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Identification of RNA Sequences and Structural Elements Required for Assembly of Fission Yeast SRP54 Protein with Signal Recognition Particle RNA

DAVID SELINGER, PATRICK BRENNWALD,† XIUBEI LIAO,‡ AND JO ANN WISE*

Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

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Signal recognition particle (SRP) is a ribonucleoprotein composed of six polypeptides and a single RNA molecule. SRP RNA can be divided into four structural domains, the last of which is the most highly conserved and, in *Schizosaccharomyces pombe*, is the primary location to which deleterious mutations map. The ability of mammalian SRP54 protein (SRP54p) to bind *Escherichia coli* 4.5S RNA, a homolog of SRP RNA which contains only domain IV, suggested that SRP54p might interact directly with this region. To determine whether domain IV is critical for SRP54p binding in fission yeast cells, we used a native immunoprecipitation-RNA sequencing assay to test 13 mutant SRP RNAs for the ability to associate with the protein in vivo. The G156A mutation, which alters the 5′ residue of the noncanonical first base pair of the domain IV terminal helix and confers a mild conditional growth defect, reduces assembly of the RNA with SRP54p. Mutating either of the two evolutionarily invariant residues in the bulged region 5′ to G156 is more deleterious to growth and virtually abolishes SRP54p binding. We conclude that the conservation of nucleotides 154 to 156 is likely to be a consequence of their role as a sequence-specific recognition element for the SRP54 protein. We also tested a series of mutants with nucleotide substitutions in the conserved tetranucleotide loop and adjoining stem of domain IV. Although tetraloop mutations are deleterious to growth, they have little effect on SRP54p binding. Mutations which disrupt the base pair flanking the tetraloop result in conditional growth defects and significantly reduce association with SRP54p. Disruption of the other two base pairs in the short stem adjacent to the tetranucleotide loop has similar but less dramatic effects on SRP54p binding. These data provide the first evidence that both sequence-specific contacts and the structural integrity of domain IV of SRP RNA are important for assembly with SRP54p.

Signal recognition particle (SRP) is a small cytoplasmic ribonucleoprotein which recognizes and targets presecretory proteins to the endoplasmic reticulum (reviewed in reference 30). Mammalian SRP is an 11S particle composed of a 300-nucleotide RNA (7SL) and six polypeptides (28, 29). Previous work in several laboratories led to the discovery of a ribonucleoprotein in the fission yeast *Schizosaccharomyces pombe* that is similar in size to mammalian SRP and contains a 254-nucleotide homolog of human SRP RNA (3, 16, 18). Disruption of the *srp7* gene demonstrated that this RNA is essential for viability in *S. pombe* (3, 18). The fission yeast homolog of mammalian SRP54 protein (SRP54p) (6) is also required for vegetative growth (1). Native immunoprecipitation experiments demonstrate that, in addition to 7SL RNA and SRP54p, the fission yeast particle contains five other polypeptides comparable in size to components of mammalian SRP (22).

The secondary structure of SRP RNA can be divided into four domains (17; see Fig. 6A for a schematic representation of the fission yeast homolog). Domain IV, the 3′-most of the two internal stem-loop structures, is the only region present in all identified SRP RNAs, including examples from eubacteria, archaebacteria, fungi, and plants (reviewed in reference 9). In all homologs except those from plants, this domain, which is highly conserved in both sequence and structure, terminates in a GNRA tetranucleotide loop. The presence of a tetraloop that conforms to this consensus significantly enhances the stability of synthetic RNA hairpins (2). Recently, the solution structure of a GNRA tetranucleotide loop was solved by nuclear magnetic resonance methods, revealing that its unusual stability arises from a highly compact architecture containing many intraloop hydrogen bonds (8). The phenotypes conferred by single point mutations and en bloc substitutions in the domain IV tetraloop (nucleotides 160 to 163) of fission yeast SRP RNA are strongly correlated with the degree of predicted disruption of the tetraloop structure (10, 22). Flanking the tetraloop is a stem containing three Watson-Crick and one or two noncanonical base pairs, whose integrity is also important for SRP RNA function (10, 22). The terminal domain IV hairpin is preceded by a bulge containing nucleotides which are invariant through evolution (9).

The results of partial reconstitution experiments suggested that all of the mammalian SRP proteins directly contact the RNA subunit with the exception of SRP54p, which could bind only in the presence of SRP19p (29). However, more recent studies reveal that human SRP54p can, in the absence of SRP19p, bind to *Escherichia coli* 4.5S RNA, a homolog of SRP RNA which contains only domain IV (21, 31). Also, SRP19p was found to be dispensable in the low-ionic-strength buffer used in a GTPase assay that requires SRP54p, SRP receptor, and 7SL RNA, suggesting that under these conditions, SRP54p directly contacts the mammalian RNA (15). Taken together, these data suggest that SRP54p is an RNA-binding protein whose association with mammalian 7SL is facilitated by SRP19p. Evidence for cooperation

* Corresponding author.
† Present address: Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.
‡ Present address: Department of Molecular Biology, Research Institute of Scripps Clinic, San Diego, CA 92037.

between the two proteins during SRP assembly is provided by the observation that overexpression of *Saccharomyces cerevisiae* SRP54p suppresses a temperature-sensitive allele of the *SEC65* gene, which encodes a putative SRP19p homolog in *S. cerevisiae* (7, 24). In RNase footprinting experiments, the domain IV tetranucleotide loop, as well as the 5′ side of the stem and preceding bulge, is protected from nuclease cleavage by canine SRP19 protein in both mammalian and fission yeast SRP RNAs (16, 23). Although the pattern of protection did not change upon addition of SRP54p (23), in view of the 4.5S data, this finding likely reflects the limited digestion of the RNA by α-sarcin, the RNase used in these studies, rather than a lack of contact between the protein and domain IV.

We have recently analyzed an extensive set of randomly generated mutations in the *S. pombe srp7* gene, which revealed that point mutations displaying conditional or lethal phenotypes cluster in domain IV (11), the region homologous to *E. coli* 4.5S RNA. Among these mutations was a single base change at position 156, the 5′ residue of the noncanonical first base pair of the domain IV terminal hairpin. The evolutionary conservation of this residue, together with the data from other laboratories discussed in the last paragraph, prompted us to investigate whether the mild conditional growth defect conferred by this mutation might be a consequence of reduced binding to the *S. pombe* homolog of SRP54p. We used a native immunoprecipitation assay to show that the mutant RNA is, indeed, defective in assembling with the protein in vivo. Since the last two nucleotides of the bulge preceding G156 are invariant in SRP RNA homologs from bacteria to humans (reviewed in reference 9), we altered them by site-directed mutagenesis and tested the mutants for SRP54p binding. These mutations conferred severe conditional growth defects and produced more significant decreases in SRP54p binding than did changing G156. Thus, our data suggest that all three residues establish base-specific contacts with the protein. We also used the native immunoprecipitation assay to test a number of other RNAs carrying nucleotide substitutions in domain IV. Single point mutations in the tetranucleotide loop have no detectable effect on SRP54p binding, while en bloc replacements reduce binding to a modest or intermediate degree, depending on the identity of the closing base pair. In contrast, mutations which disrupt the stem adjacent to the tetranucleotide loop, especially the base pair flanking the tetraloop, show more dramatic defects in binding to the protein. These data provide the first evidence that both sequence-specific contacts and the structural integrity of domain IV of SRP RNA are important for assembly with SRP54p.

MATERIALS AND METHODS

Materials. Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories. Novozyme for digesting the *S. pombe* cell wall was from NovoIndustry. Deoxynucleoside triphosphates for primer extension RNA sequencing were from Pharmacia. Reverse transcriptase was from Life Sciences, Inc. Reagents used for site-directed mutagenesis reactions were part of a kit from Amersham. Radioactively labeled compounds ([γ-^{32}P]ATP and [α-^{32}P]dCTP were from ICN Pharmaceuticals, Inc. Oligonucleotides were synthesized at the University of Illinois Biotechnology Center.

Creation and phenotypic analysis of mutations at nucleotides 154 and 155 of SRP RNA. Single-stranded phagemid pWEC4.2 (11) template was prepared from *E. coli* NM522 with helper phage M13K07. Mutagenesis reactions were performed by using a kit based on the method of Eckstein (14, 25). The mutagenic oligonucleotide (5′-GTTTCCAACC *T*CCATCG-GTAC-3′; designated AGN), was designed to mutate positions 154 (T*) and 155 (C*) in SRP RNA to each of the other three nucleotides. Site-directed mutations were confirmed by DNA sequencing.

Plasmids carrying mutant alleles of the *srp7* gene were introduced into *S. pombe* RM2a (3), a diploid which is heterozygous for disruption of the *srp7* gene, by lithium acetate transformation. Random spore analysis was performed to assess whether the mutant genes could complement the null allele (10, 11). Viable mutants (those which could support growth as the sole copy in a haploid) were tested for conditional growth defects (sensitivity to 20 or 25% glycerol at 37°C, designated an OTS [severe osmotically induced temperature-sensitive] phenotype) as previously described (10).

Preparation of RNA from a cell fraction enriched in SRP. For most experiments, cells were grown in YEL (5) at 30°C to late log phase (optical density at 600 nm of 2.5 to 3.0) and converted to spheroplasts as previously described (3). Then 500 ml of cells were harvested by centrifugation, spheroplasted, washed, and resuspended in lysis buffer (200 mM sucrose, 50 mM triethanolamine acetate [TEA] [pH 7.5], 50 mM potassium acetate [pH 7.4]. 5 mM magnesium acetate, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 20 μg of aprotinin per ml, 0.05% Nonidet P-40). The cells were lysed by mechanical disruption in a glass Dounce homogenizer, and the lysate was layered over a cushion identical to the lysis buffer except that the sucrose was increased to 0.5 M. Nuclei were removed by spinning at 8,000 rpm (10,000 × *g*) in a Beckman JS-13 rotor for 10 min. The postnuclear supernatant was brought to 0.5 M potassium acetate and subjected to three to five strokes in the Dounce homogenizer. This solution was layered over a cushion containing 0.5 M potassium acetate and spun at 47,000 rpm (151,000 × *g*) for 2 h in a Beckman Ti70.1 rotor at 4°C. The resulting postribosomal supernatant is referred to as a SEC (salt extract of the cytoplasm) prep. Alternatively, the cells were disrupted mechanically in a bead beater. After being spun down, washed once with water, and resuspended in lysis buffer, the cells were transferred to a 10-ml bead beater chamber containing glass beads and disrupted by four 30-s pulses. The bead-beaten extracts were then processed in a manner identical to that used for the spheroplast preparations.

To extract RNA from the SEC prep, EDTA was added to 20 mM and the sample was extracted with hot (60°C) phenol. After a second extraction with phenol-chloroform-isopentyl alcohol (49:49:1), the aqueous phase was precipitated with 2.5 volumes of ethanol overnight at −20°C. The RNA was pelleted by centrifugation for 30 min at 4°C in a microcentrifuge, washed with 70% ethanol, dried briefly under vacuum, and resuspended in H_2O.

Preparation of RNA from samples immunoprecipitated with anti-SRP54p. Immunoprecipitations of native fission yeast SRP were performed by a modification of the method described previously (20). Seventy-five microliters of a 1:1 suspension of protein A-Sepharose was preloaded with 30 μl of a polyclonal rabbit antiserum directed against a TrpE-SRP54p fusion protein containing the C-terminal M domain (amino acids 300 to 522) (22); 400 μl of NET-2 buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.05% Nonidet P-40) was added, and the sample was incubated for 1 to 2 h at 4°C with

rocking. The beads were collected by brief centrifugation in a microcentrifuge (3 to 5 s), washed three times with 1 ml of NET-2, and resuspended in 400 μl of NET-2. An aliquot of a SEC fraction (see above) was spun in a microcentrifuge for 15 min at 4°C, and 400 μl of the supernatant was added to the beads. After a 2-h incubation at 4°C with rocking, the beads were collected by centrifugation, washed four to five times with NET-2, and then resuspended in 300 μl of NET-2.

RNA was extracted from the resuspended beads with hot phenol as described above. After a second extraction with phenol-chloroform-isopentyl alcohol, potassium acetate was added to 0.4 M, 20 μg of glycogen was added as the carrier, and the mixture was ethanol precipitated and resuspended as described above.

RNA sequencing. Primer extension RNA sequencing was carried out either on RNA from the total SEC prep or on immunoprecipitated RNA, using the oligonucleotide 7STx as the primer (10). An LKB Ultroscan XL densitometer was used to scan the gels when quantitation was required.

Integration of mutant alleles at the *srp7* locus and suppression analysis. The G160A and G159U mutants, described elsewhere (10, 22), were marked with *LEU2* and used to replace the *srp7*::*ura4* allele of *S. pombe* RM2a (see above). For G160A, which was originally constructed in pWEC10 (10), we first introduced an *Xho*I site 130 nucleotides downstream from the *srp7* gene by site-directed mutagenesis, using the mutagenesis primer XhoDS (5′-TCGCTAACCTC GAGACTATATGGTA-3′, which alters the underlined nucleotides located at +130 to +133 [numbering according to reference 3]). A *Bam*HI-*Eco*RI fragment containing the *srp7* gene was isolated and inserted into the same sites of pTZ19U. An *Xho*I-*Sal*I fragment containing the *S. cerevisiae* *LEU2* gene was then inserted at the *Xho*I site. For G159U, which was originally constructed in pWEC4.2 (11), an *Xho*I site was created as described above, and an *Asu*II-*Eco*RI fragment containing the mutant portion of the *srp7* gene and ca. 650 bp of 3′ flanking sequence was inserted into the same sites of pWEC2 (3). The *LEU2* marker was then introduced as described above. The plasmids were digested either with *Bam*HI and *Pvu*II (for G160A) or with *Bam*HI, *Pst*I, and *Pvu*II (for G159U), and the appropriate linear fragment (5 to 10 μg) was introduced into strain RM2a by spheroplast transformation (3); the cells were then allowed to grow on minimal medium (EMM2) plates (13) containing 1.2 M sorbitol and supplemented with adenine and uracil (100 μg/ml). To test for stability, Leu^+ Ura^- transformants were grown in rich liquid medium (YEL [5]) to saturation and spread onto nonselective (malt extract [13]) and selective (EMM2 supplemented with adenine and uracil) plates at ca. 200 to 300 cells per plate. If similar numbers of colonies grew on the two plates after 7 to 10 days of incubation, the *LEU2* marker was considered to be integrated into the genome. Random spore analysis (see above) was then performed to assess the phenotypes of the integrated mutant alleles.

S. pombe strains carrying integrated mutant *srp7* alleles were transformed with plasmid pSP54-U, carrying the wild-type *srp54* gene (3), or with a complete genomic library in plasmid pFL20 (12). Transformants were tested for conditional growth defects as previously described (10).

Determination of growth rates. Rich medium cultures (50 ml) were inoculated to an optical density at 600 nm of approximately 0.2 and monitored to saturation, with readings taken on a Beckman DU-64 spectrophotometer every hour. After 6 h of growth, half of each culture was removed, and the cells were pelleted at room temperature and resuspended in 50 ml of 25% glycerol medium prewarmed to 37°C. After 2 h of growth under nonpermissive conditions, aliquots from each culture containing approximately 200 cells were plated to determine the fraction of viable cells and assess the thermoreversibility of the growth defects.

RESULTS

We devised a native immunoprecipitation-RNA sequencing assay to determine the extent to which mutant SRP RNAs are assembled with fission yeast SRP54p in vivo. Because some of the alleles analyzed are unable to support growth in the absence of a wild-type copy of the *srp7* gene, we prepared samples from diploid strains heterozygous for disruption at the chromosomal locus and carrying the mutant gene on a high-copy-number plasmid. An advantage of this strategy is that the wild-type RNA provides an internal control for quantitation. To obtain an SRP-enriched extract, we prepared a SEC fraction (see Materials and Methods) from each diploid strain. An aliquot of this fraction was immunoprecipitated under native conditions with a polyclonal serum directed against *S. pombe* SRP54p. With use of an identical protocol, the other protein components of SRP are coprecipitated along with SRP RNA (22). RNA samples from both the total SEC prep and the immunoprecipitate were sequenced with a 7SL-specific primer, and the relative amounts of mutant and wild-type RNA in each fraction were quantitated by densitometry. Data from experiments with all 13 mutants analyzed are presented in Table 1; the values reported in the fifth column provide a measure of the efficiency with which each mutant RNA associates with SRP54p in vivo. Some examples of the sequencing gels used for quantitation are shown in Fig. 1 to 3. The secondary structures of the relevant portions of the mutant SRP RNAs are shown in Fig. 4, grouped according to their degree of association with SRP54p. Three of the mutants were analyzed in independent experiments to test the reproducibility of the assay. In each case, the fractions of mutant RNA in the immunoprecipitate were very similar between trials, indicating that the percentage bound to the protein was relatively constant. The consistency of these numbers is in contrast to the rather large variation in the fraction of mutant RNA in the total sample, particularly for G159A. In fact, we have been unable to correlate the amount of mutant relative to total SRP RNA in diploid cells with either haploid phenotype or plasmid copy number, suggesting that variations in synthesis or stability may play a role in determining this ratio. The sole exception is the A161C mutation, for which the value reported here is very close to that previously found (10). This observation may be related to the fact that A161C is the only mutant assayed that exhibits growth indistinguishable from that of an isogenic wild-type strain under all conditions tested.

The highly conserved nucleotides A154, G155, and G156 are important both for growth and for SRP54p binding. One of the conditional alleles which emerged from screening an extensive series of randomly generated mutations in the fission yeast *srp7* gene was a G-to-A change at position 156, which produces a mild growth defect under nonpermissive conditions (11). This nucleotide, which is highly conserved among SRP RNA homologs, can form a noncanonical base pair to close the domain IV terminal hairpin at the end opposite the tetranucleotide loop. Since G156 is located in the region homologous to *E. coli* 4.5S RNA, which has recently been shown to bind SRP54p (15, 19), we suspected that the phenotype resulting from changing this nucleotide might arise from a defect in protein binding. The indirect

TABLE 1. Quantitation of primer extension sequencing data from binding assays

Row no.	Mutant allele	Phenotype[a]	% of mutant RNA in[b]:		% of mutant RNA bound/total[c]
			SEC prep	Immunoprecipitate	
1	G156A	Mild OTS[d]	62 ± 9	33 ± 9	53 ± 6
2	A154U	Severe OTS	85	2	2
3	G155A	Severe OTS	50	2	4
4	A161C	Wild type	94	94	100
5	G160A	Moderate OTS[e]	82	92	112
6	^{160}UUCG^{163}	Severe OTS[f]	78	13	16
7	^{159}CUUCGG^{164}	Moderate OTS[f]	46 ± 18	37 ± 17	81 ± 5
8	G159C,C164G	Severe OTS, CS[f]	39	35	80
9	G159A	Severe OTS[e]	46 ± 35	2.7 ± 2.5	5.5 ± 1.3
10	G159A,G160A	Lethal[e]	58	4	7
11	U157G,A165G	Mild OTS[f]	77	52	68
12	A166C	Moderate OTS[f]	89	43	48
13	U158C,A166C	Lethal[f]	80	2	2

[a] Sensitivity to the combination of high temperature and elevated osmotic strength, as defined in reference 10.
[b] The gel autoradiograms were densitometrically scanned. The value reported is (integrated curve area for the mutant band)/(area of mutant + area of wild-type band). Values with standard deviations are the results of two experiments.
[c] The value reported is (percentage of mutant RNA in the immunoprecipitate)/(percentage of mutant RNA in the SEC prep).
[d] Reference 11.
[e] Reference 10.
[f] Reference 22.

immunoprecipitation assay was used to test whether the mutant RNA associates with SRP54p; the results (Table 1, row 1) indicate that the level of G156A in the immunoprecipitated sample is somewhat reduced. Since the mutation converts a G · U base pair to an A-U, presumably not altering in any significant way the secondary structure of the region, it is likely that this residue provides a sequence-specific contact for SRP54p. The nucleotides at positions 154 and 155 of *S. pombe* SRP RNA are invariant through evolution and, as is frequently the case for residues recognized by RNA-binding proteins, are located in a single-stranded region. We therefore hypothesized that they might also be contact sites for SRP54p. Oligonucleotide-directed mutations at these two positions were constructed and assayed for their effects on viability and growth (Table 2). Interestingly, changing the wild-type A at position 154 to a G is lethal, while a transversion to U is viable on standard media at 30°C. At position 155, only the G-to-A transition was recovered, and it displays the same severe conditional growth phenotype as did A154U. Both alleles carrying double mutations at these nucleotides are lethal. Since our previous work revealed that most point mutations in fission yeast SRP RNA are phenotypically silent (11), the sensitivity to mutation of these two positions suggests that they are indeed important for function. The results of in vivo binding assays on the viable mutants (Table 1, rows 2 and 3; Fig. 1) indicate that the defect is likely to be the inability of the mutant RNAs to bind SRP54p, since both A154U and G155A fail to detectably associate with the protein.

Assembly of mutant RNAs with SRP54p is not significantly reduced by mutations in the tetranucleotide loop. The observation that mutations in nucleotides 154 to 156 reduce binding to SRP54p prompted us to test additional domain IV changes. The next series of mutant RNAs assayed for association with SRP54p in vivo contained either point mutations in the domain IV tetranucleotide loop and closing base pair or en bloc substitutions of the same region (10, 22). The results of these experiments (Table 1, rows 4 to 8)

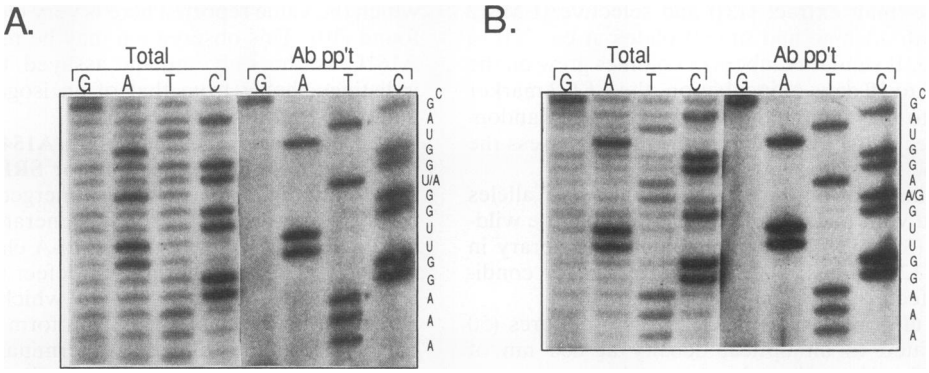

FIG. 1. RNA sequencing data for SRP RNA mutants containing substitutions in the invariant residues A154 and G155. RNA from an SRP-enriched fraction, prepared from diploid cells as described in Materials and Methods, was sequenced with an oligonucleotide complementary to SRP RNA. The relevant region of a primer extension sequencing gel is shown for total and immunoprecipitated RNA from each mutant indicated. Lanes are marked at the top with the dideoxynucleotide incorporated. Alongside each gel is the sequence of fission yeast SRP RNA, with positions of mutant and wild-type heterogeneity as shown. (A) A154U; (B) G155A. Ab pp't, antibody precipitation.

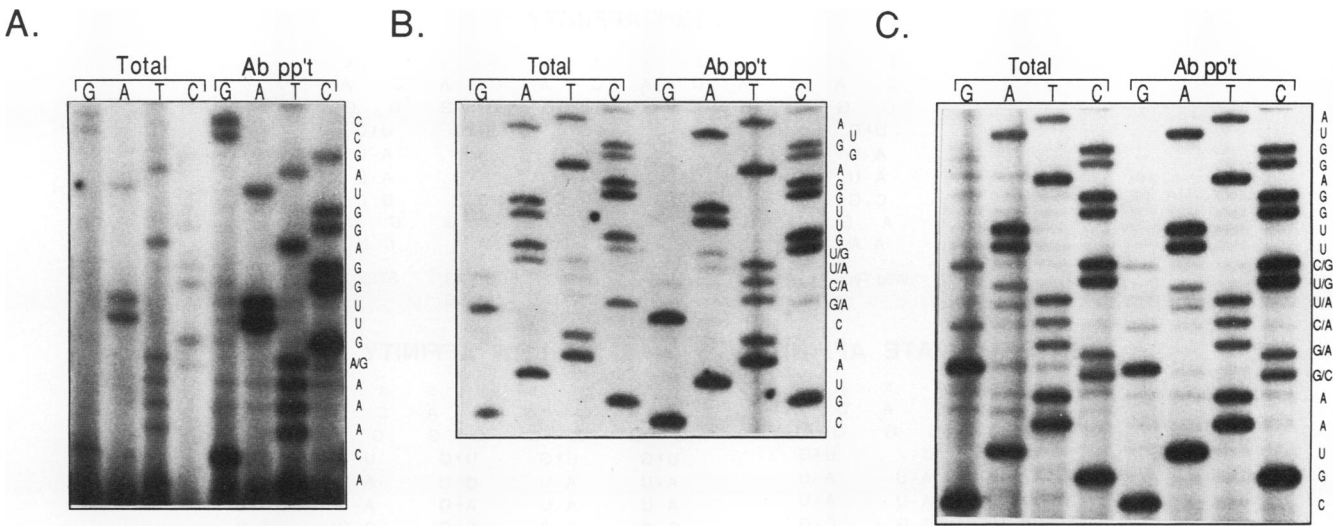

FIG. 2. RNA sequencing data for SRP RNA mutants containing domain IV tetranucleotide loop substitutions. RNA samples were prepared and processed as described in the legend to Fig. 1. (A) G160A; (B) ^{160}UUCG^{163}; (C) ^{159}CUUCGG^{164}. Ab pp't, antibody precipitation.

indicate that the phenotypic consequences of these mutations are not strongly correlated with their effects on SRP54p binding. We anticipated that the A161C mutant RNA, which confers no growth defect when it is present as the sole copy, would be efficiently assembled with SRP54p even in the presence of wild-type 7SL RNA. Indeed, this RNA is present in the immunoprecipitated RNA at the same level as in the total SEC prep RNA (Table 1, row 4), demonstrating that the mutation has no detectable effect on SRP54p binding. Surprisingly, G160A, which produces a moderate conditional growth defect (10), coimmunoprecipitates with the protein at least as well as does A161C (Table 1, row 5; Fig. 2A). This finding indicates that despite the predicted disruption of the tetranucleotide loop structure by this mutation (see reference 8), it has no detectable effect on the binding of SRP54p. Interestingly, replacement of the GAAA tetranucleotide loop with a different stabilizing tetranucleotide loop, UUCG, reduces SRP54p binding to different degrees, depending on the identity of the closing base pair. The mutant ^{159}CUUCGG^{164} which, like G160A, exhibits a moderate conditional phenotype, has a slightly reduced affinity for SRP54p (Table 1, row 7; Fig. 2C), whereas ^{160}UUCG^{163}, which confers a more severe conditional growth defect, displays a much greater decrease in binding to the protein (Table 1, row 6; Fig. 2B). A less pronounced closing base pair effect is observed with the wild-type GAAA tetraloop; the RNA containing an inverted C-G base pair bound at 80% the level of the wild-type, G-C pair (Table 1, row 8; Fig. 3C). Since neither a point mutation in nor a complete replacement of the tetranucleotide loop eliminates binding to SRP54p, we conclude that it does not contain determinants crucial for recognition by the protein.

The structure of the stem adjacent to the tetranucleotide loop is important for SRP54p binding. Our previous mutational analysis implicated the base pair flanking the tetranucleotide loop as critical for SRP RNA function and revealed that the other two base pairs in this short stem are also important, but less so than the closing pair (10, 22). The results of quantitating RNAs coimmunoprecipitated with SRP54p from extracts of cells harboring mutants altered in the stem flanking the tetraloop (Table 1, rows 8 to 13) suggest that their phenotypic effects may be, at least in part, a result of defects in protein binding. The G159A mutant RNA, which has a disrupted closing base pair and confers a severe

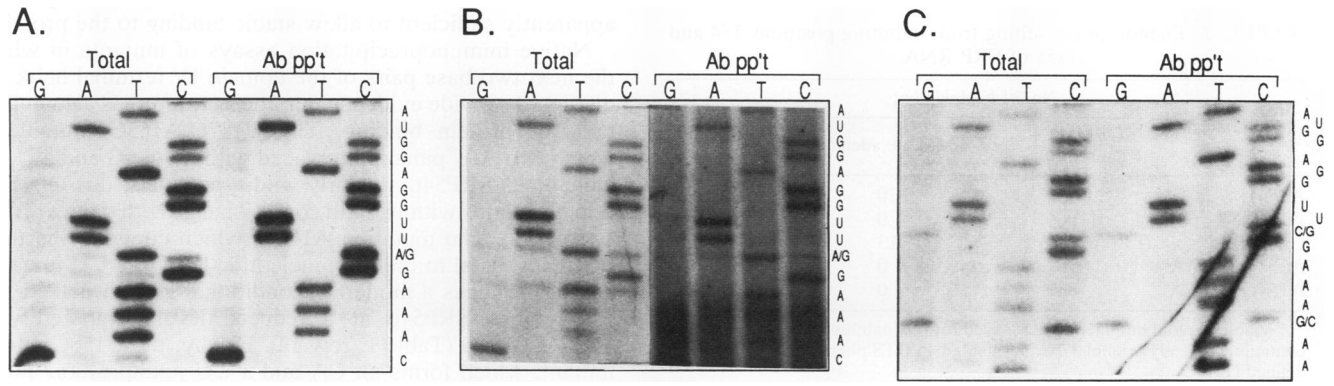

FIG. 3. RNA sequencing data for SRP RNA mutants containing mutations in the base pair closing the domain IV tetraloop. RNA samples were prepared and processed as described in the legend to Fig. 1. (A) G159A, diploid; (B) G159A, haploid; (C) G159C,C164G. Ab pp't, antibody precipitation.

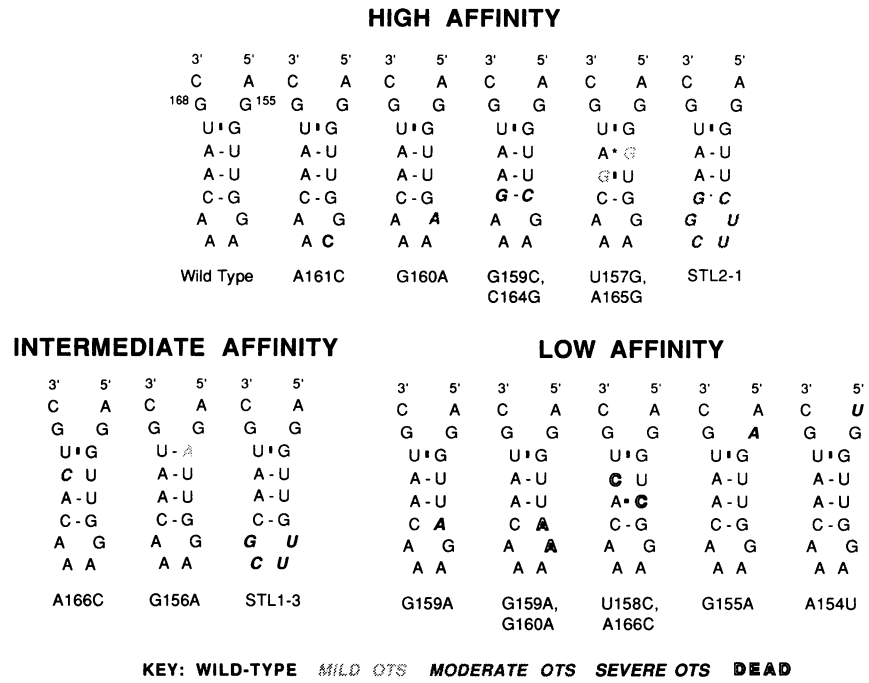

FIG. 4. Summary of the effects of mutations on the binding of SRP54p to 7SL RNA. The 13 mutant RNAs tested are divided into three groups according to the ability to bind SRP54p in vivo. Mutations which bind at 60 to 100% of the wild-type level are considered to be stably associated, those which bind at 11 to 59% are classified as intermediate, and those which show 10% or less of the wild-type level of binding are designated as binding weakly. The phenotypes of the mutations are indicated by different type faces, as shown in the key.

conditional growth phenotype, does not detectably associate with SRP54p in the diploid (Table 1, row 9; Fig. 3A). Since a cell carrying the G159A mutant as the sole copy of 7SL is viable under the growth conditions used for the diploid, we examined its binding to SRP54p in a haploid cell lacking the wild-type RNA. In this case, G159A does bind to the protein, as assayed by immunoprecipitation (Fig. 3B). This observation suggests that the base change reduces the affinity of the mutant RNA for SRP54p such that it is unable to compete effectively with the wild-type RNA in a diploid. Although we have not tested the other conditional mutants for protein binding in haploids, it seems likely that they, too, will be capable of assembling with SRP54p in the absence of competing wild-type RNA, since the protein is required for growth in *S. pombe* (1) and a particle lacking it would be nonfunctional.

TABLE 2. Phenotypes resulting from mutating positions 154 and 155 of SRP RNA

Mutant allele	No. of haploids on:		Conditional phenotype[a]
	EMM2 + adenine + uracil[b]	EMM2 + adenine[c]	
A154U	95	20	Severe OTS
A154G	90	0	Lethal
G155A	97	13	Severe OTS
A154C,G155A	78	0	Lethal
A154G,G155A	88	0	Lethal

[a] Viable haploid mutants were assayed for the inability to grow on medium containing 20% glycerol at 37°C, designated an OTS phenotype as previously defined (10).

[b] Growth indicates the presence of the plasmid containing the mutant *srp7* gene, which carries a *LEU2* marker.

[c] Growth indicates the presence of both the plasmid and the *srp7::ura4* chromosomal deletion allele.

The lethal double mutant G159A,G160A is not present at a detectable level in the immunoprecipitated RNA (Table 1, row 10), which is as expected from the results described above for G159A alone. The failure of G159A to bind the protein could be a direct consequence of disrupting a sequence-specific contact or might arise as an indirect effect of perturbing the structure of this region. To distinguish these possibilities, we tested an RNA with a different nucleotide at position 159 but also carrying a compensatory change at position 164 to restore base pairing. The G159C,C164G mutant, which, like G159A, confers a severe conditional growth defect, binds quite well to SRP54p (Table 1, row 8; Fig. 3C). Thus, the presence of a base pair at this position, even one that is inverted relative to the wild type, is apparently sufficient to allow stable binding to the protein.

Native immunoprecipitation assays of mutants in which the next two base pairs of the domain IV terminal helix are disrupted provide evidence that their structure is also important for protein binding. The U157G,A165G mutant, in which two A-U pairs are replaced with an A · G and a U · G pair, binds SRP54p at nearly wild-type levels; this observation correlates with its mild conditional growth defect (Table 1, row 11). The mutation A166C, which converts the third base pair distal to the tetranucleotide loop to a CU juxtaposition, produces a moderate conditional growth defect; this RNA binds SRP54p at a reduced level compared with U157G,A165G (Table 1, row 12). Finally, the U158C,A166C mutant, which forms an CA and a CU juxtaposition and is lethal, fails to detectably bind SRP54p (Table 1, row 13). Thus, disrupting either of these two base pairs has a similar but less pronounced effect on SRP54p binding compared with

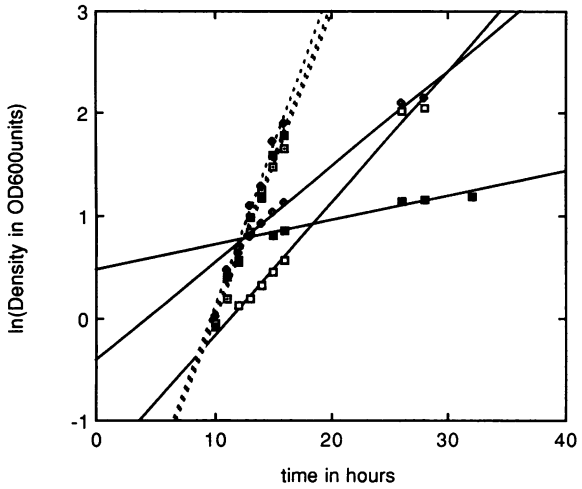

FIG. 5. Growth curves for the A154U and G160A mutants compared with those for wild-type cells under permissive and nonpermissive growth conditions. Cultures were propagated and shifted to prewarmed high-osmotic-strength medium as described in Materials and Methods. For each strain, the growth curve under permissive conditions is shown as a broken line, and that under nonpermissive conditions is shown as a solid line. Symbols: open squares, wild-type cells; closed squares, A154U; diamonds, G160A. OD600, optical density at 600 nm.

disrupting the closing base pair, but unpairing both simultaneously apparently reduces the stability of the helix sufficiently to decrease binding to near the limits of detection. Taken together, these results suggest that the sequence of this region of the RNA is not crucial for recognition by SRP54p, but that the integrity of the stem, particularly the closing base pair flanking the tetranucleotide loop, is important for stable association with the protein.

The A154U mutant exhibits a rapid response to nonpermissive growth conditions. A plate assay revealed that haploid cells harboring only the A154U mutant SRP RNA failed to produce even microcolonies upon incubation under nonpermissive conditions (25% glycerol medium, 37°C) and displayed increased uptake of the vital stain phloxin B. In this same assay, all of our other SRP RNA mutants with OTS phenotypes appear to grow for several divisions and do not rapidly lose viability as measured by uptake of phloxin B. To more accurately characterize the A154U growth defect, we performed a growth experiment in liquid media. Figure 5 shows growth curves for the A154U strain, as well as wild-type cells and G160A, a mutant with a less severe conditional growth defect. All three strains grow at virtually identical rates in normal-osmotic-strength medium at 30°C. Upon a shift into prewarmed, 25% glycerol medium, all three cell types lag for about 4 h before resuming growth. At 37°C in high-osmotic-strength medium, wild-type cells grow at 43% of their normal rate (see also reference 10). Under these conditions, the G160A mutant doubles at 73% of the wild-type rate and the A154U strain appears to grow at 18% of the wild-type rate, as measured by increased optical density of the culture. However, unlike G160A cells, A154U cells begin to lose viability within 2 h of the shift and fail to reach saturation. Only 61% of the A154U cells form colonies when plated after a 2-h incubation under nonpermissive conditions, while 95% of the G160A cells remain viable under the same regimen.

To determine whether the rapid cessation of growth in the A154U haploid might be due to dissociation of existing SRP, we used the native immunoprecipitation protocol described above to ascertain how much of the mutant RNA is stably associated with SRP54p. The G160A mutant, which despite its moderate conditional growth defect exhibits no reduction in binding to the protein even in the presence of the wild-type RNA in diploid cells, was used as a control. For each strain, a culture was harvested after growth under permissive conditions or after a 2-h shift to nonpermissive conditions. The SRP-enriched fraction prepared from these cells was immunoprecipitated with anti-SRP54p antibodies and then subjected to RNA extraction and analysis on a denaturing polyacrylamide gel. SRP RNA was successfully isolated from G160A cells propagated under both conditions, but no band was visible in either A154U sample (data not shown). The failure of A154U RNA to coimmunoprecipitate with SRP54p indicates that even under permissive conditions, their interaction is greatly weakened and cannot withstand the high-salt extraction required to release the particle from ribosomes and endoplasmic reticulum membranes. From these observations, it seems likely that the combination of high intracellular osmotic strength (glycerol can freely cross the membrane) and high temperature may indeed disrupt the particle in this mutant.

Domain IV mutants, even those which do not reduce SRP54p binding, can be suppressed by overexpression of the protein. In addition to assaying the effects of mutating SRP RNA on assembly with SRP54p, we examined the phenotypic consequences of overexpressing the protein in the presence of domain IV mutations. Two conditional alleles, G159U and G160A, were tested for suppression by the *srp54* gene on a high-copy-number plasmid. The mutant *srp7* genes were first integrated into the chromosome (see Materials and Methods), and their phenotypes were determined. In both cases, the integrated mutant confers a conditional growth defect very similar to its effect when present on a high-copy-number plasmid (data not shown). The strains harboring mutant chromosomal *srp7* alleles were transformed with plasmid pSP54-U, which carries the wild-type *S. pombe srp54* gene (1). After growth at 30°C (permissive condition), two transformants from each strain were tested for conditional phenotypes on 25% glycerol plates incubated at 37°C. Each grew as well as did an isogenic wild-type strain, indicating that elevated levels of SRP54p can suppress defects in domain IV of 7SL RNA. We also transformed the strain containing the integrated G160A allele with a genomic library and recovered six plasmids which suppress the conditional growth defect. The insert carried by one of these included a complete copy of the gene encoding SRP54p (22), providing independent corroboration that this protein interacts with domain IV of SRP RNA. In the case of G159U, which confers the same moderate conditional growth defect as does G159A (described above), the suppression is likely to result from an increase in SRP54p concentration compensating for a reduction in affinity for the mutant RNA. On the other hand, the G160A mutant, which in the native immunoprecipitation assay binds to SRP54p at wild-type levels, is suppressed by a more complicated mechanism probably involving SRP19p (see Discussion).

DISCUSSION

We have used a native immunoprecipitation assay to show that certain changes in the sequence of domain IV of fission yeast SRP RNA affect its binding to SRP54p, while others do not. Most importantly, our work provides evidence that the

evolutionarily conserved residues 154 to 156, which lie in or near the bulged region located just 5′ to the domain IV terminal hairpin, establish sequence-specific contacts with SRP54p. Single point mutations at the invariant nucleotides 154 and 155 result in both severe conditional growth defects and a dramatic decrease in SRP54p binding, to nearly undetectable levels. The importance of the A at position 154 is further underscored by the fact that changing it to a U not only abolishes the mutant's ability to compete with wild-type RNA for SRP54p binding in a diploid but also destabilizes the RNA-protein interaction to such a degree that they apparently separate during fractionation of extracts from haploid cells. This is in contrast to the behavior of the G159A mutant, which exhibited reduced binding in the diploid but could be coimmunoprecipitated with SRP54p from haploid cells. Another unique feature of the A154U mutant is that it displays a rapid response to nonpermissive growth conditions, which probably results from dissociation of SRP54p and SRP RNA in vivo. In contrast, the slow response to nonpermissive conditions observed in our other conditional SRP RNA mutants suggests that in these cells, particles assembled before the shift remain stable, but new, functional particles cannot be formed (see also reference 11). Evidence that the single-stranded nature of the bulged region, as well as the identity of nucleotides 154 and 155, is functionally important is provided by comparing the effects of the A154G and A154U mutations. Transitions are expected to be more readily tolerated in a protein binding site than are transversions, yet the A154U mutant is viable while A154G is lethal. The change from A to G at position 154 allows formation of a Watson-Crick base pair with the C at position 169, dramatically altering the structure of the region. Mutating G156, a highly conserved but not invariant residue, to an A, which replaces a G · U pair with an A-U, reduces SRP54p binding by half, suggesting that increasing the length or stability of the domain IV terminal stem is deleterious. Taken together, these results demonstrate that both the identity and structural context of nucleotides 154 to 156 are important for SRP54p binding. Since both the evolutionary conservation of these residues and the phenotypic consequences of mutating them correlate strongly with our SRP54p binding data, it is likely that both arise from their roles as sequence-specific contacts for the protein.

Mutations in the next two base pairs of the domain IV stem, which are canonical Watson-Crick combinations, have less impact both on growth (22) and on binding of SRP54p than do changes in the bulged region. However, the phenotypes conferred by these base changes also correlate quite well with the ability of the mutant RNAs to bind SRP54p (Table 1, rows 11 to 13). The variant with a mild conditional phenotype (U157G,A165G) binds best to SRP54p, while the variant with the most severe phenotype (U158C,A166C) fails to bind at a level detectable above background. This correlation suggests not only that the structure of this region is important for SRP54p binding but also that the cause of the growth defects in the mutants is probably the decreased ability of the RNAs to bind SRP54p under nonpermissive conditions.

In contrast, the phenotypes conferred by mutations in the tetranucleotide loop and closing base pair of the domain IV helix do not correlate very well with their quantitative effects on SRP54p binding, suggesting that they primarily affect the interaction of SRP RNA with some component other than SRP54p. The binding of G160A RNA, in which the first residue of the tetraloop is mutated, is indistinguishable from that of A161C, a phenotypically wild-type mutation which changes a phylogenetically variable position. While these results imply that the sequence, and thus the structure, of the tetranucleotide loop is not an important determinant of SRP54p binding, the fact that replacing the entire tetraloop with the sequence UUCG produces a marked reduction in assembly with the protein suggests the converse. The wild-type tetraloop is GAAA, one of two sequences which has been shown to enhance the stability of synthetic RNA hairpins (2). The G160A change is predicted to disrupt the tetraloop structure, since it prevents formation of a critical intraloop hydrogen bond (8). The other sequence which has been shown to stabilize synthetic RNA hairpins is UUCG (26). This tetraloop adopts a structure in vitro which is very similar to that of a GAAA tetraloop (4). Thus, the effects of these mutations on SRP54p binding are exactly the opposite of what would be predicted if they arose from perturbation of the tetraloop structure. We therefore conclude that SRP54p does not directly recognize this region of the RNA.

Why, then, does substituting a UUCG tetraloop diminish binding of the protein? We believe that the reduction in association of SRP54p with this RNA is an indirect consequence of a decrease in the thermodynamic stability of the domain IV hairpin. This idea is supported by the nearly wild-type level of binding exhibited by another en bloc substitution mutant, CUUCGG (row 7, Table 1), which, in addition to replacing the tetraloop, inverts the closing base pair. Studies using synthetic RNAs show that a hairpin containing a UUCG tetraloop with a C-G closing base pair has a T_m of >10°C higher than that of the same hairpin with a G-C closing base pair (71.7°C versus 60.1°C [2]). The base pair closing the wild type GAAA tetraloop is G-C, and a severe conditional growth phenotype results from reversing it to C-G. Of the two UUCG substitution mutants, the one closed by a G-C pair confers a more serious growth defect. These observations argue against the idea that the deleterious effects of inverting the wild-type base pair are due to disruption of a sequence-specific contact. In contrast to the wide discrepancy in SRP54p binding to the two UUCG tetraloop mutants with inverted closing base pairs, the substitution of C-G for G-C in the context of the GAAA tetraloop has little effect on SRP54p binding. The fact that both UUCG replacement mutants exhibit conditional growth defects may indicate that subtle differences in structure from the wild-type GAAA loop reduce the binding efficiency of another factor, possibly SRP19p, which has been shown to protect residues in this region from RNase cleavage (16, 23). The lack of correlation between the effects of both the G160A substitution and reversal of the closing base pair on growth and SRP54p binding is consistent with the idea that these mutations also principally affect interactions with SRP19p or another component involved in SRP function.

Overexpression of SRP54p is expected to suppress the conditional growth defects conferred by mutant RNAs whose phenotypes correlate with defects in binding the protein. We therefore expected suppression of G159U, since this mutation affects the same residue as does G159A, which reduced SRP54p binding in our assays. We believe that in this case, overexpression directly drives the association of SRP54p with the mutant RNA by compensating for a reduction in affinity resulting from the change in RNA structure. In contrast, the G160A mutant RNA binds SRP54p at wild-type levels, and thus its suppression by overexpression of the protein was unexpected. We propose that this effect occurs by a more complex mechanism involving SRP19p. In both human and *S. pombe* SRP RNA molecules, the canine SRP19p protects from RNase cleavage the region of domain

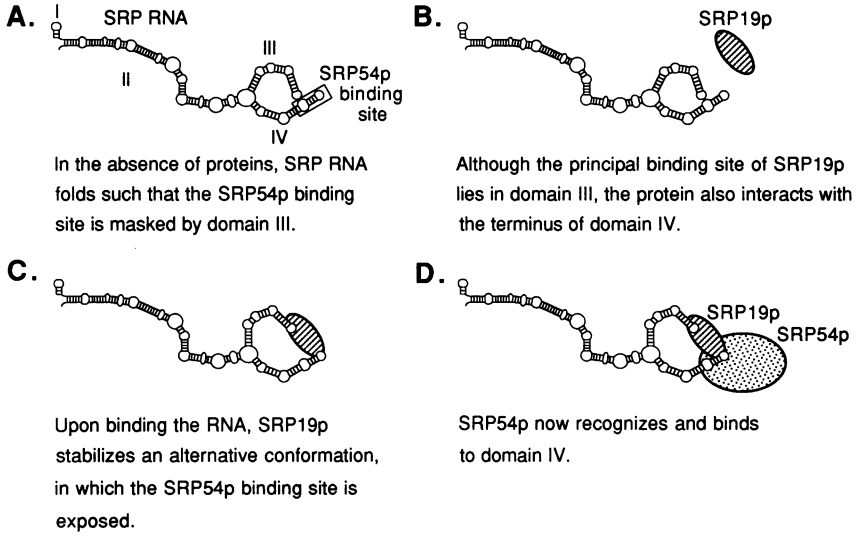

FIG. 6. Model for interactions between SRP54p and SRP19p during assembly of SRP. The sequence of events depicted is based on the data presented here, together with earlier results from several other laboratories (7, 16, 23, 24, 32).

IV extending from the invariant A on the 5′ side of the terminal bulge to the first G residue of the tetraloop (positions 154 to 160 in the *S. pombe* homolog; Fig. 4) and is required for binding of SRP54p to the mammalian RNA in vitro under high-ionic-strength conditions (16, 23). However, in the absence of SRP19p, SRP54p can bind to a domain IV-type structure, *E. coli* 4.5S RNA (15, 19), and deletion of the entire domain IV helix had little effect on the affinity of SRP19p for human SRP RNA (32).

A model consistent with all of these observations is presented in Fig. 6. We hypothesize that in the absence of SRP19p, the RNA adopts a structure in which the SRP54p binding site on domain IV is somehow masked by domain III. In particles containing full-size SRP RNA homologs, at least one function of SRP19p may be to bind both domains and stabilize an alternative conformation (presumably corresponding to the structure adopted at low ionic strength in vitro), in which the SRP54p interaction site is exposed. Thus, even though the primary (high-affinity) binding site for SRP19p lies in domain III, the protein also contacts domain IV, thereby explaining its protection of residues in the tetraloop region from RNase digestion. According to this model, if the G160A mutation reduces SRP19p occupancy of a domain IV binding site, there will be less time available for SRP54p to bind SRP RNA before SRP19p dissociates. Increasing the concentration of SRP54p may drive assembly by compensating for the decreased window of opportunity for binding to the mutant RNA. In vivo studies in *S. cerevisiae*, which showed that overexpression of SRP54p suppressed *sec65-1*, a temperature-sensitive mutant of SRP19p (7, 24), are also consistent with this model.

Because our assay for binding of 7SL variants to SRP54p is indirect, we cannot rule out that the observed effects are mediated through or influenced by the remaining SRP proteins or other components with which the particle interacts. The simplest interpretation of our data, however, is that the SRP54p binding site on SRP RNA consists of at least two elements: sequence-specific contact sites at nucleotides 154 to 156, and the presence of a stable helix distal to these residues. The virtual elimination of binding which results from mutating the invariant nucleotides A154 and G155, and the significant reduction upon changing the conserved G at position 156, identify these residues as likely base-specific contacts. Since mutations which severely reduce the thermodynamic stability of the adjoining helix decrease SRP54p binding, disruption of base pairing may prevent the stem structure from fitting into a binding pocket on SRP54p; alternatively, these effects may arise from altering the structural context of nucleotides 154 to 156 and thus be only an indirect consequence of opening the helix. The data presented here suggest that the tetranucleotide loop itself probably serves only as a stabilizing element in the context of SRP54p binding, but the severe consequences of tetraloop mutations indicate that its sequence is important for some other role. Our evidence that a tetraloop mutation can be suppressed by overexpression of SRP54p, in conjunction with earlier data from other laboratories, suggests that the tetraloop is contacted by another component of the ribonucleoprotein particle which in turn influences SRP54p binding, most likely SRP19p.

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REFERENCES

1. **Althoff, S., S. Stevens, and J. A. Wise.** Unpublished data.
2. **Antao, V. P., S. Y. Lai, and I. Tinoco, Jr.** 1991. A thermodynamic study of unusually stable RNA and DNA hairpins. Nucleic Acids Res. **19**:5901–5905.
3. **Brennwald, P., X. Liao, K. Holm, G. Porter, and J. A. Wise.** 1988. Identification of an essential *Schizosaccharomyces pombe* RNA homologous to the 7SL component of signal recognition particle. Mol. Cell. Biol. **8**:1580–1590.
4. **Cheong, C., G. Varani, and I. Tinoco.** 1990. Solution structure of an unusually stable RNA hairpin, 5′GGAC(UUCG)GUCC. Nature (London) **346**:680–682.
5. **Gutz, H., H. Heslot, U. Leupold, and N. Loprieno.** 1974. *Schizosaccharomyces pombe*, p. 395–446. *In* R. C. King (ed.), Handbook of genetics. Plenum Publishing Corp., New York.

6. **Hann, B. C., M. A. Poritz, and P. Walter.** 1989. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* contain a homologue to the 54-kD subunit of the signal recognition particle that in *S. cerevisiae* is essential for growth. J. Cell Biol. **109:**3223–3230.
7. **Hann, B. C., C. J. Stirling, and P. Walter.** 1992. *SEC65* gene product is a subunit of the yeast signal recognition particle required for its integrity. Nature (London) **356:**532–533.
8. **Heus, H. A., and A. Pardi.** 1991. Structural features that give rise to the unusual stability of RNA hairpins containing GNRA loops. Science **253:**191–194.
9. **Larsen, N., and C. Zwieb.** 1991. SRP-RNA sequence alignment and secondary structure. Nucleic Acids Res. **19:**209–215.
10. **Liao, X., P. Brennwald, and J. A. Wise.** 1989. Genetic analysis of *Schizosaccharomyces pombe* 7SL RNA: a structural motif that includes a conserved tetranucleotide loop is important for function. Proc. Natl. Acad. Sci. USA **86:**4147–4142.
11. **Liao, X., D. A. Selinger, S. Althoff, A. Chiang, D. Hamilton, and J. A. Wise.** 1992. Random mutagenesis of *Schizosaccharomyces pombe* SRP RNA: lethal and conditional mutations cluster in presumptive protein binding sites. Nucleic Acids Res. **20:**1607–1615.
12. **Losson, R., and F. Lacroute.** 1983. Plasmids carrying the yeast OMP decarboxylase structural and regulatory genes: transcriptional regulation in a foreign environment. Cell **32:**371–377.
13. **Mitchison, J. M.** 1970. Physiological and cytological methods for *Schizosaccharomyces pombe*. Methods Cell Physiol. **4:**131–165.
14. **Nakamaye, K. L., and F. Eckstein.** 1986. Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis. Nucleic Acids Res. **14:**9679–9698.
15. **Poritz, M. A., H. D. Bernstein, K. Strub, D. Zopf, H. Wilhelm, and P. Walter.** 1990. An *E. coli* ribonucleoprotein containing 4.5S RNA resembles mammalian signal recognition particle. Science **250:**1111–1117.
16. **Poritz, M. A., V. Siegel, W. B. Hansen, and P. Walter.** 1988. Small ribonucleoproteins in *Schizosaccharomyces pombe* and *Yarrowia lipolytica* homologous to signal recognition particle. Proc. Natl. Acad. Sci. USA **85:**4315–4319.
17. **Poritz, M. A., K. Strub, and P. Walter.** 1988. Human SRP RNA and *E. coli* 4.5S RNA contain a highly homologous structural domain. Cell **55:**4–6.
18. **Ribes, V., P. Dehoux, and D. Tollervey.** 1988. 7SL RNA from *Schizosaccharomyces pombe* is encoded by a single copy essential gene. EMBO J. **7:**231–237.
19. **Ribes, V., K. Römisch, A. Giner, B. Dobberstein, and D. Tollervey.** 1990. *E. coli* 4.5S RNA is part of a ribonucleoprotein particle that has properties related to signal recognition particle. Cell **63:**591–600.
20. **Riedel, N., S. Wolin, and C. Guthrie.** 1987. A subset of yeast snRNAs contains functional binding sites for the highly conserved Sm antigen. Science **235:**328–331.
21. **Römisch, K., J. Webb, K. Lingelbach, H. Gausepohl, and B. Dobberstein.** 1990. The 54-kD protein of signal recognition particle contains a methionine-rich RNA binding domain. J. Cell Biol. **111:**1793–1802.
22. **Selinger, D. S., and J. A. Wise.** Unpublished data.
23. **Siegel, V., and P. Walter.** 1988. Binding sites of the 19 kD and 68/72 kD SRP proteins on SRP RNA as determined by protein-RNA footprinting. Proc. Natl. Acad. Sci. USA **85:**1801–1805.
24. **Stirling, C. J., and E. W. Hewitt.** 1992. The *S. cerevisiae SEC65* gene encodes a component of yeast signal recognition particle with homology to human SRP19. Nature (London) **356:**534–537.
25. **Taylor, J. W., J. Ott, and F. Eckstein.** 1985. The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA. Nucleic Acids Res. **13:**8765–8785.
26. **Tuerk, C., P. Gauss, C. Thermes, D. R. Groebe, M. Gayle, N. Guild, G. Stormo, Y. d'Aubenton-Carafa, O. C. Uhlenbeck, I. Tinoco, Jr., E. Brody, and L. Gold.** 1988. CUUCG hairpins: extraordinarily stable RNA secondary structures associated with various biochemical processes. Proc. Natl. Acad. Sci. USA **85:**1364–1368.
27. **Walter, P., and G. Blobel.** 1980. Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **77:**7112–7116.
28. **Walter, P., and G. Blobel.** 1982. Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. Nature (London) **299:**691–698.
29. **Walter, P., and G. Blobel.** 1983. Dissassembly and reconstitution of signal recognition particle. Cell **34:**525–533.
30. **Walter, P., and V. R. Lingappa.** 1986. Mechanism of protein translocation across the endoplasmic reticulum membrane. Annu. Rev. Cell Biol. **2:**499–516.
31. **Zopf, D., H. D. Bernstein, A. E. Johnson, and P. Walter.** 1990. The methionine-rich domain of the 54 kd protein subunit of the signal recognition particle contains an RNA binding site and can be crosslinked to a signal sequence. EMBO J. **9:**4511–4518.
32. **Zwieb, C.** 1991. Interaction of protein SRP19 with signal recognition particle RNA lacking individual RNA-helices. Nucleic Acids Res. **19:**2955–2960.