Svp26 facilitates ER exit of mannosyltransferases Mnt2 and Mnt3 in *Saccharomyces cerevisiae*

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Introduction

In eukaryotic cells, many secretory proteins and membrane-spanning proteins are translocated across or inserted into the ER membranes and then transported to the early Golgi by COPII vesicles (Barlowe and Miller, 2013; Noda and Yoda, 2013). Selective incorporation of the cargo molecules into COPII vesicles is facilitated either by direct interaction of cargo proteins with COPII coat proteins or by ER exit adaptor proteins which mediate the interaction of cargo proteins with COPII coat proteins. Svp26 is one of the ER exit adaptor proteins in yeast *Saccharomyces cerevisiae*. ER exit of several type II membrane proteins have been reported to be facilitated by Svp26. We demonstrate here that efficient incorporation of Mnt2 and Mnt3 into COPII vesicles is also dependent on the function of Svp26. Mnt2 and Mnt3 are Golgi-localized α-1,3-mannosyltransferases with type II membrane topology involved in protein O-glycosylation. Immunoisolation of the yeast Golgi subcompartments quantitatively showed that Mnt2 and Mnt3 are more abundant in the early Golgi fraction than in the late Golgi fraction. Subcellular fractionation and fluorescence microscopy showed that deletion of the *SVP26* gene results in the accumulation of Mnt2 and Mnt3 in ER. Using an *in vitro* COPII vesicle formation assay, we further demonstrate that Svp26 facilitates incorporation of Mnt2 and Mnt3 into COPII vesicles. Finally, we showed that Mnt2 and Mnt3 were co-immunoprecipitated with Svp26 from digitonin-solubilized membranes. These results indicate that Svp26 functions as an ER exit adaptor protein of Mnt2 and Mnt3.

Key Words: endoplasmic reticulum; Golgi; mannosyltransferase; membrane traffic; *Saccharomyces cerevisiae*

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membrane-spanning domains that we discovered as an abundant membrane protein in the purified early Golgi fraction (Inadome et al., 2005). It has been shown so far that Svp26 is an ER-exit adaptor protein for Ktr3, Ktr1, Kre2, Mnn2, Mnn5, Pho8, Gda1, all of which are type II membrane proteins (Anand et al., 2009; Bue and Barlowe, 2009; Inadome et al., 2005; Noda and Yoda, 2010; Noda et al., 2014). Svp26 was later shown to be identical to the Vps26 that Svp26 functions to facilitate the ER exit of Mnt2 and Mnt3, the Golgi-localized mannosyltransferases involved in the early stage of the secretory pathway. We report here that Svp26 facilitates ER exit of mannosyltransferases Mnt2 and Mnt3 in Saccharomyces cerevisiae.

For tagging Mnt2, Mnt3 and Ktr3 with three tandem copies of the HA epitope at their C-termini, an appropriate DNA fragment containing a promoter region and the ORF of the KTR3 was amplified by PCR reactions for plasmid construction were confirmed by DNA sequencing. Svp26 was similarly tagged with FLAG epitope by homologous recombination. Strains used for the purification of early Golgi and late Golgi membrane fractions (Fig. 1) were derived from the strains described previously (Inadome et al., 2005). To create a UR3 CEN plasmid expressing Mnt2-SFP2 or Mnt3-SFP2, a DNA fragment containing a promoter region and the ORF of the MNT2 or MNT3 was amplified by PCR, and the resulting product was ligated into the expression vector, pYT28, which was created based on YFP2-N.

Yeast cells were grown in YPD [1% Bacto yeast extract (BD Biosciences, Franklin Lakes, NJ, USA), 2% Bacto peptone (BD Biosciences), and 2% glucose] or SD [0.17% yeast nitrogen base without amino acids (BD Biosciences), 0.5% ammonium sulphate, 2% glucose, and appropriate supplements] medium at 30°C. Escherichia coli DH5α (F-, supE44 ΔlacU169::Tn5 Δ(lac-proB) M15 hsdR17 recA1 endA1 gyrA96 thi-1 relA1) was used in plasmid preparation. E. coli was grown in an LB [1% Bacto tryptone (BD Biosciences), 0.5% Bacto yeast extract (BD Biosciences) and 1% NaCl] medium. Digitonin and anti-FLAG M2 affinity gel were purchased from MilliporeSigma (St. Louis, MO, USA). Dynabeads Protein A was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Chymostatin, aprotinin, leupeptin, pepstatin A, and antipain were purchased from MilliporeSigma (St. Louis, MO, USA). Phenylmethylsulfonyl fluoride (PMSF) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

### Materials and Methods

**Strains, plasmids, media, and reagents.** *S. cerevisiae* strains used in this study are listed in Table 1. All strains are derivatives of KA31a, with genes disrupted or epitope tagged by homologous recombination. All DNA fragments amplified by PCR reactions for plasmid construction were confirmed by DNA sequencing.

For tagging Mnt2, Mnt3 and Ktr3 with three tandem copies of the HA epitope at their C-termini, an appropriate DNA fragment of the 3’-region of each ORF was amplified by PCR and cloned in pYN03 (LEU2 marker) as described previously (Noda and Yoda, 2010). pYN03 carries a coding sequence for triple HA followed by a TDH3 terminator. The sequences of primers used are available upon request. The plasmids were linearized by cutting at a unique restriction site present within a cloned region of each ORF, and were transformed into yeasts to obtain strains with chromosomally tagged genes by homologous recombination. Svp26 was similarly tagged with FLAG epitope by homologous recombination. Strains used for the purification of early Golgi and late Golgi membrane fractions (Fig. 1) were derived from the strains described previously (Inadome et al., 2005).

### Table 1. *S. cerevisiae* strains used in this study.

| Strain | Genotype | Source |
|--------|----------|--------|
| KA31a  | MATaΔhis3Δleu2Δtry1Δura3 | Laboratory stock |
| YTY3   | MATaMNT2-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY6   | MATaMNT3-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY7   | MATaΔMYC-SED5::URA3MNT2-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY8   | MATaΔMYC-TLG2::HIS3MNT2-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY9   | MATaΔMYC-SED5::URA3MNT3-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY10  | MATaΔMYC-TLG2::HIS3MNT3-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY14  | MATaΔSvp26MNT2-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY15  | MATaΔSvp26MNT3-3HA::LEU2Δhis3Δleu2Δtry1Δura3 | This study |
| YTY38  | MATaMNT2-3HA::LEU2SVP26-FLAG::HIS3Δhis3Δleu2Δtry1Δura3 | This study |
| YTY40  | MATaMNT3-3HA::LEU2SVP26-FLAG::HIS3Δhis3Δleu2Δtry1Δura3 | This study |
| YTY68  | MATaKTR3-3HA::LEU2SVP26-FLAG::HIS3Δhis3Δleu2Δtry1Δura3 | This study |
**Antibodies, immunoblotting and indirect immunofluorescence.** Rabbit antisera against Erv46 and Mnn1 were gifts from Dr. Charles Barlowe (Dartmouth medical school, USA) and Dr. Todd Graham (Vanderbilt University, USA), respectively. Anti-Van1 antisera were produced in our laboratory as previously described (Hashimoto and Yoda, 1997). The peptide CLVPFGSDLM, which corresponds to the C-terminal amino acid sequence of Sec61, was chemically synthesized and coupled to keyhole limpet hemocyanin (KLH). Two rabbits were injected with KLH-peptide conjugate to produce the anti-Sec61 antiserum. Anti-HA (12CA5) and anti-FLAG monoclonal antibodies were purchased from MilliporeSigma (St. Louis, MO, USA). For immunoblotting, each antiserum was used at a dilution of 1/1000. Western blot signals were visualized by the LI-COR Odyssey system using fluorescently conjugated Goat anti–Rabbit (IRDye 680RD) or Goat anti–Mouse (IRDye 800CW) secondary antibodies (LI-COR Biosciences, Nebraska, USA).

**Immunosoliation of the Golgi compartments.** Immunosoliation of the Sed5 or Tlg2 compartments was performed essentially as described previously (Inadome et al., 2005), with some modifications. For Mnt2, strains YTY7 (6MYC::SED5::URA3 MNT2-3HA::LEU2), YTY8 (6MYC::TLG2::URA3 MNT2-3HA::LEU2), and YTY3 (MNT2-3HA::LEU2, as a negative control) and for Mnt3, strains YTY9 (6MYC::SED5::URA3 MNT3-3HA::LEU2), YTY10 (6MYC::TLG2::URA3 MNT3-3HA::LEU2), and YTY6 (MNT3-3HA::LEU2, as a negative control), were used. They were grown in 100 ml of YPD medium at 30°C to an optical density at 600 nm (OD600) of 1.0. Cells were converted to spheroplasts by incubation with recombinant lyticase in 0.1 M potassium phosphate, pH 7.5, 1 mM MgCl2, 1.2 M sorbitol, and 0.1% 2-mercaptoethanol for 30 min at 30°C. The spheroplasts were suspended in ice cold B88-500 (20 mM HEPES, pH 6.8, 500 mM potassium acetate, 5 mM magnesium acetate, 200 mM sorbitol) buffer supplemented with protease inhibitors (1 μg/ml each of chymostatin, aprotinin, leupeptin, pepstatin A, and antipain and 1 mM phenylmethylsulfonyl fluoride) and homogenized by several strokes of a Dounce homogenizer (tight fitting; Wheaton). Following a short centrifugation (5 min at 1,000 × g) to remove the unbroken cells, the lysate was centrifuged at 10,000 × g for 20 min to remove the nuclei, ER, and mitochondria. The anti-Myc mAb 9E10 was added to the supernatant and incubated for 1 hour in a cold-room. Dynabeads Protein A (Thermo Fisher Scientific, Waltham, MA, USA) were washed with B88-500 buffer and incubated with B88-500 buffer containing 10 mg/ml bovine serum albumin at 4°C for 1 h for blocking. Dynabeads were then added to the cell lysate and incubated in a cold-room for 1 h with gentle rotation. Dynabeads were collected by centrifugation at 2,000 × g for 5 min. The pelleted Dynabeads were washed five times with ice-cold B88-500 buffer. The membrane compartments that precipitated with Dynabeads Protein A were solubilized by the addition of B88-500 buffer containing 1% Triton X-100 with incubation on ice for 15 min. Dynabeads were removed by centrifugation at 2,000 × g for 5 min. The supernatant was collected as a Sed5 vesicle fraction, a Tlg2 vesicle fraction, or a control fraction and analyzed by immunoblotting. Bands were visualized using the LI-COR Odyssey system and subsequently quantitated with LI-COR Odyssey software.

**Fluorescence microscopy.** Exponentially growing yeast cells were fixed for 15 min in the phosphate buffer (pH 7.5) containing 3.6% (w/v) paraformaldehyde, and washed 3 times with the phosphate buffer. Cells were imaged with confocal laser-scanning microscopes (FV1200, Olympus, Tokyo, Japan) equipped with a 100 × 1.40 NA or 60 × 1.35 NA oil-immersion lens, and Fluoview or FV10-ASW software (Olympus). Images were obtained at 0.25 μm intervals in the z-axis. Z-stack images were constructed with Fluoview or FV10-ASW software.

**Subcellular fractionation.** Subcellular fractionation in a sucrose density gradient was performed basically as described previously (Noda and Yoda, 2010). Briefly, cells lysates were prepared by suspending frozen yeast spheroplasts corresponding to the 25 OD unit cells by several strokes in a Dounce homogenizer in an ice-cold sucrose solution (10 mM HEPES, 12.5% (w/v) sucrose, 1 mM EDTA, pH 7.4 containing protease inhibitors (1 μg/ml each of chymostatin, aprotinin, leupeptin, pepstatin A, antipain, 1 mM benzamidine and 1 mM phenylmethylsulfonyl fluoride), and unlysed cells were removed by a centrifugation at 400 × g for 3 min. An aliquot of the supernatant was diluted in a 2% SDS solution and absorbance at 280 nm was measured. After diluting the supernatant to A280 = 0.45, 0.2 ml was loaded onto a sucrose step gradient, which was generated using the following steps [all sucrose solutions were made (w/v, %) in 10 mM HEPES-KOH, pH 7.4, 1 mM MgCl2]: 0.5 ml 30% sucrose solution (10 mM HEPES-KOH, pH 7.4, 1 mM MgCl2); 0.5 ml 60%, 1.25 ml 48%, and 0.25 ml 18% sucrose. After 2.5 h of centrifugation in a Beckman TLS55 rotor at 100,000 × g, 6 fractions of 0.35 ml were collected from the top of the gradient. Aliquots of each fraction were mixed with an SDS sample buffer, and proteins were resolved by SDS-PAGE and detected by immunoblotting using anti-HA, anti-Van1 and anti-Sec61 antibodies. Bands were visualized using the LI-COR Odyssey system and subsequently analyzed with LI-COR Odyssey software. Data represent the mean and SD of triplicate experiments.

**In vitro COPII vesicle generation assay.** Purification of COPII coat components Sar1, Sec23/24 and Sec13/31, and the vesicle budding assay were performed as previously described (Noda and Yoda, 2010; Shimoni and Schekman, 2002). Microsomal membranes were prepared from YTY3 (MNT2-3HA::LEU2 SVP26), YTY14 (MNT2-3HA::HIS3 Δsyp26), YTY6 (MNT3-3HA::LEU2 SVP26), and YTY15 (MNT3-3HA::LEU2 Δsyp26), were used as donor membranes in the budding reactions and incubated either in the absence or presence of purified COPII coat components. After the incubation, aliquots representing the “total fraction” were transferred to separate tubes. The remaining reaction mixture was centrifuged at 10,000 × g for 5 min to recover medium speed supernatant (MSS), which retains COPII vesicles generated by the in vitro reaction. MSS was further centrifuged at 100,000 × g for 1 h to collect COPII vesicles. Pelleted vesicles, which were then resuspended in a SDS-PAGE sample buffer and heated at 100°C for 1 min, and total fractions were separated by
Svp26 facilitates ER exit of mannosyltransferases Mnt2 and Mnt3 in *Saccharomyces cerevisiae*

SDS-PAGE followed by immunoblotting with anti-HA, anti-Erv46 (a positive control) and anti-Sec61 (a negative control) and packaging efficiency was quantified from band intensities using the LI-COR Odyssey system. The signal obtained in the negative control reactions (-COPII components) was subtracted from the signal found in COPII vesicle fractions (+COPII components) and then incorporation efficiencies of Mnt2-3HA or Mnt3-3HA normalized to those of Erv46 were calculated and graphed. Averages from 3 independent experiments were plotted with standard deviations.

**Co-immunoprecipitation.** Prior to the start of the experiment, a 10% digitonin stock solution was prepared by adding 100 μg digitonin to 1 ml water, which was then heated at 95°C for 10 min, as described (Anderson et al., 2017).

Logarithmically growing yeast cells (100 OD_{600} units)
grown in YPD were collected, washed in water and resuspended in 750 μl B88 buffer (20 mM HEPES, 150 mM potassium acetate, 5 mM magnesium acetate, 250 mM sorbitol, pH 6.8) supplemented with protease inhibitors. Cell lysates were prepared by rigorously agitating with glass beads using a Multi-beads shocker (Yasui Kikai, Osaka, Japan) three times for 1 min with 1-min intervals at 4°C between each burst and the supernatant was recovered. Remaining beads were washed in 550 ml B88 buffer supplemented with protease inhibitors and combined with the initial supernatant. Unbroken cells were removed by centrifugation at 4,000 ¥ g for 5 min. The supernatant (900 ml) was mixed with 100 ml of 10% (w/w) digitonin (final 1%) and kept on ice for 4 h. Unsolubilized material was next removed by centrifugation at 100,000 ¥ g for 45 min and clarified lysates (input) were incubated with anti-FLAG affinity resin (10 ml bed volume) for o/n at 4°C with gentle rotation. The anti-FLAG resin was washed four times with B88 containing 1% digitonin and bound proteins were eluted with 40 μl of B88 containing 1 mg/ml FLAG peptide by rigorous shaking for 40 min at 4°C. All samples were mixed with 4x SDS sample buffer [0.5 M Tris-HCl, pH 6.8, 4% SDS, 40% glycerol, 20%(v/v) 2-mercaptoethanol] and heated at 65°C for 5 min prior to SDS-PAGE.

**Fig. 3.** Subcellular fractionation of Mnt2 and Mnt3 in the wild-type and Δsvp26 strain.
The cell lysates were fractionated on a sucrose density gradient composed of 0.5 ml 60%, 1.25 ml 48%, and 0.25 ml 18% sucrose. After 2.5 h centrifugation in a Beckman TLS55 rotor at 100,000 x g, 6 fractions of 0.35 ml were sequentially collected from the top of the gradient. Aliquots of each fraction were separated by SDS-PAGE and analyzed by immunoblotting using anti-HA, anti-Van1 (a Golgi marker protein) and anti-Sec61 (an ER marker protein) antibodies. Bands were visualized using the LI-COR Odyssey system and subsequently analyzed with LI-COR Odyssey software. The top of the gradient corresponds to fraction number 1 and the bottom corresponds to fraction number 6.

**Results and Discussion**

**Mnt2 and Mnt3 mainly localize to the early Golgi**

We have studied the mechanism of ER exit and Golgi localization of mannosyltransferases involved in O-linked or N-linked glycosylation. ER exit of several of those mannosyltransferases is reported to be facilitated by the functions of Svp26 or the Erv41-Erv46 complex (Inadome et al., 2005; Noda and Yoda, 2010; Noda et al., 2014). Mnt2 and Mnt3 are mannosyltransferases that add the 4th and the 5th mannose to the O-linked glycan in the Golgi and their ER exit adaptor protein is unknown so far (Romero et al., 1999). Based on their sequence similarities and the suggested function as a mannosyltransferase, Mnt2 and Mnt3 were predicted to localized in the Golgi. However, distribution of these proteins between the Golgi compartments is unknown. We assumed that such information is generally valuable for the understanding of the Golgi localization mechanism of membrane proteins, we first examined the abundance of Mnt2 and Mnt3 in the early or late Golgi fraction. We previously devised an immunoisolation method which enables separate purification of early Golgi-enriched and late Golgi-enriched membrane fractions (Inadome et al., 2005). This method exploits the fact that, in *S. cerevisiae*, Golgi cisternae do
Mnt2 and Mnt3 are mislocalized to the ER in the absence of Svp26

We assumed that known COPII vesicle proteins may act as ER exit adaptor proteins for Mnt2 and Mnt3. Mnt2-
sGFP or Mnt3-sGFP were expressed from a low-copy CEN plasmid in several mutant yeast strains with deletions of the genes of known adaptor proteins and localizations of the GFP fusion proteins were assessed by fluorescence microscopy. In wild-type cells, Mnt2-sGFP and Mnt3-sGFP were observed as punctate patterns similar to the immunofluorescence patterns of known Golgi-localized proteins, which is consistent with the proposed functions of Mnt2 and Mnt3 and the result of Fig. 1. In contrast, by deleting the SVP26 gene, localization of Mnt2-sGFP2 and Mnt3-sGFP changed dramatically to the ER patterns. Mnt2-sGFP2 and Mnt3-sGFP2 in the Δsvp26 strain completely regained their normal Golgi localizations by introduction of the SVP26 gene by a low-copy plasmid (data not shown), indicating that mislocalization of Mnt2-sGFP2 and Mnt3-sGFP2 to the ER was caused by the absence of Svp26. We next performed subcellular fractionation in a sucrose density gradient to further validate the microscopic observations. In the wild-type cells, Mnt2 and Mnt3 displayed similar distribution patterns to that of Van1, a Golgi membrane protein (Fig. 3). In contrast, in Δsvp26 cells, distribution patterns are shifted closer to that of Sec61, an ER-resident membrane protein. These results are consistent with those of fluorescence microscopy observation, suggesting that the normal Golgi distribution of Mnt2 and Mnt3 is dependent on the function of Svp26.

**In vitro COPII vesicle generation assay**

Considering the known function of Svp26 as an ER exit adaptor of several Golgi-localized membrane proteins, it seems likely that accumulation of Mnt2 and Mnt3 in the ER caused by the deletion of the SVP26 gene is also the result of decreased packaging efficiency into the COPII vesicles. To test whether Svp26 would facilitate the ER exit of Mnt2 and Mnt3, *in vitro* COPII vesicle generation assays were performed. Microsomes prepared from cells expressing Mnt2-3HA or Mnt3-3HA from the construct integrated into the chromosomes were incubated either with or without the purified COPII coat components, and packaging efficiencies of Mnt2-3HA or Mnt3-3HA into COPII vesicles were assessed by Western blotting. The signal obtained in the negative control reactions (-COPII components) was subtracted from the signal found in the COPII vesicle fractions (+COPII components) and then incorporation efficiencies of Mnt2-3HA or Mnt3-3HA normalized to those of Erv46 are calculated and graphed. Averages from 3 independent experiments were plotted with standard deviations. As shown in Fig. 4, packaging of Mnt2-3HA or Mnt3-3HA into COPII vesicle fractions generated using the microsomal membranes derived from Δsvp26 cells were much lower than when wild-type membranes were used. These results strongly suggest that Svp26 functions as an adaptor protein to facilitate the packaging of Mnt2 and Mnt3 into COPII vesicles.

**Co-immunoprecipitation of Mnt2 and Mnt3 with Svp26**

Co-immunoprecipitation experiments were next performed to test if Svp26 would bind to Mnt2 and Mnt3 proteins. Cells expressing Svp26-FLAG and Mnt2-3HA or Mnt3-3HA, both from the construct integrated into the chromosomes were disrupted by agitation with glass-beads and the membrane proteins were solubilized with 1% digitonin. Svp26-FLAG was immunoprecipitated with an anti-FLAG affinity gel, and the immunoprecipitates were examined for the presence of Mnt2-3HA or Mnt3-3HA using an anti-HA monoclonal antibody. A strain expressing Ktr3-3HA was included as a positive control because the ER exit of Ktr3 is Svp26-dependent and Ktr3 is co-immunoprecipitated with Svp26 efficiently, as we showed previously (Inadome et al., 2005). As seen in Fig. 5, the co-immunoprecipitation of Mnt3-3HA with Svp26-FLAG was almost as efficient as that seen for Ktr3-3HA with Svp26-FLAG. Mnt2-3HA was co-immunoprecipitated with Svp26-FLAG less efficiently than Mnt3, but with reproducibility. Mnn1, whose Golgi localization is not dependent on the presence of Svp26, was not co-immunoprecipitated with Svp26, indicating the specificity of the experiment. These results further support the function for Svp26 as an ER exit adaptor protein of Mnt2 and Mnt3.

In this paper, we have identified Mnt2 and Mnt3 proteins as the new cargos of Svp26. As ER exit of the 7 proteins, Ktr3, Ktr1, Kre2, Mnn2, Mnn5, Pho8 and Gda1, has been previously reported to be dependent on Svp26, it now becomes an ER exit adaptor protein of 9 proteins. Ktr3, Ktr1 and Kre2 are members of the Kre2 mannosyltransferase family and are involved in O-linked protein glycosylation together with Mnt2 and Mnt3. Interestingly, although Mnt2, Mnt3 and Mnn1 belong to the Mnn1 family, ER exit of Mnn1 in not dependent on the function of Svp26. A mutational analysis based on the similarities and/or differences in their sequences may provide clues as to the motif involved in the recognition by Svp26.

To narrow down the region involved in the recognition by Svp26, we conducted preliminary domain switching experiments by exploiting the type II membrane topology and the sequence similarities among mannosyltransferases. However, the chimeric construct we produced were all accumulated in the ER even in the wild-type yeast strain. Substitution of amino acids conserved only across the mannosyltransferases, whose ER exit are dependent on Svp26, could help to identify the regions or sequences involved in the recognition by Svp26.

During the course of this study, we noticed that the C-terminally truncated version of Svp26 co-immunoprecipitated with its cargo proteins with much higher efficiency. Mnt2, Mnt3, Ktr3 and Mnn2 were tested for co-immunoprecipitation with Svp26 and they all exhibited stronger co-immunoprecipitation with the C-terminally truncated Svp26 than with the full-length Svp26. Bue and Barlowe (2009) showed that deletion of C-terminal tail region of Svp26 lead to reduced packaging efficiency of itself into the COPII vesicles. We suspected that this might allow more chance or time for Svp26 to interact with its cargos, thus facilitating the detection of the interaction between the proteins. By exploiting this result, we are currently searching for the cargo proteins of Svp26 more widely in the immunoprecipitates with C-terminally truncated Svp26.
Svp26 facilitates ER exit of mannosyltransferases Mnt2 and Mnt3 in *Saccharomyces cerevisiae*

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