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A Link between Secretion and Pre-mRNA Processing Defects in *Saccharomyces cerevisiae* and the Identification of a Novel Splicing Gene, *RSE1*

ESTHER J. CHEN, ALISON R. FRAND, ELIZABETH CHITOURAS, AND CHRIS A. KAISER*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Secretory proteins in eukaryotic cells are transported to the cell surface via the endoplasmic reticulum (ER) and the Golgi apparatus by membrane-bounded vesicles. We screened a collection of temperature-sensitive mutants of *Saccharomyces cerevisiae* for defects in ER-to-Golgi transport. Two of the genes identified in this screen were *PRP2*, which encodes a known pre-mRNA splicing factor, and *RSE1*, a novel gene that we show to be important for pre-mRNA splicing. Both *prp2-13* and *rse1-1* mutants accumulate the ER forms of invertase and the vacuolar protease CPY at restrictive temperature. The secretion defect in each mutant can be suppressed by increasing the amount of *SAR1*, which encodes a small GTPase essential for COPII vesicle formation from the ER, or by deleting the intron from the *SAR1* gene. These data indicate that a failure to splice *SAR1* pre-mRNA is the specific cause of the secretion defects in *prp2-13* and *rse1-1*. Moreover, these data imply that Sar1p is a limiting component of the ER-to-Golgi transport machinery and suggest a way that secretory pathway function might be coordinated with the amount of gene expression in a cell.

The isolation of conditional mutants has greatly facilitated study of the secretory pathway in the yeast *Saccharomyces cerevisiae*. Operationally, the defining characteristic of secretory pathway mutations is that they allow protein synthesis to continue but block the export of newly synthesized secretory proteins at some stage during their transit from the ER to the plasma membrane. The first secretion mutants were isolated from mutagenized cells that had been enriched for high density, a property of cells that continue to make protein and RNA but cannot increase their surface areas. Temperature-sensitive *sec* mutants were then identified by detection of their inability to deliver invertase, acid phosphatase, and sulfate permease to the cell surface. This screen yielded a set of mutants that were defective at different steps along the secretory pathway and remarkably specific for functions involved in vesicle trafficking (29, 30). Secretion mutants have also been found by a selection for mutants that fail to incorporate high levels of mannose into N-linked carbohydrate chains in the Golgi apparatus by a [^{3}H]mannose suicide technique (25) and by a colony immunoblot screen for mutants that fail to carry out Golgi processing of pro-α-factor (42). Many of the genes isolated in these screens were represented by only a single allele, indicating that additional *SEC* genes may have been overlooked.

We have used a brute-force approach to find new mutants with ER-to-Golgi transport defects by screening a large collection of random temperature-sensitive mutants for the conditional accumulation of the ER forms of both invertase and the vacuolar protease CPY. Surprisingly, among the new secretion mutants isolated in this screen were mutants primarily defective in pre-mRNA processing. These have mutations in *PRP2*, a known pre-mRNA splicing factor, or in a novel gene, *RSE1* (named for RNA splicing and ER-to-Golgi transport), which we show here to have a role in pre-mRNA processing. We show that the secretion defects of *prp2-13* and *rse1-1* mutants appear to result from the decreased activity of *SAR1*, an intron-containing gene that encodes a small GTPase required for vesicle formation from the ER (24). The secretion defects in these mutants can be suppressed by either increasing the amount of *SAR1* or deleting the intron from the chromosomal *SAR1* locus. Thus, pre-mRNA splicing mutants exert their effects on the secretory pathway by perturbing Sar1p synthesis.

* Corresponding author. Mailing address: Department of Biology, Room 68-533, 77 Massachusetts Ave., Cambridge, MA 02139. Phone: (617) 253-9804. Fax: (617) 253-8699. E-mail: ckaiser@mit.edu.

MATERIALS AND METHODS

Strains, media, and plasmids. *S. cerevisiae* strains used in this study are listed in Table 1. Preparation of rich medium (YEP) and minimal medium (SD), standard genetic manipulations, and yeast transformations were performed as described (1).

pRH262 carries *SAR1* (the A364A allele; see Discussion) in pRS316. pAF52 is a pCT3 library plasmid containing *PRP2*. pAF56 contains *PRP2* within an *Eco*RI fragment in pRS306. For the linkage analysis of *PRP2*, pAF56 was linearized with *Sna*BI and transformed into wild type (CKY294). When integrants were crossed to CKY570, the temperature sensitivity and uracil auxotrophy were completely linked in 16 tetrads each from crosses with two different transformants. A 1.8-kb *Cla*I fragment adjacent to *RSE1* from the original rescuing YCp50 library clone, pEC2, was inserted into pRS306 to generate pEC12. To test linkage of the cloned gene to the *RSE1* locus, pEC12 was linearized with *Sna*BI and transformed into CKY567. When integrants were crossed to wild type, the temperature sensitivity was linked to uracil prototrophy in 15 of the 16 tetrads dissected (8 each from two different crosses).

To construct an allele of *SAR1* without an intron (*SAR1*-Δ*i*), a cDNA copy of the *SAR1* gene was digested with *Bam*HI and *Cla*I and ligated into *Bam*HI- and *Cla*I-digested pRH262, creating pEC23. The insert in pEC23 was sequenced to verify the presence of a wild-type *SAR1* gene with the intron precisely deleted. pEC23 was digested with *Hin*dIII and *Kpn*I, and the fragment containing the entire intronless *SAR1* gene was ligated into a pRS306 derivative with the polylinker from the *Eco*RI site to the *Not*I site deleted, forming pEC24. pEC24 was cut with *Bam*HI and integrated into the chromosome of CKY294, and the chromosomal *SAR1* allele in CKY294 was replaced with *SAR1*-Δ*i* using two-step gene replacement (1). Clones were screened for a chromosomal copy of *SAR1*-Δ*i* by comparing the sizes of PCR fragments from the *SAR1* gene (intronless *SAR1* is 139 bases shorter than genomic *SAR1*) and by testing for the absence of the *Bgl*II site internal to the *SAR1* intron. Once the wild-type strain containing intronless *SAR1* (CKY566) was generated, it was crossed to both the *rse1-1* (CKY567) and *prp2-13* (CKY570) strains to generate the sister spores *rse1-1* (CKY568) and *rse1-1 SAR1*-Δ*i* (CKY569) and the sister spores *prp2-13* (CKY571) and *prp2-13 SAR1*-Δ*i* (CKY572).

Protein extracts and immunoblotting. Cultures were grown to exponential phase at 24°C in YEP plus 2% glucose (YPD) or in SC medium lacking the appropriate auxotrophic supplements plus 2% glucose. Cells (4×10^7) were collected by centrifugation, suspended at 2×10^7 cells/ml in YEP plus 0.1% glucose to induce invertase, and shifted to restrictive temperature for 2.5 h. Protein extracts were prepared by boiling in sample buffer (80 mM Tris-HCl [pH

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source or reference
CKY294	*MAT***a** *ura3-52 leu2-3,112*	6
CKY39	*MAT*α *sec12-4 ura3-52 leu2-3,112*	Kaiser lab collection
CKY566	*MAT***a** *SAR1-*Δ*i ura3-52 leu2-3,112*	This study
CKY567	*MAT*α *rse1-1 ura3-52 leu2-3,112*	This study
CKY568	*MAT***a** *rse1-1 ura3-52 leu2-3,112*	This study
CKY569	*MAT*α *rse1-1 SAR1-*Δ*i ura3-52 leu2-3,112*	This study
CKY570	*MAT*α *prp2-13 ura3-52*	This study
CKY571	*MAT***a** *prp2-13 ura3-52 leu2-3,112*	This study
CKY572	*MAT***a** *prp2-13 SAR1-*Δ*i ura3-52 leu2-3,112*	This study
368	*MAT***a** *prp2-1 ade1 ade2 ura1 his7 tyr1 gal1*	YGSC[a] (a)
125	*MAT***a** *prp3-1 ade1 ade2 ura1 his7 tyr1 gal1*	YGSC (a)
108	*MAT***a** *prp5-1 ade1 ade2 ura1 his7 tyr1 gal1*	YGSC (a)
382	*MAT***a** *prp11-1 ade1 ade2 ura1 his7 tyr1 gal1*	YGSC (a)
YAK22	*MAT***a** *ura3 leu2 trp1 ade2* Δ*CUP1* Δ*U1:HIS3 [TRP* CEN U1-C8U*]*	A. Kistler (UCSF)

[a] YGSC, Yeast Genetic Stock Center.

6.8], 2% sodium dodecyl sulfate [SDS], 1.5% dithiothreitol, 10% glycerol, 0.1% bromophenol blue), lysing by agitation with glass beads, and diluting to a total volume of 0.1 ml with sample buffer. For Sar1p Western blotting, 4×10^7 cells grown to exponential phase at 24°C were either harvested (at $t = 0$) or collected by centrifugation, suspended in YPD at concentrations of 5×10^6 to 8×10^6 cells/ml, and incubated at restrictive temperature. After 2 or 4 h at restrictive temperature, 4×10^7 cells were harvested to make protein extracts as described above.

Protein extracts (15 μl) were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to nitrocellulose filters. For Western blot detection, the following antibodies were used: rabbit anti-invertase at 1:1,000 dilution, rabbit anti-CPY antibody at 1:7,000 dilution, rabbit anti-Sar1p antibody (gift of A. Nakano) at 1:500 dilution, and horseradish peroxidase-coupled donkey anti-rabbit immunoglobulin G (Amersham) at 1:10,000 dilution. Western blots were developed using chemiluminescence (ECL kit; Amersham).

Northern blotting. Cultures (25 ml) were grown at 24°C in YPD to exponential phase and were either used immediately (at $t = 0$) or diluted with 25 ml of pre-warmed YPD at 48°C and incubated in a 36°C bath for 3 h. Cells were harvested by centrifugation in prechilled tubes, and total RNA was extracted by agitation with glass beads (31, 35).

For Northern blots, RNA (~20 μg) was electrophoresed on 2% agarose-formaldehyde gels (34) and transferred to Hybond N nylon membrane with 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). ^{32}P-labeled gene-specific DNA probes were synthesized by random priming (Multiprime DNA labeling; Amersham) of a *Cla*I-*Eco*RI fragment from *SAR1*. Hybridization was done using the method of Church and Gilbert (3).

β-Galactosidase assays. Cells were grown to exponential phase in the appropriate SC media plus 2% raffinose overnight at 24°C. Cells were shifted to a 37°C water bath for 15 min before galactose was added to 2%. Cells were further incubated at 37°C for 1.5 h before 0.8-ml aliquots were removed and β-galactosidase activity was assayed in cells permeabilized with SDS and chloroform (1). Background readings obtained with untransformed cells were subtracted, and β-galactosidase activity was expressed in Miller units (21): $1{,}000 \times OD_{420}$/min of reaction per 1 ml of culture at OD_{600} of 1. In at least two assays, three independent transformants were tested in parallel, and the results were averaged for each data point.

Invertase assays. Cultures grown to exponential phase in YPD at 24°C were collected by centrifugation, suspended in YEP plus 0.1% glucose at 2×10^7 cells/ml, and aerated at restrictive temperature for 2 h to induce invertase. Cells were washed with a mixture of 50 mM Tris-HCl (pH 7.5) and 10 mM NaN_3, washed with distilled water, incubated in 0.3 ml of 100 mM Tris-SO_4 (pH 9.4)–50 mM β-mercaptoethanol for 10 min, washed with spheroplasting buffer (1.2 M sorbitol, 10 mM Tris-HCl [pH 7.5]), and converted to spheroplasts by incubation for 30 min at 30°C in 60 μl of spheroplasting buffer containing 60 U of recombinant lyticase. Efficient spheroplasting was judged to be present when there was >85% lysis upon dilution with 1% Triton X-100. Centrifugation at $500 \times g$ for 5 min yielded a supernatant fraction containing extracellular invertase and a spheroplast pellet. The spheroplast pellet was washed once more with spheroplasting buffer. Then, both the supernatant fraction and the spheroplast pellet were diluted to yield a final volume of 1 ml in a mixture of 10 mM Tris-HCl (pH 7.5) and 1% Triton X-100. Invertase activity was assayed in 5 μl of each sample (7).

RESULTS

Splicing mutants are defective in secretion. To identify new secretion mutants, we screened a collection of 1,200 temperature-sensitive mutants (10) by Western blotting for the intracellular accumulation of ER forms of invertase and CPY. After backcrossing until the secretion defect and temperature sensitivity cosegregated and complementation testing against our collection of known *sec* mutants, temperature-sensitive mutations in several new genes required for ER-to-Golgi transport were identified. Two of the new mutants are described in this report.

Strain CKY570 failed to grow at temperatures above 30°C. In this strain, invertase accumulated in the core-glycosylated form (Fig. 1, lane 3), and CPY accumulated in the ER form at temperatures above 30°C (data not shown). To identify the affected gene, CKY570 was transformed with a genomic library in the vector pCT3 (38) and screened for rescue of temperature sensitivity. From among 40,000 transformants, 8 rescuing clones representing two different library plasmids were iso-

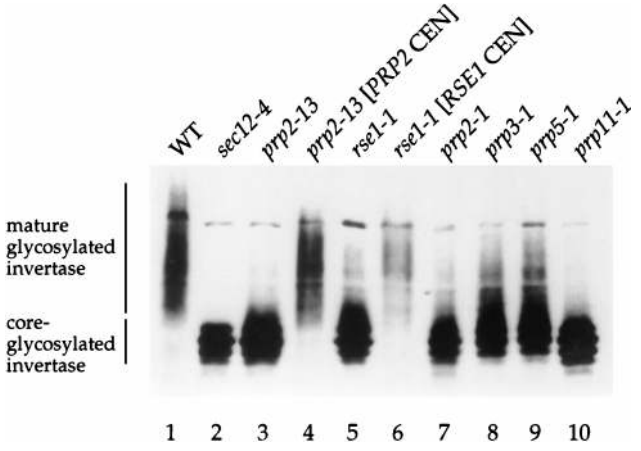
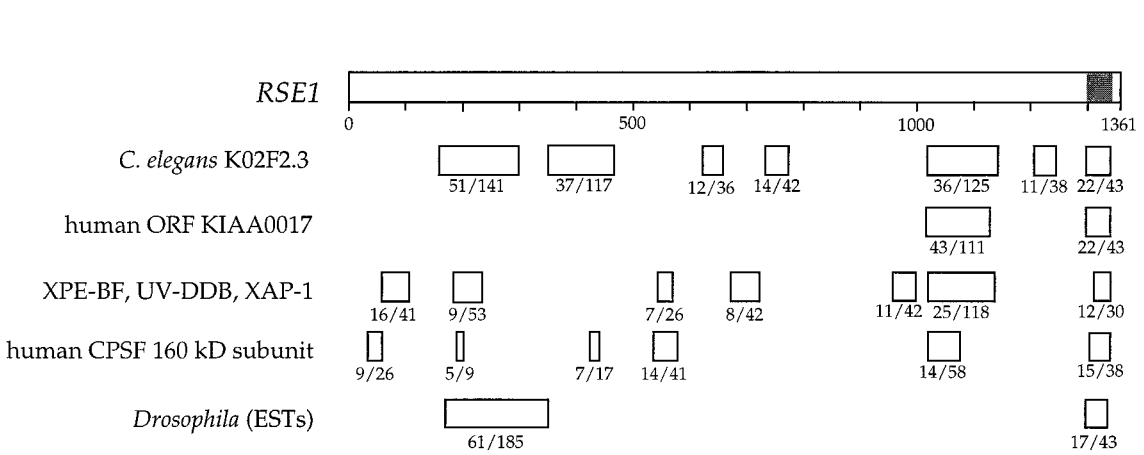


FIG. 1. Splicing mutants accumulate the ER form of invertase at restrictive temperature. Wild-type strain (CKY294) (WT) and *sec12-4* (CKY39), *prp2-13* (CKY570), *prp2-13* [*PRP2* CEN], *rse1-1* (CKY567), *rse1-1* [*RSE1* CEN], *prp2-1*, *prp3-1*, *prp5-1*, and *prp11-1* mutants were shifted to 37°C for 2.5 h in YEP plus 0.1% glucose to induce invertase. Invertase was detected in protein extracts by SDS-PAGE and immunoblotting with anti-invertase antibody. Mature glycosylated invertase migrates heterogeneously at ~140 kDa. Core-glycosylated invertase, the ER form, migrates at ~90 kDa.



B

scRSE1	1299	ILGRDHQEYRSYYAPVRKVIDGDLCENFLRLSLNEQEFLAKNL	1341
ceK02F2.3	1158	LCGRDHLAYRSYYAPCKSVIDGDICEQFSLMDTQKQKDVAEEL	1200
hKIAA0017	337	LCGRDHLSFRSYYFPVKNVIDGDLCEQFNSMEPNKQKNVSEEL	379
hCPSF160kD	1379	AFRMLHVDRRTLQNAVRNVLDGELLNRYLYLSTMERSELAKKI	1421
hXPE-BF	1070	HSFWRSFHTERKTEPATGFIDGDLIESFLDISRPKMQEVVANL	1112
dmEST		LCGRDHXSYRXSYYPVKNVXDGDXCEQYXSIEAAKQKSIAGDM	

FIG. 2. *RSE1* contains conserved domains related to mRNA processing and DNA repair proteins. (A) Under each box indicating a region of homology to the *RSE1* gene, the number of identical amino acids and the total number of amino acids in the boxed region are given. The amino acids in the shaded area of the *RSE1* gene are shown in the alignment below. The *C. elegans* and mammalian homologies were identified by using BLAST. The *Drosophila* sequences (GenBank accession nos. AA44069, AA393017, AA263279, and AA142215) were identified in a BLAST search against the NCBI database of expressed sequences tags. (B) At the C terminus of the sequence for the *RSE1* gene is a highly conserved domain that may represent a novel motif. The alignment of the amino acids at positions 1299 to 1341 for the *RSE1* gene with corresponding sequences for *S. cerevisiae* (sc), *C. elegans* (ce), human (h), and *Drosophila melanogaster* (dm) is shown. Residues that are identical in three or more of the sequences are highlighted. The sequence labelled "dmEST" is a predicted translation from GenBank accession no. AA142215.

lated. Both plasmids contained the same 2.2-kb genomic segment which rescued the temperature sensitivity and secretion defect of CKY570 (Fig. 1, lane 4). *PRP2* was shown to be the complementing gene in this genomic segment by the following two criteria. In a complementation test in which CKY570 was mated to a known *prp2-1* mutant (9), the resulting diploid strain was temperature sensitive. In a linkage test, we found complete linkage between the temperature sensitive mutation and a *URA3* marker integrated at the *PRP2* locus. Since 12 other *prp2* alleles have been reported (9, 39), we named this allele *prp2-13*.

PRP2 encodes an RNA-dependent ATPase that interacts directly with pre-mRNA before the first cleavage-ligation reaction of splicing (14, 19, 37). Given the known function of Prp2p as a nuclear splicing factor, it seemed unlikely that *PRP2* plays a direct role in ER-to-Golgi transport, and we asked whether a general connection between pre-mRNA splicing and protein secretion might exist. To test whether other pre-mRNA processing mutants have secretion defects, we examined invertase maturation in several other *prp* mutants (9). From the Yeast Genetic Stock Center, we obtained 4 of the 10 original *prp* mutants (previously called *rna* mutants), *prp2-1*, *prp3-1*, *prp5-1*, and *prp11-1*, which are defective in different steps of spliceosome formation (33). All four mutants accumulated the ER form of invertase at 37°C, indicating that each had a defect in ER-to-Golgi transport (Fig. 1, lanes 7 to 10). Thus, defects in protein secretion may be a general feature of pre-mRNA processing mutants.

***RSE1* is involved in pre-mRNA splicing.** A second gene identified in our screen was a novel gene we named *RSE1* (for RNA splicing and ER-to-Golgi transport). *rse1-1* mutants grew poorly at temperatures above 30°C and accumulated core-glycosylated invertase (Fig. 1, lane 5) and p1 CPY (data not shown) at 37°C, indicating an ER-to-Golgi secretion defect. To identify the affected gene, 15,000 transformants of the YCp50 library (32) were screened, and three complementing clones were isolated. Two of the clones were identical, and the sequence of the third clone overlapped those of the first two clones by about 7 kb. By subcloning, YML049c, a 4.1-kb open reading frame (ORF), was identified as the complementing gene which rescued the temperature sensitivity and secretion defect of the *rse1-1* strain (Fig. 1, lane 6). We confirmed that YML049c is *RSE1* by demonstrating tight linkage between *rse1-1* and a *URA3* marker integrated adjacent to the YML049c locus.

As shown in Fig. 2A, *RSE1* has regions of homology to a *Caenorhabditis elegans* ORF (GenBank accession number 2804455), a human ORF, the genes for putative human and monkey DNA repair proteins (XPE-BF, UV-DDB, and XAP-1), and the genes encoding the 160-kDa subunits of human and bovine cleavage and polyadenylation specificity factor (CPSF), which is involved in 3′ processing of pre-mRNA (11, 12, 16, 23, 28, 36). Rse1p has no known protein motifs, but the most highly conserved region of Rse1p, 43 amino acids located near the C terminus, may represent a novel motif. An alignment of this region with the corresponding regions from the other sequences is shown in Fig. 2B. The sequence relationships shown in Fig. 2 suggest a function for Rse1p in DNA repair or pre-mRNA processing. The *rse1-1* mutant appeared to have a normal capacity for DNA repair since it exhibited sensitivity to UV light identical to that of an isogenic wild-type strain (data not shown). To test whether *rse1-1* was deficient in mRNA

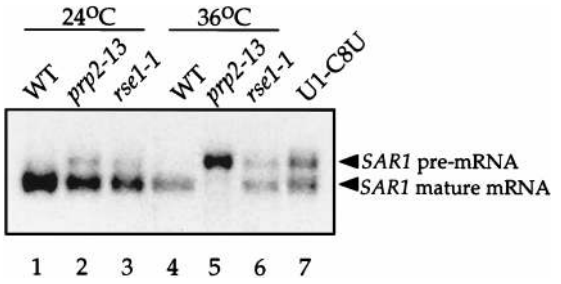


FIG. 3. Intron-bearing *SAR1* pre-mRNA accumulates in *rse1-1* and *prp2-13* mutants. Wild-type strain (CKY294) (WT) and *prp2-13* (CKY570) and *rse1-1* (CKY567) strains were grown overnight at 24°C (lanes 1 to 3) or shifted to 36°C for 3 h (lanes 4 to 6). Lane 7, a U1-C8U mutant strain (YAK22) grown at 30°C provides a positive control for a *SAR1* pre-mRNA splicing defect. Total RNA was prepared, and ~20 μg was loaded per lane on a 2% agarose-formaldehyde gel. RNA was transferred to a nylon membrane with 10× SSC, and the blot was probed with a ^{32}P-labelled *Cla*I-*Eco*RI fragment of the *SAR1* gene.

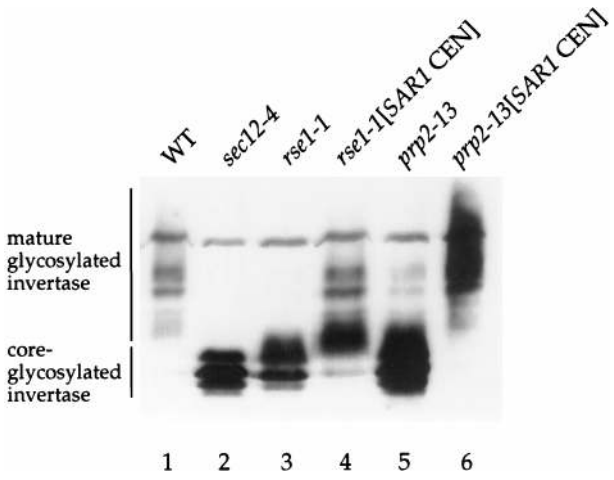


FIG. 4. Increased *SAR1* dosage suppresses the invertase secretion defect in *rse1-1* and *prp2-13* strains. Wild-type (CKY294) (WT), *sec12-4* (CKY39), and *rse1-1* (CKY567) strains were incubated at 37°C for 2.5 h in YEP plus 0.1% glucose to induce invertase; the *prp2-13* (CKY570) strain was shifted to 30°C for 2.5 h in YEP plus 0.1% glucose. Invertase was detected in protein extracts by SDS-PAGE and immunoblotting with anti-invertase antibody.

processing, we examined the processing of *SAR1* mRNA in an *rse1-1* mutant at 24 and 36°C. The *SAR1* gene, which contains a single short intron, encodes a small GTP-binding protein required for COPII vesicle formation from the ER (24). As shown in Fig. 3, lane 6, the *rse1-1* mutant accumulated intron-bearing *SAR1* pre-mRNA after 3 h at 36°C. The defect in *SAR1* pre-mRNA splicing in the *rse1-1* mutant is similar in strength to the defect seen in a U1-C8U snRNA mutant strain, which was included as a control. The *prp2-13* mutant also has a defect in *SAR1* pre-mRNA splicing which is stronger than the defect seen in either of the other two mutants.

To test whether *rse1-1* had more general effects on splicing, we examined its effects on two other introns in a splicing reporter assay. The splicing reporters contain *lacZ* interrupted by either the ribosomal protein 51a (*RP51a*) intron or *Acc*, an artificial intron with an intrinsically low splicing efficiency derived from the *RP51a* intron (17, 18). Since active β-galactosidase is expressed only when the intron has been spliced correctly, the efficiency of splicing for a given strain can be evaluated by comparing β-galactosidase activity from an intronless construct to the activities from constructs with introns. As shown in Table 2, the *rse1-1* mutant at restrictive temperature was about three times less efficient at splicing the *RP51a* intron, and about six times less efficient at splicing the *Acc* intron, than an isogenic wild-type strain. In agreement with the results of the Northern analysis of *SAR1* splicing, the splicing reporter assay showed that the *rse1-1* mutant had a substantial splicing defect, although it was not as strong as the defect in the *prp2-13* mutant at restrictive temperature. Thus, *RSE1* appears to have a general role in pre-mRNA splicing.

TABLE 2. Splicing defects in *rse1-1* and *prp2-13* strains in a reporter assay

Host strain	% β-galactosidase activity[a] of:		
	Construct with no intron	*RP51* intron	*Acc* intron
Wild type	100	37.3 ± 2.9	10.6 ± 0.6
rse1-1	100	13.1 ± 2.8	1.7 ± 0.2
prp2-13	100	0 ± 0	0 ± 0

[a] β-Galactosidase activity is expressed as the percentage of activity from the intronless β-galactosidase construct under the same conditions. The means and standard deviations for at least two assays of each of three different transformants are given. The absolute values of β-galactosidase activity for the intronless β-galactosidase transformants in a typical experiment were as follows: wild-type, 640 units; *rse1-1*, 830 units; and *prp2-13*, 1,000 units. One β-galactosidase unit is defined as 1,000 × OD_{420}/min of reaction for 1 ml of culture at OD_{600} of 1.

Pre-mRNA splicing and 3′ end formation have been shown to be coupled processes in mammalian cells (8, 20, 26, 27, 40), and the homology of *RSE1* to the genes encoding the 160-kDa subunits of human and bovine CPSF suggested that the primary defect in *rse1-1* mutants could be in 3′ end formation. *CUP1* mRNA has previously been shown to be a particularly sensitive reporter for 3′ processing defects, since unprocessed *CUP1* transcripts accumulate quickly and migrate much more slowly in gels than processed transcripts (4). On a Northern blot, the mobility of *CUP1* mRNA was not affected by *rse1-1* (data not shown). Given this result, it seems unlikely that *RSE1* is directly involved in 3′ end processing. However, we cannot exclude the possibility that 3′ end formation of genes other than *CUP1* is affected in *rse1-1* mutants.

***SAR1* is limiting for secretion in *prp2-13* and *rse1-1*.** When we screened plasmids for rescue of *prp2-13* and *rse1-1*, we found that the *SAR1* gene on a centromere plasmid could partially rescue the temperature sensitivity of both mutants. The extra copies of *SAR1* increased the threshold temperature for growth of both strains from 30°C to 33°C (data not shown). To evaluate the effect of *SAR1* overexpression on the secretion defect in splicing mutants, we assayed invertase maturation in *prp2-13* and *rse1-1* strains containing *SAR1* on a centromere-containing plasmid (pRH262). As shown in Fig. 4 (lanes 4 and 6) and Table 3, the increased amount of the *SAR1* gene restores invertase secretion in both mutants, allowing the mature glycosylated form to be produced and secreted at 30°C for the *prp2-13* strain and at 37°C for the *rse1-1* strain. The transport of CPY to the vacuole in both strains was also restored by inclusion of an extra copy of *SAR1* (data not shown).

The finding that extra copies of *SAR1* could partially suppress the temperature sensitivity of splicing mutants suggests that Sar1p is the limiting component for secretion when mRNA processing is blocked. To test this idea, we removed the intron from the *SAR1* gene to bypass the dependence of Sar1p synthesis on pre-mRNA splicing. *SAR1* in our wild-type strain (CKY294) was replaced with the intronless *SAR1* allele (*SAR1-Δi*) by two-step gene replacement, forming CKY566. *prp2-13 SAR1-Δi* (CKY572) and *rse1-1 SAR1-Δi* (CKY569) double mu-

TABLE 3. Restoration of invertase secretion in *rse1-1* and *prp2-13* strains by overexpression of *SAR1*[a]

Strain	% Invertase secreted	Total invertase activity (units/OD_{600})[b]
Wild-type	87	0.26 ± 0.03
sec12-4	5	1.31 ± 0.07
rse1-1	50	0.50 ± 0.06
rse1-1 [*SAR1* CEN]	80	0.33 ± 0.02
prp2-13	42	1.40 ± 0.15
prp2-13 [*SAR1* CEN]	83	0.66 ± 0.04

[a] To induce invertase, wild-type (CKY294), *sec12-4* (CKY39), and *rse1-1* (CKY567) strains were shifted to 37°C for 2 h in YEP plus 0.1% glucose and the *prp2-13* (CKY570) strain was shifted to 30°C for 2 h in YEP plus 0.1% glucose.
[b] The means and standard deviations from four assays (two each from two independent cultures) are given. One unit of invertase activity releases 1 μmol of glucose from sucrose at 30°C.

tants were then generated by crossing either CKY570 or CKY567 to CKY566.

As expected, the intronless allele of *SAR1* suppressed the ER-to-Golgi transport defects of both mutants. Mature glycosylated invertase was secreted in *prp2-13 SAR1-*Δ*i* at 30°C and in *rse1-1 SAR1-*Δ*i* at 37°C (Fig. 5A, lanes 4 and 6, and Table 4). CPY was processed at temperatures that would be restrictive for the single mutants (Fig. 5B, lanes 4 and 6). Also, the substitution of *SAR1-*Δ*i* for intron-containing *SAR1* partially rescued the temperature sensitivities of the *prp2-13* and *rse1-1* strains; the threshold temperature for growth of the *prp2-13* strain was increased from 30 to 33°C, and the threshold temperature of the *rse1-1* strain was increased from 30 to 37°C (Fig. 5C). Thus, it appears that the secretion defects in *prp2-13* and *rse1-1* mutants resulted directly from the failure of these strains to process pre-mRNA from *SAR1*. Although delivery of invertase to the cell surface was restored in the *rse1-1 SAR1-*Δ*i* mutant (Table 4), the invertase produced by this strain (Fig. 5A, lane 4), as well as by the *rse1-1* [*SAR1* CEN] strain (Fig. 4, lane 4), appeared to have less outer-chain glycosylation than invertase expressed in wild-type cells. This residual glycosylation defect suggested that the *rse1-1* mutation alters the function of the Golgi in a way that is not corrected by uncoupling *SAR1* expression from pre-mRNA splicing.

TABLE 4. Restoration of invertase secretion in *rse1-1* and *prp2-13* strains by intronless *SAR1* (*SAR1-*Δ*i*)[a]

Strain	% Invertase secreted	Total invertase activity (units/OD_{600})[b]
Wild type	87	0.26 ± 0.03
sec12-4	5	1.31 ± 0.07
rse1-1	48	0.42 ± 0.03
*rse1-1 SAR1-*Δ*i*	80	0.42 ± 0.05
prp2-13	29	1.28 ± 0.07
*prp2-13 SAR1-*Δ*i*	90	0.81 ± 0.10

[a] To induce invertase, wild-type (CKY294), *sec12-4* (CKY39), and *rse1-1* (CKY568 and 569) strains were shifted to 37°C for 2 h in YEP plus 0.1% glucose and *prp2-13* (CKY571 and 572) strains were shifted to 30°C for 2 h in YEP plus 0.1% glucose.
[b] The means and standard deviations from four assays (two each from two independent cultures) are given. One unit of invertase activity releases 1 μmol of glucose from sucrose at 30°C.

***SAR1* protein levels decline in splicing mutants.** To evaluate the effects of splicing mutations on levels of *SAR1* protein, we measured the amount of Sar1p in splicing mutants that had been grown at the restrictive temperature for 0, 2, or 4 h. In *prp2-13* and *rse1-1* mutants grown at permissive temperature, Sar1p was present at lower steady-state levels than in an isogenic wild-type strain (Fig. 6, lanes 1, 4, and 7). When growth was continued at restrictive temperature, Sar1p levels in the *prp2-13* and *rse1-1* mutants decreased even further. After 4 h at restrictive temperature, the Sar1p levels in the mutants declined to about one-quarter of the levels in the wild-type strain as determined by quantitative Western blotting and densitometry (Fig. 6, lanes 3, 6, and 9). Four repetitions of this experiment gave similar results. The finding that Sar1p levels decline within 2 h after Sar1p synthesis is blocked by a splicing defect implies that Sar1p is a moderately unstable protein. More direct measurements of the half-life of Sar1p were attempted in pulse-chase experiments, but Sar1p turnover could not be reliably detected by this protocol. Nevertheless, the low levels of Sar1p in *prp2-13* and *rse1-1* mutants at restrictive temperature indicate how a decrease in Sar1p synthesis could quickly result in an ER-to-Golgi transport defect.

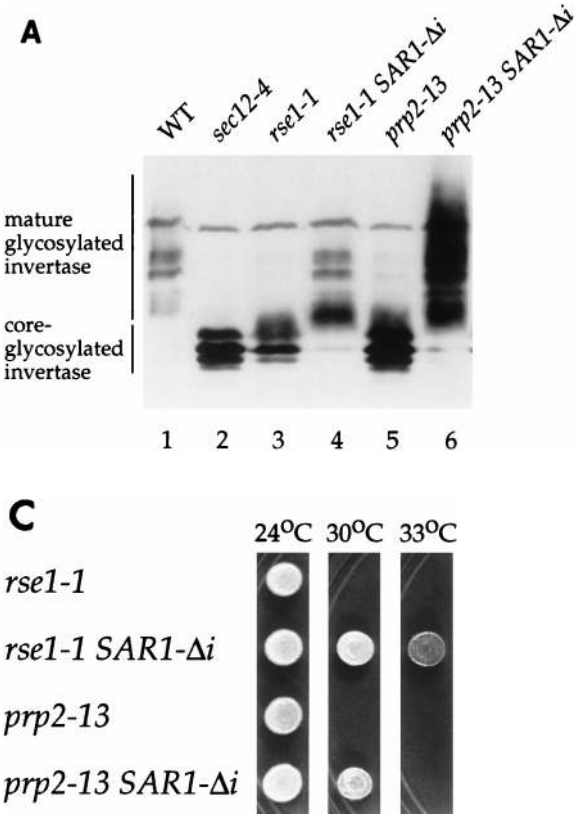
DISCUSSION

In this report, we show that (i) *RSE1* is a novel gene involved in pre-mRNA splicing, (ii) mutants with defects in pre-mRNA processing, such as *rse1-1* and *prp2-13* mutants, have ER-to-Golgi secretion defects, and (iii) these splicing mutants exert their effects on secretion specifically because they fail to process *SAR1* pre-mRNA.

The observed link between splicing defects and secretion defects can be explained by the fact that Sar1p appears to be moderately unstable in *rse1-1* and *prp2-13* mutants; consequently, a block of Sar1p synthesis in these mutants allows Sar1p levels to fall. The secretory pathway responds rapidly to a reduction of Sar1p synthesis (the block of secretion caused by a failure to splice the *SAR1* intron occurs in about 2 h, well before translation ceases due to a failure to splice the introns in ribosomal subunit genes). Thus, Sar1p may normally be present at a level close to the threshold level required for continued ER-to-Golgi transport.

Previously, it was shown that overexpression of *SAR1* could suppress the cold-sensitivity of a U1 snRNP mutation, indicating that Sar1p was limiting for growth in the U1 mutant at restrictive temperature (13). This finding suggested that the *SAR1* intron may be very inefficiently processed. However, the possibility that *SAR1* processing may be particularly sensitive to leaky splicing defects cannot explain the rapid loss of *SAR1* function that occurs in splicing mutants, since a block in secretion occurs for both tight splicing mutants, such as the *prp2-13* mutant, and leaky mutants, such as the *rse1-1* mutant. Apparently it is either the stability or the activity of the *SAR1* gene product itself that is limiting for secretory pathway function.

In addition to *SAR1*, nine other genes that are involved in secretion also contain introns (*SEC17*, *BET1*, *BOS1*, *SEC27*, *SEC14*, *ERD2*, *SFT1*, *APS3*, and *SNC1*). Inefficient splicing of pre-mRNA from one or more of these nine genes may also affect secretion in splicing mutants, since incomplete glycosylation of invertase is observed in an *rse1-1 SAR1-*Δ*i* mutant and in an *rse1-1* mutant that overexpresses *SAR1* (Fig. 4, lane 4, and Fig. 5A, lane 4). However, our data indicate that *SAR1* is the key secretion gene affected by splicing defects. In this report, we show that secretion can be largely restored in splicing mutants by deleting the intron from *SAR1* or by increasing



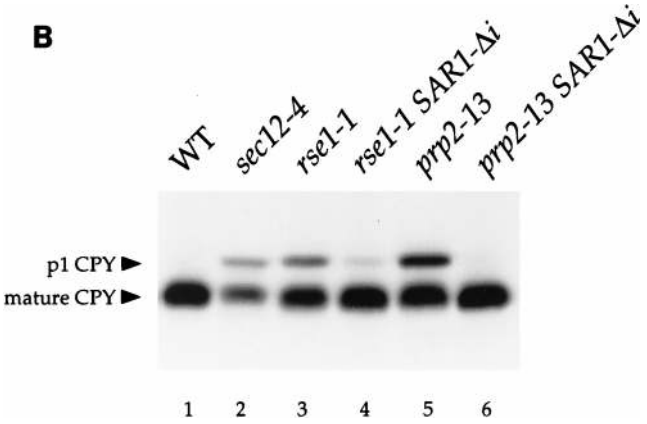


FIG. 5. An intronless *SAR1* allele suppresses the secretion defects of *rse1-1* and *prp2-13* strains. (A and B) *rse1-1* (CKY568), *rse1-1 SAR1-Δi* (CKY569), *prp2-13* (CKY571), and *prp2-13 SAR1-Δi* (CKY572) strains were grown to exponential phase at 24°C and were shifted to 37°C (or 30°C for the *prp2-13* strains) for 2.5 h in YEP plus 0.1% glucose to induce invertase. Invertase and CPY were detected in protein extracts by SDS-PAGE and immunoblotting with either anti-invertase or anti-CPY antibody. p1 CPY (ER form) and mature CPY (vacuolar form) are indicated. (C) Suspensions of cells at 10^7 cells/ml were spotted onto YPD plates and photographed after incubation at 24, 30, or 33°C for 3 days. None of the strains were viable at 37°C. WT, wild type.

the amount of *SAR1*. These results indicate that, of the intron-containing secretion genes, *SAR1* is the one whose function is most rapidly compromised after a splicing defect has been imposed. Moreover, the connection between splicing defects and secretion defects mediated by Sar1p is consistent with the putative regulatory role for Sar1p in vesicle budding from the ER, the first step in vesicle trafficking.

Our finding that secretion slows within 2 h after a block in *SAR1* pre-mRNA processing raises the possibility that inhibition of other steps in Sar1p synthesis might also lead to a defect in ER-to-Golgi transport. The existence of a more general connection between *SAR1* expression and the function of the secretory pathway suggests a mechanism by which the membrane flux within the secretory pathway could be coordinated with the rate at which new cellular proteins are synthesized. Accordingly, we have tried to examine the effect of a general decrease in the rate of translation on ER-to-Golgi transport. However, we were unable to detect the accumulation of ER precursors of secretory proteins after slowing translation by growing cells in sublethal concentrations of cycloheximide or by starving cells for amino acids. A technical problem with this type of experiment is that reducing the rate of translation also inhibits synthesis of the marker proteins used to monitor the rate of transit through the secretory pathway. The observed effect of splicing defects on secretion may depend on their unique property of causing an immediate block in Sar1p synthesis while allowing the synthesis of CPY and invertase to continue unabated for at least several hours. Ultimately, it may be necessary to find a way to assay transport through the secretory pathway that does not rely on the de novo synthesis of marker proteins in order to address a possible connection between the rate of ER-to-Golgi transport and the rate of protein synthesis.

Our findings show how splicing mutations can affect the function of the secretory pathway. A deeper connection between secretion and protein synthesis is implied by the results obtained by Mizuta and Warner, who showed that blocks at various steps in the secretory pathway cause a dramatic reduc-

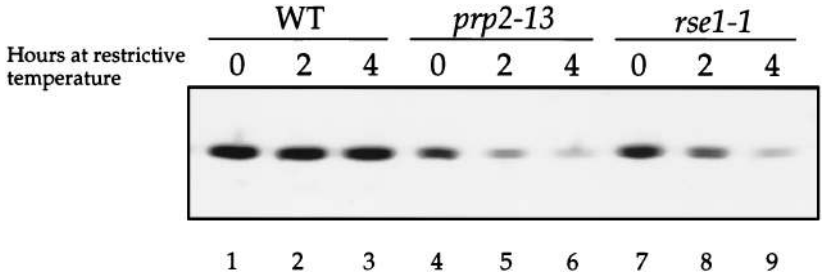


FIG. 6. *prp2-13* and *rse1-1* have reduced amounts of Sar1p at restrictive temperature. Wild-type (CKY294) (WT), *prp2-13* (CKY570), and *rse1-1* (CKY567) strains were grown to exponential phase at 24°C. Cells were either harvested (at $t = 0$) or shifted to 37°C (30°C for *prp2-13*) for 2 or 4 h. Protein extracts were prepared and proteins were resolved by SDS-PAGE on a 12% gel. Protein extract from cells (0.3 OD_{600} equivalent) was loaded in each lane. Sar1p was detected by immunoblot with anti-Sar1p antibody.

tion in the transcription of rRNA and ribosomal protein genes (22). Decreased transcription of ribosomal protein genes also resulted from treatment with the transport inhibitor brefeldin A. The regulatory interactions that make ribosome synthesis sensitive to the activity of the secretory pathway are not yet understood.

Given that many genes are known to be required for pre-mRNA processing in *S. cerevisiae*, one may ask why mutations in these genes were not isolated in previous screens for secretion mutants. The most likely explanation stems from the fact that our screen was carried out in a different genetic background than that used in previous screens for secretion mutants. The parent strain used in our screen was A364A, which carries a different allele at the *SAR1* locus than the S288C background used for most studies of the secretory pathway (2). The endogenous A364A allele, which our lab previously described as *sar1-5*, differs from the S288C allele by three nucleotide substitutions: a T instead of a G at position 533, changing Met^{42} to Ile; an A instead of a G at position 318, in the intron; and an A instead of a G at position 836, preserving an Ala^{143} codon (6). Based on work in our laboratory (2, 6), the allele of *SAR1* in A364A appears to have less activity than the corresponding allele in S288C. Thus, our screen for new *SEC* genes was likely more sensitive than previous screens to mutations that compromise the expression of *SAR1*. We first became aware of the importance of genetic background in our secretion assays when we found that the secretion defect of *prp* mutants did not segregate cleanly in crosses between A364A and S288C strains. Once we had traced the source of this genetic heterogeneity to the *SAR1* locus, we were careful to keep the *SAR1* allele constant in all subsequent genetic manipulations. In the work presented here, all of the *prp2-13* and *rse1-1* strains were constructed so that they carry the endogenous allele found in A364A (the *sar1-5* allele).

RSE1 shows homology to several genes, and regions near the N and C termini of *RSE1* show homology to the predicted translation of *Drosophila* expressed sequence tag (EST) sequences. The known mammalian proteins related to *RSE1* are all thought to bind to nucleic acids. One of these proteins, the 160-kDa subunit of human CPSF, is the subunit which binds the AAUAAA polyadenylation signal in pre-mRNA, but this subunit contains no clear match to a known nucleic-acid-binding domain (23). UV-DDB, XAP-1, and XPE-BF, which appear to be the same protein, have the most extensive similarity to *RSE1*, but their functions are not well defined. UV-DDB, which was isolated from a monkey cDNA library, has high affinity for UV-damaged DNA, although its sequence lacks any known DNA binding motifs (36). XPE-BF, the human counterpart of UV-DDB, is deficient in a subset of patients in xeroderma pigmentosum complementation group E and also binds to damaged DNA (11). The same protein, called XAP-1, was identified in a two-hybrid screen for proteins that interact with hepatitis B virus X protein (16). And recently, XAP-1 has been found to be closely related to BRF-2, a transactivator of the apolipoprotein B gene (15). Taken together, the mammalian homologies to *RSE1* suggest that Rse1p also interacts with nucleic acids, perhaps through a novel nucleic-acid-binding domain, which could lie in the highly conserved region shown in Fig. 2B. Recently, *RSE1* was identified as an ORF that interacts with *PRP9* in a two-hybrid assay (5). Since Prp9p is required for U2 snRNP addition during spliceosome assembly (41), Rse1p might also act at an early step in spliceosome assembly with Prp9p. The homologies and two-hybrid interaction are consistent with a role for Rse1p in pre-mRNA processing as described in this report, but the specific role of Rse1p in pre-mRNA processing remains to be elucidated.

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