The β Subunit of the Sec61p Endoplasmic Reticulum Translocon Interacts with the Exocyst Complex in *Saccharomyces cerevisiae*§

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**The exocyst is a conserved protein complex proposed to mediate vesicle tethering at the plasma membrane.** Previously, we identified *SEB1/SBH1*, encoding the β subunit of the Sec61p ER translocation complex, as a multicopy suppressor of the sec15-1 mutant, defective for one subunit of the exocyst complex. Here we show the functional and physical interaction between components of endoplasmic reticulum translocon and the exocyst machinery. We show that overexpression of *SEB1* suppresses the growth defect in all exocyst sec mutants. In addition, overexpression of *SEC61* or *SSE1* encoding the other two components of the Sec61 complex suppressed the growth defects of several exocyst mutants. *Seb1p* was coimmunoprecipitated from yeast cell lysates with *Sec15p* and *Sec8p*, components of the exocyst complex, and with *Sec4p*, a secretory vesicle associated Rab GTPase that binds to *Sec15p* and is essential for exocytosis. The interaction between *Seb1p* and *Sec15p* was abolished in *sec15-1* mutant and was restored upon *SEB1* overexpression. Furthermore, in wild type cells overexpression of *SEB1* as well as *SEC4* resulted in increased production of secreted proteins. These findings propose a novel functional and physical link between the endoplasmic reticulum translocon complex and the exocyst.

The targeting and fusion of transport vesicles with plasma membrane is mediated by a molecular machinery highly conserved in evolution. Prior to the fusion at the plasma membrane the secretory vesicles are recognized by the exocyst complex, which is proposed to function as a tethering factor for the vesicles at plasma membrane in *Saccharomyces cerevisiae*. This protein complex, composed of *Sec3p*, *Sec5p*, *Sec6p*, *Sec8p*, *Sec10p*, *Sec15p*, *Exo70p*, and *Exo84p* (1, 2), has a central role in establishing cell polarity in yeast (3, 4). The mammalian homologue of the exocyst, the octameric Sec6-Sec8 complex, is represented in Table I. Disruptions of *SEC61* and *Ssh1p* ER translocation complexes, respectively (20–22). The α subunits of the Sec61p and the Ssh1p complexes are *Sec1p* and *Ssh1p*, respectively, which have been shown to form the protein conducting channel (23) and the ribosome binding site at the ER (24). The γ subunit in both complexes is *Ssa1p* (22, 25). These complexes have been shown to function in cotranslational translocation and the Sec61p complex also in the posttranslational translocation (21, 22, 26). The exact functions of the β subunits of the ER translocation complexes are not known.

We have now extended our previous finding on the genetic link between *SEB1* and *sec15-1* mutation and provide biochemical and functional evidence for protein-protein interactions between the ER translocation complex β subunit and components of the exocyst complex and Sec4p.

**EXPERIMENTAL PROCEDURES**

**Strains, Media, and Culture Conditions**—The yeast strains are presented in Table I. Disruptions of *SEB1* and *SSE1* genes in NY179 were done as described (20). To obtain strain H1982, the double *sec15-1* disruptive strain, H1239, was converted to Ura⁻ by transformation with a *Sac1-NsiI* fragment from pBUP and selecting for growth in the presence of 5-fluoroorotic acid. The strain NY1427 (1) was similarly converted to Ura⁻ (H2647) by disrupting the *URA3* with *Spel*-*XhoI* fragment from pJL164 (27). To obtain H1256 the DBY746 strain was transformed with the integrating plasmid YIpLac204a linearized by *BstXI* digestion within the *TRP1* and selected for *Trp* prototrophy. Yeast cells were grown in either YPD (yeast extract peptone dextrose) or SCD (synthetic complete dextrose) (28). For plasmid selection, SCD lacking leucine, tryptophan, or uracil was used. For temperature shift-up experiments, the transformants were grown in selective liquid medium to the early logarithmic growth phase. The cultures were divided in half, and cells were pelleted and resuspended into fresh growth medium. One culture was incubated at 24 °C and the other at the restrictive temperature.

*The abbreviations used are: ER, endoplasmic reticulum; ts, temperature sensitive; HA, hemagglutinin; Pip, 1,4-piperazineethanesulfonic acid.*

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§ The abbreviations used are: ER, endoplasmic reticulum; ts, temperature sensitive; HA, hemagglutinin; Pip, 1,4-piperazineethanesulfonic acid.
Plasmids and DNA Methods—Plasmids YEpHA-SEB1 and pBSE1 have been described (20). The SEB1 cDNA covering the open reading frame was transferred from pBSE1 under TPI1 promoter in pTX212 multicopy vector (R&D Systems) as an EcoRI-XhoI fragment yielding plasmid YEpT-SEB1U. YEpBSE1U is pRS425 (29) containing the SEC62 gene in YEp352 (37) was obtained from Randy Schekman. Standard DNA methods were used for cloning. The yeast cells were transformed as described (20).

| Strain       | Genotype                                                                 | Source or reference |
|--------------|---------------------------------------------------------------------------|---------------------|
| s350-14Da    | Mata sec1-1 his4-s80 leu2-3,112 trp1-289 ura3-52                           | R. Schekman         |
| NY3          | Mata sec1-1 ura3-52                                                       | P. Novick           |
| NY15         | Mata his4-619 ura3-52                                                     | P. Novick           |
| NY179        | Mata leu2-3,112 ura3-52                                                   | P. Novick           |
| NY770        | Mata sec2-41 leu2-3,112 ura3-52                                          | P. Novick           |
| NY772        | Mata sec3-2 leu2-3,112 ura3-52                                          | P. Novick           |
| BY43         | Mata leu2 his3 ura3 sec3-101                                             | Haarer et al. (37)  |
| NY774        | Mata sec4-8 leu2-3,112 ura3-52                                          | P. Novick           |
| NY776        | Mata sec5-24 leu2-3,112 ura3-52                                         | P. Novick           |
| NY778        | Mata sec6-4 leu2-3,112 ura3-52                                          | P. Novick           |
| NY780        | Mata sec8-9 leu2-3,112 ura3-52                                          | P. Novick           |
| NY782        | Mata sec9-4 leu2-3,112 ura3-52                                          | P. Novick           |
| NY784        | Mata sec10-2 leu2-3,112 ura3-52                                         | P. Novick           |
| CKY46        | Mata sec13-1 his4-619 ura3-52                                            | C. Kaiser           |
| NY786        | Mata sec15-1 leu2-3,112 ura3-52                                          | P. Novick           |
| NY1427       | Mata sec8::URA3 leu2-3,112::(LEU2, SEC8-3x-c-myc) ura3-52 L-A-o           | TerBush et al. (1)  |
| BY55         | Mata sec17-1 ura3-52                                                     | P. Brennwald        |
| m BY12-6Da   | Mata sec18-1 his leu2-3,112 trp1-289 ura3-52                               | R. Schekman         |
| NY1213       | Mata sec19-1 leu2-3,112 ura3-52                                          | P. Novick           |
| H1063        | Mata soo2-1 soo1::HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 | Jantti et al. (31)  |
| YW21-1A      | Mata ade2 his3 leu3 tyr1 trp1 3 amin5 (slp1):LEU2                          | Y. Wada and Y. Anraku|
| 312A         | Mata sly1 ade2-1 leu2-3,112 trp1 ura3-1                                    | J. W. Walker        |
| MS177        | Mata kar2-159 ade2-101 ura3-52                                          | M. Rose             |
| MS1378       | Mata kar2-133 ade2-101 ura3-52                                          | M. Rose             |
| RSY151       | Mata sec62-1 leu2-3,112 ura3-52                                         | R. Schekman         |
| RSY285       | Mata sec61-2 ade2 leu2-3,112 pep4-3 ura3-52                               | R. Schekman         |
| RSY355       | Mata sec61-3 his4 leu2-3,112 trp1-1 ura3-52                               | R. Schekman         |
| RSY529       | Mata sec62-1 his4 leu2-3,112 ura3-52                                     | R. Schekman         |
| H1047        | Mata seb1::URA3 leu2-3,112 ura3-52                                       | This study          |
| H1239        | Mata seb1::URA3 seb2::KanMX4 leu2-3,112 ura3-52                           | This study          |
| H1256        | Mata his3::leu112 ura3-52 trp1-298::YEpplac204a (TRP1) ura3-52 CYH#        | This study          |
| H1679        | Mata seb1::URA3 leu2 ura3                                               | This study          |
| H1982        | Mata seb1::URA3::ura3 seq2::Kon ura3-52 ura3-52                           | This study          |
| DBY746       | Mata his3::leu112 ura3-52 trp1-298::URA3 eg3-52 CYH#                      | D. Botstein         |

Interactions between Seb1p, the Exocyst, and Sec4p

Yeast strains used in this study

| Strain       | Genotype                                                                 | Source or reference |
|--------------|---------------------------------------------------------------------------|---------------------|
| MATa sec1-1  | his4-s80 leu2-3,112 trp1-289 ura3-52                                       | R. Schekman         |
| MATa sec1-1  | his4-s80 leu2-3,112 trp1-289 ura3-52                                       | R. Schekman         |
| H1982        | seb1::URA3::ura3 seq2::Kon ura3-52                                         | This study          |
| MATa sec1-1  | his4-s80 leu2-3,112 trp1-289 ura3-52                                       | This study          |

Multicopy Suppression—The mutant strains were transformed with the multicopy plasmid overexpressing the gene under study or with the pertinent vector control. The multicopy suppression was first screened by replica plating freshly grown patches on selective plates and on YPD and growing them at different temperatures. Growth was monitored for 3 successive days. The suppression test was then done by dotting 10⁶ cells and three 10-fold dilutions thereof on selective plates and incubating the plates at different temperatures for 3 days. The patching method was slightly more sensitive and rescued the growth at a slightly higher temperature or detected in some cases weak suppression that was not detected by dotting.

Determination of α-Amylase and Invertase Activity—Yeast cell growth, preparation of the periplasmic and cytoplasmic fractions, and determination of the α-amylase activity were done as described previously (42). Invertase activity was determined as described (42) except that the reaction was terminated with addition of 1 Tris, pH 8.5, after which the cells were pelleted instead of filtering and the glucose liberated from sucrose by invertase was measured from the supernatant.

Antibodies and Protein Methods—Antibodies against an N-terminal peptide of Seb1p have been described (20). The antibodies against Sec4p and Sec15p were raised in rabbits injected with bacterially (Escherichia coli BL-21de) expressed and purified His₆-Seb1p and His₆-Sec15p (amino acids 1–241). HA 125A5 and Myc tag antibodies (9E10) were purchased from Roche Applied Science and Santa Cruz Biotechnology, respectively. For detection of Sec4p in Western blots, an IgG heavy chain-specific secondary antibody (Jackson Immunoresearch Laboratories Inc.) was used to reduce signals from closely migrating IgG light chain specific bands. Other antibodies were generous gifts from Howard Riezman (antibodies against Gas1p) and Romano Serano (anti-Fma1p antibodies). The proteins were separated in SDS-PAGE using the buffer system of either Laemmli (43) or Schagger and von Jagow (44) with acrylamide concentrations from 7.5 to 15% as appropriate for the proteins analyzed and transferred into Hybrid-P filter (Amersham Biosciences). The protein bands were visualized with the ECL detection system (Amersham Biosciences).

Preparation of Yeast Cell Lysates and Immunoprecipitations—Transformants of strains H2647 and NY15 were grown overnight in SCD-Ura to early logarithmic growth phase of A₆₀₀ = 1 and pelleted. The cells were washed with 10 mM Na₂SO₄ and resuspended in spheroplasting
buffer (1.4 M sorbitol, 20 mM triethanolamine, pH 7.5, 40 mM β-mercaptopro- 
thanol) containing 10 mM NaCl. Cells were converted to spheroplasts with 150 
µg/ml zymolase (Seikagaku Corp.). The spheroplasts were cooled on ice, pelleted, 
washed with spherowash (20 mM triethanolamine, pH 7.5, 1.4 M sorbitol), and 
gently lysed in either homogenization buffer (20 mM Hepes, pH 7.4, 100 mM 
NaCl 1 mM EDTA 1% Nonidet P-40) or buffer B (20 mM Pipes, pH 6.8, 100 mM 
NaCl, 1 mM EDTA, 1% dithiothreitol, 1% Nonidet P-40) by repeated pipetting 
and 30 min of incubation on ice. Buffer B was utilized to improve the 
viability of the Sec15-Sec8 complex (45). Seb1p was coprecipitated with 
aSe1p antibodies with equal efficiency from cell lysates prepared 
using either homogenization buffer or buffer B. The cell homogenates 
were centrifuged at 10,000 x g for 10 min at 4 °C, and the supernatant 
was used for immunoprecipitations. 

1.1 mg of total protein of cell lysates in homogenization buffer or 
buffer B was precleared with protein G-Sepharose (Amersham Biosciences) 
for 30 min at 4 °C and centrifuged at 20,000 x g for 15 min. 
Precleared lysate containing 1 mg of total protein was subjected to 
overnight immunoprecipitation with 3 
Precleared lysate containing 1 mg of total protein was subjected to 
immunoprecipitation with 3 

RESULTS

Overexpression of SEB1 Suppresses Temperature-sensitive
(ts) Defects of Exocyst Mutants—SEB1 encoding the β subunit 
of the Sec61p complex was isolated as a multicopy suppressor of the temperature sensitive sec15-1 mutation (20). As Sec15p is a component of the exocyst complex, it was of interest to see 
whether overexpression of SEB1 can suppress temperature-sensitive 
mutations in the other components of the complex. The exocyst 
sec mutant strains were transformed with a 
multicopy plasmid encoding SEB1 or with an empty vector, and 
their ability to grow at the permissive temperature was 
tested. Growth of all the exocyst sec mutants was rescued at the 
restrictive temperature at least partially by SEB1 overexpression 
(Fig. 1). The growth of sec8-9 and sec15-1 was rescued near 
or at the level of growth at the permissive temperature, whereas partial rescue was obtained for the rest of the 
mutants. Possible suppression of the secretion defect of sec8-9 and 
sec15-1 cells at the restrictive temperature by SEB1 overexpression was also tested. Overexpression of SEB1 ameliorated 
defective secretion of invertase by these mutants but did not 
fully restore it to the level of wild type cells (data not shown).

We also studied possible synthetic interactions between 
seb1Δ or seb1Δ seb2Δ and the exocyst mutants (sec3-101, sec5- 
24, sec6-4, sec8-9, sec10-2, and sec15-1). Deletion of either SEB1 or its close homologue SEB2 does not create a phenotype, but 
simultaneous deletion of both genes results in temperature sensitivity at 38 °C (20, 22). All of the exocyst mutants were 
crossed with seb1Δ or with seb1Δ seb2Δ strains followed by 
tetrad analysis. No interactions were observed between seb1Δ and 
exocyst mutants. The triple mutants seb1Δ seb2Δ sec10-2 
and seb1Δ seb2Δ sec15-1 derived from crosses of the double 
deletant seb1Δ seb2Δ together with sec10-2 or with sec15-1 mutation were 1-2 °C more temperature-sensitive than the 
seb1Δ seb2Δ double mutant or the single mutants sec10-2 and 
sec15-1 (data not shown). The suppression data and the syn- 
thetic interactions demonstrate multiple genetic interactions 
between the Sec61p and the exocyst complex.

Interactions between Seb1p, the Exocyst, and Sec4p
The restrictive temperatures for the mutant strains are: sec3-2, 36°C; sec5-24, 34°C; sec15-1.

Seb1p-MA did not suppress the temperature sensitivity of transformants was monitored at different temperatures. The studied genes encoding components of the exocytosis machinery was investigated. The effects in the ER translocation components by overexpression of SEB1, SEC3, SEC15, SOS1, or SOS2 gene (data not shown). The double deletion of both SEB1 and SEB2 results in impaired growth at 38°C (20, 22). The growth of this double mutant was not rescued by overexpression of any of the late-acting secretion genes tested. Thus, the suppression is unidirectional; only defects in the exocytosis machinery are corrected by increased amounts of the ER translocon components and not vice versa.

The Seb1p Transmembrane Domain Is Required for sec15-1 Suppression—Seb1p is a membrane protein with a single C-terminal transmembrane domain (20). To test whether the suppression activity of Seb1p requires the transmembrane domain and thus tight membrane association, a mutant version of Seb1p lacking the hydrophobic C-terminal membrane anchor was created. The sec15-1 cells were transformed with the plasmid encoding the mutant, YEpSEB1-MA, the plasmid encoding the wild type SEB1, or the empty vector, and the growth of the transformants was monitored at different temperatures. The Seb1p-MA did not suppress the temperature sensitivity of sec15-1 even at 35°C, the lowest temperature restrictive for sec15-1 cells (Fig. 3A). The Seb1p-MA mutant did not affect the cell growth at the permissive temperature (24°C), indicating that its expression is not harmful (Fig. 3A). The lack of a phenotype for the Seb1p-MA overexpression could be because of instability of the mutant protein. To test this possibility, lysates were prepared from the transformants used in the suppression experiment, and the amounts of Seb1p and Seb1p-MA were studied by Western blotting with Seb1p-specific antibodies. Quantitation of the blots showed that the wild-type Seb1p was ∼14 times and the Seb1p-MA 10 times overexpressed in comparison to the endogenous Seb1p level (Fig. 3B). This indicates that Seb1p-MA is stably expressed in sec15-1 cells but fails to multicopy suppress this mutant.

Seb1p Coimmunoprecipitates with the Exocyst Components Sec8p and Sec15p in Yeast Cell Lysates—The genetic interactions between SEB1 and the exocytosis machinery prompted us to study protein-protein interactions between Seb1p and the exocyst proteins. For detection of Sec8p, a yeast strain, H2647, was used in which the only chromosomal copy of SEC8 gene encodes a protein tagged with three Myc epitopes at its C terminus (1). This strain was transformed either with a multicopy plasmid expressing HA-tagged SEB1 from the ADH1 promoter (20) or with an empty vector. In parallel, wild type strain NY15 was transformed with the same plasmids. The HA-tagged form of Seb1p is fully functional such that its overexpression suppresses the ts growth defects of sec15-1, sec10-2, and sec8-9 like the wild type gene (data not shown) and was utilized in these experiments to detect the overexpressed Seb1p. Antibodies against the N-terminal peptide of Seb1p coprecipitated the Myc-tagged Sec8p (Fig. 4, top panel). The

Fig. 2. SEC61 and SSS1 multicopy suppression of the exocyst mutants. Mutant cells carrying pBW65 or the empty vector pRS425 were dotted on SCD-Leu plates (A), and those carrying FKp53 or the empty vector YEp24H were dotted on SCD-Ura plates (B) as described under “Experimental Procedures” and grown at various temperatures. The restrictive temperatures for the mutant strains are: sec3-2, 36°C; sec5-24, 34°C; sec6-4, 34°C; sec8-9, 35°C; sec15-1, 36°C.

Fig. 3. The Seb1p membrane anchor is required for sec15-1 multicopy suppression. sec15-1 cells carrying YEpSEB1-MA, YEpT-SEB1U, or the appropriate vector control were grown on SCD-Ura plates at different temperatures. Seb1p lacking the transmembrane domain cannot suppress the temperature-sensitive growth phenotype of sec15-1 in contrast to the full-length Seb1p (A). The expression levels of Seb1p-MA and Seb1p were evaluated (B). Cell lysates were prepared from the sec15-1 cells used in the suppression experiment. 10 μg of total protein of each lysate were separated in 15% SDS-PAGE, transferred to nitrocellulose filter, and probed with anti-Seb1p antibodies and quantitated by densitometry. Similar amounts of Seb1p and Seb1p-MA are expressed in sec15-1 cells. Molecular weight markers are shown on the left.
Seb1p antibodies precipitated 1.6% of the Sec8-Myc protein present in the cell lysate used for precipitation. The coprecipitation of Sec8p-Myc was genuinely due to the presence of the Seb1p antibodies as evidenced by the absence of Sec8p-Myc in the mock precipitations carried out in the absence of the Seb1p antibodies (data not shown) or in the presence of the preimmune serum (Fig. 4, top panel, pis IP). In the Western blots the anti-Myc antibodies also detected a slightly faster migrating protein band, which further indicates that Seb1p is brought down nonspecifically with immunocomplexes formed in the yeast cell lysates, two cell surface proteins not related to the exocytosis machinery were used as controls. Thus, immunoprecipitations with antibodies against Pma1p, an abundant plasma membrane protein (50), and Gas1p, a glycosylphosphatidylinositol-anchored plasma membrane cell wall protein (51), were performed. Neither one was able to coprecipitate Seb1p (data not shown), which further indicates that Seb1p immunoprecipitation together with the exocyst components and Sec4p is not because of nonspecific cross reactions.

Overexpression of SEB1 Recovers Seb1p-Sec15p Interaction in sec15-1 Cells—As SEB1 was isolated originally as a multicy copy suppressor able to rescue the growth of sec15-1 cells at the restrictive temperature, we next tested whether the putative Seb1p-Sec15p complex is present in the sec15-1 mutant cells. The sec15-1 cells overexpressing SEB1 or those carrying the empty vector plasmid were lysed and subjected to immunoprecipitations with antibodies against Sec4p. Seb1p coimmunoprecipitated with Sec4p as did the Sec8p-Myc, indicating that the complex that is formed by the exocyst components and Sec1p also contains Sec4p (Fig. 4, bottom panel). The preimmune serum did not precipitate Seb1p, Sec8p-Myc, or Sec4p.

To rule out the possibility that Seb1 protein is brought down nonspecifically with immunocomplexes formed in the yeast cell lysates, two cell surface proteins not related to the exocytosis machinery were used as controls. Thus, immunoprecipitations with antibodies against Pma1p, an abundant plasma membrane protein (50), and Gas1p, a glycosylphosphatidylinositol-anchored plasma membrane cell wall protein (51), were performed. Neither one was able to coprecipitate Seb1p (data not shown), which further indicates that Seb1p immunoprecipitation together with the exocyst components and Sec4p is not because of nonspecific cross reactions.

Overexpression of SEB1 or SEC4 Increases Production of Secreted Proteins in Wild Type Yeast—The above genetic inter-
action results suggest that SEB1 overexpression facilitates exocytosis. We have shown previously that overexpression of the SSO1 and SSO2 genes encoding plasma membrane t-SNAREs (target-soluble N-ethylmaleimide-sensitive factor attachment protein receptors), essential for exocytosis, increases the production of secreted proteins (42). We therefore proceeded in studying the effect of SEB1 overexpression on production of secreted proteins in wild type cells. Secretion of the yeast endogenous enzyme invertase was studied in DBY746 cells grown in 2% sucrose medium to allow expression of the glucose repressed SUC2 gene. Secretion of invertase to the cell surface was analyzed at several time points during growth in liquid medium, and reproducibly, up to 40% higher invertase activity was detected with SEB1 overexpression. This is the same level that is obtained with SSO2 overexpression from the endogenous SUC2 gene (42).

We also studied the effect of SEB1 overexpression on secretion of a heterologous reporter protein, Bacillus α-amylase (42). The α-amylase gene was integrated into the yeast genome at the TRP1 locus. This strain, H1256, was transformed with YEpHA-SEB1 or the empty vector, pVT102U. The transformants were grown in shake flasks in selective medium, and the amount of α-amylase secreted into the culture medium was determined. In comparison with the control transformant, up to 3-fold more α-amylase activity was found in the medium of SEB1 overexpression strain depending on the cultivation time point analyzed (Fig. 6A). The increased α-amylase activity in the medium was due to increased amount of the enzyme as determined by Western blotting (data not shown). To verify whether the increased production of α-amylase was due to increased transcription of the gene, the α-amylase mRNA levels were determined by Northern blotting. The α-amylase transcript level was increased slightly in the SEB1 overexpressing strain, being about 1.5-fold compared with that in the control strain. We next studied the effect of overexpression of SEC4 on α-amylase production. Sec4p is a small GTPase that is essential for regulation of exocytosis and binds to the post-Golgi components of the exocyst complex (20). This unexpected finding prompted us to study the genetic interactions in more depth and to search for potential physical interactions between the protein components functioning at these early and late steps of secretion.

Here we show that overexpression of SEB1 suppresses the ts mutations in all SEC genes encoding components of the exocyst complex. A functional link between the ER translocon and the exocyst complex is further supported by the increased temperature sensitivity detected in seb1Δ seb2Δ sec10-2 and in seb1Δ seb2Δ sec15-1 triple mutants. Although this synthetic effect was not strong, it may be important, because Sec10p and Sec15p form a subcomplex in vivo suggested to bridge the transport vesicle with the remaining exocyst components (49).

Overexpression of the other two genes encoding components of the Sec61p complex, SEC61 and SSS1, also suppressed the ts defects in several of the exocyst mutants. The other late-acting mutations suppressed by SEB1 overexpression were sec2-41 and sec19-1 (data not shown). The Sec2p and Sec19p are regulators of the Sec4p, a Rab GTPase that interacts physically with Sec15p (49). These genetic interactions strongly suggest
closely functional connections between the ER translocation complex and the exocytosis machinery than was surmised before. The role of the nonessential β subunits of the Sec61p and Ssh1p complexes in translocation is not known. Disruption of both SEB1 and SEB2 results in only a slight reduction in protein translocation and Gas1p maturation (22). The mammalian Sec61β is not essential for translocation but has been shown to facilitate this process (52). In yeast, overexpression of SEB1 suppresses the sec61 mutations that destabilize the Sec61p structure (20, 33, 53). However, mutations that do not affect the stability of Sec61p, but render it nonfunctional in Sec61p structure (20, 33, 53). This suggests an accessory or stabilizing role for the β subunit in the complex.

The overexpression suppressions reported here show strict directionality; only the ER translocon components can rescue the function of the mutated exocyst components and not vice versa. Thus, the observed genetic interactions and the rescued physical interaction of Seb1p with Sec15p-1 mutant protein in SEB1 overexpressing cells (Fig. 5) suggest that increased Seb1p level facilitates directly or indirectly the function of the destabilized (2, 55, 56) exocyst complex in the mutant cells. Cosegregation of Seb1p with exocyst components Sec8p and Sec15p, and the Sec15p-interacting protein Sec4p, implies that these proteins can be present in the same protein complex within the yeast cell. Notably, these coprecipitations were detected also in cells expressing the endogenous level of Seb1 protein. Under the experimental conditions used here the proportion of Seb1p communoprecipitating with Sec8p or Sec15p was low. The interactions were, however, specific as shown by the control experiments performed. The low communoprecipitation efficiencies may be due in part to the fact that Seb1p may not need to exist in a stable complex with exocyst components to fulfill its biological function. We favor the view that the observed interaction reflects the existence of a regulatory signal between the two endpoints of the secretory pathway. Such interactions are likely to be transient by nature and do not necessarily require simultaneous association of a large proportion of the cellular Seb1p with exocyst subcomponents. We would also like to stress that the fact that Seb1p overexpression rescued the interaction with the mutant Sec15p and the cell growth indicates a direct or indirect functional interaction between these molecules. Additional support for the interaction is provided by the extensive genetic data and the observed secretion enhancement, which collectively point to an in vivo interaction of these processes.

The interactions between the ER translocon and the exocyst components are intriguing because functionally the Sec61p translocation complex and the exocyst are located at opposite ends of the secretory pathway. It has, however, been shown that the cortical ER membrane and the plasma membrane frequently run in parallel in yeast cells (57, 58). Thus, at least

a portion of the Sec61p and the exocyst complexes can be in close proximity with each other within a cell. Lipschutz et al. (Ref. 65; accompanying article) demonstrate that ER translocation machinery components can exist in a complex with components of the exocyst also in Madin-Darby canine kidney cells. Interactions between the exocyst and ER resident proteins are supported also by the observed communoprecipitation and partial colocalization of the mammalian Sec69p with the inositol 1,4,5-trisphosphate receptor, IP3-R (59).

The physical interactions with the exocyst complex both in yeast and mammalian cells were studied only with the β subunit of the ER translocons. In these experiments several components (Sec6, Sec8, Sec10, and Sec15) of the exocyst complex were detected. Although our genetic data suggest that all components of these complexes may have roles in these interactions, the β subunit clearly seems to play the most important role. Increased production of secreted proteins was achieved by overexpression of SEB1 only, not with that of SEB1 or SEB2. The overexpression of SEB1 was most efficient also in the suppression of the exocyst mutations. Overexpression of a mutant form of Seb1p lacking the transmembrane domain failed to suppress sec15p-1 temperature-sensitive growth. This suggests that association of Seb1p in the ER membrane may be required to establish the functional link with the exocyst complex. Presently, we do not know the proportion of Seb1p in the translocating Sec61p complexes in SEB1 overexpressing cells. As the amount of Sec61p does not increase in an Seb1p-overexpressing strain (data not shown), a portion of Seb1p may exist outside of these complexes under these conditions. The possible contribution of the unassembled Seb1p to the interaction remains to be studied. It may be that Seb1p interaction with the exocyst is not direct but is mediated by other, presently unknown factors.

In addition to the observed physical and genetic interactions, an additional functional link was observed between the early and late processes of protein secretion. Both overexpression of Seb1p and the Sec15p-interacting protein, Sec4p, resulted in enhanced protein secretion in yeast. Increased production of secreted proteins in yeast can occur as a result of ER proliferation caused by overexpression of a heterologous ER membrane protein (60, 61). On the basis of previous morphological experiments, even the strongly overexpressed Seb1p is localized in the ER (20). When analyzed by electron microscopy, SEB1 overexpression does not cause ER membrane proliferation nor does overexpression of Seb1p increase the levels of Sec61p or Sec15p (data not shown). Lipschutz and colleagues (62) have shown that hsc10 overexpression in Madin-Darby canine kidney cells increases the amount of secreted and basolateral membrane proteins. Similarly, we show here that overexpression of Sec4p, a Sec15p-interacting protein, results in enhancement of protein secretion. The overexpression of SEC15 is toxic

Supplementary Table II

| Control strain | SEB1 transformant | SEB1/control |
|---------------|------------------|--------------|
|               | α-Amylase | %      | α-Amylase | %      | % |
| Culture medium | 22.7 (15.2–27.7) | 70.7 | 52.4 (47.4–64.5) | 87.6 | 2.3 |
| Periplasm      | 1.3 (0.6–2.3)    | 4.0  | 1.6 (0.9–2.9)   | 2.7  | 1.2 |
| Cytoplasm     | 8.1 (4.2–12.7)   | 25.3 | 6.2 (2.4–10.6)  | 10.3 | 0.8 |
| Total         | 32.1 (23.0–41.3) | 100 | 60.2 (55.8–76.3) | 100 | 1.9 |

α-Amylase activity (units × 10–5/h), a The ratio of α-amylase activity between SEB1 transformant and control strain. b The variation in independent experiments.

The values represent a mean from two duplicate SEB1 overexpression or control plasmid transformant of DBY746 strain from three independent cell fractionations.

a J. Toikkanen and S. Keränen, unpublished observation.
to yeast cells. Thus, its possible enhancing effect on secretion could not be studied. In an accompanying article, Liptschutz et al. (65) show an unaltered transcript level of proteins with increased secretion levels. Likewise, enhanced production of α-amylase in SS10 or SS12-overexpressing cells is not because of increased transcription of the α-amylase gene (42). Taken together, these results suggest that the increased production of secreted proteins in the overexpression strains occurs at post-transcriptional level.

In mammalian cells the Sec61β subunit has been shown to bind nontranslating ribosomes (63). Thus, it is possible that increased amount of the β subunit facilitates ribosome binding to ER and thereby increases the number of ribosomes that are available for binding and translation of the messengers of secreted proteins in SEB1 overexpressing cells. This perhaps explains the observed small increase in the α-amylase transcript level in SEB1 overexpressing yeast cells. Efficient ribosome binding and ER targeting have been shown to increase the half-life of mRNAs in yeast (63, 64). Although a link between the ER translocation machinery and exocyst is observed both in yeast and in mammalian cells, differences in forming and maintaining this link are likely to exist because of the different cellular signals that these cells encounter in their growth environments.

The functional and physical interactions between the ER translocon and the exocyst complex indicate the existence of a regulatory circuit that may be used for adjusting the level of translation to the secretory capacity of the cell. Increasing the level of a component involved in this regulation could lead to enhanced production of secreted proteins in wild type cells. Along the same lines, this cross-talk would be compromised by a decreased amount of (or defective) regulatory component, e.g. destabilized exocyst complex in the mutant cells. Increasing the amount of an interacting component, in this case Seb1p, could restore sufficient communication even in mutant cells and allow growth at the nonpermissive temperature. This view is supported by the fact that Sec15-1p and Seb1p were found in the same protein complex only when SEB1 was overexpressed (Fig. 5).

Taken together, our data reported here reveal novel links between molecular complexes of the ER translocon and the exocyst complex, implying new regulatory circuits between the ER and plasma membrane in the control of protein secretion in yeast.

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