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Review

Transport of Amino Acids across the Vacuolar Membrane of Yeast: Its Mechanism and Physiological Role

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In yeast cells growing under nutrient-rich condition approximately 50% of total amino acids are accumulated in the vacuoles; however, the composition of amino acids in the cytosol and in the vacuoles is quite different. The vacuoles, like lysosomes, degrade proteins transported into their lumen and produce amino acids. These amino acids should be quickly excreted to the cytosol under nutrient starvation condition and recycled for de novo protein synthesis. These suggest that specific machineries that transport amino acids into and out of the vacuoles operate at the vacuolar membrane. Several families of transporter involved in the vacuolar compartmentalization of amino acids have been identified and characterized using budding yeast Saccharomyces cerevisiae. In this review, we describe the vacuolar amino acid transporters identified so far and introduce recent findings on their activity and physiological function.

Key words vacular amino acid transporter; autophagy; starvation; yeast

1. INTRODUCTION

The concentration of cytosolic free amino acids should be strictly controlled responding to the nutrition condition for the effective synthesis of proteins, by changing the uptake activity of amino acids from the extracellular environment, synthesis and degradation of amino acids, synthesis of proteins, and proteolysis.

In the budding yeast Saccharomyces cerevisiae, the plasma membrane transporters involved in the uptake of amino acids have been extensively investigated.1,2) Seventeen transporters that belong to the amino acid–polyamine–organocation (APC) transporter family participate in the uptake of extracellular amino acids with various specificity and affinities for each substrate,3) and in any case, these plasma membrane transporters are responsible for the unidirectional uptake of amino acids from the extracellular environment into the cytosol. Several transporters, such as the general amino acid permease Gap1 (accession: DAA09192) and a basic amino acid permease Can1 (accession: DAA07591), are downregulated by ubiquitin-dependent endocytosis in response to the nitrogen source or the extracellular substrates.3) In addition, the transcription of GAP1 and CAN1 is subjected to nutrient catabolite repression, in which easily metabolized nitrogen sources, such as glutamine or ammonia, act as a repressor. On the other hand, some APC transporters, such as Agpl (accession: DAA07460) that uptakes asparagine and glutamine and also recognizes other amino acids with lower affinity, and Dip5 (accession: DAA11171) that uptakes glutamate, aspartate, serine, asparagine, glutamine, glycine, and alanine, are transcriptionally induced via Ssy1-Ptr3-Ssy5 (SPS) nutrient sensor system in response to extracellular amino acids.1,4) The other APC transporters are also regulated at transcriptional and/or post-translational level to maintain appropriate concentration of each amino acid in the cytosol.1,5)

The other important determinants for the cytosolic amino acid level are the vacuoles/lysosomes. The vacuoles/lysosomes are organelles containing various hydrolytic enzymes that are active at acidic pH and degrade macromolecules such as proteins, nucleic acids, and lipids.5) Under nutrient starvation, the bulk of intracellular organelles and cytoplasmic proteins are delivered by autophagy and subsequently degraded in the vacuoles/lysosomes. At the lysosomal membrane in higher eukaryotic cells, LYAAT-1 (PAT1, SLC36A1, accession: NP_001336669) was identified as an amino acid transporter involved in the export of small neutral amino acids from the lysosomes.6) Very recently, SNAT7 (SLC38A7, accession: NP_060701) was identified as a lysosomal transporter that is highly specific for glutamine and asparagine and was suggested to be involved in the export of glutamine derived from proteolysis in the lysosomes.7) In the case of fungi and plants, not only the digestive function as mentioned above, but the vacuoles also contribute to serve as temporary storage compartments for amino acids. In yeast cells growing under nutrient-rich condition, approximately 50% of total amino acids are accumulated in the vacuoles.8) Under starvation condition, these accumulated amino acids with those derived from a result of massive protein degradation are considered to be excluded from the vacuoles to the cytosol, and recycled for de novo protein synthesis. Therefore as the cellular uptake of amino acids across the plasma membrane, the amino acid transport into and out of the lysosomes/vacuoles must play a critical role for the maintenance of cytosolic amino acid levels.
2. ACTIVE TRANSPORT OF AMINO ACIDS ACROSS THE VACUOLAR MEMBRANE

Although half of total cellular amino acids are compartmentalized in the vacuoles of S. cerevisiae cells growing under nutrient-rich condition, the amino acid composition is quite different between in the cytosol and in the vacuolar lumen. Approximately 70–90% of cellular basic amino acids (lysine, histidine, and arginine) are accumulated in the vacuoles, whereas 90% of acidic amino acids (aspatic acid and glutamic acid) are excluded from the organelles to the cytosol. Such differential distribution of amino acids implies the presence of active and specific transport machineries at the vacuolar membrane. The vacuolar transport of amino acids has been mainly investigated using S. cerevisiae. Ohsumi et al. established an isolation method of right-side-out vacuolar membrane vesicles, and suggested that basic amino acids were actively taken up into the vacuolar membrane vesicles depending on a proton concentration gradient established by the action of vacuolar-type H\(^+\) -ATPase (V-ATPase). Kinetic analysis using vacuolar membrane vesicles revealed that 10 amino acids (arginine, lysine, histidine, phenylalanine, tryptophan, tyrosine, glutamine, asparagine, isoleucine, and leucine) were actively taken up into the vesicles, and the exchange activity of histidine and arginine was also detected. The ATP-dependent uptake activities of lysine and arginine showed saturation kinetics with apparent \(K_t\) values of approximately 0.6 mM, which is 100 times higher than those of the transport activities of the plasma membrane. The uptake of amino acids into vacuoles may function to sequester and preserve free amino acids in the vacuoles and to maintain the cytosolic concentration of amino acids at appropriate level.

3. CHARACTERIZATION OF VACUOLAR AMINO ACID TRANSPORTERS

By reverse genetics and in vitro experiments using isolated vacuolar membrane vesicles, several families of amino acid transporters have been identified. These transporters use a proton concentration gradient across the vacuolar membrane as driving force, which is mainly established by the action of V-ATPase.

Table 1. Vacuolar Amino Acid Transporters in Yeasts

| Superfamily | Family       | Saccharomyces cerevisiae | Schizosaccharomyces pombe |
|-------------|--------------|--------------------------|---------------------------|
|             | Transporter  | Accession                | Substrate                 | Accession                |
| AAAP        | Avt1         | NP_012534                | Neutral amino acids, His  | In                       |
|             | Avt2         | NP_010850                | Neutral amino acids, His  | In                       |
|             | Avt3         | NP_012776                | Basic, neutral amino acids| Out                      |
|             | Avt4         | NP_014298                | Basic, neutral amino acids| Out                      |
|             | Avt5         | NP_009464                | Acidic amino acids        | Out                      |
|             | Avt6         | NP_011044                | Neutral amino acids       | Out                      |
|             | Avt7         | NP_012178                | Neutral amino acids       | Out                      |
|             | Vba1         | NP_013806                | Lys, His                  | In                       |
|             | Vba2         | NP_009852                | Basic amino acids         | In                       |
|             | Vba3         | NP_009864                | Lys, His                  | In                       |
|             | Vba4         | NP_010404                | Quinidine,azole          | In                       |
|             | Vba5\(^c\)  | NP_013031                | Arg, 4-NQO\(^a\)         | —                        |
|             | Azr1         | NP_011740                | His                      | —                        |
|             | Sge1         | NP_015524                | Crystal violet            | —                        |
|             | Atg22        | NP_009892                | Leu, Tyr                  | Out                      |
| TOG         | Ypq1         | NP_014549                | Lys, Arg                  | In                       |
|             | Ypq2         | NP_010639                | Arg                       | In                       |
|             | Ypq3         | NP_009705                | His                       | In                       |
|             | Ers1         | NP_010000                | Unknown                   | Unknown                  |

\(^a\) 4-NQO; 4-nitroquinoline-N-oxide. \(^b\) in; uptake into the vacuoles, out; export from the vacuoles. \(^c\) The transporters that localize to the plasma membrane are indicated in the parentheses.
vacuolar membrane vesicles, several gene families for transporters have been identified and characterized. We briefly describe the vacuolar amino acid transporters in *S. cerevisiae* and also the homologs in the fission yeast *Schizosaccharomyces pombe* (Fig. 1 and Table 1).

### 3.1. Amino Acid/Auxin Permease (AAAP) Superfamily

From sequence analysis of the complete genome of *S. cerevisiae*, seven genes have been found that are related to the γ-aminobutyric acid–glycine transporters, *Caenorhabditis elegans* UNC-47 (accession: P34579) and rat vesicular neurotransmitter transporter VGTAV/VAAT (accession: O35458) (SLC32 family), which belong to the AAAP superfamily, and designated as AVT family.14) In experiments using vacuolar membrane vesicles of *S. cerevisiae*, Russnak et al.14) showed that Avt1 is required for the uptake of glutamine, isoleucine, and tyrosine. Avt3 and the related Avt4 were indicated to be involved in the export of these amino acids from vacuoles, and Avt6 is involved in the export of glutamate and aspartate. Under nitrogen-starvation conditions, proteins delivered to vacuoles by autophagy are degraded, and the resulting amino acids should be recycled to the cytosol by vacuolar amino acid transporters for de novo protein synthesis. We analyzed amino acid composition in the vacuolar fractions extracted by cupric ion treatment method.15) The amounts of amino acids in the vacuolar fraction were greatly decreased under the starvation condition in the wild-type cells, whereas the content of various neutral amino acids in the fraction was maintained at a higher level in the avt3Δavt4Δ cells.16) Interestingly, the avt4Δ cells, but not the avt3Δ cells, retained significantly larger amounts of basic amino acids in the vacuoles than the wild-type cells. These results suggest that both Avt3 and Avt4 show broad substrate specificity for neutral amino acids and that Avt4 characteristically also recognizes basic amino acids.16) The transcription of AVT4 has been suggested to be induced on nitrogen starvation, whereas that of AVT3 is constitutive.17) Avt3 and Avt4 are suggested to function in a partially redundant manner in the export of amino acids produced by autophagy.

Several neutral amino acids such as alanine, valine, and threonine had been considered not to be actively taken up into vacuoles.12) The vesicles of avt3Δavt4Δ cells, however, uptake them in an ATP- and AVT1-dependent manner.18) By competitive inhibition assay of isocitrate uptake using the vesicles of AVT1-expressing cells, it was indicated that Avt1 recognizes various amino acids with broad specificity for its substrate, including not only neutral amino acids but also histidine.19) The effect of protonophore on the activities of Avt1, Avt3, and Avt4 suggest that these transporters operate dependent on a proton concentration gradient across the vacuolar membrane.16,18) We have also suggested that other members in the AVT family, Avt6 and Avt7, are involved in the efflux of acidic amino acids and neutral ones, respectively.19,20) The substrates of Avt2 and Avt5 remain unknown.

### 3.2. Major Facilitator Superfamily (MFS)

VBA family transporters (Vba1, Vba2, and Vba3) were identified as vacuolar transporters involved in the uptake of basic amino acids,21) but so far these transporters are suggested to have broad specificity for their substrates including basic amino acids, azaoles, and quinidine.22) Another member of vacuolar MFS, Atg22, was first identified as a factor required for the breakdown of autophagic bodies derived from the fusion of autophagosomes with vacuoles during autophagy.23) It is believed that Atg22 exports amino acids out of the vacuole, because the neutral amino acids increased in the vacuoles of atg22Δ strain under nutrient-rich conditions.24) All of the AVT3, AVT4, and ATG22 are required for the viability under the starvation condition.24) Further verification is required for the transport activity of Atg22 using vacuolar membrane vesicles and the relation between export of amino acids and degradation of autophagic body should be addressed.

### 3.3. PQ-Loop Proteins

The PQ-loop proteins, which belong to the lysosomal cystine transporter (LCT) family in the transporter/opsin/G protein-coupled receptor (T/G) superfamily, possess putative 7 transmembrane helices and conserved proline-glutamine dipeptides in the motifs termed as PQ-loop.25) Recently, members of this family in rat and *C. elegans*, PQLC2 (accession: B0BMY1) and LAAT-1 (accession: NP_493686), respectively, have been reported to be involved in the transport of basic amino acids in the lysosomes.26,27) In the genome of *S. cerevisiae*, six genes belonging to the LCT family have been found. Among them, vacuolar membrane transporters Ypq1, Ypq2, and Ypq3, which are related to PQLC2, were proposed to function in the homeostasis of basic amino acids based on higher resistance of ypq1Δ and ypq2Δ mutants to L-arginine analog, canavanine, and expression pattern of YPQ3 in the presence of lysine.28) By the transport assay using vacuolar membrane vesicles, we found that ypq1Δ mutation resulted in a greatly decreased ATP-dependent uptake of lysine, as well as in a partial impairment of that of arginine.28) Recently, we also indicated that ATP-dependent uptake of arginine and histidine by vacuolar membrane vesicles is decreased by ypq2Δ and ypq3Δ mutations, respectively, and the vesicles of quadruple mutant ypq1Δypq2Δypq3Δavt1Δ completely lost the uptake activity of all basic amino acids.29) These results from in vitro experiments suggest that the Avt1 and Ypq proteins are major determinants of the uptake activity of basic amino acids into vacuoles. However, based on the measurement of vacuolar basic amino acid contents, additional transporter(s) seems to operate for uptake of these amino acids into vacuoles. Recently, it was suggested that Ypq1 is selectively degraded in the vacuolar lumen responding to the limitation of its substrate, lysine.30) The physiological function and regulation of vacuolar PQ-loop proteins should be further investigated.

### 3.4. Homologs in *Schizosaccharomyces pombe*

Few homologous genes of vacuolar amino acid transporters are found in the genome of *S. pombe* (Table 1), which imply less redundancy among their functions, and so *S. pombe* is expected to be advantageous for clarifying the physiological roles of vacuolar amino acid transporters. Mukaiyama et al.31) indicated that *S. pombe* Avt3 (SpAvt3) is involved in spore formation under starvation condition. We observed that vacuolar amino acid contents were greatly increased in *S. pombe* avt3Δ mutants and were decreased by the overexpression of avt3Δ gene, indicating that SpAvt3 is involved in the export of vacuolar amino acids and important for amino acid compartmentalization in *S. pombe* cells.32) Although the amino acid sequence of SpAvt3 is more closely related to that of *S. cerevisiae* Avt3 than Avt4, SpAvt3 recognizes both neutral and basic amino acids, which is similar to *S. cerevisiae* Avt4.32) It has been reported that a PQ-loop protein Stml functions as a G protein-coupled receptor in the plasma membrane of *S. pombe*,33) however, the Stml fused with green fluorescent protein localized to the vacuolar membrane when expressed both
in *S. cerevisiae* and *S. pombe* cells (unpublished data). Thus it is plausible that the Stm1 also participates in vacuolar amino acid compartmentalization in *S. pombe* cells.

4. PHYSIOLOGICAL ROLE OF VACUOLAR AMINO ACID TRANSPORTERS

In yeast cells growing in nutrient-rich media, free amino acids are actively taken up and compartmentalized into the vacuoles to maintain the cytosolic level of amino acids appropriately. On nitrogen starvation, recycling of amino acids from

![Fig. 2. Maintenance of Cytosolic Amino Acid Level by Inward and Outward Transport across the Vacuolar Membrane](image)

Under nutrient-rich condition, amino acids are compartmentalized into vacuoles by their uptake through the vacuolar amino acid transporters. On nutrient starvation, the accumulated amino acids with those derived from autophagic degradation of proteins are exported out of the vacuoles and recycled for *de novo* protein synthesis. Various amino acid transporters contribute to maintenance of the cytosolic concentration of amino acids at appropriate level responding to the nutritional condition.

![Fig. 3. Avt3/4 Homologs in Fungi, Mammal, and Plant](image)

Phylogenetic tree of Avt3/4 homologs from *S. cerevisiae* (Avt3 and Avt4), *Schizosaccharomyces pombe* (SpAvt3), *Histoplasma capsulatum* (EGC45590), *Coccidioides immitis* (XP_001239967), *Aspergillus flavus* (XP_002378633), *Aspergillus fumigatus* (EDP50154 and EDP53486), *Cryptococcus neoformans* (OXB37745), *Fusarium oxysporum* (FoAvt3, BAO57296), *Candida albicans* (KHC67782), *Arabidopsis thaliana* AtAvt3A and AtAvt3B (Q9FKY3 and F4LY79), and *Homo sapiens* PAT1 and PAT4 (XP.016872663). The alignment was obtained from ClustalX software and tree was visualized using NJplot software. Topology models of these homologs predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) were indicated with total number of amino acid residues.
the vacuoles is considerably important for cellular response to starvation, since autophagy-deficient mutants of *S. cerevisiae* rapidly decrease survival rate as protein synthesis declines\(^{34}\) (Fig. 2). Sporulation is one of the starvation responses in yeasts, and sporulation efficiency was greatly decreased in the autophagy-deficient mutant of *S. pombe*.\(^{35}\) The *S. pombe* mutant avt3\(^5\), avt5\(^\Delta\), and avt3\(^\Delta\)avt5\(^\Delta\)satg22\(^\Delta\), in which the gene(s) of vacuolar amino acid transporter(s) were disrupted, showed a partial decrease in sporulation efficiency.\(^{36}\) Also in the case of *S. cerevisiae*, sporulation efficiency was significantly decreased by avt3\(^\Delta\)avt4\(^\Delta\)avt7\(^\Delta\) mutation.\(^{37}\) These results suggest that cells use amino acids produced by autophagic degradation of proteins in the vacuoles, and the recycling of amino acids by vacuolar exporters is required for efficient protein synthesis under nitrogen starvation (Fig. 2).

The genes that are homologous to *S. cerevisiae* Avt3/Avt4 and *S. pombe* Avt3 are widely distributed in fungi, including pathogenic ones such as *Histoplasma capsulatum* and *Candida albicans*, and phytopathogenic ones such as *Fusarium oxysporum* (FoAvt3).\(^{38}\) These fungal-type Avt3/Avt4 homologs characteristically possess a large hydrophilic region (200–400 residues) at their N-terminus (Fig. 3). Although the region of SpAvt3 is dispensable for its transport activity of amino acids, overexpression of avt3\(^3\) without the region (avt3\(^\Delta\)vl-avt7\(^\Delta\)) could not fully complement the defect of sporulation efficiency of avt3\(^\Delta\) mutant cells.\(^{36}\) The results suggest that the N-terminal region of SpAvt3 is involved in the regulation of its function under starvation condition. Such regulatory role through the large N-terminal region is likely a common feature for fungal-type Avt3/Avt4 homologs. In higher eukaryotic cells, Avt3/Avt4 homologs of *Arabidopsis thaliana*, AtAvt3A, AtAvt3B, and AtAvt3C, function as vacuolar exporters for neutral amino acids,\(^{37}\) and the LYAT-1 (PAT1, SLC36A1) and SNAT7 (SLC38A7) have been suggested to be involved in the export of amino acids from lysosomes.\(^{6,7}\) Since these homologs in higher eukaryotes lack the long hydrophilic N-terminal region (Fig. 3), it is considered that developing new drugs specific for pathogenic fungi may be achieved using this region of fungal-type Avt3/Avt4 homologs as a target molecule.

5. CONCLUSION

A large number of transporters are involved in the uptake of amino acids from the extracellular environment with diverse substrate specificity and regulatory mechanisms. In addition, it is now becoming evident that various transporters at the vacuolar membrane bidirectionally operate for the vacuolar compartmentalization of amino acids (Fig. 2). The molecular mechanism of their regulation in response to nutritional conditions, however, still remains largely unknown. It has been reported that lysosomal amino acid transporters PAT1 (SLC36A1)\(^{38}\) and SNAT9 (SLC38A9)\(^{39}\) are involved in the regulation of mammalian Tor kinase complex 1 (mTORC1), which regulates cell proliferation and growth as the sensor kinase of nutritional condition. Although the regulation of TOR signaling by vacuolar amino acid transporter(s) in yeast has not been elucidated, vacuoles may function as an origin of nutritional signals similarly to lysosomes, as well as the storage and digestive compartment. Exploring the relations between the activity of vacuolar amino acid transporters and the intracellular amino acid level would provide a step forward into the understanding of vacuolar function on amino acid homeostasis.

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Conflict of Interest The authors declare no conflict of interest.

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