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Mechanisms of Golgi-localization of Mannosyltransferases

Yoichi NODA

Department of Biotechnology, University of Tokyo

Reseach aims

The Golgi apparatus comprises of multiple compartments with different components and functions. These compartments are successively formed by the maturation of the earliest compartment derived from the ER through homotypic fusion of the COPII vesicles (1). The maturation of compartments is believed to occur by removing the components of the earlier compartments and acquiring those of the later ones in a COPI vesicle-dependent manner (2, 3).

Svp26 was discovered as a novel function-unknown protein in our global inspection of membrane proteins in the early Golgi compartment (4). Svp26 is an integral membrane protein with 4 predicted transmembrane segments. Although Svp26 is dispensable for viability of the yeast, we found abnormal hyper-mannosylation of N-glycosyl chains and mislocalization of Golgi mannosyltransferase Ktr3 to the ER in the Δ*svp26* disruptant cell. Immunoprecipitation from the cleared lysate using 1% Triton X-100 indicated specific binding between Ktr3 and Svp26. These results suggest that Svp26 functions to ensure the correct localization of Ktr3 in the Golgi compartments. In this study, we aimed to reveal the molecular function of Svp26 and to discover new proteins that regulate the Golgi-localization of mannosyltransferases the localization of which are not dependent on Svp26.

Methods

Construction of yeast strains expressing triple HA tagged mannosyltransferase. For tagging mannosyltransferases with three copies of the HA epitope at their C-termini, an appropriate DNA fragment of the 3′-region of each mannosyltransferase was amplified by PCR and cloned in pYN497 (*HIS3* marker) or pYN503 (*LEU2* marker). These plasmids carry a coding sequence for triple HA followed by a *TDH3* terminator. The resulting plasmids were linearized by cutting at a unique restriction site located within a cloned region of each ORF, and used for the transformation of yeast to obtain desired strains with chromosomally tagged genes by homologous recombination.

In vitro COPII vesicle budding assay. Purification of COPII coat components Sar1, Sec23/24 and Sec13/31, and the vesicle budding assay were performed as described previously. Microsomal membranes were prepared from the *SVP26* or Δ*svp26* cells expressing either *KTR3-3HA* or *MNN2-3HA*. Microsomal membranes were washed once with 0.5 M NaCl in B88 and then 2 times with B88 containing protease inhibitors, and incubated for 30 min at 25°C in the presence or absence of the purified coat components. After the incubation, vesicles were separated from the donor membranes by centrifugation at 10,000 g for 5 min. The supernatant was further centrifuged at 100,000 g for 1 hour to collect the COPII vesicles. The pellets were analyzed by SDS-PAGE and immunoblotting.

RESULTS

Golgi localization of mannosyltransferases Mnn2 and Mnn5 depends on Svp26. The most significant phenotypes of Δ*svp26* cells found previously were hyper-mannosylation of N-glycosyl chains on several glycoproteins including invertase and mislocalization of Ktr3 from the Golgi to ER (4). As Ktr3 is an O-mannosyltransferase, we examined if the Golgi localization of N-mannosyltransferases depends on the presence of Svp26, which may cause excess N-mannosylation. All Golgi N-mannosyltransferases are type II membrane proteins. We observed the localization of tagged proteins encoded by the modified structural genes that were integrated in the yeast chromosome of the wild-type or Δ*svp26* cells by immunofluorescence microscopy. In both the Δ*svp26* cells and wild-type cells the localization of Mnn1 and Mnn9 were punctate, as reported previously, which is a typical pattern of Golgi-localized proteins. The N-mannosyltransferases Mnn2 and Mnn5 had double ring signals in most of the Δ*svp26* cells as we previously reported for Ktr3, which indicates mislocalization from the Golgi to ER. These results indicate a clear difference in the dependence of N-mannosyltransferases on Svp26 for their Golgi localization.

Svp26-dependent Golgi proteins Mnn2 and Mnn5 bind to Svp26. We examined the interaction between Mnn2 or Mnn5 and Svp26 by co-immunoprecipitation in lysate containing 1% digitonin in order to observe any weak or transient interactions. A significant amount of Svp26 co-immunoprecipitated with Mnn2, although less than the amount that co-immunoprecipitated with Ktr3. No Svp26 was detected in the immunoprecipitate of Mnn1 which does not depend on Svp26 for its Golgi localization. The amount of Svp26 that was found in the Mnn2 immunoprecipitate was comparable to amount seen in the immunoprecipitate of Mnn5.

Svp26 stimulates in vitro incorporation of Ktr3 and Mnn2 proteins in COPII Vesicles. There are two potential mechanisms to maintain the localization of a Golgi protein, i.e., to facilitate the arrival of the protein to, or to prevent its exit from the Golgi. To examine the first possibility, we ex-

amined if the efficiency to incorporate the mannosyltransferases in COPII vesicles is affected by the presence or absence of Svp26. To test this, we used the in vitro COPII vesicle formation system with purified coat subunits and an ER-enriched membrane preparation. When the wild-type ER-enriched membrane fraction was used, Ktr3 and Mnn2 are released from the ER efficiently by the addition of purified coat subunits. Without the addition of COPII coat components, much less Ktr3 and Mnn2 are released from the ER, compared to the positive control, Erv25. The negative control Sec61 was not released in either condition. Therefore, we conclude that Ktr3 and Mnn2 were efficiently incorporated in the COPII vesicles. In contrast, very little Ktr3 was released from this Δ*svp26* ER fraction by the addition of purified COPII subunits. As Erv25 is released in both the Δ*svp26* and wild-type fractions, the formation and release of COPII vesicles occur normally. We basically obtained the similar results as for Mnn2. These results suggest that the presence of Svp26 strongly stimulates the incorporation of Ktr3 and Mnn2 into COPII vesicles.

Svp26 recognizes the lumenal domain of mannosyltransferase. All Golgi mannosyltransferases identified so far in *S. cerevisiae* are type II membrane proteins. Their polypeptides can be divided into three parts, i.e. N-terminal cytoplasmic, short transmembrane and large lumenal domains. The lumenal domain is believed to contribute to their enzyme activity. We sought to determine which domain is responsible for the differences in Ktr3 or Mnn2 (that depend on Svp26 for Golgi localization) and Mnn1 (that does not need Svp26 to localize in the Golgi) by exchanging their domains. In the wild-type cell, all chimera proteins were observed in cytoplasmic punctae by immunofluorescent staining. This suggests that they are all localized to Golgi compartments. In the Δ*svp26* cells, Mnn1-Ktr3 and Mnn1-Mnn2 chimeras are clearly mislocalized to the ER, while Ktr3-Mnn1 and Mnn2-Mnn1 localized in the Golgi. The localization was rescued by the introduction of the wild-type *SVP26* by a CEN plasmid. As the ER mislocalization of chimera proteins corresponds to the origin of the lumenal domain, this domain is responsible for the Svp26-dependent localization of these proteins strain [5].

Golgi-localization of Kre2 family proteins in the absence of Svp26. Kre2 family comprises of 9 homologous proteins including Ktr3 and they are thought to catalyze the protein O-mannosylation in the Golgi. We examined the localization of Kre2 family proteins in the wild type strain and Δ*svp26* strain. We found that Kre2 and Ktr1 were mislocalized to ER in the absence of Svp26. We currently investigating the mechanism by which Svp26 regulates the localization of these two proteins.

Conclusions

In this report, in addition to Ktr3, we find that two other Golgi mannosyltransferases, Mnn2 and Mnn5 are also mislocalized and found in the ER in the absence of Svp26 and that localization of other mannosyltransferases including Mnn1 are not affected. Mnn2 and Mnn5 bind to Svp26 in vivo as Ktr3 does. Using an in vitro budding assay, the incorporation of Ktr3 and Mnn2 in the COPII vesicles is greatly stimulated by the presence of Svp26. As Svp26 itself is an efficient cargo, Svp26 is likely to support selective incorporation of a set of mannosyltransferases into COPII vesicles, by working as their adaptor protein. The domain switching between Svp26-dependent Mnn2 or Ktr3 and Svp26-independent Mnn1 suggests that the lumenal domain of mannosyltransferases, but not the cytoplasmic or transmembrane domain, is responsible for recognition by Svp26. These results may be valuable for the future effort to understand the function of the Golgi apparatus in more detail.

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