Sfb2p, a Yeast Protein Related to Sec24p, Can Function as a Constituent of COPII Coats Required for Vesicle Budding from the Endoplasmic Reticulum*

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The COPII coat is required for vesicle budding from the endoplasmic reticulum (ER), and consists of two heterodimeric subcomplexes, Sec23p/Sec24p, Sec13p/Sec31p, and a small GTPase, Sar1p. We characterized a yeast mutant, anu1 (abnormal nuclear morphology) exhibiting proliferated ER as well as abnormal nuclear morphology at the restrictive temperature. Based on the finding that ANU1 is identical to SEC24, we confirmed a temperature-sensitive protein transport from the ER to the Golgi in anu1-1/sec24-20 cells. Overexpression of SFB2, a SEC24 homologue with 56% identity, partially suppressed not only the mutant phenotype of sec24-20 cells but also rescued the SEC24-disrupted cells. Moreover, the yeast two-hybrid assay revealed that Sfb2p, similarly to Sec24p, interacted with Sec23p. In SEC24-disrupted cells rescued by overexpression of SFB2, some cargo proteins were still retained in the ER, while most of the protein transport was restored. Together, these findings strongly suggest that Sfb2p functions as the component of COPII coats in place of Sec24p, and raise the possibility that each member of the SEC24 family of proteins participates directly and/or indirectly in cargo-recognition events with its own cargo specificity at forming ER-derived vesicles.

In eukaryotic cells, protein transport along the secretory pathway is mediated by vesicle budding from a donor membrane and by specifically fusing the formed vesicle to an acceptor organelle. Vesicle budding is driven by the recruitment of specific coat proteins to a donor membrane (1, 2). The COPII coat, that is required for the vesicle budding from endoplasmic reticulum (ER),1 consists of heterodimeric protein complexes, Sec23p/Sec24p, Sec13p/Sec31p, and a small GTPase Sar1p.

COPII-coated vesicle formation begins with recruitment of Sar1p to the ER membrane where Sar1p-GDP is converted to Sar1p-GTP by a specific guanine nucleotide exchanging factor, Sec12p. Subsequently, Sec23p/Sec24p binds a membrane-bound Sar1p-GTP to form Sar1p-Sec23p/Sec24p prebudding complex. Finally, Sec13p/Sec31p binds the prebudding complex to cross-link them, resulting in vesicle budding (3–6). An additional protein, Sec16p, is also required for COPII-coated vesicle budding. Sec16p is a peripheral membrane protein tightly associated with the ER, and can bind COPII coat components, Sec23p, Sec24p, and Sec31p via its distinct domains. Thus Sec16p is considered to serve as a scaffold to recruit and/or assemble COPII coat components (5).

In addition to secretory and membrane proteins (cargo proteins), vesicle machinery proteins, such as v-SNAREs (vesicle targeting proteins) and cargo receptors are also specifically concentrated into COPII-coated vesicles (7–11). Cargo receptors are membrane proteins cycling between the ER and Golgi that interact with specific cargo molecules and facilitate their uptake into transport vesicles (12). For instance, several yeast proteins belonging to the p24 family are known to be involved in the transport of a subset of cargo proteins (9, 10, 13, 14). At least eight members of the yeast p24 family have been identified as putative cargo receptors, i.e. EMP24, ERV25, and ERP1-ERP6, all encoding type I integral membrane proteins (9, 10, 14). Genetic and biochemical studies revealed that Emp24p, Erv25p, Erp1p, and Erp2p function in a heteromeric complex (10, 14), and are responsible for the efficient and selective export of invertase and/or Gas1p out of the ER (9, 10, 13, 14). However, clear cut evidence for a direct interaction between these cargo proteins and p24 heteromeric complexes has not been observed yet.

Several lines of evidence strongly suggest that selective export from the ER and COPII-coated vesicle formation should be coupled. Matsuoka et al. (15) demonstrated that v-SNAREs but not ER resident proteins are concentrated in synthetic COPII-coated vesicles in a reconstituted liposome budding assay. It was also demonstrated in both yeast and mammals that some membrane cargo and vesicle machinery proteins, such as v-SNAREs, Emp24p, and vesicular stomatitis virus glycoprotein (VSV-G), specifically interact with Sar1p-Sec23p/Sec24p prebudding complex (16–18).

Although Sec24p has binding domains for Sec23p, Sec31p, and Sec16p (19–21) and a zinc finger-like domain essential for

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‡ The abbreviations used are: ER, endoplasmic reticulum; PCR, polymerase chain reaction; CPY, carboxypeptidase Y; SNARE, soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor; v, vesicular; COPII, coat protein complex II; BiP, immunoglobulin heavy chain-binding protein; UPR, unfolded protein response; UPRE, unfolded protein response element; PAGE, polyacrylamide gel electrophoresis.
its function (21), the precise role of Sec24p itself in vesicle formation and cargo selection remains to be elucidated. The Saccharomyces genome database (22) shows that there are two additional genes related to SEC24, designated SFB2 (YNL049c; 56% identity) and SFB3 (YHR098c; 23% identity). While neither SFB2 nor SFB3 is essential for growth (23), the zinc-finger-like domain is conserved among the SEC24 family of proteins. Recently, SFB3 was identified as LST1, the gene exhibiting synthetic lethal interactions with SEC13 and all of other COPII genes. Lst1p has an ability to bind Sec23p, and SEC13 and SEC24 specific inhibition of proteins. Recently, zinc finger-like domain is conserved among the heterogeneous COPII coats. While neither SFB2 nor SFB3 is essential for growth (23), the zinc-finger-like domain is conserved among the SEC24 family of proteins. Recently, SFB3 was identified as LST1, the gene exhibiting synthetic lethal interactions with SEC13 and all of other COPII genes. Lst1p has an ability to bind Sec23p, and SEC13 and SEC24 specific inhibition of proteins. Recently, zinc finger-like domain is conserved among the heterogeneous COPII coats.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—pAN1 is a YCp50-based plasmid harboring a 8.0-kilobase pair Sau3A1 yeast genomic DNA fragment containing SEC24 (ANU1), which was screened from a YCp50-based (CEN vector; URA3) yeast genomic library (26). A 2.9-kilobase pair SnaBI genomic fragment containing SEC24 was subcloned into the Smal site of pRS314 (27) to produce pAN1. pAN1 was generated as follows: sec24-20 (anu1-1) gene was cloned from TK3 strain by plasmid gap repair method (28) using pAN1. The resultant sec24-20 plasmid was digested with SnaBI, and a 2.9-kilobase pair genomic fragment containing sec24-20 was subcloned into the Smal site of pRS314 to produce pAN12. Plasmids pSF1, pSF2, and pSF11 were generated as follows: the SFB2 gene was obtained by polymerase chain reaction (PCR) amplification of chromosomal DNA isolated from *Saccharomyces cerevisiae* strain FY23 (generous gifts from Dr. W. Winston, Harvard Medical School, Boston, MA) using oligonucleotides, SFB2-5 (5'-AGAGAGAGATCCGATGTTTTGTCGAGGATCCTCC-3') and SFB2-3 (5'-AGAGAGAGACATTTAGAGGCAAACTTGTATCTTATGTCAAAGC-3'), which correspond to nucleotides, 285 to 286, and nucleotides 2980 to 2985, respectively. SFB2-5 and SFB2-3 contain a BamHI and a HindIII site, respectively, allowing for insertion into BamHI and HindIII endonuclease sites of pBS426 and pBS424 (29) to produce pSF1 and pSF11, respectively. The SFB3 gene was also obtained by PCR using SFB3-5 (5'-AGAGAGAGATCCGATGTTTTGTCGAGGATCCTCC-3') and SFB3-3 (5'-AGAGAGAGACATTTAGAGGCAAACTTGTATCTTATGTCAAAGC-3'), which correspond to nucleotides, 844 to 821, and 3144 to 3121, respectively, SFB2-5 and SFB3-3 contain a BamHI and a XhoI site, respectively, allowing for insertion into BamHI/XhoI sites of plasmid pRS426 to produce pSF2. pCYZ1 is a 2-μm based plasmid containing KAR2 UPRE (unfolded protein response element of KAR2)-CYC1 promoter-LacZ fusion gene and URA3 selectable marker, kindly provided by K. Mori (30). pUPR3 was generated as follows: a XhoI-EcoRI fragment of pCYZ1 containing UPRE-CYC1-LacZ was inserted into XhoI/EcoRI sites of pRS316 (27), and a synthetic 52-base pair oligonucleotide encoding 2×UPRE was inserted into its XhoI site of the resultant plasmid to obtain pUPR3 containing 3×UPRE-CYC1-LacZ.

The plasmids for yeast two-hybrid assay, pGAD-SEC24, pGAD-sec24-20, pGAD-SFB2, and pGBD-SEC23 were generated as follows: open reading frames of SEC24, sec24-20, SFB2, and SEC23 were obtained by PCR amplification of chromosomal DNA using following primer sets: TH24-5 corresponding to nucleotides 0 to 21 of SEC24, and TH24-3 (5'-AGAGAGAGCTCGAGAGGCTTATTTGCTAATTCTGGCTTTCATG-3') corresponding to nucleotides 2784 to 2756, TH2-5 corresponding to nucleotides 0 to 30 of SEC24, and TH2-3 (5'-AGAGAGAGCTCGAGAGGCTTATTTGCTAATTCTGGCTTTCATG-3') corresponding to nucleotides 2631 to 2600, and TH23-5 corresponding to nucleotides 0 to 26 of SEC23, and TH23-3 (5'-AGAGAGAGCTCGAGAGGCTTATTTGCTAATTCTGGCTTTCATG-3') corresponding to nucleotides 2284 to 2253, respectively. PCR fragments containing SEC24, sec24-20, and SFB2 were digested by Xhol and then cloned into Smal/SauI sites of pGAD-C1 to produce pGAD-SEC24, pGAD-sec24-20, and pGAD-SFB2, respectively. Similarly, the PCR fragment containing SEC23 was digested and cloned into pGBD-C1 to produce pGBD-SEC23.

**Strains, Media, and Growth Conditions**—Yeast strains were grown in rich (YPD) or synthetic dextrose (SD) media, and standard genetic manipulations were performed as described previously (32). For some experiments, cells were grown in synthetic complete (SC) media (32) buffered to pH 7.0 with 0.1 M potassium phosphate buffer or adjusted to pH 4.0 with HCl. The *Escherichia coli* strain DH5α (33) was used for manipulation of recombinant DNA and was grown in LB media (1% NaCl, 1% peptone, and 0.5% yeast extract) containing 100 μg/ml ampicillin. All yeast strains used in this study were listed in Table I, and
yeast strains except for PJ69-4A were derived from FY strains (generous gifts from Dr. F. Winston). Diploid strain DFD24 was sporulated to obtain a KFY1 strain. To obtain YKHK1 (Δsec24Δ) and YKHK2 (Δsec24Δ) strains from TKO1, gene deletions were performed by PCR amplification of the URRA3 gene with oligonucleotides that encoded 45 base pairs of the start codon. The amplified fragments were integrated into the genome of TKO1 by homologous recombination. Strains YKHK4 and YKHK6 were obtained from the experiment represented in Fig. 4 (see figure legend of Fig. 4).

Electron and Immunofluorescence Microscopy—Electron microscopic analysis was performed as follows: preparation of a thin section of yeast cells was carried out by the freeze-substituted fixation method as described previously (35), except that Reichart KF80 was used to freeze the cells. Thin sections were viewed on a JEO100CX electron microscope (JEOL, Tokyo, Japan) at 80 kV. For Kar2p staining, cells were fixed by direct addition of formaldehyde to the culture to a final concentration of 3.7% and incubated at room temperature for 2 h. Fixed cells were washed with potassium phosphate buffer (0.1 M potassium phosphate, pH 6.5) and converted into spheroplasts by incubation with 50 µg/ml zymolyase-100T (Seikagaku Corp., Tokyo, Japan) in 1.2 M sorbitol, 0.1 M potassium phosphate, pH 6.5, and 0.2% β-mercaptoethanol at 30°C for 1–2 h. Immunofluorescence was performed essentially as described previously (36). The rabbit anti-yeast Kar2p antiserum prepared as described previously (37) was used as the primary antibody and rhodamine-conjugated goat anti-rabbit IgG antibody (Cappel Research Products, ICN, Inc., Tokyo, Japan) was used as the secondary antibody. DNA was stained with 1 µg/ml 4,6-diamino-2-phenylindole (DAB, Japan) at 1:500 dilution. Preparations were viewed on a Axiohot fluorescence microscope (Zeiss, Jena, Germany).

β-Galactosidase Assay—Assays of β-galactosidase activity in yeast extracts were carried out as described previously (38). β-Galactosidase activity was expressed as units defined as (A420 × 1000)/(A600 × t × v), where A420 is the absorbance at 420 nm of the reaction mixture, after t minutes, A600 is the turbidity of the culture at the time of harvest, t is the number of minutes for which the reaction mixture was incubated, and v is the volume of the sample in milliliters. The values are averages of four independent yeast transformants, and error bars are presented (Figs. 2C and 5E).

Pulse-Chase Experiments—Pulse-chase experiments were performed as described previously (39) with the following modifications. Cells were grown exponentially in SC, pH 4.0, medium, lacking methionine and cysteine. For invertase transport assay, cells were transferred to SC, pH 4.0, media containing 0.1% glucose and incubated for 30 min to induce the expression of invertase before pulse labeling. Immunoprecipitations were performed using anti-carboxypeptidase Y (CPY) antibody (Rockland Immunocchemicals, Inc., Gilbertsville, PA), anti-invertase antibody (kindly provided by Dr. H. Riezman, University of Basel, Switzerland) at 1:500 dilution. Proteins were dissolved in Laemmli sample buffer containing 0.1 M dithiothreitol, incubated at 95°C for 3 min, and then subjected to SDS-PAGE (8%). Assays for general secretion competence were performed as described (40) with the following modifications. Before labeling, cells were grown exponentially in SC, pH 4.0 or 7.0, lacking methionine and cysteine. Cells were pulse-labeled with 4 MBq/ml [35S]methionine + cysteine, at 5 A600/ml in SC, pH 4.0 or 7.0, media containing 100 µg/ml α2-macroglobulin and 250 µg/ml bovine serum albumin, and then chased for 45 min by adding excess amounts of unlabeled methionine and cysteine (final concentrations of 1 and 0.8 mM, respectively). Proteins equivalent to 0.25 A600 were loaded on SDS-PAGE (8%), and autoradiographed with a BioImage BAS2000 analyzer (Fuji Photo Film, Tokyo, Japan).

Yeast Two-hybrid System—The interaction between Sec23p and Sfh2p, Sec24p, or sec24-24 gene product was tested by the yeast two-hybrid method as described (31). Open reading frames of SFB2, SEC24, anu1, and SEC23 were cloned into vectors pGAD-C1 and pGBD-C1 in-frame with the GAL4 activation or binding domain as described under “Plasmid Construction.”

RNA Preparation and Northern Analysis—Cells were grown exponentially in SC, pH 4.0, medium, and then harvested by centrifugation, washed, frozen by liquid nitrogen and stored at −80°C. Total RNA of cells was isolated by hot phenol extraction (41). RNA was quantified by absorbance at 260 nm and the integrity of the RNA was confirmed by ethidium bromide staining of RNA in agarose gels. For Northern blotting...
ting, 5 or 10 µg of each RNA sample was subjected to electrophoresis in 1.0% formaldehyde/agarose gel, followed by transferring onto a nylon membrane (Hybond N+, Amersham Pharmacia Biotech) and hybridization analysis as described (42). Probes used for hybridization were the open reading frames of SEC24 and SFB2, obtained by PCR amplification of chromosomal DNA using the primer sets TH24-5 and TH24-3 and TH2-5 and TH2-3, respectively. Probes were labeled by Random primer labeling kit (TaKaRa, Kyoto, Japan). The probed membranes were autoradiographed and analyzed with a BioImage BAS2000 analyzer.

RESULTS

Accumulation and Severe Extension of the ER in anu1-1/sec24-20 Mutants—In our previous study on nuclear morphology, we obtained four mutants, anu1-1, anu1-ana4, exhibiting abnormal nuclear morphology at the restrictive temperature (25). Unexpectedly, ANU1 was identical to SEC24, which is known to be involved in the early secretory pathway rather than the construction of nuclei (25). Electron microscopic analysis revealed that anu1-1/sec24-20 cells cultured at the restrictive temperature of 37 °C for 4 h contained morphologically aberrant and apparently fragmented nuclei (Fig. 1, B, D, and E). Accumulated membranes having appearances like an extension of the ER because of their continuity with the outer nuclear membrane were also observed in sec24-20 cells (Fig. 1, A, B and D). In addition, irregular invagination of the ER was often observed in sec24-20 cells cultured at the restrictive temperature (Fig. 1C). These results suggested that some abnormalities of the ER led to the aberrant nuclear morphology in sec24-20 cells.

Abnormal Phenotype in the ER at the Restrictive Temperature—sec24-20 cells barely grew at 30 °C, and died at 37 °C (Fig. 2A). To identify some other abnormalities related to the ER in sec24-20 cells, we investigated the distribution of Kar2p/ BiP, a well characterized ER resident chaperone, by indirect immunofluorescence microscopic analysis. Contrary to wild-type cells, punctate staining of Kar2p was observed in 90–95, 60–70, and <5% of sec24-20 cells incubated at 37, 30, and 23 °C, respectively (Fig. 2B). The Kar2p distributions in sec24-20 cells resembled those termed BiP bodies (43) observed in various mutants defective in the transport between ER and Golgi. Since the unfolded protein response (UPR) activates transcription of ER resident chaperones in response to the accumulation of un- or mal-folded proteins in the lumen of the ER accompanied by the blockade of ER-to-Golgi vesicle transport (44–48), we next investigated whether the UPR was activated in sec24-20 cells. To assess the activation of the UPR, β-galactosidase activity was determined in wild-type, sec24-20, and sec18 (anu1)-containing cells using a UPR (UPR element of KAR2)-CYC1 promoter-LacZ reporter plasmid (pCZY1). LacZ expression from pCZY1 was under the control of UPR, the 22-base pair cis-acting element necessary and sufficient for transcriptional induction of Kar2p by the UPR (30, 38). At the semipermissive temperature of 30 °C, but not at 23 °C, β-galactosidase activity of sec24-20 and sec18 cells was 3–4-fold higher than that of wild-type cells, indicating that the UPR was activated in these two mutants (Fig. 2C).

To further confirm secretion defects in sec24-20 cells, we first compared the profiles of secreted proteins secreted in the media of sec24-20 cells with that of wild-type cells by a pulse-chase experiment. In contrast to wild-type cells, a temperature-sensitive secretion was observed in sec24-20 cells (Fig. 3A). In sec24-20 cells, some proteins appeared to be secreted in more reduced kinetics than other proteins at 30 °C (Fig. 3A, lane 6, arrows), whereas the overall secretion was almost blocked at 37 °C. We further investigated whether sec24-20 cells had ER-to-Golgi vesicle transport defects by a transport assay of CPY. CPY is detected in the ER as the p1 form (67 kDa), further modified to the p2 form (69 kDa) in the Golgi, and then processed proteolytically to the mature form (m; 61 kDa) in the vacuole (49). Unlike wild-type cells, sec24-20 cells exhibited a temperature-sensitive maturation of CPY to accumulate the p1 form even after the 60-min chase at 37 °C (Fig. 3B). Thus, sec24-20 cells are defective in ER-to-Golgi vesicle transport at the restrictive temperature causing the activation of the UPR.

Suppression of sec24-20 Phenotypes by the Overexpression of a SEC24 Homologue, SFB2—The Saccharomyces genome database contains two genes related to SEC24, SFB2 (56% identity) and SFB3 (23% identity) (22). We therefore investigated whether the overexpression of either gene suppressed growth and/or vesicular transport defects of sec24-20 mutant. As shown in Fig. 2A, the overexpression of SFB2 suppressed the growth retardation of sec24-20 cells at 30 °C, but not the growth inhibition at 37 °C. On the contrary, the overexpression of SFB3 did not affect the growth of sec24-20 cells (Fig. 2A). In addition, at 30 °C but not at 37 °C, the overexpression of SFB2 also restored the secretion competence of sec24-20 cells to the same extent as that of sec24-20 cells complemented with a...
SEC24 plasmid (pAN1; URA3, CEN) (Fig. 3A). Consistent with this observation, the overexpression of SFB2 similarly improved the maturation rate of CPY in sec24-20 cells (Fig. 3B). These results indicated that both growth and ER-to-Golgi vesicle transport defects of sec24-20 cells were partially suppressed by the overexpression of SFB2.

**Suppression of the Chromosomal Deletion of SEC24 by Overexpression of SFB2**—To investigate whether the overexpression of SFB2 suppresses the lethality derived from a sec24 null mutation, we constructed a yeast strain (YKH3) harboring a null allele at the SEC24 chromosomal locus that was rescued by the SEC24 plasmid (pAN1; URA3, CEN). The Δsec24 strain was transformed with each of the following plasmids, SFB2 (pSF11; TRP1, 2 μ), SEC24 (pAN11; TRP1, CEN), or sec24-20 (pAN12; TRP1, CEN). The clones obtained by selection on SD (+5-fluoroorotic acid) plates, where only the Δsec24 cells that lose the SEC24 plasmid (pAN1, URA3) can grow, were examined. Interestingly, the Δsec24 cells containing a 2μ-based SFB2 plasmid (pSF11) grew well on the SD (+5-fluoroorotic acid) plates as well as those containing either SEC24 (pAN11) or sec24 (pAN12) plasmid (Fig. 4A), indicating that overexpression of SFB2 suppressed the lethality caused by the absence of SEC24. Furthermore, we examined the growth of the resultant Δsec24 cells rescued by pAN11 (SEC24, CEN), designated as Δsec24(SEC24, CEN) (YKH4) and those rescued by pSF11 (SFB2,2 μ), designated as Δsec24(SFB2,2 μ) (YKH6), respectively. A, 10-fold serial dilutions (starting from Δsec24 of 0.1) of Δsec24(SEC24, CEN) and Δsec24(SFB2,2 μ) were spotted on the indicated plates and incubated at 23 °C for 4 days. We designated Δsec24 cells rescued by pAN11 and those rescued by pSF11, Δsec24(SEC24, CEN) (YKH4) and Δsec24(SFB2,2 μ) (YKH6), respectively. B, 10-fold serial dilutions (starting from Δsec24 of 0.1) of Δsec24(SEC24, CEN) and Δsec24(SFB2,2 μ) were spotted on the indicated plates and incubated at 23 °C for 4 days. The pH of the YPD is 6.2, approximately.

*Cargo-specific ER-to-Golgi Protein Transport Defects in Δsec24(SFB2,2 μ) Cells*—To analyze the protein transport of Δsec24(SFB2,2 μ) cells, we first performed the assay for general secretion competence. As shown in Fig. 5A, the secretion profile of Δsec24(SFB2,2 μ) cells was almost indistinguishable from that of Δsec24(SEC24, CEN) cells, suggesting that the overexpression of SFB2 rescued almost all of the protein transport. However, at 23 and 37 °C, the protein band indicated by the arrowhead (Fig. 5A, lanes 2 and 6) was more apparent in Δsec24(SFB2,2 μ) cells than in Δsec24(SEC24, CEN) cells. Subsequently, the ER to Golgi protein transport of Δsec24(SFB2,2 μ) cells was examined by the CFP transport assay. The maturation of CFP was greatly retarded in Δsec24(SFB2,2 μ) cells cultured at 23 °C (Fig. 5B), indicating that Δsec24(SFB2,2 μ) cells were cold-sensitive for the ER-to-Golgi transport of CFP. We further analyzed the ER-to-Golgi protein transport of Δsec24(SFB2,2 μ) cells by an invertase transport assay. Invertase is detected in the ER as several core-glycosylated forms (core; 79–83 kDa) that upon arrival to Golgi are further modified to generate heterogeneous hyperglycosylated mature forms (mature; 100–150 kDa), and then secreted into the periplasmic space (50–52). As shown in Fig. 5C, comparing to Δsec24(SEC24, CEN) cells, core-glycosylated ER forms remained in Δsec24(SFB2,2 μ) cells even after the 45-min chase at any culturing temperature, suggesting that Δsec24(SFB2,2 μ) cells were originally defective in the efficient ER-to-Golgi transport of invertase. Moreover, no obvious mature form of invertase in the medium was observed in Δsec24(SFB2,2 μ) cells, confirming that a hyperglycosylation of invertase was defective in these cells (data not shown). Such maturation defects of invertase have been observed in ΔEMP24 mutant cells (9). To clarify ER-to-Golgi protein transport defects as seen in the transport of invertase in Δsec24(SFB2,2 μ) cells, we analyzed the transport of the glycosylphosphatidylinositol-anchored plasma membrane protein Gas1p. Gas1p is synthesized in the ER as a 105-kDa glycosylphosphatidylinositol-anchored precursor that carries N- and O-linked oligosaccharides, and upon arrival to the Golgi, outer chain glycosylation residues are added, generating the 125-kDa mature form (53, 54). As shown in Fig. 5D, compared to Δsec24(SEC24, CEN) cells, maturation of Gas1p was greatly retarded in Δsec24(SFB2,2 μ) cells at any culturing temperature, indicating that efficient ER-to-Golgi transport of Gas1p was impaired in Δsec24(SFB2,2 μ) cells. Taken together, despite restoration of almost all protein transport, Δsec24(SFB2,2 μ) cells still have some cargo-specific ER-to-Golgi protein transport defects. Since the UPR was activated in response to the accumulation of un- or mal-folded proteins in the lumen of the ER associated by the reduced ER-to-Golgi protein transport (Fig. 2C), β-galactosidase activity of Δsec24(SFB2,2 μ) cells harboring the reporter plasmid was determined to assess the UPR activation. β-Galactosidase activity of Δsec24(SFB2,2 μ) cells was approximately 2.5-fold higher...
than that of Δsec24(SEC24,CEN) cells at both 23 and 30 °C, indicating that the UPR was activated in Δsec24(SEC24,SFB2,2μ) cells (Fig. 5E).

**Two-hybrid Interaction between SFB2 and SEC23—**Sec24p was first identified as a complex with Sec23p (55). Because of the similarity to Sec24p, Sfb2p is supposed to bind Sec23p and act as a component of COPII coat. To investigate whether Sfb2p is similar to Sec24p, Sfb2p is supposed to bind Sec23p and was first identified as a complex with Sec23p (55). Because of the similarity to Sec24p, Sfb2p is supposed to bind Sec23p and act as a component of COPII coat. To investigate whether Sfb2p (YKH2) cells, Δsfb2 (YKH1) cells could grow as well as the wild-type cells at any culturing temperature or pH of media (Fig. 7A). When the general secretion competence of Δsfb2 cells was examined, all of the secretion profiles at 37 °C (Fig. 7B, lanes 9–12) were dramatically different from those presented in Fig. 3A because of culturing cells in SC, pH 7.0, a neutral medium suitable for Δsfb3 cells. The secretion profile of Δsfb2 cells, however, was indistinguishable from that of wild-type or Δsfb3 cells at any culturing temperature (Fig. 7B). This was consistent with the observation (23) that chromosomal deletion of SEC24 homologues did not affect the transport of either CPY or invertase. Finally, to obtain the information about the expression of SFB2, Northern blot analyses were carried out with total RNA isolated from wild-type cells. To examine the transcriptional level of SFB2 in comparison to that of SEC24, the RNA blots were hybridized with SFB2 and SEC24 probes of comparable specific activity and were exposed for the same period of time. As shown in Fig. 7C, the transcription level of SFB2 is much lower (approximately 1/8, at any culturing temperature) than that of SEC24, consistent with the fact that chromosomal deletion of SEC24 is lethal (note the expression level of SFB2 that compensates the chromosomal deletion of SEC24).

**DISCUSSION**

In this study, we describe some phenotypes of a temperature-sensitive mutant anu1-1, previously screened for abnormal nuclear morphology (25), and the partial suppression of anu1 phenotypes by overexpression of the SEC24 homologue, SFB2. At the restrictive temperature, anu1-1 cells exhibited the proliferated ER and the punctate distribution of Kar2p. Consistent with the fact that ANU1 is identical to SEC24 (25), anu1-1/ sec24-20 cells exhibited the temperature-sensitive ER-to-Golgi
vesicle transport. Both temperature-sensitive growth and transport were partially restored by overexpression of SFB2. Moreover, the overexpression of SFB2 suppressed the lethality derived from the chromosomal deletion of SEC24, depending on culturing conditions. The resultant Δsec24(SFB2,2μ) cells exhibited some cargo-specific ER to Golgi transport defects, while their secretion competence appeared to be recovered to the same extent as that of wild-type cells. A yeast two-hybrid assay revealed that like Sec24p, Sfb2p could bind Sec23p.

The abnormal nuclear morphology of sec24-20 cells (Fig. 1) has also been observed in various mutants defective in vesicular transport between the ER and the Golgi such as sec28/anu2, sec13/anu3, sec18/anu4, sar1, sec12, sec16, and uso1 (25, 56). Transport defects between the ER and the Golgi are considered to trigger a proliferation of ER, perturbing the nuclear structure because of the continuity of the nucleus with the ER (25). Consistent with the retardation of the ER to Golgi protein transport (Fig. 3), the BiP body-like distribution of Kar2p and the activation of the UPR were also observed in sec24-20 cells at 30 °C (Fig. 2, B and C). Since the UPR was also activated in sec18 cells (Fig. 2C), the activation of the UPR in sec24-20 cells is considered to represent the accumulation of proteins in the ER owing to the reduced ER-to-Golgi vesicle transport. On the other hand, the punctate distribution of Kar2p in sec24-20 cells is probably identical to the BiP body, the site where secretory proteins accumulate when ER to Golgi vesicle transport is blocked (43). It is thought that BiP may escort secretory proteins to keep them transport-competent until the transport between the ER and Golgi is resumed (43).

The precise role of Sec24p itself in vesicle formation and cargo selection yet remains unclear. The suppression of the Δsec24 mutation by overexpression of SFB2 (Figs. 4 and 5) in addition to the two-hybrid interaction between Sfb2p with Sec23p (Fig. 6 and Table II), strongly suggest that Sfb2p can replace Sec24p in the COPII-coated vesicle formation and in the transport of most proteins. This is supported by the difference in the growth rate between Δsec24(SFB2,2μ) and sec24-20(SFB2,2μ) cells. sec24-20(SFB2,2μ) cells grew slower than Δsec24(SFB2,2μ) cells at 30 °C, but faster at 23 °C (see “Results”). Moreover, the overexpression of SFB2 could rescue the growth defect of Δsec24 cells but not that of sec24-20 cells at 37 °C (Figs. 2A and 4B). Like Sec24p, Sfb2p and the sec24-20 gene product can bind Sec23p (Fig. 6 and Table II). Taken together, these results suggest that Sfb2p competes with the functionally impaired sec24-20 gene product for the binding to Sec23p in sec24-20(SFB2,2μ) cells at high culturing temperature.

The secretion assay in Δsec24(SFB2,2μ) cells revealed that most of the protein transport appeared to be recovered to the same extent as that of Δsec24(SEC24,2μ) cells (Fig. 5A). This implies that vesicle machinery proteins such as v-SNAREs, are packaged or at least included in the ER-derived vesicles of Δsec24(SFB2,2μ) cells. On the other hand, transport assays of several cargo proteins revealed that not all of the secretory proteins were efficiently exported from the ER in Δsec24(SFB2,2μ) cells (Fig. 5, B-D). In particular, Δsec24(SFB2,2μ) cells exhibited markedly slow transport kinetics of invertase and Gas1p at any culturing temperature (Fig. 5, C and D), suggesting that Sec24p, rather than Sfb2p, is indirectly responsible for concentrating them into ER-derived vesicles. It is known that the efficient ER to Golgi transport of invertase and Gas1p requires p24 proteins, the putative cargo receptor expected to serve as an adaptor linking the vesicle-forming machinery to soluble cargo recruitment (9, 10, 12–14). In the study using ER-derived microsomes and purified COPII components, Emp24p, a member of the p24 family, was found to associate with the Sar1p-Sec23p/Sec24p prebudding complex, suggesting that the prebudding complex is responsible for sorting Emp24p into COPII-coated vesicles (17). According to this observation, p24 proteins appear to interact efficiently with the Sar1p-Sec23p/Sec24p rather than the Sar1p-Sec23p/Sfb2p complex, emerging the difference between Δsec24(SEC24,2μ) and Δsec24(SFB2,2μ) cells in the kinetics of invertase and Gas1p transport. Alternatively, a reduced packaging of machinery proteins required for the Golgi to ER retrograde vesicle transport (such as Golgi to ER v-SNAREs) into ER-derived vesicles of Δsec24(SFB2,2μ) cells may retard the retrieval of p24 proteins from the Golgi to ER, resulting in the reduced kinetics of invertase and Gas1p transport. On the other hand, the cold-sensitive transport of CPY in Δsec24(SFB2,2μ) cells (Fig. 5B) suggests that COPII-like coated vesicles including Sfb2p are cold-sensitive in the selection and/or the concentration of CPY. Since the UPR was activated in Δsec24(SFB2,2μ) cells at both 23 and 30 °C (Fig. 5E), some other cargo proteins such as invertase and Gas1p might be retained in the ER independently of culturing temperature. The conditional growth of Δsec24(SFB2,2μ) cells may be due to the temperature-dependent and/or -independent ER retention of proteins affecting the cell growth, such as those required for the growth under neutral conditions.

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**Table II**

|        | SFB2 | SEC24 | sec24-20 | No fusion |
|--------|------|-------|----------|-----------|
| **SEC23** | 54.0 ± 3.1 | 47.3 ± 5.5 | 66.6 ± 0.5 | 4.7 ± 0.2 |
| **No fusion** | 15.0 ± 0.9 | 15.3 ± 1.5 | 14.8 ± 1.7 | 8.9 ± 0.4 |

a β-Galactosidase activities of PJ69–4A containing each plasmid combinations as in Fig. 6.

Sec24p, rather than Sfb2p, is indirectly responsible for concentrating them into ER-derived vesicles. It is known that the efficient ER to Golgi transport of invertase and Gas1p requires p24 proteins, the putative cargo receptor expected to serve as an adaptor linking the vesicle-forming machinery to soluble cargo recruitment (9, 10, 12–14). In the study using ER-derived microsomes and purified COPII components, Emp24p, a member of the p24 family, was found to associate with the Sar1p-Sec23p/Sec24p prebudding complex, suggesting that the prebudding complex is responsible for sorting Emp24p into COPII-coated vesicles (17). According to this observation, p24 proteins appear to interact efficiently with the Sar1p-Sec23p/Sec24p rather than the Sar1p-Sec23p/Sfb2p complex, emerging the difference between Δsec24(SEC24,2μ) and Δsec24(SFB2,2μ) cells in the kinetics of invertase and Gas1p transport. Alternatively, a reduced packaging of machinery proteins required for the Golgi to ER retrograde vesicle transport (such as Golgi to ER v-SNAREs) into ER-derived vesicles of Δsec24(SFB2,2μ) cells may retard the retrieval of p24 proteins from the Golgi to ER, resulting in the reduced kinetics of invertase and Gas1p transport. On the other hand, the cold-sensitive transport of CPY in Δsec24(SFB2,2μ) cells (Fig. 5B) suggests that COPII-like coated vesicles including Sfb2p are cold-sensitive in the selection and/or the concentration of CPY. Since the UPR was activated in Δsec24(SFB2,2μ) cells at both 23 and 30 °C (Fig. 5E), some other cargo proteins such as invertase and Gas1p might be retained in the ER independently of culturing temperature. The conditional growth of Δsec24(SFB2,2μ) cells may be due to the temperature-dependent and/or -independent ER retention of proteins affecting the cell growth, such as those required for the growth under neutral conditions.

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2. A. Hirata, unpublished observations.

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**Fig. 6. Two-hybrid interaction between SFB2 and SEC23.** Either pGAD-SEC23 or control plasmid (pGAD-C1) was co-transformed with pGAD-SFB2, pGAD-SEC24, pGAD-sec24-20, or control plasmid (pGBD-C1), into host strain PJ69-4A. The resulting clones containing each plasmid combinations were replica-plated onto SD (+His, +Ade) and control SD (+His, +Ade) plate, and incubated at 30 °C for 4 days.
The fact that some cargo proteins (such as invertase and Gas1p) require Sec24p but not Sfb2p for their efficient export from the ER suggests that Sec24p itself is responsible for the cargo recognition. Another Sec24p homologue, Lst1p, is involved in the export of restricted cargo proteins (only Pma1p is known) from the ER (24). Based on Δsfb2 phenotypes and the transcriptional level of SFR2 in wild-type cells (Fig. 7), Sbh2p may be involved in the efficient transport of specific cargo molecules like Lst1p rather than the efficient transport of many cargo proteins required for their growth like Sec24p. Hence, each of the SEC24 family of proteins may function as a component of COPII coats and serve its own cargo selectivity. We could not find the unique role of Sbh2p in the cargo selection, but demonstrated that in the ER-to-Golgi vesicle transport Shb2p could function as the component of COPII coats. The identification of Shb2p (this study) and Lst1p (24) as the component of COPII coats suggests that the coats of ER-derived vesicles may be heterogeneous. We are interested in whether one ER-derived vesicle contains one member of the SEC24 family of proteins, or alternatively, how the components of the ER shared between the components of the ER are different. If all members of the SEC24 family of proteins participate in the cargo selection event in concert with Sec23p, Sar1p, and others, the composition of the SEC24 family of proteins in the cell may affect that of cargo proteins transported by ER-derived vesicles. Further experiments are required to elucidate the roles of Sec24p and Sbh2p in cargo selection.

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