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Structure and Function of V-ATPase in Higher plants

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The vaocular H^+-ATPase (V-ATPase) has been identified in a variety of intracellular compartments of enkaryotic cells, including clathrin coated vescles, chromaffim granules, lysosomes and cetral vacuoles of yeast, Neurospora crassa and Plants[1]. V-ATPase function to provide the proton motive force for active transport of solutes across the vacular membrane [2], pH gradient formation on tonoplast is central to fundamental role of vacuolar in higher plantcell, such as in generation, maintenance and regulation of cell turgor and transport and storage of various ions and metabolites [3]. In receptor-mediated endocyiosis V-ATPase acidify endosomeal environment for active hydrolysis enzymes to dissociation of ligand-receptor complex.

一 Structure and Function of V-ATPase

1 Structure and Function of V_1 sector

Subunit A (67-73 kDa) was first cloned from plants Neurospora crassa and encoded by the yeast *VMA* (vucuolar membrane ATPase)1 gene. This subunit cotains nucleotide-binding site and is inhibited by NEM and NBD-Cl.. ATP and DTT protect subunit A from the inhibition of NEM and NBD-Cl. Subunit B (55-60 kDa) was first cloned from *Neurospora crassa* and encoded by the yeast *VMA* 2. The sequence analysis suggest that Subunit B also containts ATP-binding site which is noncatalytic and may be required for enzyme activity. Mutagenesis studies of yeast V-ATPase showed that this subunit contributes key residues to the catalytic site[4]. Subunit C (40-42 kDa) was first cloned from bovine aderenal medulla and encoded by the yeast *VMA* 5 gene. This subunit have no putative transmembrane helies and conbine with subnuit E (27-33) to form a complex which is required for CaATPase activity of V_1 [5]. Subunit E (27-33kDa) was first cloned from bovine kidney and encoded by the yeast *VMA* 4 gene. Subunit D (32-34 kDa) was first cloned from boyine aderenal medulla and encoded by the yeast VMA 8 gene. Its function may be in coupling ATP hydrolysis and proton pumping. Subunit F (10-14 kDa) was first cloned from insect and encoded by the yeast *VMA* 7 gene. The studies have indicated that the subunit F can help bradge the V_1 and V_0 domains. Subunit G (13-15 kDa) was first cloned from yeast, where it is encoded by *VMA* 10 gene .This subunit play a comparably important role in coupling the V_1 and V_0. domains. Subunie H (50-57 kDa) was first cloned from yeast, where it is encoded by *VMA* 13 gene.

2 Structure and Function of V_o sector

!00 kDa accessory subunit has two function; (1) this subunit contains baflomycin binding site, which is special inhibitor of V-ATPase, (2) a possible role of 100 kDa accessory subunit help proton translocation. 16-17 kDa subunit c is a highly hydrophobic polypeptide and contains the site of reaction with DCCD, a carboxyl reagent that a inhibitor of proton transport. Although present in six copies per enzyme complex, complete inhibition occurs on modification of a single subunit c. Sze has reported that 16-17 kDa subunit c of higher plants is encoded by four genes and there are not any differences in amino acid sequence of polypeptide encoded by different genes The differences of four genes exist in nonencoded region including 5 ' and 3 ' end of gene [6]

二 The tonoplast H^+-ATPase of soybean

1 The tonoplast H^+-ATPase of proton pumping activity was stictly dependent on ATP as an energy source and Hanes-Woolf analyses yieded an apparent Km of 0.6 mM and can hydrolysis PPl slightly, not ADP and AMP. The proton pumping activity with tonoplast vesicle displayed optimum pH was 7.0-7.5 and inhibited by nitrate, NEM, NBD-CI and bafilomycin but not by Orthovanadate and azide. The concentration of DCCD that inhibited 50 % of the maximum proton pumping activity with tonoplast vesicle is 10-15 uM.

2 Purification of the tonoplast H^+-ATPase of soybean. The tonoplast vesicle was first treated by 0.5 % sodium cholate and then by 30 mM OG to remove other membrane protein and solublization from membrane. The solubilized holoenzyme was further purified by ion-exchange chromatograph (Q-Sepharose) by a HPLC system. The purified tonoplast H^+-ATPase of soybean consisted of 67, 58, 45, 38, 35, 33 and 16 kDa subunits indicated by SDS-PAGE. They are similar to the subunits of H^+-ATPase from other plant sources, such as oat, wheat et al. The purified tonoplast H^+-ATPase of soybean was reconstituted into liposome and higher proton pumping activity was obtained.

3 The reconstituted tonoplast H^+-ATPase of soybean is sensitive to DCCD,NEM,NBD-Cl and bafilomycin.

三 The questions ,for further study, are where and how V-ATPase complex is synthesized and assembled?

V-ATPase as like F-ATPase is a mulimeric comples, however, it is unclear where and how the polypeptides of V-ATPase are assembled to form an activity complex. Immunological labeling of oat root tips with a monoclonal antibody (MAb) aginst the perphiral subunit B indicated that gold particles decoratived ER as well as provacuoles. The results are

consistent with the localized of V_1 and V_0 subunits on ER and vacuolar membrane fractionated by using a sucrose gradient. The results are provided by others support that V_1 is assembled to V_0 at the ER. Recent studies with yeast and animal showes that the folding and oligomerzation of membrane and secretiry protein complexes depend on a set of proteis in ER named molecular chaperones. Available evindence suggests that molecular chaperones function primarily by premature folding and assembly to proceed more efficiently, each of molecular chaperones has the specific role.

To understand mechanism for proton pumping of V-ATPase, Scientists think to get further purified and crystal enzyme and study on its structure as like F-ATPase. The Structural Biology is an advanced research field and The meeting hold in Tsukuba university last year will promote the research of Molecular biology including the structure and function of V-ATPase

References

[1] Steveens T.H and Forgac M 1997. Structure, function and regulation of the vacuolar H^+-ATPase. Annu. Rev. Cell Dev. Biol. 13: 779-808.
[2] Rea P.,A and Sanders D. 1987. Tonoplast energization: two H^+ Pumpps, one membrane Physiol. Plant. 71: 131-141.
[3] Sze H., Ward J. M. and Lai S. 1992. Vacuolar H^+-translocation ATPases from plants: structure, function and isoforms. J. of Bioenergetics and Biomembrane. 24: 371-381.
[4] Liu Q., Kane P.M. Newman P. R . Forgac M. 1996. Site-directed mutagenesis of the yeast V-ATPase B subunit Vma2p, J. Biol, Chem, 271 : 2018-22
[5] Peng S. B. Stone D.K. 1994. Reconstitution of recombinant 40-kDa subunit of the clathrm-coated vesicle H^+-ATPase. J. Biol. Chem. 268: 23519-23
[6] Lai S. Watson J. C. Hansen J. N., Sze H. 1991. Molecular cloning and sequencing of CDNA encoding the proteolipid subunit of the vacuolar H^+-ATPase. J. Biol. Chem. 266: 16078-84