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Introduction

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The vacuolar (H^+)-ATPases are a family of ATP-driven proton pumps which acidify a variety of intracellular compartments in eukaryotic cells. These include compartments along the endocytic pathway, such as clathrin-coated vesicles, endosomes, phagosomes, and lysosomes, compartments along the secretory pathway, including Golgi-derived vesicles and secretory vesicles, and compartments specialized for storage and degradation, such as the vacuoles of plants and lower eukaryotes. In certain specialized cells, vacuolar (H^+)-ATPases are also present in the plasma membrane where they acidify the extracellular space. This volume is devoted to a series of review articles by many of the leading contributors to our current knowledge of the vacuolar (H^+)-ATPases. In addition to the articles in this volume, there are a number of previous reviews on vacuolar (H^+)-ATPases to which the reader is referred (Forgac, 1989; Nelson and Taiz, 1989; Kane *et al.*, 1989; Gluck, 1989; Brown, 1989; Stone *et al.*, 1989; Pedersen and Carafoli, 1987; Anraku, 1987; Bowman and Bowman, 1986; Mellman *et al.*, 1986; Rudnick, 1986; Schwartz and Al-Awqati, 1986; Sze, 1985).

Acidification of vacuolar compartments in eukaryotic cells plays a critical role in a variety of basic cellular processes. Acidification of endosomes during receptor-mediated endocytosis provides the signal which activates dissociation of internalized ligands from their receptors, thus allowing recycling of free receptors to the plasma membrane. Receptor recycling in turn controls such cellular parameters as the rate of cholesterol and iron uptake and the sensitivity of cells to hormones and growth factors. Endosomal acidification also provides the trigger which activates entry of the cytotoxic portions of certain envelope viruses and toxins into the cytoplasm of infected cells. Acidification of Golgi-derived vesicles plays an analogous role in the recycling of receptors for lysosomal enzymes to the trans-Golgi where they are available for multiple rounds of lysosomal enzyme binding and targeting. Vacuolar acidification is thus crucial to the normal operation of both endocytic and intracellular membrane traffic pathways.

Acidification of such compartments as lysosomes, secretory vesicles, and storage vacuoles is crucial in at least two respects. First, the low pH within these vacuoles is necessary for acid hydrolases to process and degrade macromolecules which are delivered to these compartments. Second, vacuolar (H^+)-ATPases generate both a proton chemical gradient and a membrane potential which are used to drive the coupled transport of solutes either into and out of these organelles. Vacuolar acidification is thus necessary for protein degradation in lysosomes, peptide processing in secretory vesicles, and neutrotransmitter uptake by synaptic vesicles, as well as the concentration of solutes in storage vacuoles. Proton transport across the plasma membrane of intercalcalated cells in the mammalian kidney is responsible for urinary acidification while acidification by vacuolar proton pumps in the plasma membrane of osteoclasts is involved in bone readsorption. There are even recent reports which suggest that there may be a link between vacuolar (H^+)-ATPases and cellular transformation (Jiang *et al.*, 1990; Goldstein *et al.*, 1991).

The vacuolar (H^+)-ATPases thus play an important role in a wide variety of cellular processes. While the exact relationship between the various members of the vacuolar class of (H^+)-ATPases is still unclear, certain unifying principles have emerged. All vacuolar (H^+)-ATPases are macromolecular complexes of 500–750 kDa which are composed of two structural domains. The peripheral V_1 domain processes the nucleotide binding sites which are distributed between the 70-kDa A and 60-kDa B subunits. The A subunit appears to possess the catalytic nucleotide binding

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sites while the B subunit is also believed to play a role in nucleotide binding. At least three additional lower-molecular-weight polypeptides make up the remainder of the V_1 domain and may function in attachment or coupling of the peripheral domain to the membrane-embedded sector. The integral V_0 domain contains multiple copies of a 15–17-kDa proteolipid (subunit c) which is responsible for the sensitivity of proton translocation to dicyclohexylcarbodiimide. The nature of the remaining V_0 polypeptides is uncertain, although proteins of 100, 35–40, and 20 kDa are common to many members of this class of (H^+)-ATPases. One or more of these additional V_0 subunits most likely play an important role in the translocation of protons across the membrane.

The cloning and sequencing of *c*DNAs encoding many of the V-ATPase subunits has represented a recent major advance in this area. It has become clear from sequence data as well as the results of structural studies that the vacuolar (H^+)-ATPases are evolutionarily related to the F-type (H^+)-ATPases, which normally function in ATP synthesis. Sequence homology exists for both the nucleotide binding subunits and the DCCD-reactive *c* subunit, and these families share a similar overall structural organization. Whether the "accessory" subunits in these two families carry out similar functions remains to be determined.

In addition to subunit function, a number of important questions remain to be answered concerning this family of proteins. Are there significant structural differences between the vacuolar (H^+)-ATPases present in different intracellular membranes of a given cell and, if so, what are the consequences of these differences for activity? How are the vacuolar (H^+)-ATPases assembled in the cell and how are they targeted to their appropriate cellular destination? And finally, what is the mechanism by which vacuolar acidification is controlled in cells? The information presented in the succeeding chapters provides exciting hints at the answers to these questions, but the coming years promise even greater insights.

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