The role of membrane contact sites during prospore membrane formation in budding yeast

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Report:

Prospore membrane (PSM) formation in budding yeast is a good model for *de novo* membrane formation in the cell. We showed that membrane contact sites (MCSs) are formed between the endoplasmic reticulum (ER) and the PSM and probably regulate phospholipid composition of the PSM and PSM extension.

Research aims

Recent studies have revealed that MCSs are formed between organelles or organelles and the plasma membrane and are required for lipid exchange between membranes. We have been studying PSM formation in budding yeast as a model system for new biological membrane formation in the cell and have shown that an MCS protein, Vps13, and its sporulation-specific interacting proteins, Spo71, and Spo73, colocalize on the PSM and are required for PSM extension[1) 2)]. Further, we have revealed that the PSM extension defect in *spo73*Δ is suppressed by decreasing the level of phosphatidylinositol 4-phosphate (PI4P) in the PSM, indicating the importance of the regulation of lipid composition of the PSM. It is known that the level of PI4P in the plasma membrane is decreased by MCSs formed by tether proteins[5)]. Therefore we hypothesized that MCSs were also formed between the ER and the PSM, and regulated the lipid composition of the PSM and the subsequent PSM extension. In this study, we examined the existence of MCSs between the ER and the PSM, and suggested the possible role of MCSs in PSM formation.

Methods

Tether proteins were C-terminally tagged with GFP or GFPEnvy and observed with a PSM marker Spo20$^{51\text{-}91}$-mKate2 by fluorescence microscopy in wild-type, *spo73*Δ, *spo71*Δ, and *vps13*Δ cells during sporulation.

For Split-GFP analysis, one of the tether proteins, Tcb3, was tagged with GFP strand 11, and Dtr1, a transmembrane PSM protein, was tagged with GFP strand 1-10. These fusion proteins were expressed with Spo20$^{51\text{-}91}$-mKate2 during sporulation in wild-type cells and observed by fluorescence microscopy.

Results

To examine whether tether proteins involved in the formation of MCSs between the ER and the plasma membrane were localized to the PSM, we observed their localization with a PSM marker protein during sporulation in wild type cells. We showed that three of the tether proteins, Ist2, Tcb3, and Scs2, localized to the PSM, suggesting the formation of MCSs between the ER and the PSM (Figure 1 left). We next observed these tether proteins in *spo73*Δ, *spo71*Δ, and *vps13*Δ cells. As expected, tether proteins did not localize to the PSM (Figure 1 right), indicating that their localization was dependent on Spo73, Spo71, and Vps13.

To further confirm the existence of MCSs between the ER and the PSM, one of the tether proteins Tcb3, which is a membrane protein of the ER, and a transmembrane PSM protein, Dtr1, were tested to determine whether they were in close proximity or not using split-GFP analysis (Figure 2A). GFP fluorescence along the PSM was detected (Figure 2B), indicating that they were in close proximity to each other and that MCSs were formed between the ER and the PSM.

Conclusion

In this study, we showed that tether proteins localized to the PSM and formed MCSs between the ER and the PSM, dependent on Spo73, Spo71, and Vps13. Taking previous work into consideration, our data indicate that MCSs are involved in the control of the levels of PI4P in the PSM, which is important for PSM extension. This study will contribute to the understanding of the mechanism of *de novo* membrane formation in the cell.

References

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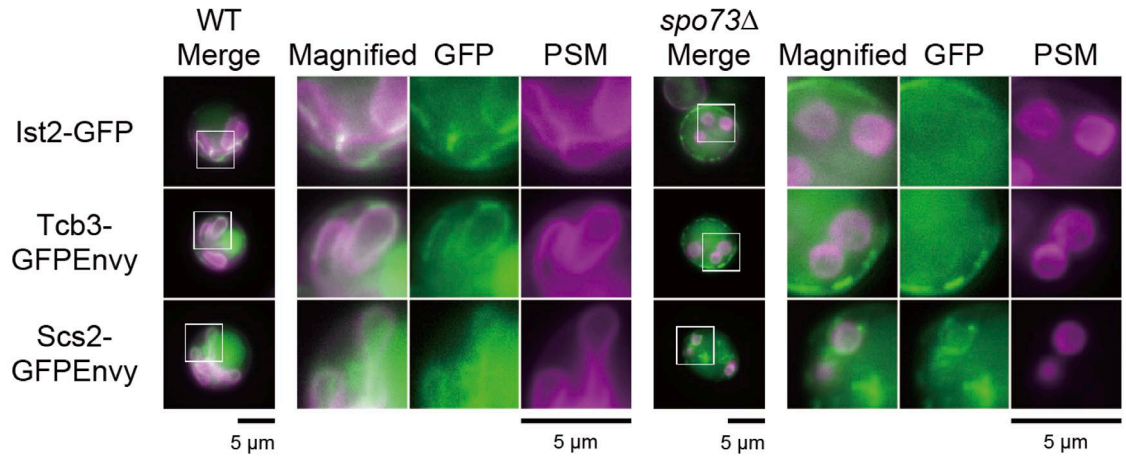

Figure 1. Localization of tether proteins during PSM formation in wild-type and *spo73*Δ cells. Ist2, Tcb3, and Scs2 tagged with GFP or GFPEnvy were observed by fluorescence microscopy in wild-type and *spo73*Δ cells with a PSM marker during sporulation.

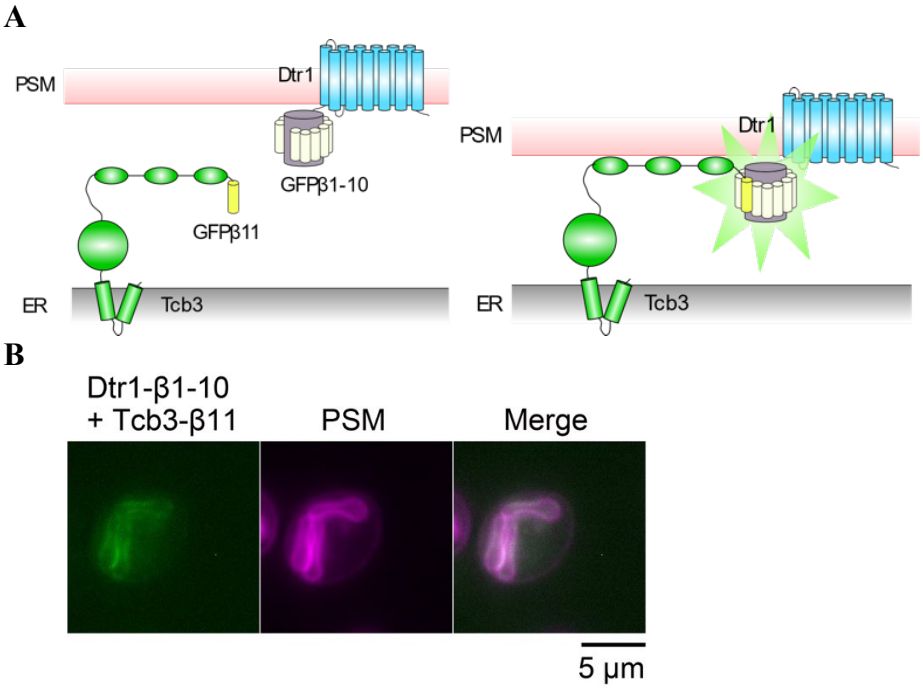

Figure 2. Split-GFP analysis of MCSs between the ER and the PSM. A. Schematic diagrams of Split-GFP analysis. GFP fluorescence can be detected only when Tcb3 is in close proximity with Dtr1. B. GFP fluorescence observed with the PSM marker during sporulation.