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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Characterization of the VBA transporter family in yeast
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In eukaryotic microorganisms, amino acids are compartmentalized in the vacuole, and this organelle is thus involved in the homeostasis of amino acids levels in cells. The intracellular distributions of amino acids suggest the existence of active transport systems on the vacuolar membrane. Two gene families, AVT (amino acid vacuolar transport) and VBA (vacuolar basic amino acid transporter), have been identified to be involved in amino acid transport across the vacuolar membrane of *S. cerevisiae*. In the VBA family, which belongs to the major facilitator superfamily (MFS), Vba1p, Vba2p, and Vba3p were involved in the vacuolar transport of basic amino acids, Azr1p and Sge1p were involved in resistance to several drugs. However, the function of Vba4p and Vba5p are still unknown.

The Vba5p is virtually a duplicate of the Vba3p. There are only five different amino acids in the transmembrane regions of these Vba proteins, and Vba5p has an extra segment at the N-terminus (124 amino acids). The GFP-Vba5p localized exclusively to the plasma membrane, and a chimera protein of Vba3p having the N-terminal extra segment of Vba5p also localized to the plasma membrane. Therefore, the N-terminal extra residues should be important for the intracellular localization of Vba5p. The uptake activities of lysine and arginine by whole cells were little affected by deletion of the *VBA5* gene, but were stimulated by overexpression of the gene. The inhibition of cell growth by canavanine or 4-nitroquinoline *N*-oxide (4-NQO) was accelerated by overexpression of *VBA5* gene, and the addition of arginine attenuated the inhibitory effect of 4-NQO. These results suggest that Vba5p is a plasma membrane protein involved in arginine uptake and drug sensitivity.

Another member of VBA family, Vba4p, exclusively localized at the vacuolar membrane. ATP-dependent uptake activity of lysine, arginine, and calcium into vacuolar membrane vesicles were increased by overproduction of Vba4p. The Vba4p is likely to be involved in uptake of basic amino acids and calcium into the vacuole. The inhibitory effect of ketoconazole on cell growth was accelerated by disruption of *VBA4* gene. It is likely that Vba4p is involved in vacuolar sequestering of toxic compounds although further investigation is necessary to determine the function of Vba4p.

In the fission yeast *Schizosaccharomyces pombe*, large amounts of basic amino acids are also compartmentalized in the vacuoles, suggesting the existence of transporter(s) for these amino acids on the vacuolar membrane, as in the *S. cerevisiae*. However, characterization of vacuolar transporters in *S. pombe* at molecular level is restricted, because a standard method for isolating the small vacuoles of *S. pombe* has not been established. From BLAST homology search in the *S. pombe* genome, we found Fnx2p, the closest of Fnx1p, and Vba2p, phylogenetically related to the Vba2p of *S. cerevisiae*. We have been suggested that these proteins were involved in vacuolar amino acid uptake. In my study, *S. pombe vba2*$^{+}$ and *fnx2*$^{+}$ genes were expressed in *S. cerevisiae* cells to evaluate the transport activity of amino acids using isolated vacuolar membrane vesicles. The GFP-fused *S. pombe* Vba2p and Fnx2p localized exclusively to the vacuolar membrane in *S. cerevisiae* cells. The amounts of lysine and arginine in the vacuolar fraction were increased by the expression of *S. pombe vba2*$^{+}$ and *fnx2*$^{+}$ genes in *S. cerevisiae* cells. ATP-dependent uptake activities of lysine and arginine were observed with vacuolar membrane vesicles isolated from Vba2p- and Fnx2p-overproducing cells. The ATP-dependent uptake of lysine was inhibited almost completely by the addition of a V-ATPase inhibitor, concanamycin A, and of a protonophore, CCCP, suggesting that *S. pombe* Vba2p and Fnx2p are vacuolar proton-coupled transporter for lysine and arginine. We showed that the *S. pombe vba2*$^{+}$ gene was also responsible for 4-NQO and quinidine sensitivity, and the addition of lysine improved the cell growth in the presence of quinidine.

In my study, I investigated some features of *S. cerevisiae* Vba5p, *S. pombe* Vba2p and Fnx2p. Characterization of these transporters at the molecular level, especially with regard to its substrate specificity, multiplicity, and regulatory mechanism, should be required for further understanding of their physiological roles in yeast.