

STRUCTURE AND MECHANISM OF THE PROTON PUMPING VACUOLAR ATPase.

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Vacuolar ATPases (V-ATPases; V1V0-ATPases) are large, membrane bound, multi subunit protein complexes which function as ATP hydrolysis driven proton pumps. The vacuolar ATPase is made of two domains: a water soluble V1, and a membrane bound V0. ATP hydrolysis taking place on the V1 is coupled to proton transport through the V0. V-ATPases and related enzymes are found in the endomembrane system of eukaryotic organisms, the plasma membrane of specialized cells in higher eukaryotes, and the plasma membrane of prokaryotes. The proton pumping action of the vacuolar ATPase is involved in a variety of vital intra- and inter-cellular processes such as receptor mediated endocytosis, protein trafficking, active transport of metabolites, homeostasis and neurotransmitter release. In the cell, the activity of the vacuolar ATPase is regulated by a substrate dependent dissociation. The dissociation is reversible and results in V1- and V0 domains which are incapable of MgATP hydrolysis and proton translocation, respectively. We have used electron microscopy and image reconstruction to generate three dimensional structural models of the V-ATPases from bovine brain and yeast. Antibody labeling and difference imaging was used to determine the binding sites of individual subunits and subunit domains in the V-ATPase. The binding positions of subunits A,H,G,C,a,d, and AC45 have been studied and will be discussed. A comparison of the structural models of intact V-ATPase and isolated V1 and V0 domains reveals that the vacuolar ATPase undergoes significant structural changes during substrate dependent dissociation. We speculate that the observed structural changes in the isolated V-ATPase domains are responsible for the silencing of the MgATPase- and proton translocation activities of the individual V1 and V0 domains.