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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：
Name Siriporn Lunprom

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

Fungal vacuoles are acidic compartments with certain similarities to plant vacuole and mammalian lysosomes. Vacuoles have various functions such as protein hydrolysis, storage of calcium, phosphate and amino acids, pH and osmotic regulation, ion homeostasis and so on (Klionsky *et al.* 1990). 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 (Wiemken and Durr 1974, Kitamoto *et al.* 1988). 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 examination 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 (Sekito *et al.* 2014). These genes must play important biological roles, such as 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, ScAvt3p homologues 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, and heterologously expressed in *S. cerevisiae*. The results showed that the GFP-SpAvt3p and also GFP-FoAvt3p localized to the vacuolar membrane in *S. cerevisiae*, like as GFP-ScAvt3p and GFP-ScAvt4p. The analysis of vacuolar amino acid contents 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 effect to ScAvt4p overproduction. The transport assay of amino acids 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 were strongly inhibited, suggesting that SpAvt3p was also involved in the export of basic amino acids, not only the neutral ones. Furthermore, overproduction of SpAvt3p fused with GFP in *S. pombe* cells reduced various neutral and basic amino acids in vacuoles. Together, these results suggest that SpAvt3p is a vacuolar transporter involved 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 (Suriapranata *et al.* 2000, Yang *et al.* 2006), a degradation process which is induced under nutrient starvation condition to supply amino acids for protein synthesis (Onodera and Ohsumi 2005, Yorimitsu and Klionsky 2005, Inoue and Klionsky 2010). In *atg22*Δ cells, the degradation of autophagic bodies, inner membrane-bound structures of autophagosomes, which are released into vacuoles, is impaired (Suriapranata *et al.* 2000). It has been suggested that ScAtg22p is involved in the release of vacuolar amino acids generated by autophagy from vacuole to the cytosol (Yang *et al.* 2006). Although ScAtg22p belongs to a transporter family, major facilitator superfamily, 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 FOXG_00164 fused with GFP localized to the vacuolar membrane like ScAtg22p when heterologously expressed 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 detecting the Ape1 maturation, which occurs in a manner dependent on the degradation of autophagic bodies. Under nutrient starvation condition, the results showed that the number of cells containing autophagic bodies in there vacuoles decreased when *FoATG22* was expressed in *S. cerevisiae atg22*Δ mutant cells. Moreover, precursor Ape1 was processed to the mature form in *FoATG22*-expressing strain. From these, it can be concluded that *FoATG22* complements the *atg22*Δ phenotypes of *S. cerevisiae*. FoAtg22p is the first homologue of ScAtg22p, which is functional in autophagy process.

In this way, two vacuolar amino acid transporter genes, *SpAVT3* and *FoAVT3* from *S. pombe* and *F. oxysporum* respectively, were identified and characterized, and one autophagy-related gene, *FoATG22* from *F. oxysporum*, was identified.

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