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Role of the fission yeast ammonium transporters in nitrogen sensing

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Research aims

Ammonium is an important source of nitrogen for many microorganisms and plants and is taken up by them from the environment to synthesize amino acids. Transport of ammonium across the cell membrane is mediated by ammonium transporters, membrane proteins conserved in bacteria, archaea, and eukarya (1). Interestingly, in the budding yeast *Saccharomyces cerevisiae* and in the plant and human pathogenic fungi *Ustilago maydis* and *Candida albicans*, ammonium transporters have been shown to be involved in a switch from yeast-form growth to filamentous growth induced by nitrogen limitation (2–4). Recently, I have functionally characterized three ammonium transporters in the fission yeast *Schizosaccharomyces pombe* and have found that one of them, Amt1, is required for ammonium-limitation induced morphological differentiation (filamentous growth underneath the surface of agar medium; Fig. 1A) (5, 6). There are two models for the role of Amt1 in the morphological differentiation in response to ammonium limitation. Amt1 might function as an ammonium sensor to generate a signal to induce filamentous invasive growth (Fig. 1B left). Alternatively, ammonium taken up by ammonium transporter or its metabolite might be a signal (Fig. 1B right). The purpose of this study is to elucidate physiological roles of ammonium transporters in *S. pombe*, focusing on their roles in nitrogen sensing.

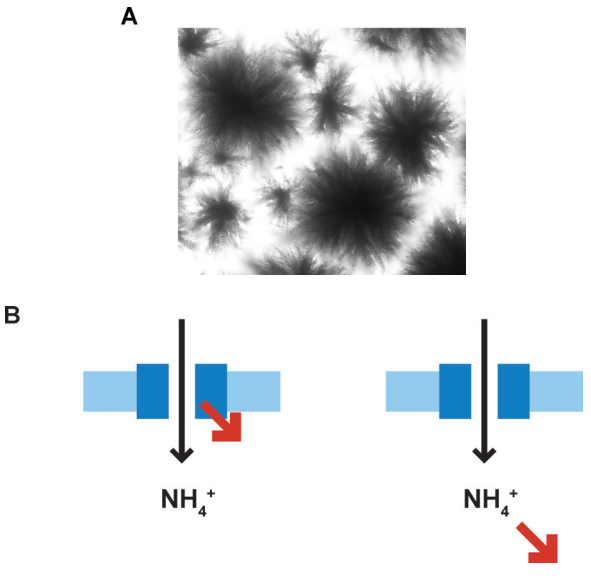

Fig. 1. Role for Amt1 in morphological differentiation of *S. pombe*. (A) Morphology of cells growing filamentously underneath the surface of the agar. (B) Two models for induction of invasive growth.

Methods

S. pombe strains used in this study were described previously (5). Invasive growth of *S. pombe* was examined on LNB medium by washing cells off the agar surface with running water (5). EMM-N medium and EMM-N medium supplemented with a nitrogen source other than ammonium were also used. Sporulation was assessed under microscope. *S. cerevisiae mep2*Δ/*mep2*Δ strain was constructed through crosses between haploid strains whose *MEP2* gene was disrupted with the *URA3* marker by a PCR-based method. Pseudohyphal growth of *S. cerevisiae* was examined on SLAD medium (2) containing various concentrations of ammonium.

Results

Role of ammonium transporter in morphological differentiation

On agar medium containing 1 mM ammonium, wild-type *S. pombe* cells exhibited invasive growth, whereas *amt1*Δ cells did not show any growth underneath the surface of the agar (Fig. 2). The model shown in Fig. 1B right suggests that the invasive growth defect of the *amt1*Δ cells could be recovered at higher concentrations of ammonium. As expected, the *amt1*Δ cells underwent invasive growth on 5 mM ammonium (Fig. 2). Next, diploid *S. cerevisiae* strain was constructed carrying disruption of the *MEP2* gene,

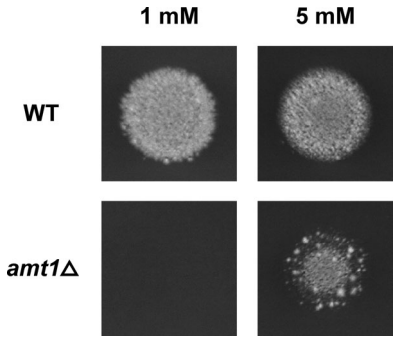

Fig. 2. Effect of ammonium concentration on the invasive growth defect of *amt1*Δ cells.

which encodes an ammonium transporter that is required for pseudohyphal growth and is proposed to function as an ammonium sensor. Unlike the invasive growth defect of the *S. pombe amt1*Δ strain, the pseudohyphal growth defect of this strain was not relieved by raising ammonium concentration in the medium (data not shown).

Role of ammonium transporter in the retention of ammonium in the cell

*S. pombe amt1*Δ *amt2*Δ *amt3*Δ cells show a defect in growth on low ammonium (5; Fig. 3). In the present study, the *amt1*Δ *amt2*Δ *amt3*Δ cells were found to have a growth defect on a nitrogen source other than ammonium as well. For example, the triple mutant grew more slowly than wild type on medium containing tryptophan as the nitrogen source (Fig. 3). Furthermore, the *amt1*Δ *amt2*Δ *amt3*Δ cells displayed slower growth compared with wild type on nitrogen-free (EMM-N) medium (Fig. 3). These results can be explained by assuming that ammonium produced by amino acid metabolism is exported or leaks out from the cell and then is taken up into the cell by ammonium transporters. The ability of the *amt1*Δ *amt2*Δ *amt3*Δ cells to undergo autophagy was assessed by testing them for sporulation. The triple mutant exhibited sporulation, although to a lesser extent than wild type, suggesting that the triple mutant can undergo autophagy. This observation supports the model in which the impaired growth of *amt1*Δ *amt2*Δ *amt3*Δ cells on EMM-N results not from a defect in autophagy but from a defect in the retention of intracellular ammonium.

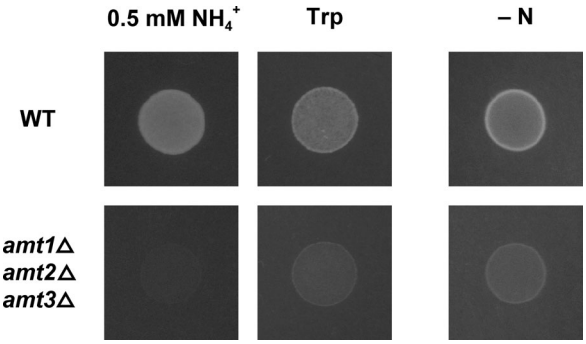

Fig. 3. Growth defects of *amt1*Δ *amt2*Δ *amt3*Δ cells on medium containing a nitrogen source other than ammonium and on nitrogen-free medium.

Conclusion

Raising ammonium concentration in the medium relieved the invasive growth defect of *S. pombe amt1*Δ cells, but not the pseudohyphal growth defect of *S. cerevisiae mep2Δ*/*mep2Δ* cells, suggesting that the roles of ammonium transporter in nitrogen sensing are different between the yeasts. *S. pombe* ammonium transporters seem to function in the retention of intracellular ammonium produced by cellular metabolism, suggesting their role in growth on a nitrogen source other than ammonium.

References

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