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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Characterization of vacuolar amino acid transporters from *Fusarium oxysporum* and *Schizosaccharomyces pombe*
Title of Dissertation

(フザリウム菌および分裂酵母における液胞アミノ酸トランスポーターに関する研究)

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In *Saccharomyces cerevisiae*, vacuoles are the largest organelles that function as digestive compartment and also serve as a major storage compartments for various amino acids. They contain about 70-90% of the basic amino acids in whole cells, whereas around 90% of acidic amino acids are found in the cytosol. This difference in the distributions of amino acids suggests the presence of specific transport systems that operate across the vacuolar membrane. So far, several genes for vacuolar amino acid transporters have been identified and characterized in *S. cerevisiae* using reverse genetics and measurement of transport activities by isolated vacuolar membrane vesicles. These include Avt3p (*YKL146w*, ScAvt3p) and Avt4p (*YNL101w*, ScAvt4p), which are involved in the extrusion of neutral amino acids, and both neutral and basic amino acids, respectively, from the vacuolar lumen into the cytosol. These genes must play important biological roles, amino acids homeostasis or recycling of amino acids. Thus the aim of this study was to identify and characterize vacuolar amino acid transporters from the organisms other than *S. cerevisiae*.

By searching genome databases, interesting proteins homologous to ScAvt3p were found. One is in *Schizosaccharomyces pombe* (*SPAC3H1.09c*, SpAvt3p) and the other one is in *Fusarium oxysporum* (*FOXG_11334*, FoAvt3p). To understand the function of these genes, *SpAVT3* and *FoAVT3* were cloned, heterologously expressed in *S. cerevisiae*, and characterized. The results showed that the GFP-SpAvt3p and also GFP-FoAvt3p localized to the vacuolar membrane in *S. cerevisiae*, as GFP-ScAvt3p and GFP-ScAvt4p. The analysis of amino acid content of the vacuolar fraction indicated that the amounts of neutral amino acids in vacuoles were greatly decreased by overproduction of SpAvt3p and FoAvt3p, and

interestingly, basic amino acids were also decreased by SpAvt3p overproduction, which was similar to ScAvt4p overproduction. The transport assay of amino acid using vacuolar membrane vesicles isolated from *S. cerevisiae* heterologously expressing *SpAVT3* demonstrated that alanine and tyrosine were exported from vesicles in an ATP-dependent manner. In addition, uptake activities of arginine was strongly inhibited in the vesicles isolated from *S. cerevisiae* cells overexpressing *SpAVT3*, suggesting that SpAvt3p was also involved in the export of basic amino acids, not only the neutral ones. Furthermore, GFP-fusion of SpAvt3p overproduced in *S. pombe* cells reduced various neutral and basic amino acids in vacuoles. Together, these results suggest that SpAvt3p is a vacuolar transporter involving in the export of amino acids from *S. pombe* vacuoles. Similarly, the ATP-dependent export activities of FoAvt3p for proline, alanine, threonine and valine were detected using isolated vacuole membrane vesicles from *S. cerevisiae* cells heterologously expressing *FoAVT3*. The analysis of the amino acid contents of the vacuolar fraction and amino acid transport activities revealed that FoAvt3p functions as a vacuolar amino acid transporter, exporting mainly neutral amino acids.

Another vacuolar membrane protein in *S. cerevisiae*, namely Atg22p (ScAtg22p), has been reported to be involved in autophagy, a degradation process which is induced under nutrient starvation condition to supply amino acids for protein synthesis. In *atg22*Δ cells, the degradation of autophagic bodies, inner membrane-bound structures of autophagosomes, which are released into vacuoles, is impaired. It has been suggested that ScAtg22p is involved in the release of vacuolar amino acids generated by autophagy from vacuole to the cytosol. Although ScAtg22p belongs to a transporter family, it is still not clear whether it transports amino acids from the vacuole to the cytosol. Two ScAtg22p homologues, FOXG_00164 and FOXG_09714, which are encoded in *F. oxysporum* genome, were characterized. Subcellular localization analysis showed that only GFP-fusion of FOXG_00164 localized to the vacuolar membrane like ScAtg22p dose in *S. cerevisiae*. Thus, only FOXG_00164 was further studied and was named “FoAtg22p”. To investigate whether *FoATG22* is involved in autophagy pathway, two experiments were carried out, *i.e.* observing the accumulation of the autophagic bodies and analysis of Ape1 maturation, which occurs in a manner dependent on the degradation of autophagic bodies. The results showed that the number of autophagic bodies decreased when *FoATG22* was expressed in *S. cerevisiae atg22*Δ mutant cells. Moreover, under nutrient starvation condition, precursor Ape1 was processed to the mature form in *FoATG22*-expressing strain. From these, it can be concluded that *FoATG22* complements the *atg22*Δ phenotype of *S. cerevisiae*. FoAtg22p is the first homologue of ScAtg22p, which is functional in autophagy process.