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*Yarrowia lipolytica TSR*1 Gene Product

A NOVEL ENDOPLASMIC RETICULUM MEMBRANE COMPONENT INVOLVED IN THE SIGNAL RECOGNITION PARTICLE-DEPENDENT TRANSLOCATION PATHWAY*

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The *tsr*1-1 mutation has been initially identified as an extragenic suppressor of the *scr*2.II-13 mutation that alters the 7SL RNA component of the signal recognition particle (SRP) and results in severe defects in protein translocation and SRP stability. We showed previously that the *TSR*1 gene was essential and that the *tsr*1-1 mutation allowed complete recovery of *scr*2.II-13-associated secretory defects. We show here that the *tsr*1-1 mutation also restores SRP stability in an *scr*2.II-13 context. The *TSR*1 gene product (Tsr1p) is stably associated with rapidly sedimenting material and cofractionates with the lumenal protein Kar2p of the endoplasmic reticulum; it behaves in protease protection assays as a transmembrane component. Coimmunoprecipitation experiments revealed a physical interaction with Kar2p and with ribosomal components associated to the 5.8S rRNA as well as with SRP components like Sec65p and 7SL RNA. We propose that Tsr1p is an important component of the endoplasmic reticulum membrane, interacting both with the SRP-ribosome complex in the cytosol and with Kar2p in the lumen of the endoplasmic reticulum.

Secretory and membrane proteins in eukaryotic cells are synthesized on ribosomes attached to the endoplasmic reticulum (ER)[1] membrane and targeted by means of the signal recognition particle (including 7S RNA, Srp19p, Srp54p, Srp68p, Srp72p, Srp9p, and Srp14p) to the translocation machinery (for recent review see Ref. 1). At the ER, the SRP-signal sequence-ribosome complex interacts via the SRP with a heterodimeric integral membrane protein called SRP receptor or docking protein, which is a complex of two GTPases, SRα and SRβ (2, 3). This interaction, followed by the hydrolysis of GTP by the SRP and the SRP receptor complex, leads to the release of the signal sequence from the SRP and its insertion into the translocation machinery and allows the dissociation of the SRP from the membrane (4).

The overall rate and selectivity of protein translocation across the ER suggest the presence of many interactions with different components of the translocation system including Sec61p complex (5, 6) and other proteins that interact either directly or are in close proximity to the newly synthesized secretory proteins, such as signal peptidase (7), glycosyltransferase (8), protein-disulfide isomerase (9), and BiP (10). Other interactions with the ribosome seem to be required for the direct transfer of the nascent chain to the protein-conducting channel. The membrane anchoring of ribosomes engaged in protein translocation is mediated by the Sec61p complex (11).

In *Yarrowia lipolytica*, substitutions at the G^{128} and A^{130} positions of the stem loop 1 of 7SL RNA, which forms the presumed binding site of Srp19p (12), have permitted selection of a thermosensitive mutant *scr*2.II-13. Defects in this mutant include a decrease in the synthesis of secretory proteins, preferential cleavage of the 7SL RNA, and a substantial instability of the SRP. We have reported the isolation of a suppressor of the *scr*2.II-13 mutation (13). This suppressor, called *tsr*1-1 (thermosensitive rescued), restores normal growth to *scr*2.II-13 mutants (*tsr*1-1(*scr*2.II-13)) at 34 °C but not to a wild type $SCR2^+$ strain (*tsr*1-1($SCR2^+$)). Complementation of the *tsr*1-1 thermosensitive phenotype has made it possible to isolate the *TSR*1 gene that encodes a serine threonine-rich protein of 461 amino acids with an amino-terminal leader sequence and a transmembrane domain.

The present report describes the stability of the SRP in the *tsr*1-1(*scr*2.II-13) context and addresses the features of the *TSR*1 gene product (Tsr1p) and its relation to the SRP and translocation apparatus. We find that Tsr1p is an ER membrane protein that interacts with the SRP in the cytosol and BiP in the ER lumen and thus could play a crucial role in the translocation pathway either as a component of the translocon or as a functional intermediate between the lumen and the cytosolic side of the ER.

EXPERIMENTAL PROCEDURES

Strains, Growth Conditions, and Materials—Strains and plasmids used in this study are listed in Table I. The YPD medium contained 1% yeast extract, 1% Bacto-Peptone, 1% glucose, and 2% agar. The selective minimal medium contained 1% glucose, 0.17% yeast nitrogen base without ammonium sulfate (Difco, Detroit, MI), and 0.1% proline as nitrogen source and was supplemented with appropriate nutrients.

All enzyme reactions, DNA and RNA extractions, and hybridization preparations were performed as described by Maniatis *et al.* (14). Transformation of *Y. lipolytica* was performed as described by Xuan *et al.* (15). Dithiobis succinimidylpropionate (DSP) was from Sigma.

Protein Immunoblotting and Immunoprecipitation—Protein extracts were prepared from yeast cells, separated through 10% SDS-polyacrylamide gel, blotted to nitrocellulose, and probed with specific antibody. Bound antibodies were visualized by enhanced chemiluminescence (Amersham Corp.). Native immunoprecipitation of the SRP was performed as described by He *et al.* (12).

The protocol of coimmunoprecipitation described by Esnault *et al.*

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[1] The abbreviations used are: ER, endoplasmic reticulum; SRP, signal recognition particle; GST, glutathione *S*-transferase; DSP, dithiobis succinimidylpropionate; PAGE, polyacrylamide gel electrophoresis; BiP, immunoglobulin heavy chain-binding protein.

TABLE I
Strains and plasmids used in this study

Strains/plasmids	Description	Source or reference
Y. lipolytica		
Wild type	*MatB, his1–1, leu 2–35, ura 3–302, SCR1::ADE1, Δ SCR2 (LEU2, SCR2)*	Ref. 12
*tsr*1–1 (*URA3, SCR2*$^+$)	*MatB, his1–1, leu 2–35, ura 3–30, tsr1–1 SCR1::ADE1, Δ SCR2 (URA3, SCR2*$^+$*)*	This work
*tsr*1–1 (*LEU2, scr2.11–13*)	*MatB, his1–1, leu 2–35, ura 3–30, tsr1–1 SCR1::ADE1, Δ SCR2 (LEU2, scr2.11–13)*	This work
pINA823	pBR322 carrying *Y. lipolytica SCR2* and *LEU*2 genes	This laboratory
pINA24	pBR322 carrying *Y. lipolytica* rDNA gene	Ref. 24
pML5	Derivative of pGEX-2T carrying the *GST-TSR*1 fusion	This work

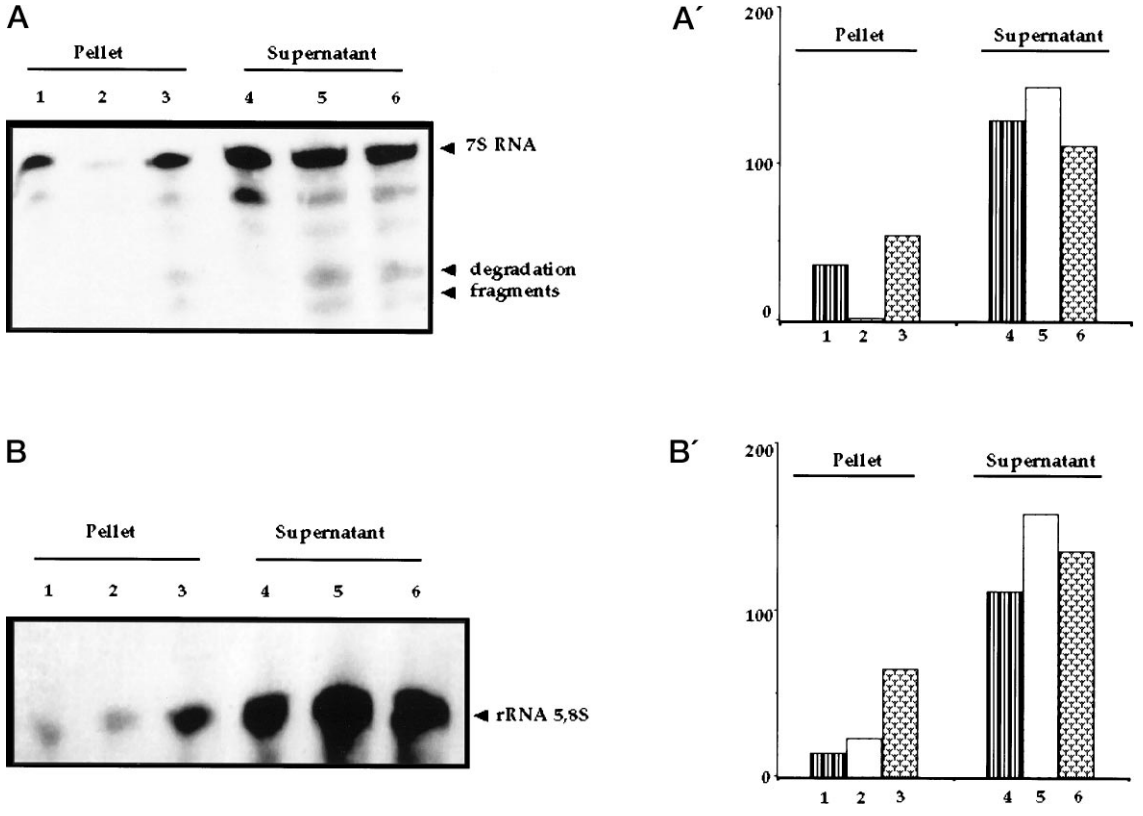

FIG. 1. ***tsr*1-1 stabilizes the SRP.** Whole cell lysate was prepared from *scr*2.II-13, *tsr*1-1(*scr*2.II-13), and wild type strains by gentle lysis of spheroplasts and immunoprecipitated with the anti-Sec65p antibody. RNA from the supernatant and the immunoprecipitates (*Pellet*) was recovered and analyzed on 6% polyacrylamide, 8.3 M urea denaturing gel. RNA was then transferred to Nylon Hybond N$^+$ membrane and hybridized either with the *Sal*I-*Cla*I fragment of pINA823 containing the *SCR*2 gene to probe the 7S RNA (*A*) or the *Bgl*II-*Eco*RI fragment of pINA24 containing the rDNA to probe the rRNA 5.8S (*B*). RNA was then scanned, and the results of the quantification of the 7S RNA (*A′*) and the rRNA 5.8S (*B′*) are reported. *Lanes 1* and *4*, wild type strain; *lanes 2* and *5*, *scr*2.II-13 strain; *lanes 3* and *6*, *tsr*1-1(*scr*2.II-13) strain.

(16) was adapted for the immunoprecipitation of the 7SL RNA, Kar2p, and Sec65p by the anti-GST-Tsr1p antibody. Cell fractionation was performed as described by Ruohola and Ferro-Novick (17).

Anti-Tsr1p Antibody Preparation—The pML5 plasmid containing the GST-*TSR*1 fusion has been obtained by cloning the *Bcl*I-*Eco*RI fragment carrying the *TSR*1 gene downstream from the GST open reading frame of the pGEX 2T vector (Stratagene). The GST-Tsr1p fusion protein was overexpressed, purified on a GST-Sepharose column (Pharmacia Biotech Inc.) and used to immunize rabbits. The sera were tested and used at 1:10000 dilution for immunoblots. In pML5 containing *Escherichia coli* cells, these antibodies recognized a single protein with the mobility expected for the GST-Tsr1p fusion.

RESULTS

The tsr1-1 Mutation Promotes a Complete Recovery of the SRP Stability Defect of scr2.II-13—It has been reported using the human autoimmune anti-SRP antibody that the stability of the SRP was greatly reduced in the *scr*2.II-13 mutant (12). To assess whether the suppressive mutation *tsr*1-1 could repair this instability, cell lysates from *scr*2.II-13, *tsr*1-1(*scr*2.II-13), and wild type strains were immunoprecipitated with an anti-Sec65p antibody. Immunoprecipitates were analyzed on 6% polyacrylamide, 8.3 M urea denaturing gel and hybridized with an *SCR*2 probe (Fig. 1*A*). Contrary to the *scr*2.II-13 mutant where immunoprecipitated 7SL RNA represented only 2–3% of the wild type, the immunoprecipitability of the 7SL RNA was completely restored in the *tsr*1-1(*scr*2.II-13) strain. These results indicate clearly that the suppressive *tsr*1-1 mutation stabilizes the mutant SRP. The level of total cellular 7SL RNA was quantitated in the three strains analyzed (Fig. 1*A′*). Nearly identical levels of 7SL RNA were observed in the revertant and wild type strains, whereas *scr*2.II-13 accumulated more RNA, probably to compensate for the decreased stability of the SRP in this mutant. Results shown in Fig. 1*A* indicate that the 7SL RNA was still cleaved in the *tsr*1-1(*scr*2.II-13) strain.

To examine whether *tsr*1-1-mediated stabilization of the SRP mutant may involve ribosomes, we analyzed by Northern hybridization the amount of 5.8S rRNA in the three immunoprecipitates. Results in Fig. 1 (*B* and *B′*) show that whereas the level of SRP-associated 5.8S rRNA was quite similar in both *scr*2.II-13 and wild type strains, this level was increased three to four times in the suppressed strain *tsr*1-1(*scr*2.II-13). From

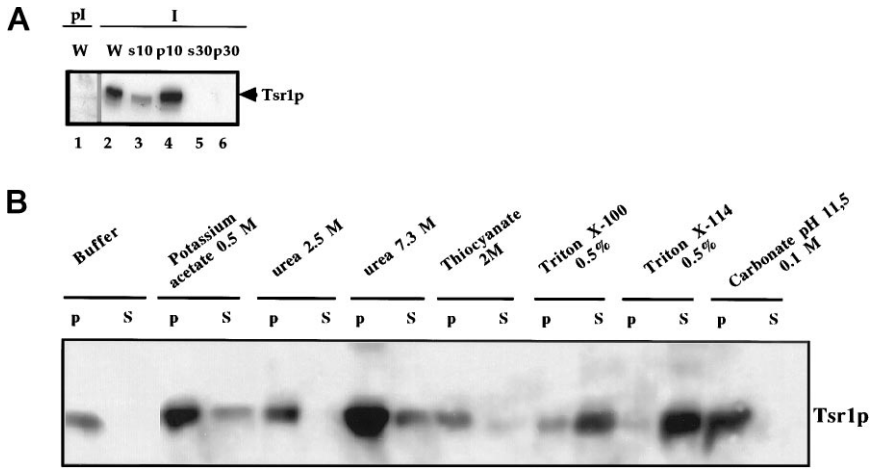

FIG. 2. **Tsr1p associates with membranes.** A whole cell extract was prepared from the wild type strain by gentle lysis of spheroplasts. *A*, the cell-free extract (*W*) was centrifuged for 10 min at 10,000 × *g*. The resulting supernatant (*s10*) was subfractionated by a 20-min centrifugation at 30,000 × *g*. The corresponding pellets (*p10* and *p30*) were each resuspended in the original volume of lysis buffer. Equal volumes of each fraction were subjected to SDS-PAGE and immunoblotted with anti-GST-Tsr1p antibody. *B*, membrane association of Tsr1p. The 10,000 × *g* pellet (*p*) was subjected to various treatments, followed by a 30-min centrifugation at high speed. Treatments included: 60 min at 24 °C with buffer alone, 30 min at 0 °C with 0.5 M potassium acetate; 120 min at 24 °C with 2.5 M or 7 M urea; 60 min at 24 °C with 2 M thiocyanate; 30 min at 0 °C with 0.1 M carbonate at pH 11.5. Fractions were subjected to SDS-PAGE and immunoblotted with anti-GST-Tsr1p antibody. Bound antibodies were visualized by ECL (Amersham Corp.). *pI*, preimmune sera; *I*, immune sera; *p*, pellet; *S*, supernatant.

these data we concluded that the suppressive effect of *tsr*1-1 may result in a tighter association of ribosomes with the mutated SRP.

Membrane Association of Tsr1p—We generated polyclonal antibodies directed against a GST-Tsr1p fusion protein (see "Experimental Procedures"). On a Western blot of total cell extract treated with 4% Triton X-100, (Fig. 2*A*), the immune serum (*lane 2*) but not the preimmune serum (*lane 1*) recognized a 50-kDa protein. To determine the *in vivo* localization of the Tsr1p, extracts obtained from wild type cells by gentle spheroplast lysis were fractionated by differential centrifugation: 10 min at 10,000 × *g* followed by 20 min at 30,000 × *g*. At each stage, the supernatants and the pellets were collected and prepared for SDS-PAGE. Proteins were tranferred to a nitrocellulose filter, and the immunoblot was decorated with Tsr1p antibodies. Fig. 2*A* (*lanes 3–6*) demonstrates that most of Tsr1p fractionated in the 10,000 × *g* pellet. The preferential association of Tsr1p with rapidly sedimenting material seems compatible with a mitochondrial or ER localization (18).

Tsr1p Is an ER Transmembrane Protein—The sequence analysis of Tsr1p highlighted a transmembrane domain (13). To confirm that Tsr1p was indeed a membrane-spanning protein, spheroplasts were gently lysed, and cellular membranes were sedimented by a 10,000 × *g* centrifugation. The resuspended membrane pellet was subjected to a set of treatments followed by a centrifugation at high speed, and evaluation of the supernatant and pellet fractions were analyzed by immunoblot using the anti-GST-Tsr1p polyclonal antibodies. Agents that release peripheral proteins associated with membranes by weak electrostatic bonds (2.5 M urea, 0.5 M acetate, 0.1 M carbonate at pH 11.5) had no effect on the fractionation of Tsr1p (Fig. 2*B*). Harsher treatments were more effective: 7.3 M urea or 2 M thiocyanate stripped 35–45% of Tsr1p from the membranes, whereas Triton X-100 and Triton X-114 stripped 85–90%. These results indicate that Tsr1p is a transmembrane protein. To examine the topology of Tsr1p in the membrane, the 10,000 × *g* membrane fraction was subjected to digestion by 0.1 mg/ml proteinase K in the presence or the absence of 4% Triton X-100. After quenching of the reaction with 25% trichloroacetic acid, samples were resolved by a 10% SDS-PAGE, transferred to nitrocellulose, and immunoblotted with the GST-Tsr1p polyclonal antibody. Results in Fig. 3 show that in the absence of

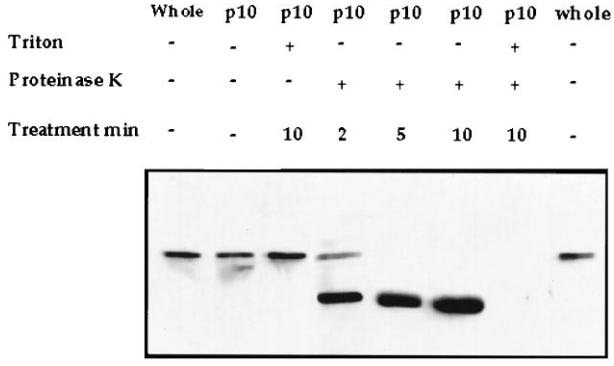

FIG. 3. **Protease protection of Tsr1p.** Cell free extract (*Whole*) was prepared from the wild type strain, and cellular membranes were sedimented by a 10,000 × *g* centrifugation (*p10*). Solubilized cells were treated with or without 0.1 mg/ml proteinase K in the presence or the absence of 4% Triton X-100. The reactions were stopped with trichloroacetic acid and processed for SDS-PAGE and immunoblotted with anti-GST-Tsr1p antibody. Bound antibody was visualized by ECL (Amersham Corp.).

detergent, Tsr1p was digested to a 34-kDa resistant fragment, which is compatible with a removal of the Tsr1p carboxyl terminus and suggests that this domain is localized on the cytosolic side. In the presence of 4% Triton X-100, Tsr1p was completely proteolyzed.

To address whether Tsr1p was an ER membrane protein, spheroplasts were gently lysed, and cellular membranes were subfractionated in a sucrose gradient into 22 fractions and analyzed using antibodies raised against Tsr1p and Kar2p. Whereas most cellular protein was found in the fractions F15 and F17 (Fig. 4*A*), results in Fig. 4*B* show that Tsr1p and Kar2p were both localized in the fractions F11 and F12. This colocalization between Tsr1p and Kar2p supports the conclusion that Tsr1p is an ER component.

Tsr1p Interacts with the SRP, Ribosomes, and BiP—To address the interactions of Tsr1p with the SRP or components of the translocation apparatus, we examined the coimmunoprecipitation of the 7SL RNA, Sec65p, 5.8S rRNA, and Kar2p using the GST-Tsr1p polyclonal antibody. Cells from wild type strain were grown overnight to early-log phase and 1 A_{600} × ml were collected. Cell lysates were treated or not with DSP and

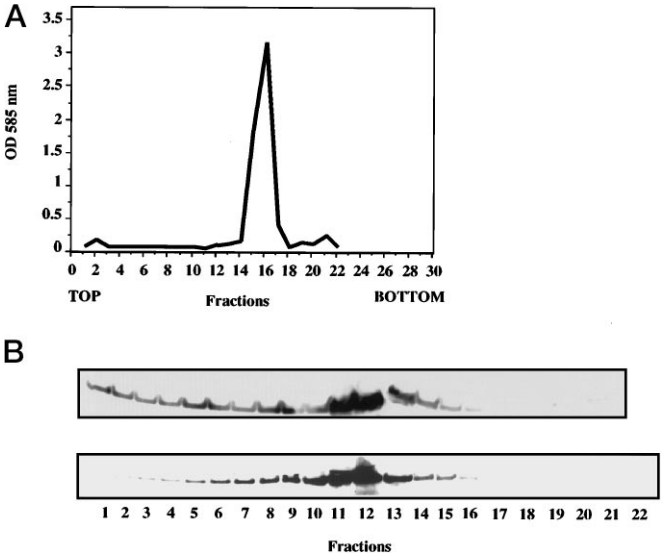

FIG. 4. **Colocalization of Tsr1p and Kar2p.** Wild type wild strain and cellular membranes were sedimented and subfractionated in a sucrose gradient. A total of 22 fractions, each 500 μl, were collected. *A*, dosage of whole proteins in each fraction. *B*, sample from each fraction was heated 5 min at 100 °C and subjected to SDS-PAGE and immunoblotted with anti-GST-Tsr1p antibody. Bound antibody was visualized by ECL (Amersham Corp.).

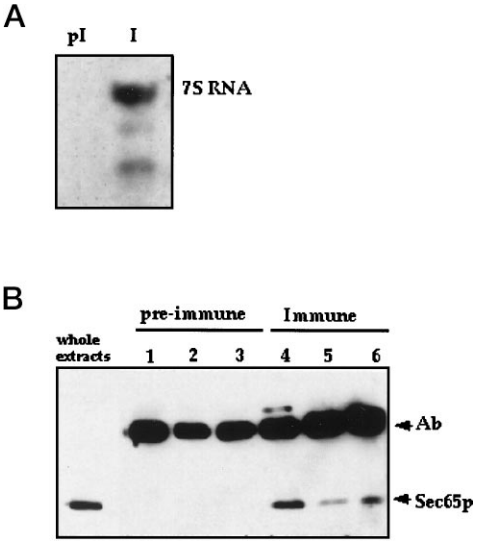

FIG. 5. **Coimmunoprecipitation of 7SL RNA and Sec65p with the Tsr1p.** *A*, whole cell lysates were prepared from a wild type strain by gentle lysis of spheroplasts (whole) and immunoprecipitated with the preimmune and the anti-GST-Tsr1p sera. RNA was recovered from the immunoprecipitates and subjected to Northern blot using to the *Sal*I-*Cla*I fragment of pINA823 containing the *SCR*2 gene as a probe. *pI*, preimmune immunoprecipitates; *I*, immune immunoprecipitates. *B*, whole cell lysate was prepared from a wild type strain by gentle lysis of spheroplasts, treated or not with DSP as indicated and immunoprecipitated with the preimmune and the anti-GST-Tsr1p serum. Solubilized proteins were recovered from the immunoprecipitates, resolved on 12.5% SDS-PAGE, and immunoblotted with anti-Sec65p serum. Bound antibody (*Ab*) was visualized by ECL (Amersham Corp.). *Lanes 1* and *4*, extracts were treated with 4% Triton X-100 for 10 min followed with DSP for additional 10 min. *Lanes 2* and *5*, extracts were treated with 0.2 mg/ml DSP for 10 min and then with 4% Triton X-100 for an additional 10 min. *Lanes 3* and *6*, extracts were treated simultaneously with 0.2 mg/ml DSP and 4% Triton X-100.

immunoprecipitated with the GST-Tsr1p polyclonal antibody. RNAs were recovered from the immunoprecipitates, analyzed on a 6% polyacrylamide, 8.3 M urea denaturing gel and hybridized with an *SCR*2 probe. Results in Fig. 5*A* show that the

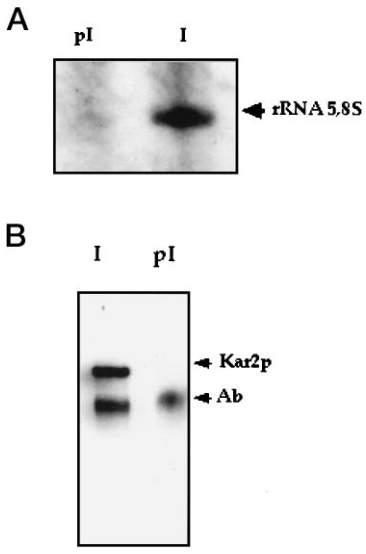

FIG. 6. **Coimmunoprecipitation of rRNA 5.8S and Kar2p.** Whole cell lysate was prepared from a wild type strain by gentle lysis of spheroplasts and immunoprecipitated with the preimmune and the anti-GST-Tsr1p sera. *A*, RNA was recovered from the immunoprecipitates and subjected to Northern blot using the *Eco*RI-*Bgl*II fragment of pINA24 containing the rDNA as a probe. *B*, solubilized proteins were recovered from the immunoprecipitates, analyzed on 10% SDS-PAGE, and immunoblotted with anti-Kar2p serum. Bound antibody (*Ab*) was visualized by ECL (Amersham Corp.). *pI*, preimmune immunoprecipitates; *I*, immune immunoprecipitates.

anti-Tsr1p antibody precipitated a small fraction of SRP RNA that is compatible with an interaction between Tsr1p and the SRP. To confirm this interpretation, proteins recovered from the immunoprecipitates were analyzed on 12.5% SDS-PAGE gels, transferred to nitrocellulose filter, and decorated with the Sec65p serum. Results in Fig. 5*B* show that a significant immunoprecipitation of Sec65p was obtained after treatment with 4% Triton X-100 followed by cross-linking using DSP. This treatment increases the accessibility of DSP to molecular complexes between Tsr1p and other proteins. Comparing the amount of Sec65p in the supernantant and in the immunoprecipitates, 65% of Sec65p has been estimated to interact with Tsr1p (data not shown). Both immunoprecipitation of 7SL RNA and Sec65p indicate clearly that Tsr1p interacts with the SRP. To understand whether this interaction is obtained with free SRP or within a SRP-ribosome complex, we have examined the coimmunoprecipitation of 5.8S rRNA with the Tsr1p. Results in Fig. 6*A* show that a clear immunoprecipitation of 5.8S rRNA is obtained with the Tsr1p antibodies, suggesting that the Tsr1p interacts with the SRP while coupled to ribosomes. To test whether the Tsr1p could interact through its lumenal domain with components of the ER lumen, we have tested the coimmunoprecipitation of Kar2p with the Tsr1p. Results in Fig. 6*B* show that in the case of Kar2p, coimmunoprecipitation is detected even without DSP treatment.

All these results show that Tsr1p interacts both with the SRP, probably during its targeting as a SRP-ribosome-nascent chain complex to the ER membrane, and with Kar2p. Its membrane localization suggests that it may involve its amino-terminal and its carboxyl-terminal domains, respectively, in each of these interactions.

DISCUSSION

We have previously described the isolation of the *tsr*1-1 mutation as a suppressor of the thermosensitivity conferred by *scr*2.II-13, a mutation of the SRP RNA that results in defects in the translocation of secretory proteins and destabilizes the SRP in the yeast *Y. lipolytica* (13). *tsr*1-1 restored normal growth to

*scr*2.II-13 mutants but resulted in a thermosensitive phenotype at 34 °C in a wild type *SCR*2 context associated with severe defects in the early steps of the translocation pathway. We further cloned and described the new and essential gene *TSR*1, whose expression restored thermoresistance and translocation to a *tsr*1-1(*SCR*2$^+$) strain. Based on these data, we concluded that the *TSR*1 gene product played an essential role in the SRP-dependent translocation apparatus. To get further insights into its function, we analyzed the product of *TSR*1 and its interactions with the components of the SRP and the translocation machinery.

Tsr1p is a serine-threonine-rich protein of 461 amino acids that shows extensive homology with two putative proteins Yhc8p from *Saccharomyces cerevisiae* and Ylu2p from *Hansenula polymorpha* (19). The presence of an amino-terminal signal sequence and of a membrane spanning domain suggested that Tsr1p was an integral membrane protein of the ER membrane. Immunoblot experiments using a Tsr1p antiserum revealed a protein with a molecular mass of ± 50 kDa in close agreement with predictions derived from sequencing data. Using cell fractionation experiments we have demonstrated that Tsr1p was associated with a rapidly sedimenting fraction and required treatment with detergents to be solubilized. Proteinase K treatment in the absence of detergent showed that the Tsr1p molecular mass shifted from 50 to 34 kDa, which is compatible with the removal of its carboxyl-terminal domain. Fractionation experiments show a significant colocalization between Tsr1p and Kar2p. Taken together, these results strongly suggest that Tsr1p is a type I transmembrane protein of the ER, exposing its carboxyl-terminal to the cytoplasm and its amino-terminal to the ER lumen.

Genetic and biochemical interactions indicated that Tsr1p interacts with components of the SRP and of the translocation apparatus. First, the *tsr*1-1 mutation was isolated as an allele-specific suppressor of a 7SL RNA mutation *scr*2.II-13. Second, immunoprecipitation analysis using anti-Sec65p antibodies showed that contrary to an *scr*2.II-13 mutant, where little 7SL RNA was coimmunoprecipitated with Sec65p, in *tsr*1-1(*scr*2.II-13) suppressed cells, the SRP stability was completely restored. Third, the Tsr1p antiserum immunoprecipitated, in a wild type strain, the SRP-ribosome complex as judged by the coimmunoprecipitation of Sec65p, rRNA 5.8S, and 7SL RNA. Coimmunoprecipitation by the Tsr1p antiserum of Kar2p, an ER lumenal chaperone protein from the HSP70 class, was detected even without cross-linking and suggests a strong interaction between these two proteins. This interaction appears very important because recent results have shown that binding of secretory precursor polypeptides to a translocon subcomplex is regulated by BiP (20) and that Kar2p can act in cooperation with some components of the translocon to stimulate translocation by pulling the secretory protein precursors across the ER membrane (21, 22).

Together our results indicate clearly that Tsr1p participates in a complex involving these components, and because *TSR*1 is an essential gene, we have to conclude that it can play an important role in the SRP-dependent translocation events at least.

How does the mutated Tsr1p suppress the *scr*2.II-13 defect? Our results show that it does not restore an almost wild type structure to an SRP containing a defective *scr*2.II-13 mutant RNA, because this RNA is still as susceptible to degradation as it was in the original *TSR*1$^+$ background. This nucleolytic cleavage seems to reflect a secondary effect of the mutation, not directly responsible for the secretory defect. We observed that in a *tsr*1-1(*scr*2.II-13) strain, a ribosomal component, the 5.8S RNA, was much more efficiently coimmunoprecipitated by antibodies directed against Sec65p, a SRP component, than it was in a *TSR*1$^+$ strain, mutated or not for the 7SL RNA. This strongly suggests that *tsr*1-1 reinforces or stabilizes interactions between SRP and the ribosome at the docking or at the translocation site. Because the primary defect of *scr*2.II-13-mutated SRP has been postulated to involve inefficient targeting of this SRP to the ER, it is tempting to speculate that *tsr*1-1 may suppress *scr*2.II-13 by increasing the half-life of the ribosome complex at the receptor or translocation site. Conversely, this would account for the deleterious effect of *tsr*1-1 in an *SCR*2$^+$ context, where the clearance time would be unduly lengthened.

What would then be the function of wild type Tsr1p? In the above scheme, Tsr1p would be required to accelerate dissociation of the SRP from the ribosome. Recent work from Bacher *et al*. (23) suggests that ribosomes play a crucial role in the GTPase cycle of the SRP, acting as GTP loading factors for SRP54 in the presence of a signal peptide and strengthening the SRP-ribosome complex. Dissociation of the complex is thought to occur at the SRP receptor, where a GTP-loaded SRP receptor α subunit activates the GTPase activity of SRP54. Activation of SRP receptor α by GTP requires an unknown partner, which may be the translocon or a translocon-associated protein. A likely place for Tsr1p in this puzzle would be at this level, where it could gauge through its lumenal domain, possibly through Kar2p interaction, the state of clearance of the translocon and control SRP-ribosome dissociation through its cytoplasmic domain. The recent identification of a *TSR*1 functional homologue in *S. cerevisiae*[2] should permit testing these possibilities.

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