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Investigation of the molecular mechanisms regulating the secretion from the endoplasmic reticulum in *Saccharomyces cerevisiae*

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Research aims

In eukaryotic cells, secretory pathways serve as the major routes for extracellular protein transport, and many questions need to be yet answered. Although the secretory pathway is initiated in the endoplasmic reticulum (ER), a number of its aspects, such as the molecular mechanisms of cargo selection and regulation, remain unclear. The cytosolic coat protein complex II (COPII) is required for the physical deformation of the ER membrane, and in combination with the small GTPase Sar1, it drives the formation of the COPII vesicles[1] (Fig. 1). COPII vesicle formation occurs at specialized membrane domains, the so-called ER exit sites (ERES), which are thought to be responsible for the regulation of ER export.[2] However, most of the molecular mechanisms underlying the assembly and regulation of the ERES remain unknown. In this study, we conducted a comprehensive investigation of the organization of the ERES.

Methods

To identify the functional domain of Sec16, which is responsible for its transport function, various deletion mutants of Sec16 were expressed and examined. To identify the Sec16 domain required for ER localization and ERES organization, the subcellular localization of these fragments (fused to fluorescent proteins) was analyzed. In addition, subcellular localization and biochemical characterization of the Sec23 homolog, Nel1, was performed. Furthermore, to investigate the steady-state organization of ERES in live cells, fluorescence confocal microscopy was carried out on cells expressing Sec24-GFP paired with the expression of one of the Sec24 isoforms tagged with mCherry, to determine whether the Sec24 isoforms are uniformly distributed at each ERES.

Results

1) Identification of the functional domain of Sec16

We found that the central conserved domain (CCD) of Sec16, which is well conserved among the Sec16 homologs in various species, is responsible for its ER localization. We also show that the C-terminal domain of Sec16, in combination with the CCD domain, is responsible for the membrane anchoring of Sec16, via ER-localized Sar1-GTP. In addition, the N-terminal domain of Sec16 is required for the functional assembly of the COPII coat in the ER.

2) Functional analysis of Nel1

We found that Nel1 overexpression cannot compensate for the loss of Sec23 function and that Nel1 does not associate with Sec24 and its isoforms, indicating that Nel1 activity is not functionally redundant with Sec23. We show

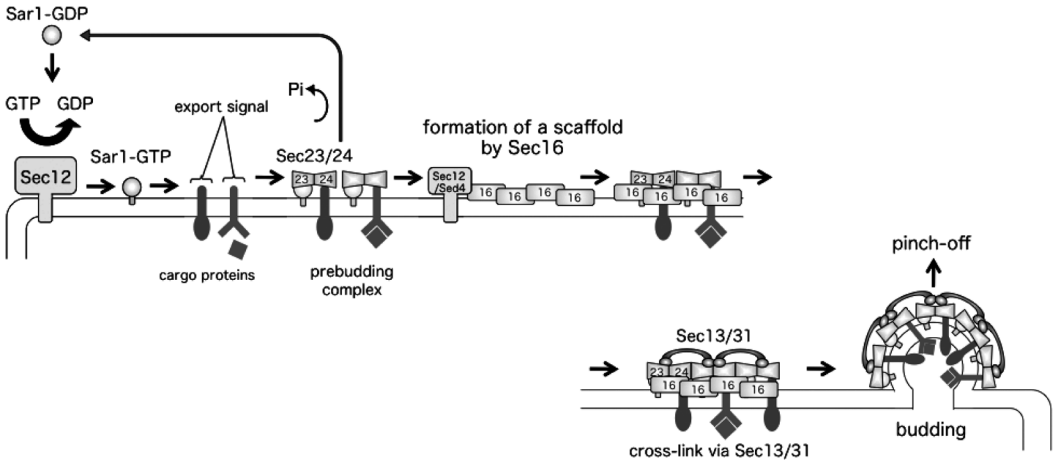

Fig. 1. COPII vesicle formation in the ER

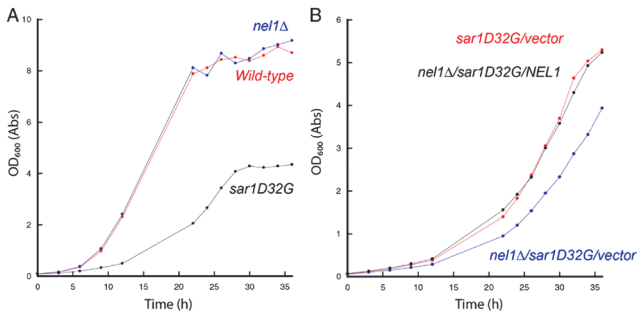

Fig. 2. *NEL1* has a genetic interaction with *SAR1*. A) Growth phenotype of the wild-type, *nel1*Δ, and *sar1D32G* strains at 23°C. B) Growth phenotype of the *sar1D32G* strain and *sar1D32G*/*NEL1*Δ double mutant strain at 23°C.

that purified Nel1 exhibits strong Sar1 GAP activity, but does not bind to Sec13/31, suggesting that Nel1 plays a role in the regulation of the GTPase activity of Sar1, but may act distinctly from Sec23 as a subunit of the COPII coat. We also demonstrate that the chromosomal deletion of *NEL1* leads to a significant growth defect in the temperature-sensitive *sar1D32G* background, suggesting a possible functional link between these proteins.[3)]

3) Analysis of the diversity of the ERES

Using live cell-imaging analyses in *S. cerevisiae*, we demonstrate that the isoforms of cargo-adaptor subunits (Sec24 and Lst1) are unequally distributed at each ERES at a steady state, suggesting that each ERES has a distinct preference for the cargo proteins. These results also suggest that ERES in *S. cerevisiae* are heterogeneous with respect to their composition.

Conclusions

Our results show that Sec16 is not only a scaffolding protein for COPII vesicle formation, but also serves to organize COPII coat assembly at ERES. We identified Nel1 as a novel homolog of Sec23 that exhibits genetic and biochemical interactions with Sar1 in *S. cerevisiae*. ERES, which are visible as 50–100 small dots in *S. cerevisiae*, are heterogeneous with respect to their functional characteristics.

References

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2) Yorimitsu, T., and Sato, K. (2012) Insights into structural and regulatory roles of Sec16 in COPII vesicle formation at ER exit sites. *Mol. Biol. Cell* **23**: 2930–2942.
3) Kodera, C., Yorimitsu, T., and Sato, K. (2014) Sec23 homolog Nel1 is a novel GTPase-activating protein for Sar1 but does not function as a subunit of the coat protein complex II (COPII) coat. *J. Biol. Chem.* **289**: 21423–21432.