Sls1p, an Endoplasmic Reticulum Component, Is Involved in the Protein Translocation Process in the Yeast Yarrowia lipolytica*

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Signal recognition particle-dependent targeting of secretory proteins to the endoplasmic reticulum membrane is predominant in the yeast Yarrowia lipolytica. A conditional lethal mutant of the SCR2-encoded 7S RNA provided the first in vivo evidence for involvement of this particle in cotranslational translocation (He, F., Beckerich, J. M., and Gaillardin, C. M. (1992) J. Biol. Chem. 267, 1932–1937). In order to identify partners of 7S RNA or signal recognition particle in their function, we selected synthetic lethal mutations with the 7S RNA mutation (sls). The SLS1 gene, cloned by complementation of the sls1 mutant growth defect, encodes a 426-amino acid polypeptide containing a NH2-terminal signal peptide and a COOH-terminal endoplasmic reticulum (ER) retention motif. The SLS1 gene product behaves as a luminal protein of the ER. Sls1p was sedimented with membrane-rich organelles and was resistant to protease degradation without prior membrane solubilization. Immunofluorescence microscopy showed a typical endoplasmic reticulum perinuclear staining. Co-immunoprecipitation revealed that Sls1p resides close to the major translocation apparatus component, Sec61p. Deletion of the SLS1 gene led to a temperature-sensitive growth phenotype. Synthesis of several secretory proteins was shown to be specifically reduced in Δsls1 cells. We propose that Sls1p acts in the preprotein translocation process, interacting directly with translocating polypeptides to facilitate their transfer and/or help their folding in the ER.

In order to enter the secretion pathway, secretory proteins of eukaryotic cells have to be transported across or inserted into the endoplasmic reticulum (ER)1 membrane. To achieve this translocation step, secretory proteins must be specifically targeted to the translocation machinery in the ER membrane and be competent for crossing this membrane (2). In higher eukaryotes, the signal recognition particle (SRP) was shown to take part into these functions (3). SRP is composed of a single 7S RNA and six polypeptides (4). When the signal sequence of a nascent secretory polypeptide is extruded from the ribosome, it is first recognized by the nascent polypeptide associating complex (5), which allows specific binding of SRP. Interaction of SRP with the nascent chain-ribosome complex causes translational slow down. After binding of SRP to its membrane-bound receptor, SRP is displaced from the complex and the nascent chain is transferred to the translocation site where crossing takes place simultaneously to translation. As soon as the polypeptide emerges in the lumen of the ER, it interacts with various proteins for processing and folding. A somewhat different picture emerged from studies on the yeast Saccharomyces cerevisiae. Indeed, several secretory proteins in this yeast appeared to be transported post-translationally, both in vivo and in vitro (6, 7), and homologues of mammalian SRP components that have been identified in this yeast and function in translocation (8–11) are not essential for cell viability. In another yeast Yarrowia lipolytica, deletion of both genes SCR1 and SCR2 encoding 7S RNA is lethal (12), and we suggested earlier that the SRP-dependent targeting may be the main pathway, as in higher eukaryotic cells.

Isolation of conditional lethal mutants in the 7S RNA provided in vivo evidence for involvement of SRP in cotranslational translocation (1, 13). In order to identify partners of SRP in this process and to better understand its molecular mechanisms, we have now selected synthetic lethal mutations with the 7S RNA mutation, called sls. In the present paper, we describe identification and characterization of one of these genes, SLS1, and of its gene product.

MATERIALS AND METHODS

Strains, Growth Conditions, and Materials—For DNA manipulation, Escherichia coli strains TG1 or DH5α were used. E. coli cells were grown at 37°C in LB medium (2.5% Luria broth base). Ampicillin was added at 100 μg/ml. The Y. lipolytica haploid mutagenized strain was MatB, scr1::ADE1, Δscr2, ura3, Ian2, his-l, containing the replicative plasmid pNA1090 carrying the scr2-11.13 allele and the URA3 gene. Replacement of the scr2-11.13 allele by the wild-type allele was done by plasmid shuffling using the replicative plasmid pNA237, which contained the SCR2 gene and the UEA2 gene. For genetic studies, the sls1 Ts mutant was mated with a Mata, scr1::ADE1, Δscr2, lys11, ura3, Ian2 strain containing the plasmid pNA398 carrying the SCR2 gene and the URA3 gene. Diploids were sporulated and analyzed as described previously (12). To isolate the SLS1 gene from a LEU2-based replicative genomic library constructed by P. Fournier, a Leu– Ts segregant from this cross was obtained. Transformation of Y. lipolytica by the lithium acetate method was performed as described previously (14). Y. lipolytica strains were usually grown at 28°C in YPD (1% yeast extract, 1% bacto-peptone, 1% glucose). For transformant selection, minimal medium (0.07% yeast nitrogen base without ammonium sulfate and without amino acids, 1% glucose, 0.1% proline) was used, and supplements were added to a final concentration of 0.01%. 5-Fluoroorotic acid (10 μg/ml) was used as selective agent. For labeling, GC medium (same as GPP but 0.2% casein instead of 3% protease peptone) was used.
Plasmids and Nucleic Acid Manipulations—E. coli plasmid pBS (Bluescript, from Stratagene) was used for DNA sequencing of a 2-kb Clal-Sall fragment carrying the SLS1 gene. pNA237 and pNA298 containing the SCR2 allele have been described previously (1). To test sls1-1 monocompend replication, a 3.4-kb XbaI-HindIII fragment was cloned between the unique sites NheI and HindIII of an integrative plasmid. The recombinant 70-kDa protein was expressed and harvested after 2 min at 450 × g. Cells were incubated for 2 h on ice; two were treated with 0.5 mg/ml of polylysine. Cells were treated with 10 mg of Zymolyase 20T for 2 min of lysis buffer and divided into three parts. Reactions were stopped by the addition of 0.2 M sorbitol, 0.1 M NaCl, 25 mM NaPi, pH 7.4, 1 mM EDTA, and 0.1 mg/ml of lysis buffer and divided into three parts. Reactions were stopped by the addition of 0.2 M sorbitol, 0.1 M NaCl, 25 mM NaPi, pH 7.4, 1 mM EDTA, and 0.1 mg/ml of 4°C during 3 h in the presence of antibodies and 10 μl of protein A-Sepharose. Extracts and PBS-washed immunoprecipitates were diluted with 2 × SDS-PAGE buffer containing 50 mM diithiothreitol and heated 10 min at 95°C before SDS-polyacrylamide gels. After migration, proteins were electrophoresed into nitrocellulose membranes (Schleicher & Schuell). Preincubation with 2% milk, antibody incubations, and washes were done in 100 mM Tris, pH 8, 150 mM NaCl, 0.05% Tween 20 buffer.

Pulse-Chase Labeling and Immunoprecipitation of AEP—Cells grown overnight in GPP medium at 18°C were concentrated to 1,000 Klett in GC medium and equilibrated at 18°C for 20 min before a 1-h shift to a nonpermissive temperature of 28°C. Cells were radiolabeled with 750 μCi of [-4,5,5-3H]leucine for 45 s and then chased with a 300-fold excess of cold L-leucine. 3-mi aliquots were withdrawn at 0, 1, 2, 5, and 5 min and mixed with 2.3 g of crushed ice plus 10 mM NaF, and 2 mM phenylmethylsulfonyl fluoride to stop protein synthesis. After centrifugation, supernatants were precipitated with 10% trichloroacetic acid on ice for 1 h. Precipitates were dissolved in 200 μl of SDS-PAGE loading buffer and neutralized with 1 M Tris, pH 9.5. Cell extracts, prepared as described previously (16), were adjusted to 1% SDS and 4% Triton X-100 before immunoprecipitation. Total incorporation of label was measured at two post-chase time points by scintillation counting of boiled trichloroacetic acid precipitates from two 100-μl samples of labeled cell suspension. Proteins were analyzed by electrophoresis in 10% polyacrylamide gels, after which they were dried and fluorographed.

Glycoprotein Precipitation with Concanavalin A-Sepharose—Cells were grown in minimal medium at 20°C, 25 optical density units of cells were resuspended in 0.8 ml of sulfate-free synthetic medium and incubated labeling with 500 μCi of Tran35S-label for 10 min at 26°C. Cells were mixed with 200 μl of 50 mM cold methionine and cysteine and 10 μl of 1 M NaF, 100 μl of the 200 μl of clarified extract was diluted with 400 μl of conconavalin A buffer (15 mM NaPi, pH 7, 150 mM NaCl, 1% Triton X-100, 0.1% SDS), and 40 μl of packed conconavalin A-Sepharose beads was added, followed by incubation at room temperature for 2 h. After four washes, bound proteins were dissociated in SDS-PAGE buffer by heating at 95°C for 5 min. Glycosylated proteins were analyzed by electrophoresis in 6% polyacrylamide gels. Gels were then dried and fluorographed.

RESULTS

Isolation of a Mutant That Displays Synthetic Lethality with the scr2-II.13 Mutant—To identify partners of the 7S RNA and/or the SRP in the translational translocation process, we looked for new mutations aggravating the phenotype of the conditional scr2-II.13 mutation. Previous studies have indicated that such synthetic lethal interactions occur between genes encoding proteins involved in a common complex process (17, 18). The scr2 mutant strain that contains chromosomal deletions of both SCR1 and SCR2 and carries the scr2-II.13 allele on a replicative plasmid is viable at 32°C, having a generation time doubled at this temperature (1). Clones unable to grow at 32°C were selected after UV mutagenesis of the scr2 mutant strain. To distinguish between mutants with a tight temperature-sensitive lethal growth phenotype and synthetic lethal ones, the scr2-II.13 allele was replaced by the wild-type allele by plasmid shuffling (selection for Leu+ Ura− transformants), and the growth phenotype produced by the second mutation alone was studied. The sls1-1 mutation (for synthetic lethal with the 7S RNA mutation) was shown to confer an extreme Ts growth phenotype only in association with the scr2-II.13 mutation (Fig. 1, 1 versus 2). However, the sls1 single mutant still displayed a temperature-sensitive growth, having its generation time doubled at 28°C in a scr2-II.13 context as compared with a wild-type strain (Fig. 1, 2 versus 3).

Isolation of the SLS1 Gene—The sls1-1 mutation was shown...
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**Primary Structure of the SLS1 Gene Product**—The 1967-bp insert was sequenced and shown to contain a single complete open reading frame of 1278 bp (Fig. 3A), potentially encoding a 426-amino acid polypeptide. A putative TATA box was found 135 bp upstream of the start codon, and a putative transcription termination element was present 14–58 bp downstream of the TAA translation stop codon (Fig. 3B). The deduced amino acid sequence shared no significant homology with proteins present in the database. However, the amino-terminal sequence of the SLS1 protein showed the features of a 17-amino-acid signal peptide, containing a stretch of 8 hydrophobic residues (19). Such an amino-terminal extension characterizes secreted proteins. A tetrapeptide motif RDEL was observed at the carboxyl terminus of the predicted sequence. A related sequence, KDEL, has been shown to be implicated in soluble protein retention in the endoplasmic reticulum (20). Because no other hydrophobic region is present, Sls1p is likely to be an ER luminal protein. No potential N-linked glycosylation site was found.

**Localization of the SLS1 Gene Product**—To verify the pre-
dictions concerning the intracellular localization of Sls1p, antibodies were raised against a large region of the Sls1 protein extending from residue 21 to residue 403, fused to the S. japonicum glutathione S-transferase protein. The serum reacted with a unique protein of approximately 55 kDa in whole extracts of wild-type cells (Fig. 4A, lane 1). This signal was amplified in cells carrying the SLS1 gene on a centromeric plasmid (Fig. 4A, lane 2). In cells expressing a carboxyl-terminal deletion of Sls1p lacking the last 23 amino acid residues, the signal appeared at a lower molecular weight (Fig. 4A, lane 4). The amount of the truncated protein was reduced compared with the wild-type protein level. In a cell fractionation and protease sensitivity experiment, Sls1p was shown to reside within a membranous cellular compartment. Sls1p was sedimented after a low speed centrifugation (10,000 × g) and remained resistant to protease degradation in the absence of detergent (Fig. 4B). However, after membranes solubilization by Triton X-100, Sls1p became accessible to proteinase K. Immunofluorescence microscopy was performed with anti-Sls1p antibodies to determine the localization of the SLS1 gene product. Wild-type cells showed a specific Sls1p perinuclear staining (Fig. 4C). Because the ER is an extension of the nuclear envelope, this staining pattern is consistent with a localization of the protein into the ER. A similar pattern was obtained in S. cerevisiae for the soluble ER resident protein Kar2p (21) or for the ER membrane protein Sss1p (22). In cells expressing the 3'9-truncated copy of the SLS1 gene, Sls1p immunoreactivity was more diffuse (not shown).

Levels of Sls1p Expression—ER lumenal proteins such as the chaperone BiP or the protein disulfide isomerase are involved in folding of translocated polypeptides and are induced under conditions leading to protein misfolding. In the yeast Schizosaccharomyces pombe or in S. cerevisiae, increased levels of the BiP/Kar2p protein result from an induction of its transcripts (21, 23). To test such a regulation for the SLS1 gene, intracellular levels of Sls1p were first examined by immunoblotting under conditions of heat shock and inhibition of glycosylation. A 2-fold increase of Sls1p intracellular amounts was observed 1 h after incubation at 34 °C compared with levels in cells incubated at 28 °C (Fig. 5A, lanes 2, 4, and 6 versus lanes 1, 3, and 5) and

**Fig. 4. Localization of the Sls1 protein.** A, characterization of the anti-Sls1p antibodies produced against a glutathione S-transferase-Sls1p fusion protein. Western blot analysis of whole cell extracts from various Y. lipolytica transformants containing different SLS1 mutant constructs. The strain in lane 1 contains the wild-type SLS1 allele on the chromosome, the strain in lane 2 also contains the SLS1 gene on a 3–4-copy plasmid, the strain in lane 3 contains the deleted Δsls1 allele on the chromosome, and the strain in lane 4 contains a 3'-truncated chromosomal copy of SLS1. Equal amounts of total protein were applied on SDS-PAGE. B, cell fractionation and protease protection of Sls1p. A whole cell extract from the wild-type strain 136463 was made by a gentle method and cleared from unbroken cells and cell wall fragments by centrifugation at 450 × g. Supernatant (S450) was re-collected and subfractionated by a 20-min centrifugation at 10,000 × g leading to two fractions (S10000 and C10000). Samples of the resuspended latter fraction were treated on ice for 1 h with 0.5 mg/ml of proteinase K in the absence or in the presence of 4% Triton X-100. Samples were analyzed by SDS-PAGE followed by Western blotting with Sls1p antiserum. C, localization of Sls1p by immunofluorescence in cells expressing the chromosomal SLS1 gene. Cells were grown overnight in rich medium, prepared for immunofluorescence, and treated with rabbit anti-Sls1p antibodies, followed by fluorescein isothiocyanate-conjugated anti-rabbit IgG to localize the Sls1 protein (a) and 4,6-diamidino-2-phenylindole to visualize nuclear DNA (b and d). The Sls1p signal was abolished by exclusion of the primary antibodies or by staining of the Δsls1 strain (c).
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Fig. 5. Levels of intracellular Sls1p after heat shock and tunicamycin treatment. A, half of an overnight culture in rich medium was incubated at 34°C (lanes 2, 4, and 6), whereas the second half remained at 28°C (lanes 1, 3, and 5). Samples were taken every 15 min for 90 min. B, half of an overnight culture in rich medium was incubated for 3 h at 23°C in the presence of tunicamycin (10 μg/ml) (lane 2), whereas the second half was left untreated (lane 1). Whole cell extracts were made and subjected to SDS-PAGE (8% polyacrylamide gel). Proteins were transferred to nitrocellulose and probed with rabbit anti-Sls1p antibodies and goat anti-rabbit IgG conjugated with peroxidase. In these experiments, equal amounts of total protein were loaded for each extract.

was maintained for at least 30 min. Half of an overnight wild-type cell culture, freshly diluted, was treated with tunicamycin for 3 h at 23°C. Levels of Sls1p detected by Western blot in cell extracts from this culture were 2–3-fold higher than amounts revealed in cells of the untreated second culture (Fig. 5B, lane 2 versus lane 1). Similar results were obtained when SLS1 induction was monitored using a SLS1::lacZ transcription fusion (not shown).

Sls1p Is Required for Growth at Elevated Temperatures—Because a short Sls1 protein (of approximately 20 kDa) is still present in the sls1-1 mutant, a sls1 null mutant was constructed to determine if the protein was required for cell viability. A 2.45-kb ClaI-HindIII fragment carrying a deletion of the first 289 amino acids and the URA3 gene (Fig. 3A) was used to transform a haploid strain carrying a SLS1 wild-type copy on a LEU2-based replicative plasmid. Ura+ transformants were selected. To eliminate clones that would have integrated the deleted fragment in the resident LEU2-plasmid, stable Ura+ clones were retained after culture in rich medium and replica plating on 5-fluoroorotic acid medium in the presence of leucine. Loss of the LEU2, SLS1 plasmid was then selected after incubation at 25°C. One of the Ura+ Leu− segregants showed a temperature-sensitive growth phenotype. Integration of the deleted copy in place of the wild-type one was confirmed by Southern blot analysis. Immunoblot of extracts from Δsls1 cells with anti-Sls1p antibodies revealed no signal (Fig. 4A, lane 3). The function of the Sls1p is therefore not required for cell viability but is essential for optimal growth at elevated temperatures.

Secretory Protein Synthesis Is Inhibited in Δsls1 Cells—Because the scr2-11.13 mutation caused a specific inhibition of the synthesis of a cotranslationally translocated secretory protein (1) and because the sls1-1 mutation conferred a synthetic growth defect with the 75 RNA mutation, we looked for similar defects in the sls1 null mutant. Δsls1 cells were compared with SLS1 cells for synthesis, maturation, and secretion rates of the AEP by a pulse-chase labeling and immunoprecipitation experiment after 1 h shift to 28°C. AEP is synthesized as a 53-kDa prepropeptide with a signal sequence and a pro-domain upstream from the mature domain. In wild-type cells, the earliest precursor immunoprecipitated (pAEP, 55 kDa) lacks the signal sequence and is core-glycosylated, as expected for a cotranslationally translocated protein (16, 24). Subsequent cleavage by a Xpr2-like Golgi endoprotease results in a 20-kDa propeptide and a 32-kDa mature form, both of which are secreted.

Δsls1 and SLS1 cells were labeled in inducing medium for 45 s and chased. Fig. 6A shows a 7-fold reduction of the amount of labeled intracellular forms in Δsls1 cells compared with SLS1 cells (lanes 1–4 versus lanes 5–8). The level of the secreted 32-kDa mature form in the growth medium of Δsls1 cells, detected after chloroacetic acid precipitation, was also lower than those revealed in supernatant of SLS1 cells (Fig. 6B, lanes 1–4 versus lanes 5–8). Total protein synthesis was similar in the two strains. Because AEP precursors in Δsls1 cells could bind to concanavalin A-Sepharose (not shown), they corresponded to translocated forms. Maximal level of both precursor and mature forms was only obtained at 2.30 min in Δsls1 cells (Fig. 6A, lane 3) compared with 1 min in SLS1 cells (Fig. 6A, lane 6). Therefore, detection of total newly synthesized AEP precursors was delayed in the absence of the SLS1 gene product. This observation suggests that translocation is affected in a Δsls1 context. In addition, a delay in AEP processing was observed. In contrast to SLS1 cells, where the mature form was predominant at 1 min in cell extracts (Fig. 6A, lane 6) and secreted at 2.30 min in the medium (Fig. 6B, lane 7), most of
and in mature form was immunoprecipitated at 2.30 min in D cell extracts (Fig. 6) in the absence of the loss of several major glycoproteins appeared to be largely reduced in the absence of the SLS1 gene product (Fig. 7, lane 2 compared with lane 1), suggesting that synthesis of these precursors was impaired. In contrast, the amount of at least one glycoprotein was increased in Δsls1 cells.

The secretion of AEP is delayed in SLS1 overexpressing cells. AEP synthesis and secretion were studied in the presence of a 3-fold higher level of the Sls1 protein, as estimated by immunoblotting (Fig. 4A), in order to detect any modification of the secretion process. Pulse-chase labeling experiments were performed under the same conditions as for the sls1 null mutant. The ratio of AEP incorporation, measured after immunoprecipitation, to total protein labeling was similar in both extracts of overexpressing cells and wild-type cells, suggesting that the Sls1 protein is not limiting in the cotranslational translocation process. However, in these cells, intracellular maturation of the 32-kDa precursor form by the endoprotease and subsequent secretion of the 32-kDa mature form in the growth medium were delayed compared with SLS1 cells (Fig. 8, lanes 5–8 versus lanes 1–4).

Sls1p is Localized Close to the Major Component of the Translocation Pore—in order to test the proximity of the Sls1 protein to the translocation apparatus as expected for a protein involved in translocation, co-precipitation of Sls1p with the major component of the translocation channel Sec61p (25) was performed. A Sec61p homolog has been recently identified in Y. lipolytica. The Y1 Sec61p amino acid sequence shares 22% identity and 37% similarity with the human Sec61p NH2-terminal region. A membrane-rich fraction was prepared from wild-type cells. Samples were subjected or not to cross-linking by the cleavable reagent dithiobis(succinimidyl propionate) (0.2 mg/ml) simultaneously to solubilization in 1% Triton X-100 and were immunoprecipitated by anti-Sls1p antibodies. Lane 1, crude extracts; lane 2, Sec61p immunoprecipitates after cross-linking; lane 3, Sec61p immunoprecipitates without dithiobis(succinimidyl propionate) treatment. Sec61p blotted with antibodies raised against a 22-amino acid NH2-terminal peptide on crude extracts (lane 1), on extracts solubilized with 1% Triton X-100 and immunoprecipitated either by anti-Y. lipolytica Sec61p antibodies (lane 2), by anti-S. cerevisiae Sec61p antibodies (lane 3), or by anti-Sls1p antibodies (lane 4). In lane 5, immunoprecipitates analyzed in lane 2 were probed with anti-Y. lipolytica Sec61p preimmune serum.

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Fig. 7. Pattern of glycosylated proteins in SLS1 cells (lane 1) and Δsls1 cells (lane 2) at 25°C. [35S]Methionine-labeled total proteins from each strain were incubated with concanavalin A-Sepharose beads. Bound proteins were eluted by heating, applied to a 6% SDS-polyacrylamide gel, and visualized after fluorography.

Fig. 8. AEP secretion in Sls1p overexpressing cells. SLS1 monocopy cells (lanes 1–4) and SLS1 multicopy cells (lanes 5–8) were pulse-labeled for 45 s and chased as previously described. Samples were taken at 0, 1, 2.30, and 5 min post chase and centrifuged. Proteins from each supernatant were trichloroacetic acid-precipitated and subjected to SDS-PAGE and fluorography. Mature AEP is signaled by an arrow.

Fig. 9. Sls1p and Sec61p co-precipitation. A, a membrane-rich fraction was prepared from wild-type cells. Samples were subjected or not to cross-linking by the cleavable reagent dithiobis(succinimidyl propionate) (0.2 mg/ml) simultaneously to solubilization in 1% Triton X-100 and were immunoprecipitated by anti-S. cerevisiae Sec61p antibodies. Precipitates were resolved on SDS-PAGE transferred to nitrocellulose, and blotted with anti-Sls1p antibodies. Lane 1, crude extracts; lane 2, Sec61p immunoprecipitates after cross-linking; lane 3, Sec61p immunoprecipitates without dithiobis(succinimidyl propionate) treatment. Sec61p blotted with antibodies raised against a 22-amino acid NH2-terminal peptide on crude extracts (lane 1), on extracts solubilized with 1% Triton X-100 and immunoprecipitated either by anti-Y. lipolytica Sec61p antibodies (lane 2), by anti-S. cerevisiae Sec61p antibodies (lane 3), or by anti-Sls1p antibodies (lane 4). In lane 5, immunoprecipitates analyzed in lane 2 were probed with anti-Y. lipolytica Sec61p preimmune serum.

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peptide of Y. lipolytica Sec61p. These antibodies revealed a same product either in crude extracts (Fig. 9B, lane 1) or in Yl Sec61p (lane 2), Sc Sec61p (lane 3), or Sls1p immunoprecipi- tates (lane 4). No signal appears in Y. lipolytica Sec61p immu-noprecipitates when blotting was done with preimmune serum (lane 5).

DISCUSSION

Genetic data indicate that major secretory proteins are tar-geted and translocated across the ER membrane in a SRP-dep-endent way in the yeast Y. lipolytica (12). This yeast therefore represents a good model for a genetic approach of cotransla-tional translocation molecular mechanisms. A SRP-deficient strain carrying the mutant scr2-II.13 allele on a replicative plasmid (1) was used to look for secondary mutations that specifically exacerbate the Ts growth phenotype displayed by the two mutations. The translation defect of the scr2-II.13 mutant could be explained by a decrease in initiation of pre-protein translocation. To explain the translation defect in the absence of Sls1p, we favor the hypothesis that Sls1p helps translocating polypeptides on the luminal side of the ER mem-brane, completing their translocation. Consistent with this scheme, a delay is observed for the appearance of total newly synthesized precursors in the absence of the SLS1 gene product. The function of mammalian ER luminal proteins in co-translational translocation, mediating the net transfer of the polypeptide into the ER lumen, has already been proposed (28). Sls1p might thus increase the translocation initiation rate on the opposite side of the ER membrane by freeing the translocon from the ER side. Co-precipitation of Sls1p with the major component of the translocation apparatus, which reveals that Sls1p resides in the vicinity of the translocation site, fits well with a function of the protein in preprotein translocation. Inter-estingly, Sls1p displays a domain similar to the COOH- terminal moiety of the family of Sec61p polypeptides (Fig. 10). Sec61p was described as a companion of Sec61p (Sec61α) in the translocon (29). We propose that this domain is involved in Sls1p binding for Sls1p. This hypothesis will be tested in the near future.

Our results are compatible with a second function of Sls1p in protein folding after translocation has been completed, its de-fect accounting for the delay observed in the processing of the 55-kDa precursor form in Sls1 cells. Additional evidence for a role of Sls1p during precursor transit through the ER was provided by the delay of precursor processing into the mature form in cells overexpressing Sls1p. Prevention of secretion of some secretory proteins has been described in mammalian cells overexpressing the BiP protein (30) and was supposed to result from the stabilization of the complex between newly translo-cated preprotein and BiP. Induction of Sls1p levels under condi-tions leading to ER accumulation of misfolded preproteins was also consistent with such a property.

Our results show that synthetic lethality may be used as a screen for isolation of new mutations and that such genetic interaction could be observed for two products that are phys-i-cally separated but involved in the same coordinated pathway.

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