S3-3

Membrane Proteins in the Golgi and Transport Vesicles in *Saccharomyces cerevisiae*

Koji Yoda

Department of Biotechnology, Graduate School of Agricultural and Biological Sciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

The yeast *Saccharomyces cerevisiae* is a microorganism that has been used in brewing alcoholic beverage and making bread ever since the dawn of human history. Currently, it also serves an excellent model of eukaryotic cells in the modern biology because most of biochemical, genetic, cellular and molecular biological research techniques are available in this unicellular eukaryote. The fundamental mechanism for cells to live and produce their progeny is essentially common among mammal, plant and yeast and a free-living unicellular microorganism has many advantage to do fine experiments to resolve the fundamental biological problems.

Vesicular transport. In eukaryotic cell, components such as nucleic acid, protein, carbohydrate and lipid are not randomly intermingled but properly localized in their place to play their functional role. A cell is divided into a number of subcompartments by lipid-bilayer membrane. While the mitochondria and chloroplasts have own systems to get their components, other membranous subcompartments have a common origin of their components. Proteins in the cell wall, cytoplasmic membrane, endosome, vacuole/lysosome, Golgi apparatus, nuclear envelope are all supplied by delivery from the endoplasmic reticulum (ER) which only has the machinery to incorporate polypeptide from the cytosol. Proteins and lipids are then distributed to various subcompartments by vesicle-mediated intracellular transport.

The proteins incorporated in the membrane or lumen of the ER are collected and incorporated inthe COPII-coated vesicles to be delivered to other cellular subcompartments. The COPII coat subunits are recruited from the cytosol to the ER membrane by interaction with GTP-bound Sar1 and cargo molecules in the ER, and then deform the ER membrane to bud and liberate the transport vesicles. The vesicles take off their coat, fuse to the earliest compartment of the Golgi apparatus and thus supply the cargo molecules. The targeting and fusion of membranes of the vesicles and acceptor compartment is mediated by specific pairing of membrane proteins, vesicular (v-) and target (t-) SNARE proteins. As the complement to this forward movement of materials, reverse movement is mediated by vesicle formation with GTP-bound Arf and COPI coat subunits followed by uncoating and fusion of vesicle to the ER or an earlier compartment of the Golgi with the aid of another pair of v- and t-SNAREs. Vesicular trafficking delivers materials to other compartments by similar mechanism within the cell (Fig. 1). The selective sorting and transport of materials in the well-balanced anterograde and retrograde trafficking should be important to keep the

functional compartmentalization of the cell.[1)]

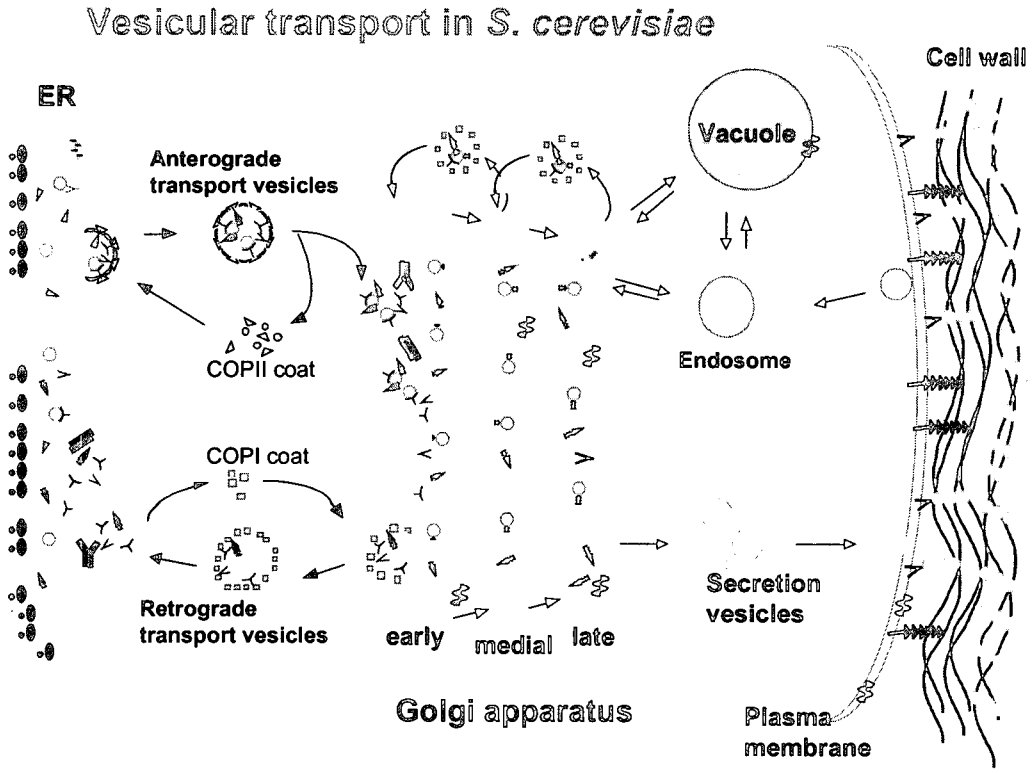

Figure 1. Intracellular trafficking in yeast. Proteins destine to exit from the ER are concentrated in the COPII vesicles and delivered to the early Golgi by anterograde transport. The proteins that should be returned to the ER or earlier Golgi compartment are concentrated in the COPI vesicles which carry out retrograde transport. Several kinds of coat proteins are working to deliver materials among the subcellular compartments. The essential mechanism is believed to be common to human cells except those related to the cell wall.

Golgi apparatus. The Golgi apparatus occupies the central position of intracellular trafficking by maturating and sorting of delivered materials. While the mammalian Golgi forms a stack of vesicular plates at the perinuclear position, the yeast Golgi is composed of a number of tubular vesicles which are dispersed in the whole cytoplasm. In spite of their morphological difference, both are considered to have similar subcompartments with different composition and function. The subcompartments can be ordered as early, medial and late from those proximal to the ER. Among the important components of the Golgi are glycosyltransferases[2,3)], nucleotide and nucleotide-sugar transporters[4,5)], processing proteases, enzymes to synthesize complex sphingolipd, and proteins that drive the vesicular transpot.[6)] Recently, the Golgi processing protease was found to have an important role to maintain the cell wall integrity.[7)]

Vig4/Vrg4 is a polytopic membrane protein that has at least ten transmembrane domains. Vig4 exists as an oligomer. Amino acid substitutions in the mutant Vig4 with reduced transporter activity were found in a C-terminal restricted region where the amino acid sequence is highly conserved among the nucleotide sugar transporters.[4)] Because this protein functions as the sole transporter of GDP-mannose encoded in the *S. cerevisiae* genome, it is essential for Golgi mannosylation and viability of the yeast. The Golgi compartments of *S. cerevisiae* consist of dispersed tubulovesicles in the cell. However, when *VIG4* was

expressed using a strong constitutive promoter of glyceroaldehyde-3-phosphate dehydrogenase (*TDH3*) on a 2-μm multicopy plasmid, stacked cisternae were found in the yeast. The overproduced Vig4 tagged with myc epitope was detected on these stacked cisternae by immunoelectron microscopy using a monoclonal antibody.[5] Although the mechanism underlying this observation is yet unclear, the amount of a membrane protein could change the morphology of intracellular membrane structures.

In order to characterize the early, medial and late compartments of Golgi apparatus and related vesicular structures, it is undoubtedly important to examine them biochemically. To purify each compartment, we recently developed a convenient immunoadsorption method.[6] The early Golgi t-SNARE Sed5 protein was tagged with myc epitope by replacement of the chromosomal gene, and the vesicles that carry myc-tagged Sed5 were collected using a monoclonal antibody 9E10 and formaldehyde-fixed *Staphylococcus aureus* cells. Analysis of the membrane proteins in the collected vesicles revealed that the early Golgi compartment was highly enriched. Novel membrane protein Svp26 which participate in sorting of selective Golgi proteins was found. This method could be applied to other marker proteins. Using myc-tagged Tlg2 as the probe, the vesicles of the late Golgi compartment and endosome were collected. Novel membrane protein Tvp38, Tvp23, Tvp18 and Tvp15 were found. The functional role of these novel proteins are currently not clear but they are in a large network of protein-protein interaction including a number of trafficking proteins.

References

1. K. Yoda and Y. Noda: J. Biosci. Bioeng. **91**, 1 (2001).
2. H. Hashimoto and K. Yoda: Biochem. Biophys. Res. Commun. **241**, 682 (1997).
3. H. Kojima, H. Hashimoto and K. Yoda: Biosci. Biotechnol. Biochem. **63**, 1970 (1999).
4. M. Abe, H. Hashimoto and K. Yoda: FEBS Lett. **458**, 309 (1999).
5. H. Hashimoto, M. Abe, A. Hirata, Y. Noda, H. Adachi and K. Yoda: Yeast 19, 1413 (2002).
6. J.-H. Cho, Y. Noda and K. Yoda: FEBS Lett. **469**, 151 (2000).
7. N. Tomishige, Y. Noda, H. Adachi, H. Shimoi, A. Takatsuki and K. Yoda: Mol. Gen. Genom. **269**, 562 (2003).