiley and Sons, Inc.

PERIN, M.S., FRIED, V.A., STONE, D.K., XIE, X.-S. AND SÜDHOF, T.C. (1991). Structure of the 116-kDa polypeptide of the clathrin-coated vesicle synaptic vesicle proton pump. *J. biol. Chem.* **266**, 3877–3881.

PUOPOLO, K., KUMAMOTO, C., ADACHI, I. AND FORGAC, M. (1991). A single gene encodes the catalytic 'A' subunit of the bovine vacuolar H^+-ATPase. *J. biol. Chem.* **266**, 24564–24572.

PUOPOLO, K., KUMAMOTO, C., ADACHI, I., MAGNER, R. AND FORGAC, M. (1992*a*). Differential expression of the 'B' subunit of the vacuolar H^+-ATPase in bovine tissues. *J. biol. Chem.* **267**, 3696–3706.

PUOPOLO, K., SCZEKAN, M., MAGNER, R. AND FORGAC, M. (1992*b*). The 40-kDa subunit enhances but is not required for activity of the coated vesicle proton pump. *J. biol. Chem.* **267**, 5171–5176.

RACKER, E. (1976). *A New Look at Mechanisms in Bioenergetics*. New York: Academic Press, Inc.

SÜDHOF, T.C., FRIED, V.A., STONE, D.K., JOHNSTON, P.A. AND XIE, X.-S. (1989). Human endomembrane H^+ pump strongly resembles the ATP-synthetase of Archaebacteria. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6067–6071.

SUTTON, R. AND APPS, D. K. (1981). Isolation of a DCCD-binding protein from bovine chromaffin granule membranes. *FEBS Lett.* **130**, 103–106.

UMEMOTO, N., OHYA, Y. AND ANRAKU, Y. (1991). *MA11*, a novel gene that encodes a putative proteolipid, is indispensable for expression of yeast vacuolar membrane H^+-ATPase activity. *J. biol. Chem.* **266**, 24526–24532.

UMEMOTO, N., YOSHIHISA, T., HIRATA, R. AND ANRAKU, Y. (1990). Roles of the *VMA3* gene product, subunit *c* of the vacuolar membrane H^+-ATPase on vacuolar acidification and protein transport. *J. biol. Chem.* **265**, 18447–18453.

WANG, S.-Y., MORIYAMA, Y., MANDEL, M., HULMES, J. D., PAN, Y.-C. E., DANHO, W., NELSON, H. AND NELSON, N. (1989). Cloning of cDNA encoding a 32-kDa protein: an accessory polypeptide of the H^+-ATPase from chromaffin granules. *J. biol. Chem.* **263**, 17638–17642.

WARD, J. M. AND SZE, H. (1992). Proton transport activity of the purified vacuolar H^+-ATPase from oats. *Plant Physiol.* (in press).

XIE, X.-S. AND STONE, D. K. (1986). Isolation and reconstitution of the clathrin-coated vesicles proton translocating ATPase complex. *J. biol. Chem.* **261**, 2492–2495.

ZIMNIAK, L., DITTRICH, P., GOGARTEN, J. P., KIBAK, H. AND TAIZ, L. (1988). The cDNA sequence of the 69 kDa subunit of the carrot vacuolar H^+-ATPase. *J. biol. Chem.* **263**, 9102–9112.