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ENERGIZING PORTERS BY PROTON-MOTIVE FORCE

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Summary

It is generally accepted that the chemistry of water was the most crucial determinant in shaping life on earth. Among the more important chemical features of water is its dissociation into protons and hydroxyl ions. The presence of relatively high proton concentrations in the ambient solution resulted in the evolution of proton pumps during the dawn of life on earth. These proton pumps maintained neutral pH inside the cells and generated electrochemical gradients of protons (proton-motive force) across their membranes. The existence of proton-motive force enabled the evolution of porters driven by it that are most probably among the more primitive porters in the world. The directionality of the substrate transport by the porters could be to both sides of the membranes because they can serve as proton symporters or antiporters. One of the most important subjects of this meeting is the mechanism by which proton-motive and other ion-motive forces drive the transport processes through porters. Is there a common mechanism of action for all proton-driven porters? Is there some common partial reaction by which we can identify the way that porters are energized by proton-motive force? Is there a common coupling between proton movement and uptake or secretion of certain molecules? Even a partial answer to one of these questions would advance our knowledge... or confusion. As my mentor Efraim Racker used to say: ‘If you are not totally confused you do not understand the issue’.

Introduction

Very early on, Nature designed a system that would interconvert chemical energy stored in energy-rich components, such as ATP or a low redox potential electron carrier, with electrochemical energy in the form of a vectorial ion gradient across membranes. Protons were selected to serve as the universal intermediary electrochemical gradient, the sodium ion being the runner-up. Two separate systems evolved that created and maintained this gradient. The first system was proton translocation coupled to downhill vectorial electron transport across membranes. The second system was a reversible ATP-dependent proton pump. Because both pumps ended up functioning in the same membranes, an electrochemical gradient of protons became the universal high-energy intermediate connecting these two distinct systems. The redox potential pump and H^+-ATPases both transport protons to the same side of the membrane. In bacteria, they pump the protons outside the cells and in mitochondria and chloroplasts they pump the protons into the interspace and lumen, respectively. In eukaryotes, there exist additional ATP-

Key words: vaculoar H^+-ATPase, proton gradient, transport, membrane, pumps.

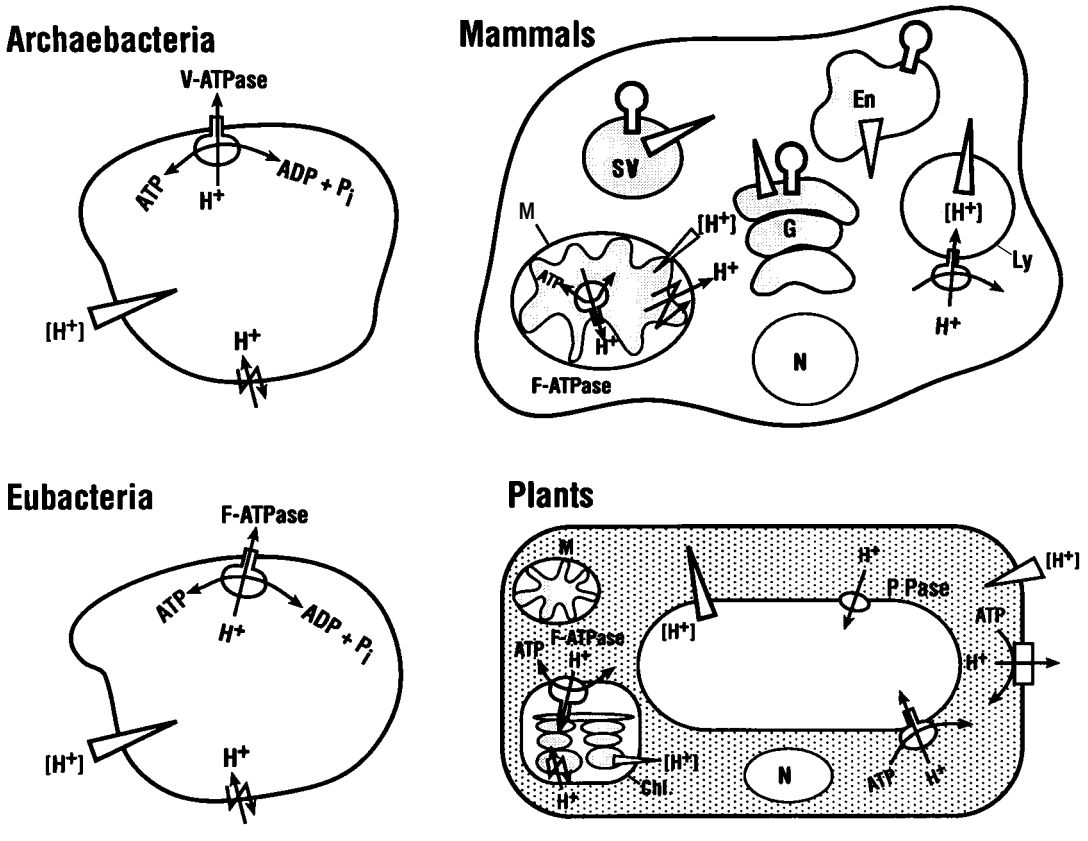

Fig. 1. Location and directionality of the major proton pumps in different organisms. In eubacteria and archaebacteria, both the respiratory electron transport chain and the H^+-ATPases pump protons outwards, resulting in greater proton concentrations outside the cells. In mitochondria, the directionality of these proton pumps is identical to that in bacteria, resulting in a low proton concentration inside the organelle. In chloroplasts, these proton pumps operated in the opposite direction, resulting in high proton concentrations in the lumen. V-ATPase in the vacuolar system pumps protons into the lumen, maintaining a lower pH than in the cytoplasm. In plant vacuoles, additional proton pumps (H^+-pyrophosphatase) pump protons into the vacuole. In plants and fungi, a P-type ATPase pumps protons towards the outside of the cells. N, nucleus; G, Golgi apparatus; SV, synaptic vesicle; En, endosome; Ly, lysosome; M, mitochondria; Chl, chloroplast.

dependent proton pumps that transport protons into the vacuolar system (V-ATPases) and out of the cells (V-ATPases and P-ATPase). Fig. 1 shows a schematic representation of the main proton pumps acting in bacteria and eukaryotic cells. The porters driven by proton-motive force utilize the energy provided by these primary pumps for the transport of numerous substances. Their structure and mechanism of action are a direct consequence of the directionality and the composition (proton gradient *versus* membrane potential) of the available proton-motive force.

Functional evolution of the primary proton pumps

Analysis of homologous sequences and extrapolation to the past can provide a relatively reliable scenario for the evolution of some biological systems during the past one billion years. The onset of the primary pumps and the first porters took place over 3.5 billion years ago. Therefore, intuition plays a major role in formulating evolutionary events in the beginning of life. The redox-potential-driven proton pumps evolved by interaction of electron carriers, such as non-heme iron proteins and cytochromes, with electron plus proton carriers, such as flavoproteins and quinones. By organizing these carriers in an arrangement that will transport electrons across membranes, proton translocation could be coupled to the transport of electrons and the primitive photosynthetic and respiratory electron transport systems were evolved (Mitchell, 1968). Concomitant with these events, ATP-dependent proton pumps emerged that function on the same membranes as the redox-potential-driven proton pumps. In this way, oxidative metabolism and photophosphorylation evolved and the bulk of the ATP was synthesized by these processes. In addition, by regulating the rates of these proton pumps, pH homeostasis could be maintained. What was the identity of the primordial ATP-dependent proton pump? Two main candidates for this job function today as V-ATPases and P-ATPases. These two ATP-dependent proton (and other ions) pumps have completely different structures and distinct mechanisms of action (Bowman and Bowman, 1986; Pedersen and Carafoli, 1987; Nelson and Taiz, 1989). P-ATPases operate with a phosphoenzyme intermediate and, consequently, are sensitive to vanadate. F- and V-ATPases function without an apparent phosphoenzyme intermediate and are relatively insensitive to vanadate. Whereas F- and V-ATPases are composed of several subunits organized separately in membrane and catalytic sectors, P-ATPases can operate with a single membrane protein of about 100 kDa. We believe that V-ATPase was the primordial proton pump and that it functions as a hexamer of a single gene product with distinct catalytic and membrane sectors (Nelson, 1992*a*,*b*). Soon thereafter, the gene was split into two separate genes that gave rise to distinct catalytic and membrane sectors (Taiz and Nelson, 1994). F-ATPases evolved from the primitive V-ATPase after photosynthesis had increased the oxygen concentration in the atmosphere, and the two subfamilies of proton pumps evolved separately (Nelson, 1989). Archaebacteria maintained the V-ATPase as their proton pump and eubacteria evolved together with their F-ATPase. In eukaryotes, F-ATPase is present exclusively in mitochondria and chloroplasts and V-ATPase is present in the vacuolar system and plasma membrane (see reviews in Harvey and Nelson, 1992). The main function of F-ATPase in eubacteria, chloroplasts and mitochondria is to synthesize ATP at the expense of the proton-motive force generated by respiration and photosynthesis. V-ATPase functions in ATP formation only in archaebacteria. In eukaryotes, it functions exclusively as an ATP-driven proton pump. This differential function may be related to the structural evolution of the main component (proteolipid) of the membrane sectors of F- and V-ATPases. As shown in Fig. 2, the ancestral proteolipid was probably a short highly hydrophobic protein of about 8 kDa that spanned the membrane twice. Soon after the eubacteria had separated from the archaebacteria and eukaryotes, the gene encoding the proteolipid underwent duplication

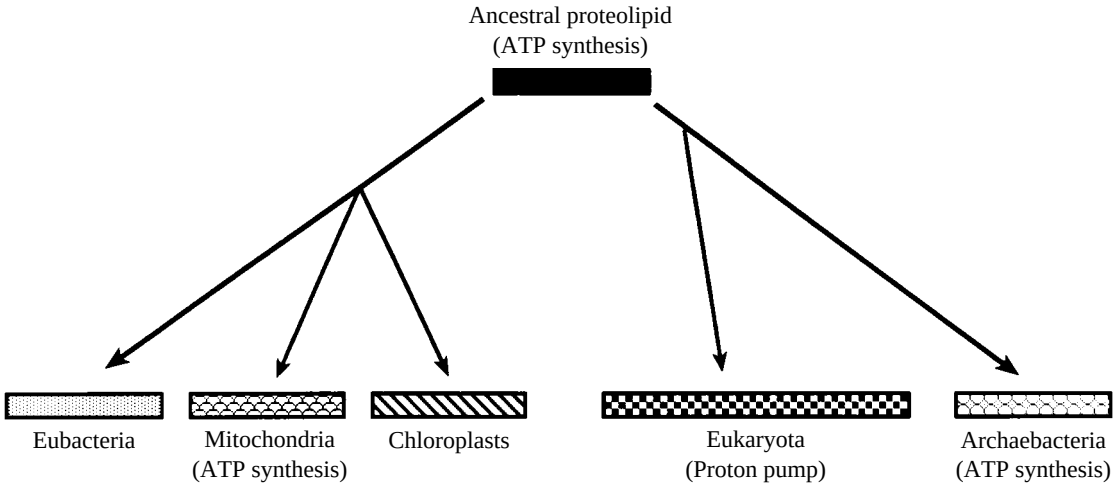

Fig. 2. Evolution of the proteolipids of F- and V-ATPase in the various organisms. The correlation between the size of the proteolipid and the potential activity of the enzyme in ATP-synthesis is depicted.

and fusion to generate a protein with four transmembrane helices (Mandel *et al.* 1988; Nelson, 1992*c*). It was proposed that these events transformed the V-ATPase in eukaryotes into an exclusive proton pump unable to function in ATP formation.

Proton pumps cannot act alone and they have to be accompanied by secondary transporters. Proton conductance across membranes might generate large membrane potentials that, in turn, could stop the flow of protons and even damage the membranes. This drawback can be circumvented by several unrelated processes. Conductance of anions alongside protons is a simple way to prevent the formation of high membrane potentials and it allows large-scale acidification of the lumen. The same effect can be achieved by proton-driven ion exchangers operating with uneven stoichiometry. Utilization of the generated proton-motive force for ATP formation and secondary uptake processes may help solve the same problem. A balance can also be maintained by the action of other primary ion pumps. We think that the P-ATPases evolved to function under circumstances in which the primordial proton pumps were not sufficient for the ion movement processes required by the emerging complex life forms. It is assumed that primordial oceans were much less salty than the oceans are today and that their sodium concentration increased constantly. The sodium that leaked into the cells had to be removed. Some F- and V-ATPases evolved to pump sodium outside certain bacteria (Hoffmann and Dimroth, 1991; Takase *et al.* 1993, 1994), but this seems not to be a favorable avenue. The evolution of sodium/proton exchangers in combination with the evolution of sodium-pumping P-type ATPases probably provided the necessary solution. P-ATPases appeared to be much more versatile than F- and V-ATPases with respect to the cations that they transport, and today they function in the transport not only of sodium, protons and potassium but also in the transport of calcium and several metal ions

that are necessary for some biochemical processes but are poisonous at high concentrations (Pedersen and Carafoli, 1987). In plants and fungi, proton-translocating P-ATPases that function on the plasma membrane provide most of the energy required for secondary uptake processes, including sugar and amino acid transport (Wach *et al.* 1992). In the plasma membrane of mammalian cells, the most abundant primary pump is the Na^+/K^+-ATPase that pumps sodium out and potassium in and generates a negative membrane potential inside the cells. Consequently, most of the secondary uptake systems in the plasma membrane of mammalian cells are driven by sodium gradients. The proton-motive-force-dependent uptake systems are restricted to the vacuolar system and plasma membranes of specialized cells.

Proton-driven exchangers and transporters

In bacteria, primary proton pumps are the main energy source for secondary transport processes and, therefore, most of their transporters are driven by proton-motive force. However, thermophilic and alkaliphilic bacteria primarily utilize sodium-driven transporters (Konings *et al.* 1992). While the preferential use of sodium by alkaliphiles is obvious, why the thermophilic bacteria prefer sodium over protons is not clear. In these bacteria, the Na^+/H^+ antiporter plays a major role in maintaining the sodium gradient across the membrane. The lactose permease of *Escherichia coli* was extensively studied by Kaback and his colleagues and was promoted as a paradigm for membrane transport proteins (Kaback, 1992). A wealth of experimental evidence from this and other laboratories supports the notion that this transporter consists of 12 transmembrane helices. Three charged amino acids in helix X were recognized as important residues either mechanistically or for substrate recognition. Remarkably, lactose permeases that were expressed in two separate parts exhibited control levels of activity. Moreover, lactose permease molecules deleted of helices III and IV were complemented by molecules deleted of helices IX and X, suggesting a functional interaction between two inactive molecules. We do not know whether other transporters of prokaryotes can be as flexible as the lactose permease and whether eukaryotic transporters can tolerate even smaller alterations. One of the most important contributions of the numerous studies on lactose permease is the lesson that was learned and implemented for unraveling the mechanism of other transporters.

Specific examples of transporters driven by proton-motive force are discussed throughout this volume. It is likely that similar mechanisms of action underlie proton-driven bacterial and eukaryotic transporters. Understanding this proton-coupled transport system may also shed light on the mechanism of sodium-driven mammalian transporters.

Ion/proton exchangers play a major role in pH homeostasis in bacteria and eukaryotes. In *E. coli* and *Bacillus* species, Na^+/H^+ and K^+/H^+ antiporters regulate the internal pH and maintain the appropriate membrane potential that can drive ATP formation even at high pH and salinity (Padan and Schuldiner, 1994; Krulwich *et al.* 1994). In mammalian cells, in which the body fluids prevent extreme conditions, ATP-driven transporters are more active in maintaining ion homeostasis. However, specific antiporters such as Ca^{2+}/H^+ and Na^+/H^+ exchangers function in the fine tuning of pH and calcium

homeostasis (Grinstein *et al.* 1993; Reeves *et al.* 1994). In plant and fungal cells, ATP-dependent proton pumps play a major role in energizing the plasma membrane and the vacuolar system. Consequently, most of their transporters are driven by proton-motive force and the pH and calcium homeostasis are maintained largely by proton antiporters (Nakajima-Shimada *et al.* 1991). Proton-motive-force-dependent systems are also widely distributed as energizers in insect gastrointestinal and sensory epithelia (reviews in Harvey and Nelson, 1992). In lepidopteran midgut, V-ATPases in parallel with K^+/H^+ antiporters (Wieczorek *et al.* 1991) generate a membrane voltage greater than 240 mV that maintains a 4 unit pH gradient (Dow, 1992) and drives massive amino acid/K^+ symport (Giordana *et al.* 1982) across the apical membrane. V-ATPase provides the bulk of the energy required for transport processes in the vacuolar system of eukaryotes (Nelson, 1992*a*,*b*). Therefore, most of the transport processes in the vacuolar system are driven by proton-motive force. Accumulation of solutes into the lumen is driven by specific exchangers that exchange specific components for protons and these transporters may be related to the bacterial ion exchangers. Some of the vesicular transporters are related to the bacterial transporters involved in drug resistance and secrete the drugs in exchange for protons (Schuldiner, 1994). Since drug-resistant bacterial strains are of considerable threat to the public health, these transporters are the focus of several research groups.

In this meeting, we have witnessed stunning progress in the perception of the machinery that underlies the action of ion-motive-driven transport across membranes, but the mechanism of this process is still elusive or even illusive.

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