Journal of Bioenergetics and Biomembranes, Vol. 31, No. 1, 1999

Introduction: V-ATPases 1992–1998

Patricia M. Kane[1]

Received November 15, 1998

The vacuolar H^+ATPases (V-ATPases) are electrogenic proton pumps found on internal membranes of all eukaryotic cells and on the plasma membrane of certain cell types. V-ATPases act to acidify intracellular compartments and to drive secondary uptake of a variety of other ions and nutrients. V-ATPases have been functionally linked to a wide variety of cellular processes, including protein sorting, receptor-mediated endocytosis, zymogen activation, pH and calcium homeostasis, and accumulation of neurotransmitters, to name only a few. As plasma membrane proton pumps, they play critical roles in nutrient uptake inects, urinary acidification, bone resorption, and cytoplasmic pH regulation, and they have even been linked to tumor progression (reviewed in Stevens and Forgac, 1997). The V-ATPases as a family were last reviewed in this journal in 1992 (Forgac, 1992). At the time that volume was prepared, the subunit composition of V-ATPases was just becoming clear. The first few subunit genes had been cloned, and the evolutionary relationship between the V-ATPases and the F-ATPases of mitochondria, chloroplasts, and bacteria was established. Since that time, there has been explosive progress in the field. This volume contains a series of reviews from laboratories that have made major contributions to this progress in recent years, and focuses on the specific contributions of each of those laboratories. For further information, the reader is referred to a number of recent reviews on vacuolar H^+-ATPases that highlight other aspects of the structure and function of this pump (Stevens and Forgac, 1997; Merzendorfer *et al.*, 1997; Harvey and Wieczorek, 1997; Nelson and Klionsky, 1996; Forgac, 1996).

[1] Department of Biochemistry and Molecular Biology, SUNY Health Science Center, 750 East Adams Street, Syracuse, New York 13210.

The similarity between V- and F-ATPases has been a fruitful source of progress in the past few years and comparisons with the archaebacterial A-ATPases, which may be progenitors of both V-and F-ATPases, have yielded further insights. The overall structure of V-ATPases strongly resembles the structure of F-ATPases; both enzymes consist of a complex of peripheral subunits containing the nucleotide-binding sites and several "stalk" subunits attached to a complex of integral membrane proteins containing the proton pore. The high-resolution structures now available for the peripheral F_1 sector of the F-ATPases (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998) have focused and accelerated studies of the catalytic mechanisms of both F- and V-type ATPases. At present, it appears that the mechanism of ATP hydrolysis is fundamentally similar between F-and V-ATPases, consistent with the evolutionary conservation of the nucleotide binding subunits, although there are certainly differences in enzyme regulation and coupling to proton transport (see below). The evolutionary relationship between with proteolipid subunits of the V-and F-type ATPases was also uncovered a number of years ago (Nelson and Nelson, 1989), but recent studies indicate that the proteolipid structure in the V-ATPases may be more complex than that proposed for F-ATPases. Archaebacterial ATPases are able to functionally accommodate proteolipid subunits that are quite variable in size and structure (Muller *et al.*, this volume) and eukaryotic V-ATPases appear to contain multiple, structurally distinct proteolipids in each ATPase complex (Oka *et al.*, 1997, 1998; Hirata *et al.*, 1997). The mechanism of coupling ATP hydrolysis to proton pumping may also show significant differences between V- and F-type ATPases. The putative "stalk" subunits implicated in structural and functional coupling of the nucleotide binding subunits and the membrane sector show little if any conservation between F -and V-type ATPases,

0145-479X/99/0200-003$16.00/0

although they are conserved among V-ATPases (Margolles-Clark et al., this volume). Positioning of the stalk subunits in the ATPase complex and definition of their specific functional roles in coupling ATP hydrolysis to proton transport, assembly of the complex, and enzyme regulation promise to be a major areas of future interest.

The groundwork for genetic approaches to V-ATPase structure and function had been laid by 1992 with the description of a well-defined Vma–phenotype, characteristic of loss of V-ATPase function, in the yeast *Saccharomyces cerevisiae* (Nelson and Nelson, 1990). Yeast has continued to be an extremely important genetic model for V-ATPases, comparable in importance to the *Escherichia coli* F-ATPase. The subunit composition of the yeast V-ATPase has been unraveled by a combination of genetic and biochemical approaches, and the Vma^- phenotype has provided both a means of uncovering new subunits and a stringent test of whether newly identified "subunits" were indeed required for V-ATPase activity (reviewed by Nelson and Klionsky, 1996; Stevens and Forgac, 1997). Site-directed and random mutagenesis of V-ATPase subunits in yeast is already in use in a number of laboratories as a basis for subunit structure–function studies. However, yeast is no longer the only genetically tractable organism available for studies of V-ATPase activity. V-ATPase subunit genes have now been disrupted in *Neurospora crassa* and *Drosophila* (Ferea *et al.*, 1996; Davies *et al*., 1996) and identification of V-ATPase subunit genes in *Caenorhabditas elegans* also opens the possibility of a genetic approach (Oka *et al.*, 1997, 1998). These systems promise to provide further insights into the physiological roles of V-ATPases, particularly the roles of plasma membrane V-ATPases, which are present in certain organs of *Drosophila* and *C. elegans*, but have not yet been identified in fungi.

The regulation of V-ATPases has been another active area of research in the past several years and it promises to be a major source of interest for many years to come (reviewed by Forgac, 1996; Merzendorfer *et al.*, 1997). V-ATPases are present in multiple intracellular compartments that must ultimately achieve distinct internal pH values. In addition, there is now substantial evidence that V-ATPase activity is regulated in response to cellular growth conditions, such as carbon source and cytoplasmic redox potential, and that trafficking of fully or partially assembled V-ATPase complexes may also be manipulated as a means of regulating or targeting enzyme activity. Unraveling the many layers of regulation of V-ATPases and their physiological implications promises to better define the diverse cellular roles of V-ATPases.

What is next for the V-ATPase field? The research areas briefly described above have each spawned a set of new questions, but there are now numerous biochemical and genetic tools available for addressing them. Comparison of V- and F-type ATPases has been very enlightening, but it also has its limits, since some features of these two classes of enzymes are very clearly different. A high-resolution structure of a V-ATPase would be extremely valuable and, given recent success in purifying large amounts of an active V_1-ATPase from *Manduca sexta* (Graf *et al.*, 1996), may not be out of reach. Direct structural information would allow a more critical evaluation of the similarities and differences between V- and F-type ATPases. The biosynthesis and assembly of V-ATPases are only beginning to be understood and are unquestionably very complex. The discovery of links between enzyme regulation and assembly state have further complicated this picture. Although a number of regulatory mechanisms have been uncovered in the past few years, it is entirely possible that there are even more modes of regulation for V-ATPases; how these regulatory mechanisms are integrated in whole cells remains unclear. Finally, it is clear that V-ATPases can be adapted to a wide array of cellular functions and the question of how V-ATPases affect cell physiology in different contexts is particularly fascinating. The reviews that follow provide hints of the answers to these questions. The next few years promise to be as interesting and surprising as the last 6 years in the V-ATPase field.

REFERENCES

Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994). *Nature* **370**, 621–628.

Bianchet, M. A., Hullihen, J., Pedersen, P., and Amzel, L. M. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 11065–11070.

Davies, S. A., Kelly, D. C., Goodwin, S. F., Wang, S-Z., Kaiser, K., and Dow, J. A. T. (1996). *J. Biol. Chem.* **271**, 30677–30684.

Ferea, T. L., and Bowman, B. J. (1996). *Genetics* **143**, 147–154.

Forgac, M. (1992). *J. Bioenerg. Biomembr* **24**, 339–340.

Forgac, M. (1996). *Soc. Gen. Physiol. Ser.* **51**, 121–132.

Graf, R., Harvey, W. R., and Wieczorek, H. (1996). *J. Biol. Chem.* **271**, 20908–20913.

Harvey, W. R., and Wieczorek, H. (1997). *J. Exp. Biol.* **200**, 2203–2216.

Hirata, R., Graham, L. A., Takatsuki, A., Stevens, T. H., and Anraku, Y. (1997). *J. Biol. Chem.* **272**, 4795–4803.

Merzendorfer, H., Graf, R., Huss, M., Harvey, W. R., and Wieczorek, H. (1997). *J. Exp. Biol.* **200**, 225–235.
Nelson, N., and Klionsky, D. J. (1996). *Experentia* **52**, 1101–1110.
Nelson, H., and Nelson, N. (1989). *FEBS Lett.* **247**, 147–153.
Nelson, H., and Nelson, N. (1990). *Proc. Natl. Acad. Sci. USA* **87**, 3503–3507.
Oka, T., Yamamoto, R., and Futai, M. (1997). *J. Biol. Chem.* **272**, 147–154.
Oka, T., Yamamoto, R., and Futai, M. (1998). *J. Biol. Chem.* **273**, 22570–22576.
Stevens, T.H., and Forgac, M. (1997). *Annu. Rev. Cell Develop. Biol.* **13**, 779–808.