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The Molecular Mechanism of Vacuolar Function in Yeast Cell Homeostasis

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Abstract : Cell homeostasis is regulated by vacuolar activity and it has been shown that lipid composition of the vacuole plays an important role in vacuolar function. The major phosphoinositide species present in the vacuolar membrane include phosphatidylinositol 3,5-biphosphate ($PI(3,5)P_2$) which is generated from PI(3)P controlled by Fab1p. Deletion of FAB1 gene reduce the synthesis of $PI(3,5)P_2$ and thus result in enlarged or fragmented vacuoles, with neutral vacuolar pH due to reduced vacuolar H^+-ATPase activity. These mutants also exhibited poor growth at high extracellular pH and in the presence of $CaCl_2$. Conversely, VPS34 regulates the synthesis of PI(3)P from phosphatidylinositol (PI), and the lack of Vps34p results in the reduction of vacuolar activity. Although the cellular observations are clear, it is still unknown about the molecular mechanism between the phospholipid biosynthesis pathway and vacuolar activity. Since both VPS34 and FAB1 are important in vacuolar activity, we hypothesize that the molecular mechanism of vacuolar function might be regulated by the transcriptional regulators of phospholipid biosynthesis. In this study, we study the role of the major phospholipid biosynthesis transcription factor, INO2, in the regulation of vacuolar activity. We first performed qRT-PCR to examine the effect of Ino2p on the expression of VPS34 and FAB1. Our results showed that VPS34 was upregulated in the presence of inositol for both WT and ino2Δ cells. However, FAB1 was only upregulated significantly in ino2Δ cells. This indicated that Ino2p might be the negative regulator for FAB1 expression. Next, growth sensitivity experiment showed that WT, vma3Δ, and ino2Δ grew well in growth medium buffered to pH 5.5 containing 10 mM $CaCl_2$. As cells were switched to growth medium buffered to pH 7 containing $CaCl_2$ WT, ino2Δ and opi1Δ showed growth reduction, whereas vma3Δ was completely nonviable. As the concentration of $CaCl_2$ was increased to 60 mM, ino2Δ cells showed moderate growth reduction compared to WT. This result suggests that ino2Δ cells have better vacuolar activity. Microscopic analysis and vacuolar acidification were employed to further elucidate the importance of INO2 in vacuolar homeostasis. Analysis of vacuolar morphology indicated that WT and vma3Δ cells displayed vacuoles that occupied a small area of the cell when grown in media buffered to pH 5.5. Whereas, ino2Δ displayed fragmented vacuoles. On the other hand, all strains grown in media buffered to pH 7, exhibited enlarged vacuoles that occupied most of the cell's surface. This indicated that the presence of INO2 may play negative effect in vacuolar morphology when cells are grown in media buffered to pH 5.5. Furthermore, vacuolar acidification assay showed that only vma3Δ cells displayed notably less acidic vacuoles as cells were grown in media buffered to pH 5.5 and pH 7. Whereas, ino2Δ cells displayed more acidic pH compared to WT at pH7. Taken together, our results demonstrated the molecular mechanism of the vacuolar activity regulated by the phospholipid biosynthesis transcription factors Ino2p. Ino2p negatively regulates vacuolar activity through the expression of FAB1.

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