Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title
Elucidation of the nutrient-dependent regulatory mechanisms that control the ammonium transporter gene *amt1*$^{+}$ in fission yeast

Student’s Name
Ng Shet Lee

Approved Digest
The uptake of nitrogen nutrition is essential for cell growth where the nitrogen element is utilized as building blocks of cellular macromolecules. Ammonium is one of the preferred nitrogen sources in many organisms. The uptake of ammonium is mediated by the Amt/MEP/Rh (Ammonium transporters/Methylammonium permease/Rhesus) proteins, a family of integral membrane proteins that are conserved from bacteria to mammalian cells. Three ammonium transporters named Amt1, Amt2, and Amt3 exist in the fission yeast *Schizosaccharomyces pombe*. While all three ammonium transporters exhibit ammonium uptake ability, only the Amt1 protein shows an increase upon nitrogen starvation. Indeed, ammonium appears to negatively regulate the expression of the Amt1 protein, as evidenced by the increased Amt1 protein in medium lacking ammonium, and a decrease of Amt1 upon the re-addition of ammonium. Nitrogen limitation localizes Amt1 from the cytoplasm to the plasma membrane, where it is likely to uptake extracellular ammonium.

The *S. pombe* GATA transcription factor Gaf1 was identified as the transcription of *amt1*$^{+}$ in response to nitrogen starvation. The transcription of *amt1*$^{+}$ is dependent on the phosphorylation status and the cellular localization of Gaf1. Upon nitrogen starvation, Gaf1 is dephosphorylated and translocated into the nucleus. The dephosphorylation and nuclear translocation of Gaf1 is largely dependent on the protein phosphatase Ppe1. As nitrogen starvation of *ppe1Δ* cells partially induces Gaf1 dephosphorylation and transcription of *amt1*$^{+}$ mRNA, it is likely that an additional protein phosphatase regulates the Gaf1-*amt1*$^{+}$ pathway. The amino acid sequence of Ppe1 shares high similarity with that of Ppa2, the major

catalytic subunits of the type 2A protein phosphatase (PP2A) complex. Overexpression of either Ppa1 and Ppa2 suppressed the cold-sensitive phenotype of the *ppe1Δ* mutant, indicating the redundant function shared between Ppe1 and PP2A. Unlike Ppe1, PP2A is dispensable for the nuclear translocation of Gaf1. On the other hand, when the PP2A activity is inhibited by the okadaic acid, the induction of *amt1*$^+$ upon nitrogen starvation was abolished, indicating that the activity of PP2A is required for the *amt1*$^+$ induction. Taken together, dephosphorylation of Gaf1 by these two types of protein phosphatases appears to play distinct roles in the nitrogen-starvation dependent regulation of Gaf1; Ppe1 controls the Gaf1 nuclear translocation, while the PP2A- dependent dephosphorylation of Gaf1 leads to the induction of *amt1*$^+$. I also found that Par1, one of the regulatory subunits of the PP2A complex, also affects the induction of *amt1*$^+$ in response to nitrogen starvation.

In eukaryotic cells, from yeast cells to mammals, the target of Rapamycin (TOR), a serine/threonine protein kinase, promotes cellular growth in response to nutrients. In fission yeast cells, there are two TOR kinases, namely Tor1 and Tor2, that assemble into TOR complex 2 (TORC2) and TOR complex1 (TORC1), respectively. TORC1 controls cellular growth by phosphorylating downstream targets to promote anabolic processes such as ribosome biogenesis, protein synthesis and translation. TORC1 is also known as the cellular target of rapamycin, an immunosuppressant drug. TORC1 is activated in response to nitrogen such as ammonium or amino acids, hence suggesting the possible involvement of the TORC1 pathway in ammonium uptake. When the TORC1 activity is compromised in the temperature- and rapamycin-sensitive mutant *tor2-287,* an increase in the *amt1*$^+$ transcription was observed, implying that the TORC1 pathway negatively regulates the transcriptional activity of *amt1*$^+$. Indeed, Gaf1 was dephosphorylated and localized to the nucleus in the *tor2-287* mutant, suggesting that inhibition of the TORC1 activity results in the dephosphorylation of Gaf1 and its translocation to the nucleus, as observed during nitrogen starvation. Deletion of the *ppe1*$^+$ gene abolished nuclear entry of Gaf1 in rapamycin-treated cells, indicating that Ppe1 is required for the entry of Gaf1 into the nucleus upon TORC1 inhibition.

This study revealed that dephosphorylation of Gaf1 by Ppe1 and PP2A in

response to nitrogen starvation is required for the regulation of the ammonium transporter Amt1 expression in fission yeast, and that nitrogen-sensing TORC1 is involved in this process.