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Identification of an Essential *Schizosaccharomyces pombe* RNA Homologous to the 7SL Component of Signal Recognition Particle

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We have cloned the gene encoding a novel small cytoplasmic RNA from the fission yeast *Schizosaccharomyces pombe*. Four lines of evidence support the idea that this RNA is a homolog of the 7SL RNA component of mammalian signal recognition particle (SRP), which targets presecretory proteins to the endoplasmic reticulum membrane. First, it shares limited but significant primary sequence homology with previously identified 7SL RNAs and can be folded into a similar secondary structure. Second, it possesses the 5′ triphosphate characteristic of unprocessed RNA polymerase III transcripts, and moreover, it is the only fission yeast RNA in this size range with such a terminus. Third, its behavior in cell fractionation experiments suggests that it is part of a small ribonucleoprotein which forms salt-labile contacts with larger structures. Fourth, the particle containing *S. pombe* 7SL RNA resembles mammalian SRP in both size (11S) and affinity for DEAE-Sepharose. Disruption of the single-copy gene, designated *slr1*$^+$, reveals that the RNA is indispensable for growth in fission yeast. This result is not surprising, since secretion is an essential cellular process.

The signal recognition particle (SRP) is a ribonucleoprotein composed of six polypeptides and one molecule of 7SL RNA (49, 52), which is required for transport of presecretory proteins into microsomal vesicles in vitro (50). Based on experiments using a heterologous system (wheat germ translation components with canine pancreas microsomes and SRP), a model was developed in which SRP is postulated to bind to the signal sequence (50, 56) as it emerges from the ribosome, causing an arrest of translation (51). When the ribosome-nascent chain-SRP complex reaches the endoplasmic reticulum (ER), SRP interacts with an integral membrane protein, known as docking protein (20) or SRP receptor (7), and translation resumes, accompanied by vectorial translocation of the nascent polypeptide.

Although the large body of biochemical data which has accumulated concerning the structure and function of SRP for the most part supports this model, some aspects, in particular translation arrest, have recently been questioned (19, 29). More directly relevant to the results described in the present paper is the fact that the details of the role played by 7SL RNA in SRP function are as yet almost completely unknown. It has been established that 7SL RNA is essential for reconstitution of a functional canine SRP from its separated components (54). A subparticle derived from reconstitution in the absence of the 9K-14K heterodimer is competent for protein translocation but does not exhibit the arrest of preprolactin synthesis demonstrated for intact SRP (36). The Alu structural domain of 7SL RNA (nucleotides 1 through 100 and 255 through 300) which associates with these proteins is also dispensable for ER targeting (37). Thus, fully half of the RNA can be removed without disrupting the signal recognition and protein translocation activity of SRP, a surprising result, since the entire length of the RNA has been conserved through evolution (8, 46).

7SL RNA appears to be in direct contact with five of the six SRP polypeptides, as judged by sucrose gradient analysis (54) and RNA footprinting studies (V. Siegel and P. Walter, personal communication). The one protein (54K) which does not associate with 7SL RNA can be cross-linked to the bovine preprolactin leader peptide during translation in the presence of a photoactivatable amino acid analog (13, 14), suggesting that it may act as an adapter between the SRP core and the nascent presecretory protein.

That the hydrophilic RNA component of SRP does not interact directly with the hydrophobic leader sequence is to be expected. There has been only speculation as to what role 7SL does play in SRP function. For example, it has been suggested that the RNA could bind a region of the SRP receptor which is rich in charged amino acids and bears a resemblance to other proteins which interact with nucleic acids (15). A second model is that 7SL might form hydrogen bonds to sequences in rRNA (52). A third proposal is that conformational changes in 7SL RNA may be important for SRP function and in particular that sequences which show a high degree of conservation with 5S rRNA might interact transiently with a similar target in the large subunit (64). Since these hypotheses are not mutually exclusive, all may describe aspects of SRP function; however, no direct evidence has yet been provided in support of any of them.

To confirm or rule out models for the in vivo role of 7SL RNA, it would be extremely useful to characterize an analog of SRP from an organism that can be manipulated genetically. We report here the identification on a *Schizosaccharomyces pombe* RNA whose physical properties and behavior in cell fractionation experiments resemble those of mammalian 7SL. Our observation that this RNA is encoded by an essential single-copy gene sets the stage for a genetic dissection of the early steps in protein secretion.

MATERIALS AND METHODS

Materials and enzymes. Restriction enzymes were obtained from Brisco, Ltd., except that *Sty*I was from New England BioLabs, Inc., Beverly, Mass., and *Asu*II was from Promega Biotec. Exonuclease III and mung bean nuclease were obtained from Stratagene; reverse transcriptase was from Life Sciences, Inc., St. Petersburg, Fla.; DNA ligase was from IBI. RNA sequencing enzymes and nucleotides for DNA sequencing were from Pharmacia, Inc., Piscataway, N.J. Sequencing primers were synthesized at the University of Illinois Biotechnology Center.

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[α-^{32}P]dCTP and [α-^{35}S]dATP were purchased from Amersham Corp., Arlington Heights, Ill., and [γ-^{32}P]ATP and $^{32}P_i$ were from ICN Pharmaceuticals Inc., Irvine, Calif. The nick translation kit and Hybond nylon membrane were from Amersham, GeneScreen was from New England Nuclear Corp., Boston, Mass., and nitrocellulose was from Schleicher & Schuell, Inc., Keene, N.H. DEAE-Sepharose CL-6B was obtained from Pharmacia. Guanidine thiocyanate was from Fluka Biochemicals; phenylmethylsulfonyl fluoride was from Sigma Chemical Co., St. Louis, Mo.; Nikkol (octaethyleneglycol-mono-*N*-dodecyl ether) was from Nikko Chemical Co., Ltd., Tokyo, Japan.

In vivo labeling and RNA isolation. Cells were grown in low-phosphate YEPD medium (33) to an optical density at 595 nm of 0.3 and then were incubated with carrier-free $^{32}P_i$ at a concentration of 0.25 mCi/ml for 4 to 5 h (ca. 2 generations). RNA was isolated by vigorous vortexing of the cell pellet with an equal volume of glass beads and GTE buffer (4 M guanidine thiocyanate, 100 mM Tris hydrochloride [pH 7.5], 10 mM EDTA) followed by a 1:10 dilution, hot phenol extraction, phenol-chloroform extraction at room temperature, and ethanol precipitation of the aqueous phase.

Nonradioactive total RNA was prepared by the same procedure with the addition of one phenol-chloroform extraction and one chloroform extraction.

Characterization of 7SL candidates from fission yeast. Electrophoresis and elution of RNAs from two-dimensional gels were carried out as previously described (59). Digestion of uniformly labeled RNA samples with nuclease P1, followed by thin-layer chromatography, was also done as described previously (59).

Cloning and sequence analysis. The *S. pombe* 7SL gene was isolated from a bank of fission yeast DNA generated by partial digestion with *Sau*3AI, followed by insertion into the vector pFL20 (17). The probe was a radioactive cDNA made from gel-purified 7SL, primed with random calf thymus hexamers as previously described (59). After subtracting out signals arising from rRNA contamination of the probe, a single positive clone remained. The 7SL coding region was located within the 12-kilobase (kb) insert by hybridization of labeled cDNA to Southern blots of various restriction enzyme digests. Two pUC8 subclones carrying the gene were used for sequence analysis: a 2.1-kb *Bam*HI-*Eco*RI fragment, pWEC2 (see Fig. 3), and a 1.0-kb *Eco*RI-*Hin*dIII fragment, pWEC9. DNA sequence analysis was carried out on double-stranded templates by modified versions (2, 42, 61) of the Sanger method with the universal primer, 7S-15 (an oligonucleotide designed from RNA sequence data) or 7S-US or 7S-DS (oligonucleotides directly abutting the gene upstream or downstream, designed on the basis of DNA sequence data); the latter three are overlined in Fig. 3. RNA sequencing was performed on both 5′- and 3′-end-labeled 7SL with RNases T1, U2, PhyM, and CL3 (3, 41); the products were resolved on 20 and 8% sequencing gels.

Cell fractionation. *S. pombe* cells were grown in YEL medium (9) containing half the normal amount of glucose, treated with Novozyme at 2 mg/ml to form spheroplasts, suspended in HMS buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.6], 5 mM magnesium acetate, 0.25 M sucrose) plus 0.1% Nonidet P-40, and lysed by mechanical disruption with a Dounce homogenizer. Nuclei were pelleted through a cushion of HMC (HMS except with 0.5 M sucrose) and were washed once with HMS while the cytoplasm was held on ice. After suspension in HMS, the nuclei were salt extracted by incubating for 15 min on ice in 0.5 M potassium acetate (pH 7.2) with occasional gentle agitation; the cytoplasmic fraction was likewise raised to 0.5 M salt and incubated for 15 min. To pellet particulate material (ribosomes and microsomes), each fraction was layered over a cushion of HMC containing 0.5 M potassium acetate and spun at 140,000 × *g* for 3 h. RNA was extracted from each fraction as described above. A Northern (RNA) blot of RNA from each fraction was made and hybridized as previously described (59).

Biochemical analysis. A fraction enriched in SRP from either fission yeast or human cells was prepared as described in the preceding section, except that HMS was replaced by buffer I (50 mM triethanolamine [pH 7.5], 50 mM potassium acetate [pH 7.2], 5 mM magnesium acetate, 250 mM sucrose, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.01% Nikkol). Postpolysomal supernatants from 1 liter of *S. pombe* cells or 500 ml of HeLa cells were diluted twofold and loaded onto separate DEAE-Sepharose CL-6B columns equilibrated in buffer IV (53). After washing with buffer I containing 0.35 M potassium acetate, the columns were eluted with steps of 0.5, 0.6, 0.7, 0.8, and 1.0 M salt. The elution profile of SRP was assayed by extracting RNA from a portion of each fraction and running a gel to detect 7SL. Samples of fractions enriched in SRP from each organism were mixed and loaded onto a 5-ml 5 to 20% sucrose gradient in buffer I with 0.5 M potassium acetate which was spun at 50,000 rpm in an SW55 rotor for 5 h. The gradient was fractionated into 350-μl aliquots from which RNA was prepared and analyzed by gel electrophoresis. A parallel gradient of naked RNA from *S. pombe* was also run. A third gradient contained marker proteins (catalase, 11.3S; horseradish peroxidase, 3.5S; and cytochrome *c*, 2.1S), and a fourth contained marker DNA fragments (one kilobase ladder, obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md.: 0.51 kb = 7S; 1.02 kb = 8.6S; 1.64 kb = 10S; 2.04 kb = 10.8S; 3.05 kb = 12.3S; 4.07 kb = 15.4S).

Gene disruption. We created a null allele of the fission yeast *slr1*$^+$ gene by a strategy similar to that of Russell and Nurse (34). The starting clone was a plasmid from a series generated by digestion with exonuclease III, in which the deletion extends to approximately 520 nucleotides beyond the *Hin*dIII site, corresponding to 175 nucleotides 3′ to the end of the gene. The upstream sequence was reconstructed by cloning in the 1.1-kb *Hin*dIII fragment from pWEC2 (see Fig. 3), creating a plasmid (pWECR19) which is missing the gene and only small amounts of flanking DNA at either end. The plasmid was linearized by cutting at the unique *Sty*I site, blunt-ended by treatment with mung bean nuclease, and ligated to a similarly treated *Sph*I fragment containing the *S. pombe ura4*$^+$ gene (derived from pUra4, a kind gift of Paul Russell and Paul Nurse). The ligation mixture was transformed into an *Escherichia coli* strain which cannot grow on medium lacking uracil (JFS116 *pyrF*::Tn*5* *leuB trpC hsdR*^{k} *araT* Δ*lacIZY rpsL srlA*::Tn*10* *recA*; a kind gift of Mike Woontner [40]); we have found that *ura4*$^+$, like *Saccharomyces cerevisiae URA3*, complements the bacterial auxotrophy. Two plasmids were used in subsequent steps: pΔWU1, containing a single copy of *ura4*$^+$ and pΔWU2 carrying a tandem head-to-tail duplication of *ura4*$^+$. From each of these, we isolated by preparative gel electrophoresis a linear *Bam*HI-*Eco*RI fragment carrying the disruption allele and transformed an *S. pombe* diploid (genotype *ura4-d18*/*ura4-d18* *leu1-32*/*leu1-32* *ade6-704*/*ade6-704* h^+/h^+; a kind gift of P. Russell and P. Nurse). We used Southern analysis to identify a strain in which a stable integration event had occurred at the *slr1*$^+$ locus. The blots were probed with pWECR19, the immediate precursor to the plasmid

used for gene disruption (see above), labeled by nick translation; hybridization conditions were as previously described (59). In all six strains analyzed which were derived from transformation with the construction carrying a single *ura4* gene, integration had occurred elsewhere in the genome, not at the *slr1* locus. Since Hayles et al. (11) observed correct integration only with a tandem duplication, we analyzed 10 strains transformed with this construction and found 1 which had the correct genomic structure. Finally, we isolated sporulation-competent (h^{90}/h^{+}) diploids (9) from this strain and the untransformed strain. Tetrads were dissected on rich medium (9) supplemented with uracil, leucine, and adenine.

RESULTS

Identification of a 7SL RNA candidate from *S. pombe*. We initially used two criteria for identifying fission yeast RNAs which were potential analogs of 7SL from higher eucaryotes: size and 5′-end structure. Mammalian 7SL is 299 nucleotides long (45) and is an unprocessed RNA polymerase III transcript (16, 62). Figure 1 shows a two-dimensional gel of total in vivo ^{32}P-labeled *S. pombe* RNA, revealing the presence of two species which were close in size but somewhat smaller than mammalian SRP RNA. The species designated 7SL possessed a GTP at its 5′ end and appeared to lack modified nucleotides, as demonstrated by thin-layer chromatography (Fig. 2); the position of the pppG moiety was identical to that of the same nucleotide resulting from P1 nuclease digestion of 5S RNA (data not shown). The other fission yeast RNA similar in size to mammalian 7SL (Fig. 1) has primary sequence homology to U3 small nuclear RNA, is capped with trimethylguanosine, and contains modified nucleotides (G. Porter, K. Holm, P. Brennwald, and J. Wise, manuscript in preparation). The two smaller RNAs visible on the gel in Fig. 1 are the fission yeast analogs of U2 and U1 small nuclear RNAs from mammals (P. Brennwald, G. Porter, and J. Wise, manuscript in preparation).

Structural homology between human and fission yeast 7SL RNAs. A gene encoding the putative 7SL analog was identified from a bank of fission yeast DNA (a kind gift of David Beach) with a cDNA probe (for details, see Materials and Methods). The restriction map of a 2.1-kb *Eco*RI-*Bam*HI subclone and the location of the gene within this fragment is shown in Fig. 3a. The boundaries of the gene were assigned by direct sequencing of the RNA. On the basis of its homology to the RNA component of SRP (see below), the locus has been designated *slr1*$^{+}$ for 7SL RNA.

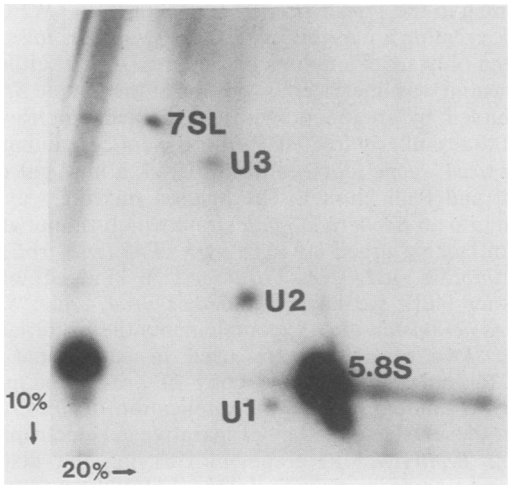

FIG. 1. Two-dimensional polyacrylamide gel pattern of in vivo ^{32}P-labeled RNA from *S. pombe*. Fission yeast RNA labeled with $^{32}P_i$ was extracted as described in Materials and Methods. A two-dimensional (10 and 20%) polyacrylamide gel, containing 4 M urea in both dimensions, provided optimal resolution of the *S. pombe* RNAs in this size range (150 to 350 nucleotides).

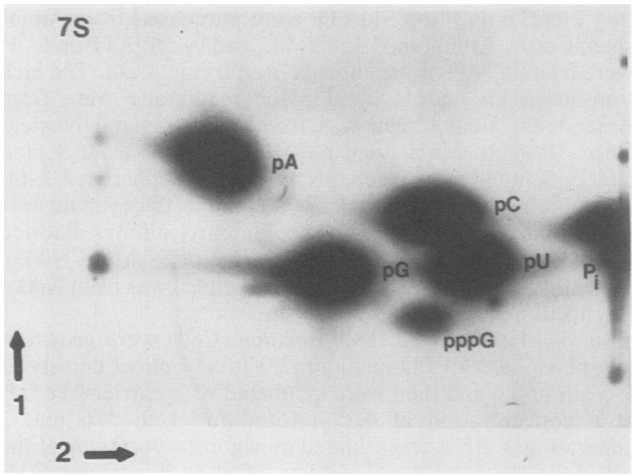

FIG. 2. 5′-end analysis of fission yeast 7SL RNA. The RNA species designated 7SL was eluted from a gel similar to that shown in Fig. 1 and was digested to completion with P1 nuclease. The products from the uniformly labeled RNA were chromatographed on a cellulose thin-layer plate in two dimensions by using the solvent systems developed by Silberklang et al. (38). The positions of nonradioactive markers (visualized by UV) are indicated.

The sequence of *S. pombe* 7SL RNA, together with that of the *slr1*$^{+}$ gene and flanking DNA, is presented in Fig. 3b. The sequence between nucleotides 1 and 10 is similar (one mismatch) to the A box which directs tRNA transcription by RNA polymerase III (44) and has been underlined in the DNA sequence; nucleotides 9 through 19 and 44 through 54 each differ in two positions from the consensus. The underlined sequence from 156 through 164 has two nucleotide differences from the consensus B box transcriptional signal (28); nucleotides 131 through 137 match the B box, except for a 1-nucleotide deletion. We have no evidence that any of the regions mentioned actually function as promoter elements in fission yeast cells, but it is perhaps relevant that the A box in human 7SL genes is more closely related to the canonical sequence than the B box is to its prototype (47). No significant conservation was observed between the fission yeast and human 7SL 5′-flanking DNA, despite the fact that sequences upstream from the human RNA coding region appear to be necessary for efficient transcription both in vivo and in vitro (48); the DNA upstream from *S. pombe* 7SL is extremely A+T rich, whereas the human 7SL 5′-flanking region is high in G+C residues. As expected, the DNA flanking the *slr1*$^{+}$ gene shows little homology to sequences upstream from several fission yeast small nuclear RNA genes which have been characterized in our laboratory (Brennwald et al., in preparation); the small nuclear RNA coding regions are preceded by typical *pol*II transcription signals.

The *S. pombe* 7SL RNA coding sequence is 254 nucleotides long and can be aligned in several different ways with human 7SL to produce approximately 50% primary se-

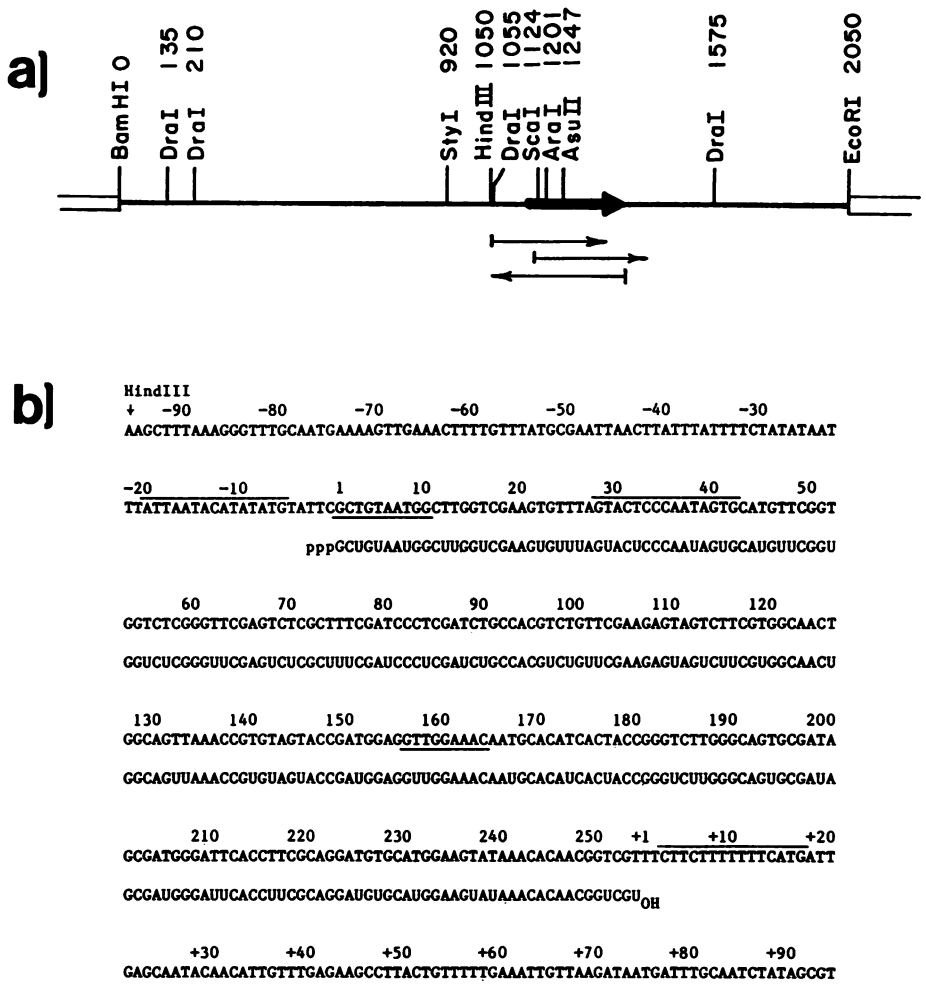

FIG. 3. (a) Restriction map and sequencing strategy for the *slr1*$^+$ gene. The gene, indicated by a heavy arrow, is located just downstream of the *Hin*dIII site in pWEC2, a pUC18 subclone carrying a 2.1-kb *Bam*HI-*Eco*RI fragment. Numbers refer to the distance in base pairs from the *Bam*HI site which marks the left boundary of the subcloned fragment. The DNA sequencing strategy is indicated by the arrows below the gene. The boundaries of the gene were determined by direct RNA sequencing on either 3'- or 5'-end-labeled *S. pombe* 7SL RNA. (b) Nucleotide sequence of the *slr1*$^+$ gene and flanking sequence. The sequence of the noncoding DNA strand, determined by the dideoxy method, is shown on the top line, and the RNA sequence, determined by partial enzymatic digestion, is shown on the bottom line. The overlined sequences are the primers used in DNA sequencing. The underlined sequences are homologous to RNA polymerase III transcription signals (see text).

quence identity. One possible alignment is shown in Fig. 4. Although the overall homology between *S. pombe* and human 7SL is rather low, we note that there are two patches of quite high sequence identity, one of which is located in the conserved central region of the higher eucaryotic RNA (46). Comparison of the *S. pombe* RNA with *Drosophila* (46) and *Tetrahymena* (J. Wise, unpublished data) 7SL analogs (results not shown) also reveals about 50% identity. Higher conservation, 66%, is observed between the fly and human 7SL RNA sequences. Comparison of the 3' 136 nucleotides of fission yeast 7SL with the sequence of an RNA of identical size from a more closely related organism, *Aspergillus nidulans*, reveals ~60% identity (J. Wise, unpublished observations). The fact that these ascomycete RNAs are 45 nucleotides shorter than higher eucaryotic 7SL RNAs accounts for about 15% of their divergence; *Tetrahymena* 7SL is also smaller than the canine RNA but by only 10 to 15 nucleotides. It has been pointed out by Zwieb (64) that there is a match in 16 of 20 positions between human 7SL (nucleotides 236 through 255) and 5S RNAs (nucleotides 84 through 103). However, *Drosophila* 7SL and 5S are less similar in sequence (64), and for fission yeast RNA, virtually no homology is observed between the equivalent regions.

If this *S. pombe* RNA is indeed analogous in function to 7SL from higher eucaryotes, it should be possible to fold the molecules into a common secondary structure. The T-shaped pattern of stems and loops generated for the fission yeast RNA is strikingly similar to the proposed structure of human 7SL RNA (Fig. 5), which was derived primarily from analysis of compensatory base changes in *Drosophila* 7SL (8, 64). A long tightly base-paired stem carries most of the free energy of the structure in both the human and *S. pombe* RNAs (−87.7 and −57.7 kcal, respectively, as calculated by the RNA folding program of Zuker and Stiegler (63). The crossbar of the T forms a domain with less hydrogen bonding in both RNAs (human, −31.7 kcal; *S. pombe*, −42 kcal). An

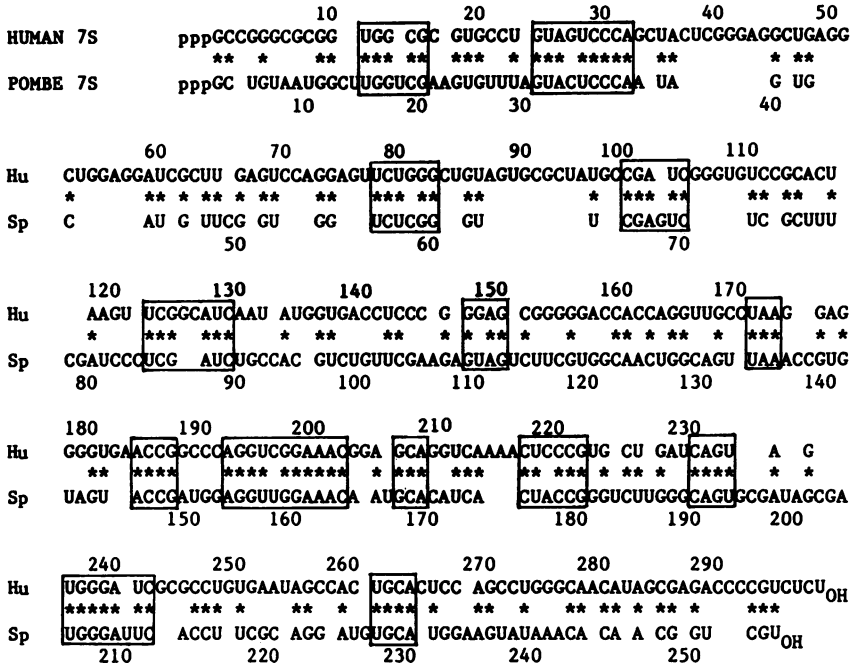

FIG. 4. Comparison of fission yeast and human 7SL primary sequences. The top line shows the human (Hu) 7SL RNA sequence (45), and the bottom line shows the *S. pombe* (Sp) *slr1*$^+$ coding sequence. Identical nucleotides are indicated (*), and regions of extended homology have been boxed.

alternative pairing for the terminal domain of the *S. pombe* RNA (Fig. 5, inset) more closely resembles the structure proposed for the same region of metazoan 7SL; note, however, that the 5′ and 3′ ends are circularly permuted with respect to the human RNA (also see Discussion). We have obtained limited data (data not shown) for both 5′- and 3′-end-labeled fission yeast 7SL cleaved under native conditions (41) with V1 nuclease (secondary and tertiary structure specific), S1 nuclease (single-strand specific), and T1 RNase (single-stranded G-specific), which is consistent with the folding derived from computer and phylogenetic analysis but does not distinguish between the two terminal structures shown. Regions of significant primary sequence homology between the fission yeast and human 7SL RNAs have been boxed in Fig. 4 and 5; they generally occur in similar positions within the secondary structures.

Behavior of *S. pombe* 7SL RNA during cell fractionation. Another compelling line of evidence that the *S. pombe* RNA we are studying is the molecule of interest is its behavior in cell fractionation experiments. As a consequence of its adapter role, a portion of the cellular SRP is physically associated with ribosomes or microsomes. These interactions can be disrupted by high salt in other cell types examined (55). The 7SL RNA, as part of an 11S ribonucleoprotein, remains in the cytoplasmic post-ribosomal supernatant after high-speed centrifugation. The gene we have cloned encodes an RNA which was approximately 70% localized to the post-ribosomal salt wash (Fig. 6b, lane CSn). The blot also demonstrated that the abundant 7S-sized bands observed on the gel (Fig. 6a) in the pellet (P), cytoplasmic (C), and total RNA (T) fractions did not hybridize to the cloned gene; they probably correspond to rRNA breakdown products. Very different results were observed when this blot was stripped and reprobed with 5′-end-labeled L15 oligonucleotide (1) which hybridizes to U2 small nuclear RNA. This species was predominantly nuclear and was found almost exclusively in the pellet fractions (P. Brennwald and J. Wise, unpublished observations). High salt is essential for efficient extraction of the 7SL RNA into the post-ribosomal supernatant, since more than half of it sedimented with the pellet if this step is omitted (Fig. 6c and d). This distinguishes the particle we are studying from a 9.6S particle implicated in secretion in *Saccharomyces cerevisiae* (58), which was found in the post-ribosomal supernatant at all ionic strengths examined (see Discussion).

Resemblance of fission yeast SRP to mammalian SRP in its physical properties. To further confirm that we are studying an RNA which is part of a structure similar to mammalian SRP, we have characterized the *S. pombe* ribonucleoprotein by three different biochemical methods. Figure 7 shows a sucrose gradient analysis which demonstrated that the particles in which human and fission yeast 7SL RNAs reside were similar in size. Both were found predominantly in fraction 9, although more fission yeast 7SL RNA appeared in fraction 8; the slightly smaller size of the *S. pombe* particle is perhaps due to the shorter length of the RNA component. The catalase marker (11.3S), as well as a 2.04-kb (10.8S) DNA fragment, also peaked in fraction 9. In a parallel gradient run on naked RNA from *S. pombe* (data not shown), the 7SL peaked in fraction 6.

A newer method for characterizing ribonucleoproteins is the gel mobility shift assay (12). The native particle containing fission yeast 7SL ran significantly more slowly than the naked RNA (data not shown). A third line of evidence that *S. pombe* 7SL is part of a ribonucleoprotein is its behavior on DEAE-Sepharose, which gives an indication of the relative amounts of RNA and protein in the particle or of the accessibility of the RNA component. The fission yeast RNP eluted completely with 0.5 M salt (data not shown), similar to recent observations for wheat germ SRP (29). In our laboratory, the HeLa particle eluted at between 0.5 and 0.6 M salt. The other major RNA in the material loaded was

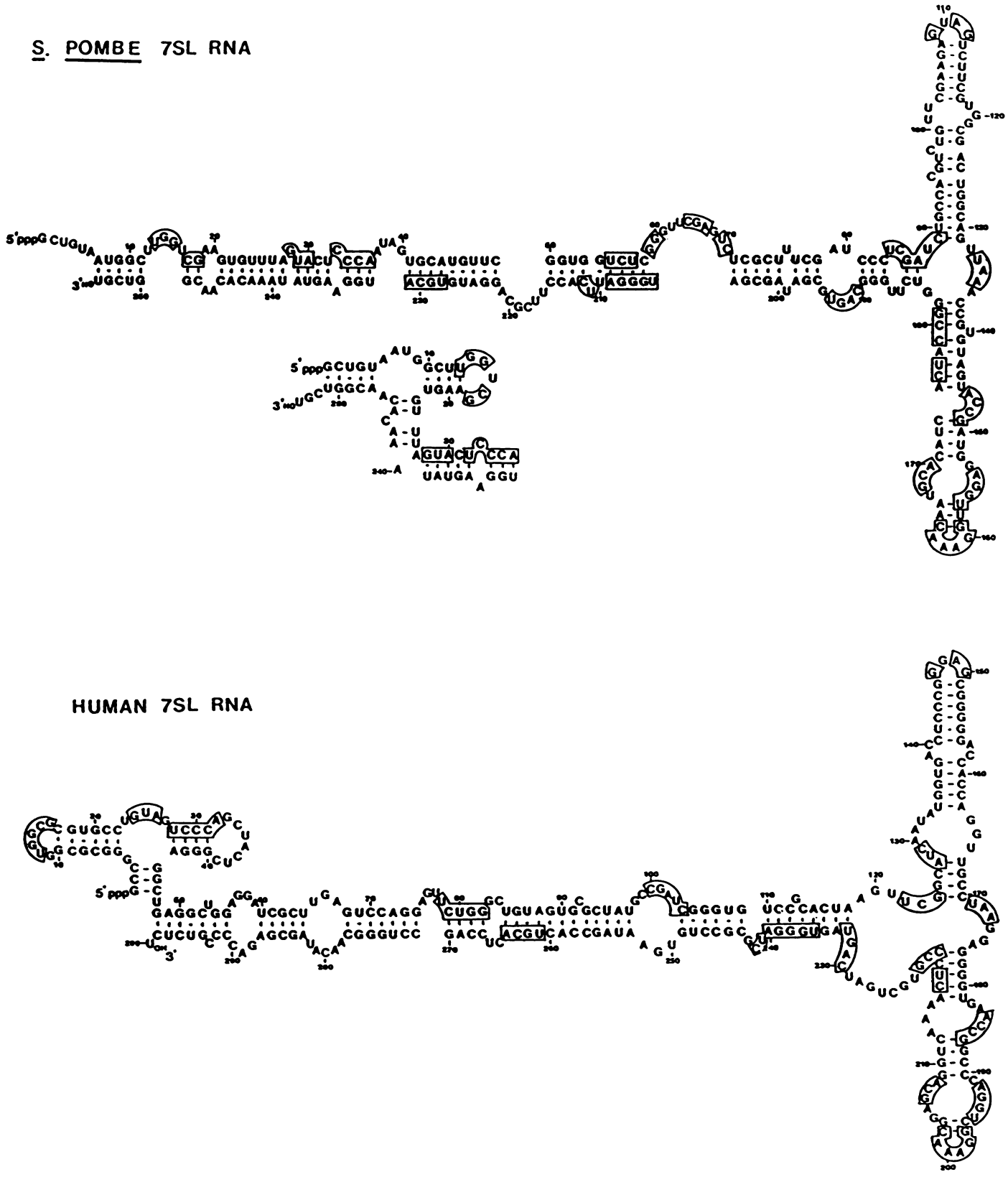

FIG. 5. Comparison of *S. pombe* and human 7SL secondary structures. The secondary structure for fission yeast 7SL is derived from a minimum free energy prediction generated by the RNA folding program of Zuker and Stiegler (63), incorporating information on primary sequence homology to the human RNA (Fig. 4) to position single-stranded regions. An alternative base pairing for nucleotides 1 through 32 and 235 through 254 is shown in the inset. The secondary structure for human 7SL RNA is taken from Zwieb (64). The same regions of extended primary sequence homology indicated in Fig. 4 have been boxed here.

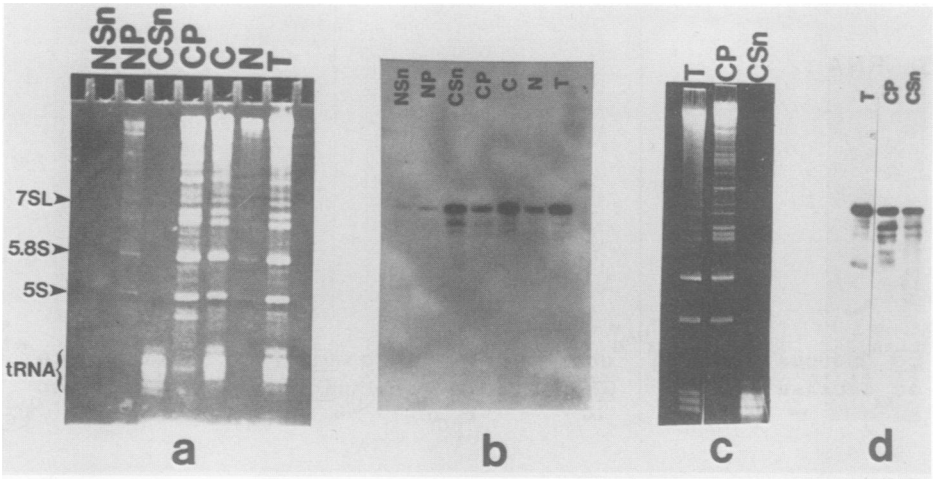

FIG. 6. Subcellular distribution of *S. pombe* 7SL RNA. Cell fractionation and RNA preparation were carried out as described in Materials and Methods. An equal number of cell equivalents of RNA from each fraction was loaded onto each lane of the gel shown in panel a. Panel b is an autoradiograph of a GeneScreen Northern blot of this gel hybridized with pWEC1 labeled by nick translation. Abbreviations: T, total RNA; N, nuclear RNA; C, cytoplasmic RNA; CP, cytoplasmic pellet RNA; CSn, cytoplasmic post-ribosomal supernatant RNA; NP, nuclear pellet RNA; NSn, nuclear post-ribosomal supernatant RNA. Panels c and d show gel electrophoretic and Hybond Northern analysis of total, cytoplasmic pellet, and cytoplasmic post-ribosomal supernatant RNA from an experiment in which the cytoplasm was not extracted with high salt before the high-speed spin. The ethidium-stained gels and autoradiograms have been aligned so that the 7SL band is in the same position on both.

tRNA, which eluted at 0.8 M salt, as would be expected for low-molecular-weight naked RNA. Taken together, the results of this experiment and the sucrose gradient analysis suggest that the protein contents of human and *S. pombe* SRP might be similar.

***S. pombe* 7SL RNA essential for viability.** Secretion has been shown to be an essential cellular process in bacteria (27; for a review, see reference 39) and budding yeast (26; for a review, see reference 35) via the isolation of temperature-sensitive lethal mutations. Furthermore, SRP is required for mammalian secretory protein targeting in vitro (50), and a functional particle cannot be reconstituted in the absence of the RNA component (54). We therefore predicted that if the *S. pombe* putative 7SL RNA is involved in secretion, it would be indispensable for viability. To test this hypothesis, we created a null allele of the gene by recombinant DNA manipulations (for details, see Materials and Methods) and used this to replace one copy of the wild-type gene in a diploid strain (32) (Fig. 8b). Figure 8c shows a genomic Southern blot, demonstrating that integration has occurred at the correct genomic locus. The band at 1.2 kb in the wild-type DNA has increased in size to 4.2 kb as a result of the presence of two copies of the *ura4*$^+$ gene, whereas the band at 3.8 kb has decreased to 3.3 kb as a result of deletion of the 7SL coding region and immediate flanking DNA. If an essential gene had been disrupted, upon sporulation, we would expect viability to segregate 2:2. That this is indeed the case is demonstrated by the tetrad data (Fig. 9). No more than two spores survived per tetrad derived from the strain heterozygous for gene disruption at the *slr1* locus, whereas the tetrads derived from the untransformed diploid generally showed 4:0 segregation for viability. In the disruption strain, all of the spores able to form colonies (Fig. 8b) were Ura$^-$, indicating that they received a wild-type copy of the *slr1* gene. Eight tetrads, in addition to those shown, have been analyzed with the same results. In the positions in which no visible colony formed, the spore underwent no more than one to two rounds of cell division. Thus, 7SL RNA appears to be absolutely required for growth in fission yeast.

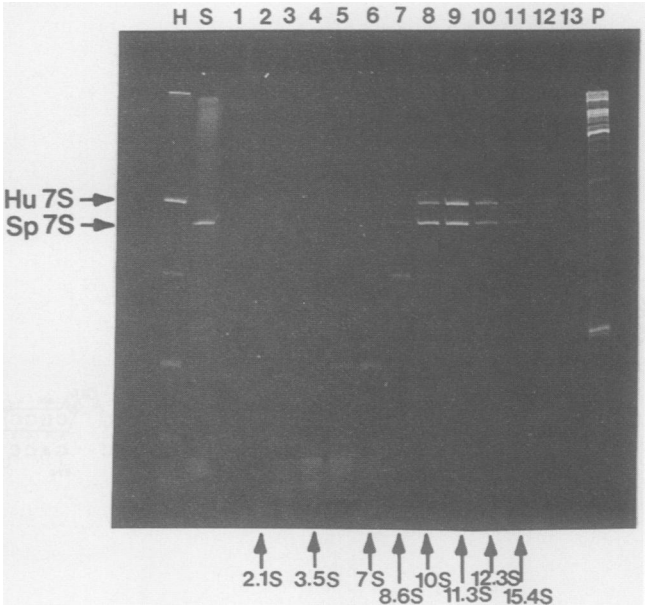

FIG. 7. Sucrose gradient analysis of human and fission yeast SRP. Samples of HeLa cell and *S. pombe* post-ribosomal salt extract which had been concentrated by passage over DEAE-Sepharose were mixed together and fractionated on a 5 to 20% sucrose gradient. RNA was extracted from each of 13 fractions and the pellet (P). The lanes marked H and S are aliquots of RNA extracted from human and fission yeast SRP concentrated on DEAE-Sepharose; the top of the gradient corresponds to lane 1. The RNAs were run on a 6% polyacrylamide–7 M urea gel and stained with ethidium bromide. S values indicated at the bottom were derived from parallel gradients containing marker proteins and DNA fragments (see Materials and Methods). Hu, Human; Sp, *S. pombe*.

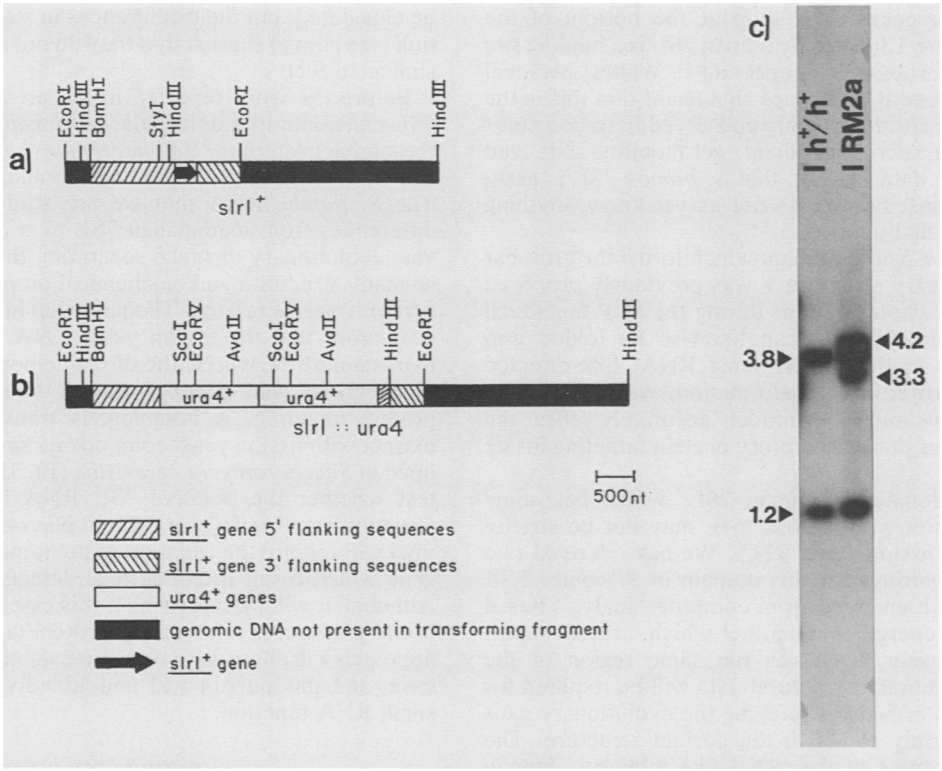

FIG. 8. Gene disruption of *slrl*$^+$. (a) Restriction map of the wild-type genomic locus. (b) The disrupted allele, created by deletion of the gene and insertion of two copies of the fission yeast *ura4*$^+$ gene at an adjacent site (see Materials and Methods for details). (c) Genomic Southern blot (43) of DNA from the parental diploid (h^+/h^+) carrying two wild-type copies of the *slrl*$^+$ gene and the diploid strain heterozygous for gene disruption at the *slrl* locus (RM2a), digested with *Hin*dIII. The blot was probed with plasmid pWECR19 (see Materials and Methods for details on how it was constructed), which has equal lengths of homology to the wild-type and disrupted alleles.

In light of the above conclusion, it is interesting that the diploid heterozygous for *slrl* gene disruption showed no apparent growth defect relative to the isogenic wild-type strain. As judged by Northern analysis (data not shown), the amount of 7SL RNA in this strain appeared to be undiminished relative to a strain with two intact copies of the 7SL coding sequence. A wild-type diploid transformed with the fission yeast 7SL gene carried on an *S. pombe ARS* high-copy-number (5) vector also appeared to have an amount of 7SL RNA virtually indistinguishable from that of cells lacking the plasmid (data not shown). The plasmid copy is functional, since it can complement the gene knockout in haploid spores derived from RM2a. Taken together, these results suggest that the *slrl*$^+$ gene may be under transcriptional regulation.

DISCUSSION

The remarkable discovery that SRP from canine pancreas contains an RNA subunit launched the search for 7SL in an organism which can be manipulated genetically. We believe that the structural and biochemical evidence presented here makes a compelling case that the *S. pombe* RNA we are studying is a bona fide 7SL analog, particularly in light of our evidence that the other small RNAs from this organism (Fig. 1) appear to be counterparts of mammalian small nuclear RNAs. Further evidence for identification of this fission yeast RNA as 7SL is provided by work from another laboratory, demonstrating that it can bind canine SRP proteins (M. Poritz, V. Siegel, and P. Walter, personal communication). Although the primary sequence homology between the *S. pombe* and human RNAs is relatively low, their secondary structures are conserved to a high degree. A notable previous example of RNAs with identical functions but virtually unrelated primary structures are the enzymatically active subunits of RNase P, M1 RNA from *E. coli*, and P RNA from *Bacillus subtilis*. As would be expected, since secondary structure is a more important determinant of functional capacity than is sequence, these RNAs, like fission yeast and human 7SL, fold into a very similar series of stems and loops (30).

The short stretches of primary sequence homology shared by human and *Schizosaccharomyces* 7SL RNAs generally fall within or extend into single-stranded regions; such a pattern has previously been observed in ribosomal RNAs from phylogenetically distant organisms (23, 60) and is thought to be a consequence of the fact that single-stranded regions are likely to interact with other RNAs or proteins and thus are subject to more evolutionary constraints on

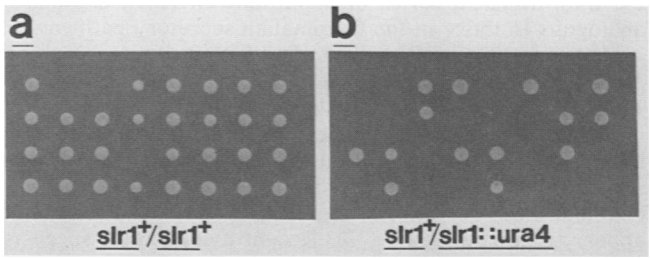

FIG. 9. Dissection of tetrads from the diploid strain heterozygous for gene disruption at the *slrl* locus (b) and of tetrads from an untransformed diploid (a).

their primary sequences. The loop at the bottom of the secondary structure T formed by human 7SL is a binding site for the 19K SRP protein (V. Siegel and P. Walter, personal communication), and it is perhaps significant that this is the center of the segment most highly conserved in fission yeast 7SL (Fig. 5). Our sucrose gradient, gel mobility shift, and DEAE-Sepharose data indicate that *S. pombe* 7SL is associated with proteins, but we do not as yet know anything about their individual nature.

The loosely base-paired domain which forms the crossbar of the 7SL secondary structure T was previously proposed to adopt alternate conformations during the SRP functional cycle in mammals (65) and can likewise be folded into different systems in the fission yeast RNA. Site-directed mutagenesis, followed by transformation, will allow us to assess whether this and other models accurately reflect the events which occur during secretory protein targeting inside the cell.

The terminal domain in human 7SL, which has some features in common with tRNAs (64), may not be strictly conserved in the fission yeast RNA. We have derived two alternative base pairings for this domain in *S. pombe* 7SL (Fig. 5), one which emerged from computer analysis based on minimum free energy, and another which, at least superficially, more closely resembles the same region of the human RNA. Additional structural data will be required for 7SL analogs from organisms bridging the evolutionary gaps before we can firmly establish the correct structure. The functional implications of the tRNA-like folding in human 7SL are not yet clear, but it may be relevant that the terminal structure is part of the region of mammalian SRP (the Alu domain of the RNA and associated proteins) thought to be responsible for the translation arrest phenomenon (37). It will, therefore, be interesting to determine whether the fission yeast particle mediates a site-specific halt in synthesis of nascent secretory polypeptides.

We undertook the present work with fission yeast because, despite extensive effort, we did not succeed in isolating such an RNA or RNA-containing particle from *Saccharomyces cerevisiae* by using the same experimental approaches. It is tempting to speculate that *S. pombe* might contain a higher abundance of secretory components, since it is more closely related to filamentous fungi, which secrete at a higher level than budding yeast. This notion is supported by the recent observation (T. Chappell and G. Warren, personal communication) of a Golgi apparatus in wild-type fission yeast cells; in contrast, electron microscopic observations of budding yeast reveal Golgi-related organelles only in mutant cells blocked in secretion at this step (25). Although the secretory pathway has not been as well studied in fission yeast as in bacteria or budding yeast, it is clear that this organism exports proteins which have classical signal sequences (5, 18) and seems likely that, as in *S. cerevisiae* (4, 24) they transit a series of membrane-enclosed organelles analogous to those in the mammalian secretory pathway.

Other investigators set out to find SRP in *S. cerevisiae* by examining the requirements for ER translocation in vitro. The functional assay has also not yet succeeded in identifying an RNA-containing particle. However, a 9.6S soluble factor which stimulates ER targeting of prepro-α-factor was isolated (58). A larger (12S) putative targeting factor was characterized several years ago from *E. coli* and also did not appear to contain RNA (22). Recent evidence (6) suggests that the bacterial and yeast factors which stimulate secretory protein transport can substitute for each other and thus may be functionally related. Their roles in this process remain to be elucidated, but their differences in structure and localization (see above) suggest that they do not act via a mechanism similar to SRP.

Before the work reported in the present paper, the sole 7SL candidate in a unicellular organism was an RNA from the archaebacterium *Halobacterium halobium* (21), which has tenuous structural homology to mammalian SRP RNA. The *S. pombe* RNA that we are studying exhibits some differences from mammalian 7SL as a consequence of the vast evolutionary distance separating them but is similar in so many structural and biochemical properties that it almost certainly seems related. The next step in providing unequivocal proof that the fission yeast RNA is a true analog of mammalian 7SL will be the direct demonstration using both biochemistry and genetics that it functions in secretory protein targeting. A homologous translation-translocation extract with fission yeast components similar to those developed in *Saccharomyces cerevisiae* (10, 31, 57) can be used to test whether the putative 7SL RNA is required for ER targeting in vitro. To prove that it plays a role in secretion in vivo will require the isolation of point mutations in the *slr1*$^+$ gene which result in conditional defects in protein export. Although it will be surprising if this essential RNA turns out not to play a role in secretory protein targeting, our genetic approach will allow us to determine its mode of action in any case, and this pursuit will undoubtedly shed new light on small RNA function.

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