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Modification of Vacuolar Function and Physiology of *Saccharomyces cerevisiae* by Expressing Mammalian V-ATPases

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Research aims

Vacuoles constitute a large compartment occupying a quarter to half of the cell volume. This large organelle has function in maintaining cellular homeostasis by transporting small and macromolecules across the membrane. Vacuolar ATPases (V-ATPase) are a class of proton pumps which hydrolyse ATP to ADP+Pi and transport protons from one side of the membrane to another. The V-ATPase is composed of multiple subunits including *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *a*, *c*, *c′*, *c″*, and *d*. In *Saccharomyces cerevisiae*, each subunit except *a* is encoded by a single gene, however, in higher organisms like mouse, multiple loci encodes isoforms for each subunit. In addition, more variants can be produced by an alternative splicing events. Therefore, the mammalian exhibits a wide variety set of V-ATPase in their subunit compositions.

These different isoforms are expressed not only in specific tissues or cell types but their expressions are regulated at developmental stages. Moreover, these subunit isoforms determines the subcellular localization of V-ATPases. These observations leads an argument that V-ATPases with distinct isoforms may exhibits distinct catalytic or transport profiles. In terms of applies sciences, mammalian isoforms is a resource for altering the biochemical and physiological function of ATPase.

In this research, we identified the multiple isoforms consituting the mammalian V-ATPase. We also established a method for expressing the subunits of mammalian origin in the yeast *Saccharomyces cerevisiae*: this system is quite useful for determining the subunit characteristics.

Methods

Expression of mammalian V-ATPase subunits in yeast

cDNA candidates for each isoforms were identified by EST database search using the corresponding yeast sequence as a probe. Full-length clones were obtained as EST clones or reverse-transcription PCR products, and the coding regions were subcloned into a yeast expression plasmid pKT10 which expresses exogeneous protein under a regulation of *TDH3* promoter.

Characterization of yeast/mammalian hybrid V-ATPase on the yeast vacuolar membrane

Yeast spheroplasts harbouring the expression plasmids were generated and further incubated in YPD+0.8 M sorbitol. Vacuoles were prepared with discontinuous Ficoll gradients and converted into vesicles. ATPase activity was measured using a coupled spectrophotomeric assay. ATP-dependent proton transport activity was measured by AMCA fluorescence quenching.

Preparation of Antibodies

Isoform-specific rabbit antibodies against synthetic peptides or bacterially expressed GST- or MBP-fusion proteins were generated and purified with affinity columns conjugated with the corresponding recombinant proteins.

Results

Temperature dependent proton transport of mammalian E subunit isoform

We have identified two novel mouse genes, *Atp6e1* and *Atp6e2*, encoding testis-specific (*E*1) and ubiquitous (*E*2) V-ATPase subunit *E* isoforms, respectively. The *E*1 transcript appears about 3 weeks after birth, corresponding to the start of meiosis, and is expressed specifically in round spermatids in seminiferous tubules. Immunohistochemistry with isoform-specific antibodies revealed that the V-ATPase with *E*1 and *a*2 isoforms is located specifically in developing acrosomes of spermatids and acrosomes in mature sperm. In contrast, the *E*2 isoform was expressed in all tissues examined and present in the perinuclear compartments of spermatocytes.

The *E*1 isoform exhibits 70% identity with the *E*2, and both isoforms functionally complemented a null mutation of the yeast counterpart *VMA4*, indicating that they are *bona fide* V-ATPase subunits. The chimeric enzymes showed slightly lower Km_{ATP} than yeast V-ATPase. Consistent with the temperature-sensitive growth of *vma4*-expressing *E*1 isoform, vacuolar membrane vesicles exhibited temperature-sensitive coupling between ATP hydrolysis and proton transport.

Lung and kidney specific isoforms and their expression in yeast

We also identified two alternative splicing variants of *C*2 subunit isoforms: *C*2-a, a lungspecific isoform containing a 46-amino acid insertion, and C2-b, a kidney-specific isoform without the insert. Immunohistochemistry with isoform-specific antibodies revealed that V-ATPase with *C*2-a is localized specifically in lamellar bodies of type II alveolar cells, whereas the *C*2-b isoform is found in the plasma

membranes of renal and intercalated cells. A chimeric yeast V-ATPase with mouse the *C*2-a or *C*2-b isoform showed a lower Km_{ATP} and lower proton transport activity than that with *C*1 or Vma5p (yeast *C* subunit).

Novel characteristic of V-ATPase: subunit rotation during the enzyme reaction

The general structure of V-ATPase is similar to that of F-ATPase (ATP synthase) which also hydrolyses ATP and forms an electrochemical proton gradient. We showed continuous rotation of purified F-ATPase. Although the physiological role and structure of the two enzymes are different, it has been proposed that V-ATPase has a rotary mechanism similar to that of F-ATPase. To examine this possibility, we focused on the V-ATPase *G* subunit. Assuming that the *G* subunit rotates relative to the *c* ring of V-ATPase, we introduced a His tag and a biotin-binding domain to *c* and *G*, respectively, of the yeast enzyme. Upon ATP hydrolysis, we observed continuous counter-clockwise rotation of an actin filament connected to the *G* subunit of V-ATPase.

References

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