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Mechanisms of regulation of membrane phospholipids metabolism and its physiological significance

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Research aims

In eukaryotic organisms, a membrane lipid bilayer is primarily composed of two groups of amphiphilic molecules: Glycerophospholipids and complex sphingolipids. The structural diversity of these groups, however, is thought to number in the thousands. Furthermore, the composition of these lipids is tightly regulated in each organelle membrane. This complex diversity and distribution of membrane phospholipid is essential for the maintenance of various cellular events, such as signal transduction, membrane trafficking, and energy metabolism. However, the molecular mechanisms that regulate membrane phospholipid metabolism are largely unknown. Previously, we screened a collection of ~4,800 *Saccharomyces cerevisiae* strains containing mutations in nonessential genes, to identify mutants exhibiting increased sensitivity to abnormal metabolism of complex sphingolipids in the membrane.[1)] We found that the double mutation of *SAC1* encoding a phosphoinositide (PIP) phosphatase and one of a specific group of non-essential sphingolipid-metabolizing enzyme gene (*CSG1*, *CSG2*, *IPT1* or *SCS7*) caused a synthetic growth defect. While the deletion of *SAC1* alone resulted in a reduction in the cellular levels of phosphatidylserine (PS), the restoration of PS levels by overexpression of PS synthase partly suppressed the synthetic growth defect. These results indicated that the reduction in PS levels might be the cause of the synthetic growth defect observed in the *SAC1* deletion strain.[1)] However, the mechanism by which PS levels are decreased in the *sac1Δ* cells remained unclear. In this study, we characterized the mechanism underlying this reduction in PS in *sac1Δ* cells.

Methods

Yeast cells were metabolically labeled with [^{32}P] orthophosphate, and the labeled phospholipids were extracted and analyzed by thin-layer chromatography (TLC). The subcellular distribution of PS was observed by expression of GFP-tagged lactadherin C2 domain (GFP-Lact-C2).[2)] Cells expressing GFP-Lact-C2 were visualized by fluorescence microscopy.

Results

The rate of PS biosynthesis is reduced in *sac1*Δ cells

To determine the rate of PS biosynthesis, wild-type and *sac1*Δ cells were pulse-labeled with [^{32}P]orthophosphate for 15, 30, and 60 min. As shown in Fig 1A, the rate of PS biosynthesis in *sac1*Δ cells was reduced by approximately 50%, compared to the wild-type control. PS can be converted to phosphatidylethanolamine (PE) by the PS decarboxylases, Psd1 and Psd2.[3)] To investigate whether the reduction in PS levels in the *sac1*Δ strain is due to the conversion of PS to PE, the rate of PS biosynthesis was examined in the *psd1*Δ *psd2*Δ and *psd1*Δ *psd2*Δ *sac1*Δ strains. As summarized in Fig. 1B, the rate of PS biosynthesis in the *psd1*Δ *psd2*Δ *sac1*Δ strain was approximately 50% lesser than that of the *psd1*Δ *psd2*Δ strain, indicating that the observed reduction in the PS levels occurs indepen-

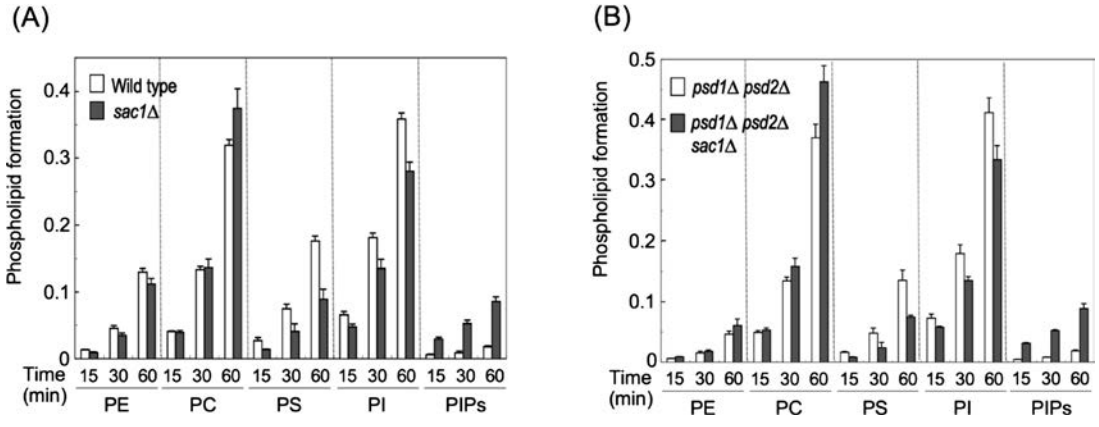

Fig. 1. Pulse radiolabelling of *SAC1*-deleted cells with [^{32}P] orthophosphate.

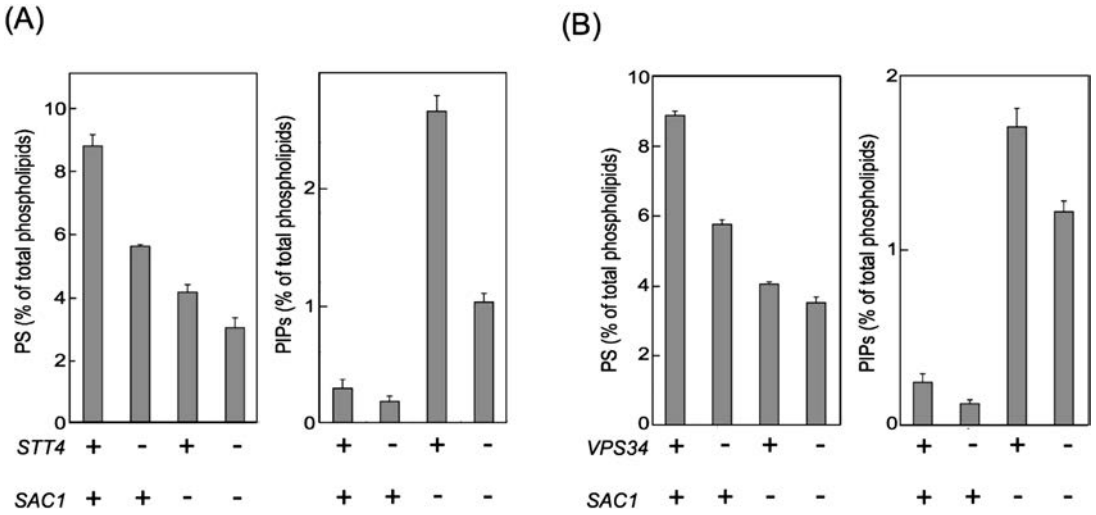

Fig. 2. Effects of *SAC1*, *STT4*, and *VPS34* mutations on PS and PIP levels.

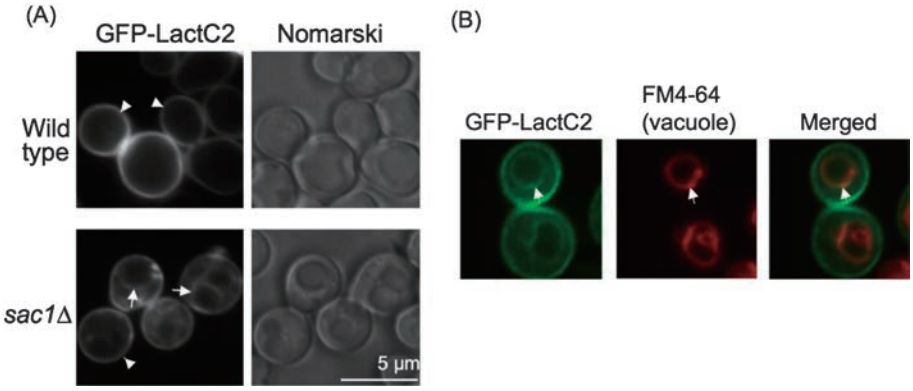

Fig. 3. Distribution of GFP–Lact-C2 in *sac1Δ* cells.

dently of PS decarboxylation. These results suggested that the biosynthesis of PS is reduced by the *SAC1* deletion.[4)]

Normal metabolism of PIPs is important for maintenance of cellular PS level

To determine whether the PIP phosphatase activity of Sac1 is necessary for the maintenance of PS levels, *SAC1-C392S*, a catalytic inactive mutant, was introduced into *sac1*Δ cells. The mutant, however, was unable to rescue the defect in PS levels in the *sac1*Δ strain. Mutation of *SAC1* causes marked accumulation of PI(4)P, the bulk of which is generated by the PI 4-kinase Stt4.[3)] Therefore, we investigated whether repression of *STT4* could suppress the observed reduction in PS levels in the *sac1*Δ strain. As depicted in Fig. 2A, the PS levels were reduced by *STT4*-repression, compared to the control. Moreover, the repression of *STT4* enhanced the reduction in the PS levels caused by the *SAC1* deletion. These results demonstrate that both repression of *STT4* and deletion of *SAC1* result in a reduction in PS levels. Sac1 can also dephosphorylate PI(3)P and PI(3,5)P_2. The *VPS34* gene, which encodes a PI 3-kinase, is responsible for the production of PI(3)P in yeast, and deletion of this gene results in depletion of PI(3)P and PI(3,5)P_2. As such, we examined the effect of a double mutation in the *VPS34* and *SAC1* genes on PS levels. Similar to the results obtained by repression of *STT4*, deletion of *VPS34* resulted in a reduction in the PS levels (Fig. 2B). Again, deletion of *SAC1* resulted in a reduction in the PS level; however, this reduction was not rescued by the deletion of *VPS34*. Collectively, these results indicate that a defect in dephosphorylation of PIPs by Sac1, a defect in the synthesis of PIPs by Stt4 and Vps34, or a defect in both the dephosphorylation and the synthesis of PIPs causes the observed reduction in PS levels.[4)]

Aberrant intracellular distribution of PS in *sac1*Δ cells

To examine the effects of the *SAC1* deletion on the intracellular distribution of PS, GFP-tagged Lact-C2, a PS-specific probe,[2)] was expressed in wild-type and *sac1Δ* cells. While GFP–Lact-C2 was observed predominantly at the plasma membrane of wild-type cells, the intracellular distribution of GFP–Lact-C2 was increased in the *sac1Δ* cells (Fig. 2A). A large portion of the intracellular GFP–Lact-C2 in the *sac1Δ* cells localized to vacuolar membranes (Fig. 2B).[4)]

Conclusion

In this study, we characterized the reduction in PS levels caused by deletion of *SAC1*. We found that the apparent rate of PS biosynthesis in *sac1Δ* cells was significantly reduced compared to that of wild-type cells. Double mutation

of *SAC1* and *STT4* or *VPS34* failed to restore the intracellular PS levels, suggesting that both the synthesis and degradation of phosphoinositides are important for the maintenance of PS. Finally, we observed an abnormal PS distribution at vacuolar membranes in *sac1*Δ cells. Collectively, these results suggest that Sac1 is involved in mediating a normal rate of biosynthesis and distribution of PS.[4)]

In yeast, PS is not an essential phospholipid; however, it contributes to a variety of cellular functions, including vesicular trafficking.[5)] At present, the physiological significance of Sac1-mediated PS maintenance remains unclear. Further investigation of the molecular mechanisms underlying the regulation of PS by Sac1, and the functional relationship between PS and Sac1, will provide new insights into the physiological significance of PS and PIPs.

References

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