Coordination of N-Glycosylation and Protein Translocation across the Endoplasmic Reticulum Membrane by Sss1 Protein*

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Secretory proteins are translocated across the endoplasmic reticulum (ER) membrane through a channel formed by three proteins, namely Sec61p, Ssh1p, and Sss1p (Johnson, A. E., and van Waes, M. A. (1999) Annu. Rev. Cell Dev. Biol. 15, 799–842). Sec61p and Sss1p are essential for translocation (Ennault, Y., Blondel, M. O., Deshaies, R. J., Schekman, R., and Kepes, F. (1993) EMBO J. 12, 4083–4093). Sec61p is a polytopic membrane protein that lines the translocation channel. The role of Sss1p is unknown. During import into the ER through the Sec61p channel, many proteins are N-glycosylated before translocation is completed. In addition, both the Sec61 channel and oligosaccharyl transferase (OST) copurify with ribosomes from rough ER, suggesting that OST is located in close proximity to the Sec61 channel (Gorlich, D., Prehn, S., Hartmann, E., Kalies, K.-U., and Rapoport, T. A. (1992) Cell 71, 489–503 and Wang, L., and Dobberstein, B. (1999) FEBS Lett. 457, 316–322). Here, we demonstrate a direct interaction between Sss1p and a subunit of OST, Wbp1p, using the split-ubiquitin system and co-immunoprecipitation. We generated mutants in the cytoplasmic domain of Sss1p that disturb the interaction with OST and are viable but display a translocation defect specific for proteins with glycosylation acceptor sites. Our data suggest that Sss1p coordinates translocation across the ER membrane and N-linked glycosylation of secretory proteins.

Protein translocation across the ER membrane is an essential process in all eukaryotes (1). Translocation is mediated by a channel formed most likely by three copies of the heterotrimeric Sec61 complex (Sec61p, Ssh1p, and Sss1p in yeast; Sec61α, Sec61β, and Sec61γ in mammals) (1, 2, 5). During cotranslational protein import through the Sec61 channel, the large ribosomal subunit binds to the channel, and channel proteins copurify with ribosomes upon solubilization of rough ER-derived microsomes (3, 4). The signal peptide is cleaved off from secretory proteins as soon as the cleavage site is sufficiently exposed on the luminal side (1). The enzyme complex responsible for cleavage, signal peptidase, is anchored to the Sec61 channel by Sec61β (6). This interaction is most likely transient, as signal cleavage is required only once early in translocation (6). The OST complex in yeast consists of eight or possibly nine subunits (Ost1p, Wbp1p, Stt3p, Swp1p, Ost2p, Ost3p, Ost5p, Ost6p, and Ost4p); the first five of the subunits listed are essential. All subunits except Ost4p, against which no antibodies are available, were shown to be assembled in a 240-kDa complex in the ER membrane (7). OST can N-glycosylate secretory proteins early during translocation into the ER and close to the luminal exit of the Sec61 channel (7–9). N-glycan acceptor sites can occur throughout a protein and may need to be modified cotranslationally before they are buried by protein folding. Close proximity of OST to the Sec61 channel is therefore required until termination of translocation. Proximity of the two complexes has also been suggested by binding of the mammalian homologues of OST subunits, Ost1p (ribophorin I) and Swp1p (ribophorin II), to ribosomes that are associated with the Sec61 channel in the ER membrane (10, 11). A direct interaction between OST and the Sec61 channel, however, has not been demonstrated to date, nor has a role for glycosylation in translocation been found.

To analyze the interaction of OST with other proteins in the ER membrane, we used the split-ubiquitin-based yeast two-hybrid system, which can be used to monitor interaction between membrane proteins in situ (12, 13). The N-terminal and C-terminal halves of ubiquitin (Nub and Cub), when fused to heterologous proteins, can interact and refold into a quasi-native ubiquitin structure (13). A mutant form of Nub, NubG, can reconstitute ubiquitin with Cub only if both NubG and Cub are fused to proteins that are in close spatial proximity (Fig. 1A) (13). If a transcriptional activator is fused to the C terminus of Cub, formation of quasi-native ubiquitin results in cleavage by cytosolic ubiquitin-specific proteases (Fig. 1A, UBP) carboxyl-terminally of Cub, diffusion of the transcriptional activator to the nucleus, and expression of reporter genes. Using this system we found an interaction between the OST subunit Wbp1p and the protein translocation channel subunit Sss1p. We generated mutants in Sss1p in which the interaction with Wbp1p is perturbed to investigate the functional significance of this interaction in vivo and in vitro.
FIG. 1. Sss1p and Wbp1p form a complex in the ER membrane. A, split-ubiquitin constructs and detection of protein-protein interactions. Cub-PLV was fused to the cytosolic C terminus of Wbp1p. NubG was fused to cytosolic termini of an enzyme that catalyzes lipid-linked glucose formation, Alg5p, the OST subunit Osl1p, proteins required for misfolded protein export from the ER and degradation (Hrd1p, Der1p, Cue1p, and Ubc7p), and the translocon subunit Sss1p. Interaction of Wbp1p-Cub-PLV with Osl1p-NubG leads to release of PLV by cytosolic ubiquitin-specific proteases (UBP). B, growth of strains expressing Nub-Sss1 and Wbp1p-Cub-PLV on minimal media without tryptophan and leucine (left side; LW) or without tryptophan, leucine, and histidine and with a chromogenic substrate of β-galactosidase (right side; LWH/X-Gal). C, lysates from strains expressing the indicated combination of proteins were separated by SDS-PAGE, and transferred proteins were detected with peroxidase-labeled G. The asterisk indicates a Wbp1p-Cub-PLV derived band that occurred independently of ubiquitin-specific protease (UBP)-mediated cleavage.

**Experimental Procedures**

**Yeast Methods and Strains**—We used standard yeast medium and methods (14). Split-ubiquitin constructs were expressed in strain YG0673 (MATa ade2-101 his3-200 leu2-1 lys2-801 trp1-Δ63 ura3-52) using H3S, KRY494 (MATa ade2-101 his3-200 leu2-1 lys2-801 trp1-Δ63 ura3-52 sss1::HIS3), pRS304 containing wild type or mutant sss1 was integrated into TRP1, the strains sporulated, and the haploid progeny carrying the TRP1-integrated genes as sole copies of SSS1 analyzed; the strains and genotypes are KRY507 (MATa ade2-101 his3-200 leu2-1 lys2-801 trp1-Δ63 ura3-52 sss1::HIS3 trp1::pRS304(sss1-1)), KRY509 (MATa ade2-101 his3-200 leu2-1 lys2-801 trp1-Δ63 ura3-52 sss1::HIS3 trp1::pRS304(sss1-1)), KRY516 (MATa ade2-101 his3-200 leu2-1 lys2-801 trp1-Δ63 ura3-52 sss1::HIS3 trp1::pRS304(sss1)), and ISWH4 (MATa his7-1 leu2-1 lys1-1 trp1-Δ63 ura3-52 WBPI-HA::kanMX).

**Plasmids**—pNub-ALG5 and pOST1-Nub are described (12). All other Nub fusions were cloned in-frame with the respective genes using PCR. Mutants in SSS1 were generated by error-prone PCR using a limiting concentration of dATP and the Nub-SSS1 plasmid as template. Inserts from plasmids that no longer supported growth of YG0673 on selective media without histidine were sequenced.

**Co-immunoprecipitations**—Microsomes were prepared from YPH501, the HA-tagged WBPI strain, and isogenic SSS1 wild type and mutant strains (15). For co-immunoprecipitations, 200 μg of microsomes were solubilized in 1 ml of GLB-T (250 mM sorbitol, 100 mM NaCl, 25 mM Hepes, pH 7.4, 1 mM MgCl2, 1% Triton X-100, and 10% glycerol) and immunoprecipitated with Sss1p antiserum or Wbp1p antiserum crosslinked to protein A-Sepharose at 4 °C. Proteins were resolved by SDS-PAGE on 10 or 18% gels and transferred to polyvinylidene difluoride membranes and detected with anti-HA antibody. Proteins in the far right lane were digested with protein N-glycanase (PNGase) prior to electrophoresis. B, wild type yeast microsomes were lysed, and Sss1p and Wbp1p were immunoprecipitated in duplicate with specific polyclonal antibodies as above. After electrophoresis and transfer, Sss1p was detected by immunoblotting. Blots shown in **panels A**, **B**, and **C** were also probed with an antibody against a signal peptidase subunit, Spc3p, but signal peptidase did not co-precipitate with either antibodies and proteinase-coupled anti-rabbit antibodies were used. For protein N-glycanase digestions, immunoprecipitates were resuspended in 50 mM NaPO4, pH 7.5, 0.5% Nonidet P-40, 2 mM EDTA, 5 μg/ml leupeptin, and 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) and incubated with 2 units of protein N-glycanase for 2 h at 37 °C.

**Pulse-Chase Experiments**—Radiolabelling, immunoprecipitations, and concanavalin A precipitations were performed as described (16, 17).

**In Vitro Translocation**—Microsomes were prepared from SSS1 wild type and mutant strains (15). Excess in vitro translated, [35S]methionine-labeled, wild type ppF or a mutant form in which the glycosylation sites had been removed by site-directed mutagenesis (pAgpFF) (106 cpm) was translocated into 0.5-μl microsomes (A280 = 25) at 10 or 20 °C for the indicated periods of time in the presence of ATP and an ATP-regenerating system (17). The amount of membranes was limiting.
in the reactions. Trichloroacetic acid-precipitated samples were resolved on 18%, 4 M urea acrylamide gels, and a translocated, glycosylated, wild type gpgF or a translocated, signal-cleaved, mutant ΔgpgF was quantified using a phosphorous imager (PerkinElmer Life Sciences). Samples were taken in duplicate. Translocated proteins were at least 90% protease-protected. Microsomes were washed with buffer containing 25 mM EDTA for 15 min and 500 mM potassium acetate for 1 h for the experiment shown in Fig. 5D as described (18).

RESULTS

Sss1p and Wbp1p Form a Complex in the ER Membrane—To identify ER membrane proteins that interact with OST, we used a chimeric protein consisting of the OST subunit Wbp1p and Cub, Protein A, and the transcriptional activator LexA-VP16 (Wbp1p-Cub-PLV) as bait (Fig. 1A) (12, 13). This bait has been well characterized and used successfully in the past to demonstrate the fidelity of the split-ubiquitin system to measure protein-protein interactions (12, 13). Interaction of this protein with a NubG fusion protein results in cleavage of the PLV moiety by cytosolic ubiquitin-specific proteases (Fig. 1A, UBP), diffusion of PLV to the nucleus, and transcription of LexA-VP16-controlled reporter genes in our strain HIS3 and LacZ.

A. Nub-Sss1p
B. Wbp1-Cub-PLV
C. LW
LWH/X-Gal

Fig. 3. Mutations in Sss1p perturb the interaction with Wbp1p. A, growth of strains co-expressing NubG-SSS1 or NubG-sss1 and Wbp1-Cub-PLV on minimal plates without leucine and tryptophan (left side, LW) or without leucine, tryptophan, and histidine and with a chromogenic substrate of β-galactosidase (right side, LWH/X-Gal). B, alignment of S. cerevisiae Sss1p amino acid sequence (S.c.) with its homologues in other organisms. Amino acid substitutions in sss1 mutants are indicated. C, helical wheel projection of cytoplasmic amino acids 1–38 of S. cerevisiae Sss1p. Hydrophobic residues are indicated in blue, hydrophilic residues in red, and positions of the amino acids mutated in sss1-1 and sss1-4 are in bold red and circled.

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strain in which neither protein was tagged (Fig. 2B). Under the lysis conditions used, Sss1p antibodies also precipitated the OST subunit Swp1p (Fig. 2C), and Wbp1p antibodies precipitated Sbh1p (Fig. 2D) and Sec61p (not shown), suggesting that both the OST complex and the Sec61 complex were stable and associated with each other. Our data demonstrate a close physical interaction between the Sec61 channel and the OST complex in the ER membrane.

Mutations in Sss1p Disrupt Interaction with Wbp1p in the Split-ubiquitin System—To investigate the functional significance of the interaction of Wbp1p and Sss1p, we used error-prone PCR to generate Sss1p mutants that no longer interact with Wbp1p in the split-ubiquitin system (Fig. 3A). We isolated two mutants, sss1-1 and sss1-4, that expressed mutant Sss1p at wild type levels (not shown). In both sss1-1 and sss1-4, a charged residue replaces a conserved hydrophobic or uncharged residue within the amino-terminal cytoplasmic α-helical domain of Sss1p (sss1-1: Q17 to R; sss1-4: V18 to D; Fig. 3, B and C); in addition, the mutants in the double mutant sss1-4 are predicted to disrupt the α-helix (Fig. 3C). Despite the complete lack of interaction of both mutant Sss1 proteins with Wbp1p in the split-ubiquitin system, both proteins still co-immunoprecipitated Wbp1p (not shown). This suggests that the sss1 mutations affect the orientation of OST and the translocon toward each other rather than completely disrupting their interaction. Haploid strains in which wild type SSS1 had been replaced with either of the mutant genes were viable, and the mutant strains showed no growth phenotype over a temperature range from 16–37 °C (not shown), suggesting that the essential function of Sss1p in protein translocation was not affected.

Mutations in Sss1p Affect N-Glycosylation and Protein Secretion—We predicted that a disturbed interaction between the translocon and OST should result in less efficient glycosylation and, hence, a reduced amount of intracellular glycoproteins after a short pulse. We labeled wild type and sss1 mutant yeast for 5 min with [35S]methionine and lysed the cells either immediately or after a 5-min chase. Intracellular glycoproteins were isolated from the lysates using the mannose-specific lectin Concanavalin A. As shown in Fig. 4A, both sss1 mutants displayed a profound reduction in glycoprotein biosynthesis compared with wild type (58% of wild type after 5 min chase). [35S]methionine incorporation was comparable in wild type and sss1 mutant strains. Most secretory proteins in yeast are N-glycosylated and, for many proteins, glycosylation is important for folding. As only fully folded proteins are packaged into ER-to-Golgi transport vesicles and secreted (20), hypoglycosylation should result in reduced protein secretion. Protein secretion in the sss1 mutants, however, was delayed rather than diminished (Fig. 4B). We observed a similar delayed secretion for individual glycoproteins (not shown). Consistent with the transient nature of the glycoprotein biosynthesis defect in the sss1 mutants, these strains displayed no increased sensitivity to tunicamycin compared with wild type yeast. Our data suggest that N-linked glycosylation and protein folding were inefficient when the interaction of OST with the Sec61 channel was suboptimal but that most proteins were eventually glycosylated and secreted.

We next examined the glycosylation status of specific proteins in the ER of SSS1 wild type and mutant cells. We found that neither carboxypeptidase Y (CPY; four N-linked glycos) nor protein disulfide isomerase (PDI; five N-linked glycans) nor the translocated ER-form of the α-factor precursor (3gpαF, three N-linked glycans) were under-glycosylated in the mutants (Fig. 4C, and not shown) (21–23). Translocation of the cytosolic α-factor precursor ppαF into the ER lumen of sss1 mutants, however, was delayed (Fig. 4C). Note that in wild type and sss1 mutant cells, the translocated, glycosylated α-factor precursor 3gpαF is transported to the Golgi with equal rapidity and proteolytically processed during the 5-min chase, suggesting that there is no defect in transport of secretory proteins in these cells (Fig. 4C, compare 0- and 5-min chase). No translocation defect was detected for the precursors of PDI, CPY, or a protein without N-glycan acceptor sites, BiP, even when the pulse time was reduced to 1 min (Fig. 4C, and not shown).

Fig. 4. Mutants in Sss1p affect glycoprotein biosynthesis and protein secretion. A, SSS1 wild type and mutant cells were pulse-labeled for 5 min and chased for 0 or 5 min. Glycoproteins were precipitated from equal amounts of radiolabeled cell lysates with Concanavalin A-Sepharose, resolved by SDS-PAGE on a 12.5% gel, and quantified using a phosphorous imager (PerkinElmer Life Sciences). The signal in the wild type lanes was set to 100%. Individual glycoproteins affected in sss1 mutants are indicated by black circles. B, cells (black squares, SSS1; open squares, sss1-1; open circles, sss1-4) were labeled for 5 min and chased for the indicated periods of time. At each time point, cells were harvested, and an aliquot of the supernatant was precipitated with trichloroacetic acid and analyzed by scintillation counting. Each time point was taken in duplicate, and the experiment was performed twice. Intracellular transport of CPY (not shown) and 3gpαF ([panel C]) was not affected in the mutants. C, SSS1 wild type and mutant cells were labeled for 5 min and chased for the indicated periods. PDI and ppαF were immunoprecipitated with polyclonal antisera and visualized after electrophoresis on 10% gels (PDI) or 18% 4–18% urea gels (ppαF) using a phosphorescent imager (PerkinElmer Life Sciences). Note that the gel system used can resolve ppαF and paF and that PDI is mildly under-glycosylated in this strain background (5gPDI, fully glycosylated).
Mutations in Sss1p Cause a Translocation Defect Specific for Glycoproteins—The N-linked glycans of α-factor precursor (ppαF) are required for protein folding (24). All three glycosylation acceptor sites of ppαF are in the N-terminal half of the protein, and, in contrast to PDI and CPY, the first is within four amino acids of the signal cleavage site (60 amino acids, PDI; 106 amino acids, CPY) (21–23). Our data suggested that glycosylation of one or more of these sites was essential for efficient ppαF translocation into the ER lumen. We examined this possibility in a cell-free system based on in vitro translated, radiolabeled ppαF and microsomes prepared from SSS1 wild type and mutant cells (25, 26). As shown in Fig. 5A, posttranslational ppαF translocation into wild type microsomes was extremely efficient with a t1/2 of less than 2 min. Translocation of ppαF into sss1 mutant microsomes was significantly slower (t1/2, 5 min), and maximal import was reduced by ~20% compared with wild type. To investigate the effect of the sss1 mutants on cotranslational import, we performed experiments with a modified version of ppαF in which the signal peptide had been replaced with a transmembrane domain (D2HoctαF) (18). Maximal import of this protein was similarly reduced in the mutants (not shown). Decreasing the assay temperature to 10 °C exacerbated the translocation defect in sss1 mutant microsomes (Fig. 5B). To examine whether this difference in import kinetics was indeed related to glycosylation of the translocation substrate, we repeated the experiment with a mutant form of ppαF in which the N-glycan acceptor sites had been removed by site-directed mutagenesis (pΔGpαF) (27). Translocation of pΔGpαF into wild type microsomes was slower than that of ppαF (t1/2 of 5 min versus < 2 min; Fig. 5, C versus A, black squares) but proceeded with identical kinetics in wild type and sss1 mutant microsomes at 20 °C (Fig. 5C). Our data suggest that the translocation defect for ppαF in sss1 microsomes was indeed due to reduced glycosylation efficiency caused by the perturbed translocon-OST association in these membranes.

A recent paper by Nikonov et al. (28) suggests that OST may stay associated with Sec61 channels after termination of translocation. To disrupt existing translocon-OST complexes, we treated SSS1 wild type and mutant microsomes with EDTA and high salt (18). This treatment removes ribosomes from the cytoplasmic face of the microsomes, which may stabilize the interaction of OST with the Sec61 channel. The high salt/EDTA wash also disrupts electrostatic protein-protein interactions mediated by cytoplasmic domains of ER proteins. Translocation of ppαF into salt-washed microsomes was slower than into untreated membranes (Fig. 5, D versus A, black squares) suggesting that the interaction of OST with the translocon had been affected by the EDTA/high salt treatment. Membranes from both sss1 mutants were severely deficient in the reformation of a functional OST/Sec61 channel complex in vitro and, hence, in the import of ppαF (Fig. 5D, open symbols).

**DISCUSSION**

We have shown here that an essential subunit of the protein translocation channel in the ER membrane, Sss1p, mediates binding of the OST complex to the channel via the OST subunit Wbp1p. The role of Sss1p in OST recruitment to the protein translocation channel is independent of its essential function in stabilizing the translocation channel, as mutants in the cytoplasmic domain of Sss1p are viable and display no general defects in protein import into the ER (29, 30) (not shown; Figs. 4 and 5). Appropriate anchoring of OST to the protein translocation channel promotes the immediate glycosylation of translocating proteins and this, in turn, accelerates protein translocation into the ER of a high proportion of proteins with N-glycan acceptor sites and, hence, results in efficient biosynthesis of glycoproteins (Fig. 4A). Using a posttranslationally imported secretory glycoprotein and a mutant version without glycan acceptor sites as a model proteins, we have demonstrated that at physiological temperature, membranes containing mutant Sss1p, which do not interact appropriately with...
Wbp1p, are fully competent for import into the ER of non-glycoproteins (pΔΔppF, Fig. 5C). Thus, the structure of the protein translocation channel itself in the sss1Δ1 and sss1Δ4 mutants is not significantly compromised. The half-time for import of a secretory glycoprotein into the ER, however, was significantly increased in these sss1 mutants (2.5×; Fig. 5A), suggesting that glycosylation contributes to efficient translocation into the ER.

Ours is the first demonstration of an effect of glycosylation on protein translocation into the ER. The acceleration of protein import by glycosylation may be due to improved folding of the luminal part of the translocation intermediate. The N-glycans of ppΔF are indeed required for its folding (24); the unglycosylated mutant form pΔΔppF is not transported efficiently from the ER to the Golgi complex but rather is retained in the ER and subsequently targeted for ER-associated degradation (24, 31). Matlack et al. have shown that ppΔF is prone to backsliding in the translocation channel and that its import into proteoliposomes, which do not contain active OST, requires binding of bulky molecules such as BiP or ppΔF antibodies in the ER lumen (32). In intact cells, glycosylation of the N-terminal prorregion of ppΔF early during translocation into the ER may fulfill a similar function. Although N-glycans themselves cannot prevent backsliding in the translocation channel (33), glycosylation-induced folding of the ppΔF prorregion may interfere with movement of the translocating chain toward the cytoplasm and thus promote vectorial transport of ppΔF into the ER lumen.

The propensity for backsliding in the translocon may be a function of the signal sequence that is recognized by the protein translocation channel itself (34). We found indeed that if the ppΔF signal peptide was substituted with the transmembrane region of dipeptidyl aminopeptidase B (DPAPB) and the protein was cotranslationally imported into microsomes, glycosylation no longer increased the speed of import into the ER, although maximal import into sss1Δ1 mutant membranes was still lower than into wild type microsomes (not shown). Most studies on protein import into the ER are done with cotranslationally imported substrates, which may explain why an effect of glycosylation on import has not been observed so far. Our observation that mutations in SSSI that disturb the OST-translocon interaction lead to a delay in biosynthesis of ∼40% of glycoproteins in Saccharomyces cerevisiae suggests that glycosylation contributes to the efficient import into the ER of a large number of proteins. In a preliminary analysis of secretory glycoproteins in the S. cerevisiae genome, we found that the majority of these contain multiple N-glycan acceptor sites, which makes it likely that their modification contributes to protein folding. In addition, a significant fraction of glycoproteins contain acceptor sites close to the N terminus, where glycosylation is more likely to have an immediate effect on vectorial transport through the protein translocation channel.

At present, we cannot exclude the possibility that additional interactions between subunits of the protein translocation channel and OST subunits contribute to the association between the two complexes. Given that mutations in SSSI alone resulted in a glycosylation-related translocation defect, however, we propose that the binding of Wbp1p to Sss1p is essential for OST-translocon interaction. Further experiments should allow us to address the mechanisms that allow the close cooperation between the Sec61 channel and OST during protein translocation into the ER.

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REFERENCES

1. Johnson, A. E., and van Waes, M. A. (1999) Annu. Rev. Cell Dev. Biol. 15, 599–642.
2. Esnault, Y., Blondel, M. O., Deshaies, R. J., Schekman, R., and Kepes, F. (1993) EMBO J. 12, 4083–4093.
3. Gorlich, D., Frehn, S., Hartmann, E., Kalies, K.-U., and Rapoport, T. A. (1992) Cell 71, 489–503.
4. Wang, L., and Dobberstein, B. (1999) FEBS Lett. 457, 316–322.
5. Beckmann, K., Spahn, C. M., Eswar, N., Helmers, J., Penczek, P. A., Sali, A., Frank, J., and Blobel, G. (2001) Cell 107, 361–372.
6. Kalies, K.-U., Rapoport, T. A., and Hartmann, E. (1998) J. Cell Biol. 141, 887–894.
7. Krauser, R., and Lehle, L. (1999) Biochim. Biophys. Acta 1426, 259–273.
8. Whittle, P., Nilsen, I. M., and von Heijne, G. (1996) J. Biol. Chem. 271, 6241–6244.
9. Popov, M., Tam, L. Y., Li, J., and Reithmeier, R. A. (1997) J. Biol. Chem. 272, 18325–18332.
10. Kreibich, G., Ulrich, B. L., and Sabatini, D. D. (1978) J. Cell Biol. 77, 446–487.
11. Kalies, K.-U., Gorlich, D., and Rapoport, T. A. (1994) J. Cell Biol. 126, 925–934.
12. Stagljar, I., Korostensky, C., Johnson, N., and te Heesen, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5187–5192.
13. Thaminy, S., and Stagljar, I. (2002) in Protein-Protein Interactions (Goelmis, E., ed.), pp. 385–405, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
14. Guthrie, C., and Fink, G. R. (1991) Methods Enzymol. 194, 3–37.
15. Lyman, S. K., and Schekman, R. (1996) J. Cell Biol. 131, 1163–1171.
16. Pilon, M., Schekman, R., and Rimsch, K. (1997) EMBO J. 16, 4540–4548.
17. Hartly, C., Strahl, S., and Rimsch, K. (2001) Mol. Biol. Cell 12, 1093–1101.
18. Ng, D. T., Brown, J. D., and Walter, P. (1996) J. Cell Biol. 134, 269–278.
19. Fu, J., Chen, M., and Kreibich, G. (1997) J. Biol. Chem. 272, 29687–29692.
20. Hurtley, S. M., and Helenius, A. (1989) Annu. Rev. Cell Biol. 5, 277–307.
21. Stevens, T., Esmen, B., and Schekman, R. (1982) Cell 30, 439–448.
22. LaMantis, M., Minura, T., Tachikawa, H., Kaplan, H. A., Lennarz, W. J., and Miznagan, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4453–4457.
23. Kurjan, J., and Herskovitz, I. (1982) Cell 30, 933–943.
24. Kaplan, S., Green, R., Rocco, J., and Kurjan, J. (1991) J. Bacteriol. 173, 627–635.
25. Hansen, W., Garcia, P. D., and Walter, P. (1986) Cell 45, 397–406.
26. Rothblatt, J. A., and Meyer, D. I. (1996) Cell 44, 619–628.
27. Mayinger, P., and Meyer, D. I. (1993) EMBO J. 12, 659–666.
28. Nikonorov, A. V., Snapp, E., Lippincott-Schwartz, J., and Kreibich, G. (2002) J. Cell Biol. 158, 497–506.
29. Esnault, Y., Feldheim, D., Blondel, M.-O., Schekman, R., and Kepes, F. (1994) J. Biol. Chem. 269, 27478–27485.
30. Wilkinson, B. M., Esnault, Y., Craven, R. A., Skiba, F., Friesi, J., KEpes, F., and Stirling, C. J. (1997) EMBO J. 16, 4459–4509.
31. McCracken, A. A., and Bradski, J. L. (1996) J. Cell Biol. 132, 291–298.
32. Matlack, K.-U., Misselwitz, B., Plath, K., and Rapoport, T. A. (1999) Cell 97, 553–564.
33. Oei, C. E., and Weiss, J. (1992) Cell 71, 87–96.
34. Jungnickel, B., and Rapoport, T. A. (1995) Cell 82, 261–270.