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Interactions between Spc2p and Other Components of the Endoplasmic Reticulum Translocation Sites of the Yeast *Saccharomyces cerevisiae**

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In yeast, the endoplasmic reticulum membrane proteins Sec11p and Spc3p are essential for the cleavage of signal peptides of nascent polypeptide chains during their passage through translocation sites. Genetic and biochemical experiments demonstrate that Sec11p and Spc3p are tightly associated with two other proteins, Spc1p and Spc2p, whose functions are largely unknown. Using anti-Spc2p antibodies, we show here that this heterotetrameric complex associates with Sbh1p and Sbh2p, the β-subunits of the Sec61p complex and the Ssh1p complex, respectively. Depletion of Spc2p decreased the enzymatic activity of the SPC *in vitro,* led to a loss of Spc1p, and led to a down-regulation of the amount of Sec11p and Spc3p in the endoplasmic reticulum. Moreover, the deletion of Spc2p also decreased the expression level of Sbh2p. These data implicate that Spc2p not only enhances the enzymatic activity of the SPC but also facilitates the interactions between different components of the translocation site.

Transport of proteins across the translocation sites in the endoplasmic reticulum (ER)[1] membrane is triggered by signal sequences that are usually located at the amino terminus of the polypeptide chain (for review, see Refs. 1 and 2). It is performed either co-translationally, with the ribosome tightly bound to the membrane, or post-translationally. In both cases the central component of the translocation site is a heterotrimeric Sec61 complex, which consists of an α-subunit, spanning the membrane 10 times, and two small single spanning proteins, the β-subunit and the γ-subunit. The complex forms the hydrophilic channels through which the protein translocation occurs and is responsible for the signal sequence recognition in the ER membrane. During co-translational translocation, the Sec61 complex is also responsible for the tight binding of the ribosome to the translocation site.

In yeast, two different types of ribosome-bound Sec61 complexes exist, which most likely are involved in the co-translational pathway: the Sec61p complex and the Ssh1p complex. The two complexes differ in both their α-subunits (Sec61p and Ssh1p) and β-subunits (Sbh1p and Sbh2p), but contain the same γ-subunit (Sss1p) (3–5). In addition, yeast contains a Sec complex consisting of the Sec61p complex and the Sec62/63 subcomplex which is essential and sufficient for post-translational translocation (4). Deletion of *SEC61*, *SSS1*, *SEC62*, or *SEC63* results in a massive accumulation of precursors of secretory proteins and is lethal (3, 6, 7). In contrast, the Ssh1p complex is not essential for cell viability, but is required for a normal growth rate. Additionally, the β-subunits of the Sec61-like complexes, Sbh1p and Sbh2p, are not essential for the function of the respective translocation sites. However, cells lacking both proteins accumulate precursors of secretory proteins in the cytoplasm, and their growth at elevated temperatures is severely impaired (5).

Another component of the ER translocation site is the signal peptidase complex (SPC), which removes signal sequences from the nascent chains during the transport process. In yeast, the SPC consists of four different polypeptides named Spc1p, Spc2p, Spc3p, and Sec11p (8). Spc3p and Sec11p have single transmembrane spans with the majority of the protein being located in the ER lumen. Both proteins are essential for yeast viability. Deletion of either protein leads to the loss of signal peptidase activity both, *in vivo* and *in vitro* (9–11). Sec11p shows some homology to the bacterial leader peptidase LepB (12, 13) and site-directed mutagenesis experiments demonstrate that it forms the active center of the SPC (14).

Nothing is known so far about the function of Spc1p and of Spc2p. Their mammalian homologues Spc12 and Spc25 probably span the membrane twice with only very few residues facing the ER lumen. Based on this topology, it was suggested that these proteins may also perform functions independent from the actual proteolytic activity of the SPC (15). This idea is supported by the fact that chicken Spc12 and Spc25 are not needed for the proteolytic activity of the SPC *in vitro* (16). Additionally, yeast *SPC1* and *SPC2* are not essential for the survival of the cells at temperatures below 38 °C. However, at 42 °C Δ*spc2* mutants display an accumulation of precursors of secretory proteins *in vivo* and a reduced cell viability (17, 18).

We show here that Spc2p is required for full enzymatic activity of the SPC *in vitro*. In addition, depletion of Spc2p decreases the expression levels of other SPC components and of Sbh2p. Immunoprecipitation also demonstrates that Spc2p is in complex with Sbh1p and Sbh2p, the β-subunits of Sec61-like complexes. Together, these data implicate that a major function of the Spc2p is to facilitate the interactions between different components of the translocation site.

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[1] The abbreviations used are: ER, endoplasmic reticulum; eq, membrane equivalent; PAGE, polyacrylamide gel electrophoresis; SPC, signal peptidase complex; YPD, yeast extract peptone dextrose; YPGal, yeast extract peptone galactose; OD, optical density; wt, wild type; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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EXPERIMENTAL PROCEDURES

General Methods—Media and cell growth conditions have been described elsewhere (4, 5). Specific modifications to these procedures are indicated in the text.

Yeast Strains—Yeast strains were as follows: DF5, *mat* ***α/a***, *trp1–1(am)/trp1–1(am), his3-Δ200/his3-Δ200, ura3–52/ura3–52, lys2–801/lys2–801, Leu2–3,-112/Leu2–3,-112* (10); SEY62.10, *mat* ***a*** *his3-Δ200 leu2–3,112 lys2–801 suc2-Δ9 trp1-Δ901 ura3–52 GAL* (19); HFY403, *mat* ***α*** *Δspc2::URA3 his3-Δ200 leu2–3,112 lys2–801 trp1-Δ901 ura3–52* (18); HFY401, *mat* ***a*** *Δspc1::TRP1 ade2–101 his3-Δ200 leu2–3,112 lys2–801 trp1-Δ901 ura3–52* (17); and HFY404, *mat* ***a*** *Δspc1::TRP1 Δspc2::URA3 ade2–101 his3-Δ200 leu2–3,112 trp1-Δ901 ura3–52* (18).

Plasmid Construction—The plasmid pHM3 used for complementation of the *Δspc2* mutation was constructed as follows. *SPC2* was amplified by polymerase chain reaction using a forward oligonucleotide (ATGAGTTCTGCTAAACCTATTATG) starting from the start codon of *SPC2* and a reverse primer (CAAGGTACCGAAGATGTTATCAAAG), which is located 680 base pairs downstream of the *SPC2* stop codon. The product was digested with *Kpn*I and the resulting fragment was cloned into a pRS414 (20) at *Sma*I/*Kpn*I site. A 500-base pair *Bam*HI fragment containing the Gal10 (21) promotor was inserted into the *Bam*HI site upstream of the *SPC2*.

Spc2p Depletion Experiment—Cells of the strain HFY403 that harbored the plasmid pHM3 were preincubated overnight in YPGal medium (10). The cells were diluted to a final concentration of 0.1 OD_{600} in YPGal. After a 1-h incubation at 30 °C, the cells were harvested by centrifugation and were resuspended in the same volume YPD medium. After 6 h, the culture was diluted again from 0.7 OD_{600} to 0.1 OD_{600} with YPD medium. After 10 h, the cells were shifted again to YPGal medium by harvesting and resuspending in the same volume of YPGal medium. At indicated time points, aliquots of the culture were collected and microsomes prepared as described (10). To obtain comparable amounts of membranes, the samples were normalized to same amounts of Sec62p by SDS-PAGE and immunoblotting, using anti-Sec62p antibodies.

In Vitro Assay for Signal Peptidase Activity—The assay was performed according to (8) using prepro-α-factor as a substrate with the following modifications. Aliquots of yeast cells growing in YPD or YPGal-rich medium were removed at distinct time points, and membranes were prepared. To obtain comparable amounts of membranes, the samples were normalized to same amounts of Spc3p or Sec62p as indicated. Digitonin extracts (4) corresponding to the indicated amount of membranes (10–40 eq (Ref. 22)) were prepared and 210 μl of buffer added. The final conditions of the assay were 50 mM triethanolamine, pH 8, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.4 mg/ml phosphatidylcholine, 0.01% SDS (from the SDS pre-treatment of the prepro-α-factor), 120 mM potassium acetate, 0.2% digitonin, and protease inhibitors (10 μg/ml leupeptin, 5 μg/ml chymostatin). The added prepro-α-factor was synthesized in a reticulocyte system in the presence of $[^{35}S]$methionine. After 2 h at 23 °C, the samples were precipitated and precursor and product were separated by SDS-PAGE. The ratio between precursor and product was determined by measuring the amount of the radioactivity using a Fuji BAS 2000 bioimager.

Antibodies, Immunoprecipitation, and Lectin Binding—Antibodies against Sec11p, the amino terminus of Spc1p and Spc2p, have been described in Ref. 18. Antibodies against Sbh2p and antibodies against Spc3p have been described in Refs. 5 and 10, respectively. Antibodies against Sec61p, Sec62p, and Sbh1p were described in Ref. 4. Antibodies raised against the carboxyl terminus of Sec72p were a kind gift of C. Unger (Berlin, Germany). New anti-Spc1p antibodies were raised against the carboxyl terminus of Spc1p using the peptide (CKIEINVDQYD). Affinity purification and immobilization were carried out as described (4). Immunoblots were performed as described earlier (4) using enhanced chemiluminescence system (Amersham Pharmacia Biotech or PerkinElmer Life Sciences). For quantification ^{35}S-labeled anti-rabbit IgG antibody (Amersham Pharmacia Biotech) was used in the immunoblots followed by analysis using a Fuji BAS 2000 bioimager.

Immunoprecipitation—Membrane fractions from wt and *Δspc2* (HFY403) strains were solubilized in extraction buffer (20 mM HEPES, pH 7.4, 375 mM potassium acetate, 10% glycerol, 1.5% digitonin, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 4 μg/ml chymostatin, 5 μg/ml pepstatin) at a final concentration of 50 eq/ml. Lysates were centrifuged at 200,000 × *g* in a TLA100.3 rotor for 60 min to divide off ribosomes, ribosome-bound proteins, and unsolubilized material. The supernatants were diluted with four volumes of 20 mM Hepes, pH 7.4, 1.5% digitonin, and immunoprecipitation was performed using affinity-purified antibodies against Spc1p and Spc2p, covalently coupled to Protein A-Sepharose beads. After incubation for 8 h at 4 °C, the beads were washed eight times with 20 mM Hepes, pH 7.4, 100 mM potassium acetate, 2.5% glycerol, 1.5% digitonin and the bound material was eluted with SDS-sample buffer. Equal amounts corresponding to the starting volume of the clarified lysates, the immunoprecipitates and the supernatants were analyzed by SDS-PAGE and immunoblotting using antibodies against Sec61p, Sec62p, Spc3p, Spc2p, and Spc1p. For the identification of Sbh1p and Sbh2p in the samples, Tricine gel electrophoresis (16.5% T, 6% C) and immunoblotting procedure were used as described (23). Immunodecorated proteins were visualized using Protein A-coupled horseradish peroxidase (Sigma) and enhanced chemiluminescence.

Concanavalin A Binding Assay—Membrane fractions from wt and *Δspc1Δspc2* (HFY404) strains were solubilized in extraction buffer (50 mM triethanolamine, pH 8.0, 375 mM potassium acetate, 10% glycerol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1.5% digitonin, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 4 μg/ml chymostatin, 5 μg/ml pepstatin) at a final concentration of 0.25 eq/μl. After a centrifugation step (174,000 × *g* for 20 min at 4 °C in a TLA100.3 rotor), the supernatants were incubated overnight at 4 °C with 10 μl of concanavalin A-Sepharose (Amersham Pharmacia Biotech). After washing of the concanavalin A-Sepharose beads (three times with the extraction buffer described above), the proteins were eluted with SDS-sample buffer. Equal amounts corresponding to the starting volume of the bound and unbound material were analyzed by SDS-PAGE and immunoblotting using antibodies against Sec11p, Spc2p, and Spc3p.

RESULTS

Deletion of Spc2p Reduces the Activity of the SPC in Vitro—*In vivo* analysis of *Δspc2* strains revealed an accumulation of precursors of secretory proteins at 42 °C (18). To investigate whether this effect of the *SPC2* deletion is directly linked to the activity of the SPC, we set out to analyze the enzymatic activity of Spc2p-free SPC *in vitro*. Signal peptidase cleavage was assayed as described in Ref. 10 using digitonin solubilized membranes from the *Δspc2* strain and prepro-α-factor as a substrate. As a control we included membranes derived from a wild-type strain, a *Δspc1* strain, and a strain with a *Δspc1*/*Δspc2* double deletion. The amount of digitonin extract added was calibrated to the same amount of Sec11p and Spc3p. We found that the deletion of Spc1p had no effect on the signal peptidase activity (Fig. 1*A*). In contrast, deletion of Spc2p reduced the activity by a factor of 2. A *Δspc1*/*Δspc2* double deletion resulted in no further reduction of the signal peptidase activity. We wondered whether the decreased signal peptide cleavage could be due to a direct effect on the catalytic center of the SPC or a diminished stability of the complex. To test whether the stability is altered in a *Δspc1*/*Δspc2* strain, a digitonin extract was bound to concanavalin A. In wild-type cells yeast SPC does not dissociate into its subunits in the presence of digitonin (18). In addition to the glycosylated Spc3p, the bound material contained the unglycosylated Sec11p, indicating that also in the absence of Spc1p and Spc2p the two remaining subunits did not dissociate (Fig. 1*C*). To confirm these results, we repeated the cleavage assay in the presence of saponin using membranes from wild-type cells and a *Δspc1*/*Δspc2* mutant strain. These conditions are probably more gentle for the enzyme, because integral membrane proteins like the SPC are not released from the lipid bilayer. Again, the activity of the SPC from the double deletion strain was only half that of wild-type SPC (data not shown). Together these data indicate that Spc2p is modulating the peptidase activity of the yeast SPC.

Influence of Spc2p on the Composition and the Steady State Level of Yeast SPC—Next we wanted to analyze the SPC composition of the deletion strains in more detail. Membranes purified from a *Δspc1* strain, a *Δspc2* strain, a *Δspc1*/*Δspc2* strain, and a corresponding wild-type strain were calibrated using to the same amount of Sec62p as a measure of the number of translocation sites. The amount of the different SPC subunits was analyzed by immunoblotting (Fig. 1*B*). We found

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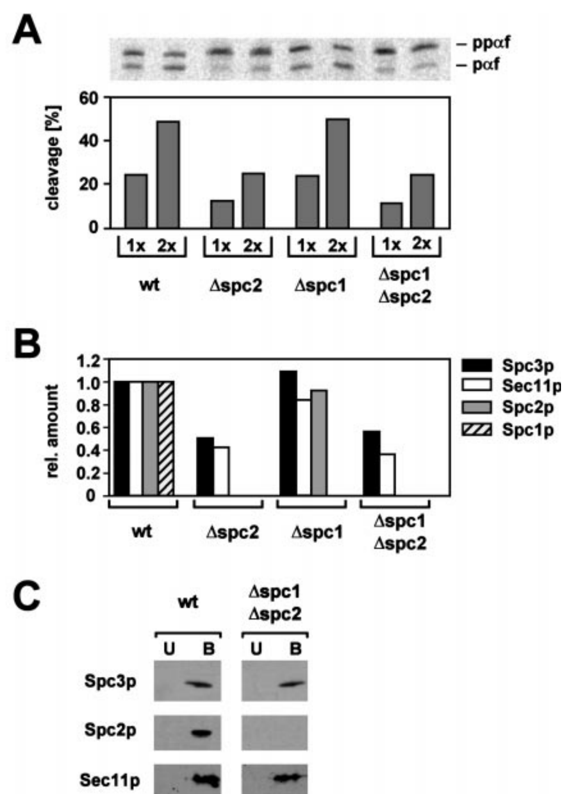

FIG. 1. **SPC composition and signal peptidase activity of yeast strains deficient in Spc1p and Spc2p.** *A*, signal peptidase activity of a wt, Δ*spc2*, Δ*spc1*, and Δ*spc1*/Δ*spc2* strain. Microsomes were purified from wt (SEY62.10), Δ*spc2*, Δ*spc1*, and Δ*spc1*/Δ*spc2* strains and normalized to similar amounts of Sec62p. Digitonin extract corresponding to 20 and 40 eq of membranes were prepared and analyzed as indicated under "Experimental Procedures" with the exception that the assay was performed for 3 h at 25 °C. Samples were separated by SDS-PAGE and analyzed by a bioimager (Fuji Bas 2000). *ppαF*, prepro-α-factor; *pαF*, pro-α-factor. *B*, expression level of Spc1p, Spc2p, Spc3p, and Sec11p. The relative amount of the different SPC subunits in the microsomes analyzed in *A* was determined by immunoblotting using ^{35}S-labeled secondary antibodies and a bioimager (Fuji Bas 2000). *C*, microsomes from a wt and a Δ*spc1*/Δ*spc2* strain were bound to concanavalin A-Sepharose. Unbound (*U*) and bound (*B*) material that corresponded to either 12 eq of membranes (Spc3p and Spc2p) or 30 eq of membranes (Sec11p) was processed by SDS-PAGE, and the presence of the different SPC subunits was determined by immunoblotting.

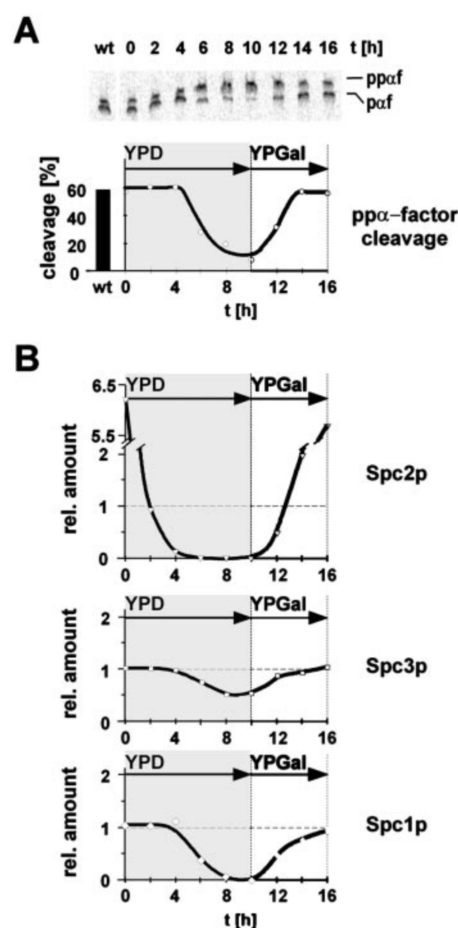

FIG. 2. **Analysis of the SPC composition and signal peptidase activity using a conditional *spc2* mutant.** A Δ*spc2* strain (HFY403) transformed with the plasmid pHM3 expressing Spc2p under control of an Gal10 promotor was preincubated in YPGal medium allowing the expression of Spc2p. The cells were then shifted to YPD medium to repress the Spc2p synthesis. After 10 h cells were shifted back to YPGal medium to reinduce the Spc2p synthesis. Aliquots of cells were removed at the time points indicated, and microsomes were prepared. wt membranes were isolated from the SEY62.10 strain. *A*, signal peptidase activity of digitonin extracts corresponding to 40 eq of microsomes were analyzed. *ppαF*, prepro-α-factor; *pαF*, pro-α-factor. *B*, amounts of Spc3p, Spc2p, and Spc1p were analyzed by SDS-PAGE and immunoblotting. Quantification of proteins with ^{35}S-labeled secondary antibody was performed as described above (Fig. 1).

that membranes of a Δ*spc2* strain did not contain Spc1p. Spc1p was also not detectable if complete cell lysates were analyzed. Nevertheless, there was no reduction in the expression of Spc1p mRNA detectable, indicating that the regulation was at the level of protein synthesis or degradation (data not shown). Moreover, we observed that strains depleted of Spc2p expressed a lower amount of Sec11p and Spc3p in relation to Sec62p (Fig. 1*B*).

To confirm that the differences between the wild-type strain and the deletion strains were not due to differences in the genetic background, we repeated the experiment using a Δ*spc2* strain that contained *SPC2* on a plasmid under control of a Gal10 promotor. Cells were first grown on galactose-containing medium. Under these conditions Spc2p expression is induced. The cells were then shifted to glucose for 10 h to inhibit Spc2p synthesis and finally re-shifted on galactose to reinduce the Spc2p synthesis. At different time points, cells were collected and a crude ER membrane fraction was prepared. The samples were calibrated to the same amount of Sec62p as a measure of the number of translocation sites. The amount of Sec62p per OD of membranes was in all samples nearly identical, indicating that the density of translocation sites did not change significantly during the experiment (data not shown). The different samples were then analyzed for their signal peptidase activity *in vitro*. Upon induction of Spc2p, the cleavage activity of the strain was identical to that of an isogenic wild-type strain (Fig. 2*A*). Depletion of Spc2p led to a decrease of signal peptidase activity to at least one fourth of original values (Fig. 2*A*). Please note that the more dramatic decrease in signal peptidase activity compared with Fig. 1*A* is due to differences in the calibration of the samples. Whereas in the experiment shown in Fig. 2*A* the samples were calibrated to the same amount of Sec62p, samples in Fig. 1A were calibrated to the amounts of Sec11p and Spc3p. As expected, depletion of Spc2p also caused the loss of Spc1p and a decrease of Spc3p and Sec11p to half compared with the level of Sec62p (Fig. 2*B*). Re-induction of Spc2p reversed these effects. In contrast to Spc1p, Spc2p is therefore crucial for the composition and the steady-state level of the SPC in the ER membrane.

Deletion of Spc2p Decreases the Expression Level of Sbh2p, the β-Subunit of the Ssh1 Complex—Although the Sec62p level roughly paralleled the overall amount of ER membranes, one cannot exclude that Sec62p is up-regulated when Spc2p is

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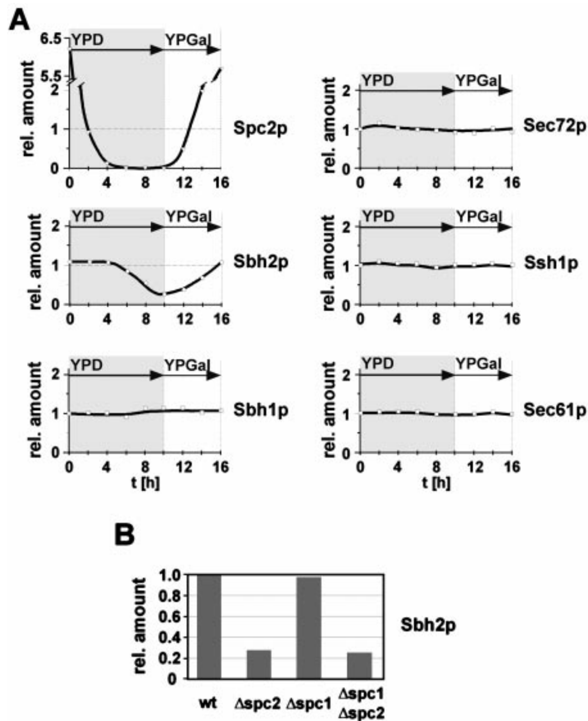

FIG. 3. **Effect of the Spc2p depletion on the amount of Sbh2p.** *A*, a Δ*spc2* strain (HFY403) transformed with the plasmid pHM3 was preincubated in YPGal medium and then shifted to YPD medium to repress the Spc2p synthesis. After 10 h cells were shifted back to YPGal medium. Aliquots of cells were removed at the time points indicated, microsomes prepared, and the amount of the different components of ER translocation sites was analyzed by immunoblotting using the antibodies indicated. The amount of the respective proteins was normalized to the amount found in membranes isolated from a wt (SEY62.10) strain. *B*, the amount of Sbh2p in a wt and a Δ*spc1*/Δ*spc2* strain were analyzed by immunoblotting using ^{35}S-labeled secondary antibodies as described above (Fig. 1).

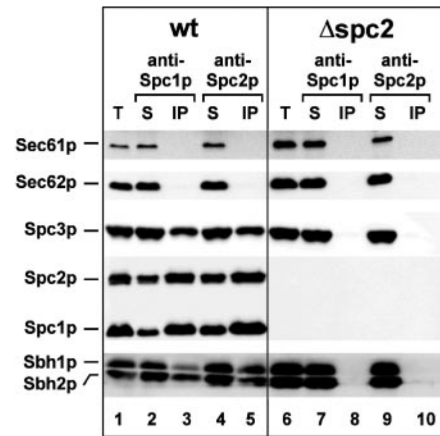

FIG. 4. **Co-immunoprecipitation of Sbh1p and Sbh2p with antibodies against Spc1p and Spc2p.** Digitonin extracts were prepared from microsomes of a wt (SEY62.10) or Δ*spc2* strain. From these extracts immunoprecipitation were performed with antibodies against Spc1p or Spc2p, respectively. The total extract (*T*), supernatant (*S*), and immunoprecipitate (*IP*) were analyzed by SDS-PAGE and immunoblotting using antibodies specific for the indicated proteins.

depleted. Therefore, we repeated the experiment and assayed the amount of other components of the translocation apparatus in the ER by immunoblotting (Fig. 3*A*). As for Sec62p, the amount of Sec72p, another component of the Sec62/63 complex, did not change in response to alterations of the Spc2p level. Similarly, the amount of components of the Sec61p complex Sec61p and Sbh1p, and the amount of Ssh1p, the α-subunit of the Ssh1 complex, did not change. However, the level of Sbh2p (the β-subunit of the Ssh1p complex) decreased to about one fifth following depletion of Spc2p and returned to the wild-type level, after re-initiation of synthesis of Spc2p (Fig. 3*A*).

These differences in the expression level of Sbh2p were also detected if membranes of a Δ*spc2* strain that carried no *SPC2* plasmid were compared with membranes of an isogenic wild-type strain (Fig. 3*B*). Deletion of *SPC1* did not decrease the Sbh2p level (Fig. 3*B*).

Sbh1p and Sbh2p Are in Proximity to the SPC—The observed effect of Spc2p on the stability of Sbh2p could be explained by a physical interaction of these two proteins in the membrane. In order to test this hypothesis, we performed immunoprecipitation experiments. Yeast rough membranes were solubilized with digitonin and depleted of ribosome-associated Sec61-like complexes by centrifugation. In addition to the components of the SPC complex, antibodies raised against Spc2p were able to precipitate both Sbh2p and Sbh1p from this extract (Fig. 4). The amount of Sbh1p and Sbh2p molecules associated to the SPC under these conditions was in the order of 5–10% of the whole population. Other components of the translocation machinery like Sec62p or Sec61p were not precipitated under these conditions. The same result was obtained using antibodies raised against Spc1p. No precipitation was observed if membranes of a Spc2p deletion strain were used. This shows that the SPC is in a complex with Sbh1p and Sbh2p.

DISCUSSION

Several data indicate that the mammalian SPC is located close to ribosome-associated translocation channels that are active in co-translational protein translocation. Signal peptides of nascent chains are cleaved off by the SPC subunits while being translocated (24). Chemical cross-linking experiments have revealed that mammalian SPC25 is in the vicinity of Sec61β in the presence of translocating ribosomes (25). However, so far, no complex between the SPC and components of the Sec61 complex has been purified. It was therefore possible that the observed proximity between SPC and the Sec61 complex is only mediated by a simultaneous interaction of both complexes with membrane-bound ribosomes. We now show that in yeast the SPC is associated with the β-subunits of the Sec61 complexes in the absence of ribosomes. We could not demonstrate that the SPC-associated β-subunits are also associated to Sec61p or Ssh1p. Moreover, antibodies raised against the amino termini of Sbh1p and Sbh2p immunoprecipitate Sec61-like complexes but not SPC components. Besides the possibility of technical limitations, these findings could also indicate that the population of Sbh1p and Sbh2p that is associated with the SPC is different from that found in the Sec61 complexes. In contrast to Sbh1p, Sbh2p becomes unstable *in vivo*, when not able to associate with an α-subunit such as Ssh1p or Sec61p (5, 26). Therefore, the fact that the depletion of Spc2p results in a comparable decrease in the steady state level of Sbh2p confirms the existence of complexes between the SPC and Sbh2p. Moreover, it indicates that interaction between the SPC and the β-subunits of the Sec61complexes is mediated by the Spc2p. Cross-linking experiments in mammals also point toward a direct interaction between SPC25 and Sec61β (25). We speculate that Spc2p and Sbh1p or Sbh2p form an interface for the association between the SPC and the Sec61 complexes.

The Spc1p/SPC12 and Spc2p/SPC25 are components of the signal peptidase that are not found in procaryotic organisms. Although chicken SPC depleted of SPC12 and SPC25 has signal peptidase activity *in vitro*, the *in vivo* analysis of a *SPC2* deletion mutant showed a weak phenotype resulting in an

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accumulation of precursors of secretory proteins at 42 °C (18). Protease protection assays revealed that the precursors are located within the ER lumen, indicating that the accumulation is not caused by a lower translocation efficiency (*e.g.* due to the lower amount of Sbh2p in these cells) (data not shown). The biochemical analysis of these mutants now demonstrates that the *in vivo* phenotype is probably a result of two effects. First, the depletion of Spc2p significantly reduces the amount of SPC per translocation site in the ER membrane. Second, SPCs that have no Spc2p and no Spc1p show a diminished cleavage activity *in vitro* at least for the substrate used in the assay. Deletion of Spc1p alone has no influence on the activity of the SPC. Interestingly, the analysis of the conditional *spc2* mutant indicates that SPCs which are only depleted of the Spc2p show also no decreased activity (Fig. 3*A*; compare 4 and 6 h after shift to glucose). To this end, it is not clear how the presence of Spc2p (and perhaps also Spc1p) enhances the activity of the catalytic subunit Sec11p. One possibility is that the membrane-spanning regions of Spc1p and Spc2p help to form the binding pocket for the hydrophobic substrate of the protein. Thus, they may maintain structural flexibility or stability of SPC needed for effective processing of signal peptides.

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REFERENCES

1. Rapoport, T. A., Jungnickel, B., and Kutay, U. (1996) *Annu. Rev. Biochem.* **65,** 271–303
2. Johnson, A. E., and van Waes, M. A. (1999) *Annu. Rev. Cell. Dev. Biol.* **15,** 799–842
3. Deshaies, R. J., and Schekman, R. (1987) *J. Cell Biol.* **105,** 633–645
4. Panzner, S., Dreier, L., Hartmann, E., Kostka, S., and Rapoport, T. A. (1995) *Cell* **81,** 561–570
5. Finke, K,, Plath, K., Panzner, S., Prehn, S., Rapoport, T. A., Hartmann, E., and Sommer, T. (1996) *EMBO J.* **15,** 1482–1494
6. Esnault, Y., Blondel, M. O., Deshaies, R. J., Scheckman, R., and Kepes, F. (1993) *EMBO J.* **12,** 4083–4093
7. Rothblatt, J. A., Deshaies, R. J., Sanders, S. L., Daum, G., and Schekman, R. (1989) *J. Cell Biol.* **109,** 2641–2652
8. YaDeau, J. T., Klein, C., and Blobel, G. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 517–521
9. Bohni, P. C., Deshaies, R. J., and Schekman, R. W. (1988) *J. Cell Biol.* **106,** 1035–1042
10. Meyer, H. A., and Hartmann, E. (1997) *J. Biol. Chem.* **272,** 13159–13164
11. Fang, H., Mullins, C., and Green, N. (1997) *J. Biol. Chem.* **272,** 13152–13158
12. Zwizinski, C., and Wickner, W. (1980) *J. Biol. Chem.* **255,** 7973–7977
13. van Dijl, J. M., de Jong, A., Vehmaanpera, J., Venema, G., and Bron, S. (1992) *EMBO J.* **11,** 2819–2828
14. VanValkenburgh, C., Chen, X., Mullins, C., Fang, H., and Green, N. (1999) *J. Biol. Chem.* **274,** 11519–11525
15. Kalies, K. U., and Hartmann, E. (1996) *J. Biol. Chem.* **271,** 3925–3929
16. Lively, M. O., and Walsh, K. A. (1983) *J. Biol. Chem.* **258,** 9488–9495
17. Fang, H., Panzner, S., Mullins, C., Hartmann, E., and Green, N. (1996) *J. Biol. Chem.* **271,** 16460–16465
18. Mullins, C., Meyer, H. A., Hartmann, E., Green, N., and Fang, H. (1996) *J. Biol. Chem.* **271,** 29094–29099
19. Robinson, J. S., Klionsky, D. J., Banta, L. M., and Emr, S. D. (1988) *Mol. Cell. Biol.* **8,** 4936–4948
20. Sikorski, R. S., and Hieter P. (1989) *Genetics* **122,** 19–27
21. Johnston, M., and Davis, R. W. (1984) *Mol. Cell. Biol.* **4,** 1440–1448
22. Walter, P., and Blobel, G. (1983) *Methods Enzymol.* **96,** 84–93
23. Schägger, H. and von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379
24. Perara, E., Rothman, R. E., and Lingappa, V. R. (1986) *Science* **232,** 348–352
25. Kalies, K. U., Rapoport, T. A., and Hartmann, E. (1998) *J. Cell Biol.* **141,** 887–894
26. Biederer, T., Volkwein, C., and Sommer, T. (1996) *EMBO J.* **15,** 2069–2076

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MEMBRANE TRANSPORT STRUCTURE FUNCTION AND BIOGENESIS:

Interactions between Spc2p and Other Components of the Endoplasmic Reticulum Translocation Sites of the Yeast *Saccharomyces cerevisiae*

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