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# The Process of Cell Wall Synthesis That Depends on Vesicular Transport

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## Research aims

The cells of microorganisms have a rigid cell wall at their surface and thus, are able to live in various harsh environments. The fungi and yeast are eukaryotic microorganisms and have a cell wall composed of complex polysaccharides and proteins. Chitin and $\beta$-1,3-glucan are synthesized by enzymes in the cell membrane directly from their soluble precursors. On the contrary, $\beta$-1,6-glucan and mannoproteins are subjected to maturation and proper localization processes after their synthesis via the intracellular vesicular transport. This starts from the endoplasmic reticulum (ER), through the Golgi apparatus and to the cell surface. These processes are yet not fully clear. In this study, we discovered a number of novel proteins from the isolated organelles or mutants with a defective cell wall, and characterized them. These results will provide much understandings as to the metabolism, growth, cellular functions and biotechnological application of fungi and yeast.

## Methods

The budding yeast *Saccharomyces cerevisiae* was routinely used in this study.

A novel gene *RCR1* was found by induction of resistance to an antifungal dye Congo red which was believed to bind to the yeast cell wall glucan and inhibit its assembly. A simultaneous loss of Gas1 and Kex2 which plays a role in the maturation of glucan and processing of secretory proteins, respectively, is synthetically lethal. The novel genes *SKG1* and *SKG6* were isolated among the multicopy suppressor genes of this phenotype. The gene products were tagged with epitope, their intracellular localization was observed by indirect immunofluorescent staining, and the phenotypes of their disruptants, chimera mutants or multicopy carriers, were examined.

The intracellular vesicles of the yeast Golgi apparatus were immunoisolated using tagged membrane proteins Sed5 or Tlg2 as the marker after mild cell disintegration. Thus the early and late Golgi compartments were successfully isolated. The protein constituents were identified by micropeptide mapping using MALDI TOF-MS. Six novel proteins were characterized by epitope tagging and gene disruption to find out their interactions and roles in vesicular trafficking.

## Results

*Rcr1 protein* The gene product of *RCR1* which induces resistance to Congo red by its multicopy introduction, was an integral membrane protein in the ER. It actually works in the wild-type cell because its null mutant showed an increased sensitivity to Congo red. By the introduction of multicopy *RCR1* in various yeast mutants with various Congo red sensitivity, the chitin content in the cell wall significantly decreased while the glucan content did not change. No alteration was found in the amount and localization of chitin synthases. The ER membrane protein Rcr1 regulates the chitin synthases at the cell membrane by mechanisms still unknown. It was confirmed that the major target of Congo red in yeast is also chitin and not glucan (Imai *et al.*, 2005).

**Skg1 and Skg6 proteins** Novel proteins Skg1 and Skg6 were discovered by our multicopy suppressor screening of the synthetic lethality of the of *kex2Δ gas1Δ* mutations. They are integral membrane proteins and localize in the growing region of the budding cell. These proteins are likely to help the polarized growth of the cell wall (Tomishige *et al.*, 2005a, 2005b).

**Svp26 protein** Although the yeast Golgi apparatus is composed of multiple subcompartments with different protein composition and function, the exact analysis of their protein composition has not been done before. We succeeded in the isolation and purification of the early and late Golgi compartments by immunoadsorption. The isolated vesicles had different transport-related proteins, glycosylation enzymes, and glucan-synthetic proteins. We discovered six novel membrane proteins. The Svp26 protein in the early Golgi compartment was an integral membrane protein. It bound to Svp26 itself and to mannosyltransferase Ktr3. In the *svp26Δ* null mutant, we found a shift in the localization of various Golgi membrane proteins. The majority of Ktr3 was found in the ER in the *svp26Δ* cell. Furthermore, the degree of outer *N*-glycosylation of secretory proteins significantly increased in the cell. Therefore, Svp26 regulates the localization of Golgi proteins and the process of *N*-glycan elongation (Inadome *et al.*, 2005).

## Conclusion

Our genetic and biochemical study of yeast discovered novel membrane proteins in the ER and Golgi, and we made important findings regarding their roles in the cell

wall chitin content, structure of glycoproteins and polarized cell growth.

## References

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