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Genetic and biochemical analysis of the fission yeast ribonucleoprotein particle containing a homolog of Srp54p

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ABSTRACT

Mammalian signal recognition particle (SRP), a complex of six polypeptides and one 7SL RNA molecule, is required for targeting nascent presecretory proteins to the endoplasmic reticulum (ER). Earlier work identified a *Schizosaccharomyces pombe* homolog of human SRP RNA and showed that it is a component of a particle similar in size and biochemical properties to mammalian SRP. The recent cloning of the gene encoding a fission yeast protein homologous to Srp54p has made possible further characterization of the subunit structure, subcellular distribution, and assembly of fission yeast SRP. *S.pombe* SRP RNA and Srp54p co-sediment on a sucrose velocity gradient and co-immunoprecipitate, indicating that they reside in the same complex. *In vitro* assays demonstrate that fission yeast Srp54p binds under stringent conditions to *E.coli* SRP RNA, which consists essentially of domain IV, but not to the full-length cognate RNA nor to an RNA in which domain III has been deleted in an effort to mirror the structure of bacterial homologs. Moreover, the association of *S.pombe* Srp54p with SRP RNA *in vivo* is disrupted by conditional mutations not only in domain IV, which contains its binding site, but in domains I and III, suggesting that the particle may assemble cooperatively. The growth defects conferred by mutations throughout SRP RNA can be suppressed by overexpression of Srp54p, and the degree to which growth is restored correlates inversely with the severity of the reduction in protein binding. Conditional mutations in SRP RNA also reduce its sedimentation with the ribosome/membrane pellet during cell fractionation. Finally, immunoprecipitation under native conditions of an SRP-enriched fraction from [^{35}S]-labeled fission yeast cells suggests that five additional polypeptides are complexed with Srp54p; each of these proteins is similar in size to a constituent of mammalian SRP, implying that the subunit structure of this ribonucleoprotein is conserved over vast evolutionary distances.

INTRODUCTION

Signal recognition particle, a soluble factor required for translocation of nascent secretory proteins into mammalian microsomal vesicles *in vitro*, is stripped from the membranes by washing in high salt (1). The observation that translocation activity can be restored by adding back the salt wash (2) provided a functional assay for purification of canine SRP. This particle was shown to be a stable 11S ribonucleoprotein (RNP) composed of six polypeptides organized into two heterodimers (Srp68/72p and Srp9/14p) and two monomers (Srp54p and Srp19p) and one ca. 300 nucleotide RNA molecule historically designated 7SL (1, 3–5). The sequence of SRP RNA was determined more than a decade ago, both directly (6) and from a cDNA clone (7). RNAs with sequence and structural homology to SRP RNA have been identified in numerous organisms representing all three major branches of the phylogenetic tree (reviewed in 8). While the sequences of cloned cDNAs encoding each of the protein components of mammalian SRP have now been reported (9–15), homologs have been identified in other organisms only for Srp19p and Srp54p. The *Saccharomyces cerevisiae SEC65* gene, which was cloned by complementation of a temperature-sensitive allele identified in a screen for secretion-defective mutants, contains two short stretches of similarity to mammalian Srp19p (16). The cloning of *SRP54* genes from virtually any source was made possible by the unexpected finding that the mammalian protein shares significant primary sequence identity with a previously uncharacterized *E.coli* open reading frame (9, 10). Homologs have been identified to date from the bacteria (formerly called eubacteria; 17) *Bacillus subtilis* (18) and *Mycoplasma mycoides*

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(19), from the yeasts *S.cerevisiae* and *Schizosaccharomyces pombe* (20), and from both the cytoplasm and chloroplast of a plant, *Arabidopsis thaliana* (21, 22).

We and others previously identified an RNA from fission yeast that closely resembles mammalian 7SL RNA in secondary structure and is part of a ribonucleoprotein similar in size and chromatographic properties to canine SRP (23–26). Data derived from both biochemical and genetic experiments suggest that *S.pombe* and mammalian SRP are organized similarly. First, RNase footprinting experiments revealed that the canine Srp19 protein protects nucleotides in the vicinity of the loops capping the two central hairpins of both fission yeast and mammalian 7SL and the Srp68/72p heterodimer protects several non-contiguous regions in the central portion of both RNAs (25, 27). Second, lethal and conditional lesions identified through random mutagenesis of the fission yeast *srp7* gene cluster in regions of the RNA secondary structure that correspond to presumptive protein binding sites (28). These include a conditional mutation in a highly conserved residue near the 5′ terminus, which in the mammalian RNA is protected from hydroxyl radical cleavage by the Srp9/14p heterodimer (29); a temperature-sensitive allele harboring mutations in and near the domain III hairpin loop, which is protected from RNase digestion by Srp19p (25) and contains nucleotides whose identity is critical for binding of this protein (30); and several conditional alleles containing mutations in the domain IV hairpin loop, which is also protected from RNase digestion by Srp19p (25). Third, mammalian Srp54p binds *in vitro* to *E.coli* 4.5S RNA (31, 32), a homolog of SRP RNA that consists essentially of domain IV (33, 34), and several conditional and lethal mutations in domain IV of *S.pombe* 7SL RNA affect assembly with the protein *in vivo* (35). Fourth, Srp19p facilitates the association of Srp54p with canine SRP RNA *in vitro* (5, 27), and the *S.pombe srp54* gene on a multicopy plasmid relieves the growth defects conferred by an allele with a point mutation in one of the regions protected by Srp19p (35).

While the structure and function of *S.pombe* SRP RNA has been extensively characterized genetically (28, 35–37), only one of the protein components of fission yeast SRP has been identified to date. A gene encoding Srp54p was isolated by using the polymerase chain reaction to amplify from *S.pombe* genomic DNA a fragment bounded by short stretches of sequence conserved between the human and bacterial homologs (20). Comparative sequence analysis, together with the results of partial proteolysis of mammalian Srp54p, suggests that this protein consists of two structurally distinct domains (32, 38). The carboxyl-terminal M domain, so named because it is rich in methionine, binds to SRP RNA (32, 38) and can be crosslinked to a signal sequence (32, 39). The amino-terminal region is designated the G domain based on the presence of conserved GTP-binding motifs common to the G protein superfamily (9, 13). The protein predicted from the sequence of the *S.pombe srp54* gene likewise contains both a G and an M domain, as does the product encoded by a homologous *S.cerevisiae* gene isolated using the same PCR primers, although both yeast proteins are extended at the C-terminus relative to other homologs (20). We have recently shown that Srp54p, like SRP RNA, is essential for viability in *S.pombe*, and that the integrity of the G domain is critical to the function of the protein *in vivo* (40).

The availability of a cloned gene encoding Srp54p made possible the generation of constructs for production of antisera and for *in vitro* expression, opening additional avenues of investigation. As expected, antibodies directed against Srp54p fusion proteins immunoprecipitate 7SL RNA, and this interaction is disrupted by mutations in domain IV. Surprisingly, however, mutations located outside the Srp54p binding site also result in defective assembly with the protein, suggesting that SRP assembly is cooperative *in vivo*. Consistent with this idea, the growth defects of conditional mutations located throughout SRP RNA are ameliorated by the *srp54* gene on a multicopy plasmid. Both *S.pombe* SRP RNA and Srp54p associate with microsomes in a salt-labile interaction, which is disrupted by mutations that reduce their interaction. Finally, immunoprecipitation under native conditions with antibodies directed against Srp54p indicates that fission yeast signal recognition particle is remarkably similar in subunit structure to its mammalian counterpart.

MATERIALS AND METHODS

Preparation of antibodies against fusion proteins containing portions of Srp54p

Recombinant DNA manipulations to create plasmids for fusion gene expression, as well as constructs described in subsequent sections, were performed according to Sambrook *et al.* (41). To prepare a TrpE–Srp54p fusion protein, we used the polymerase chain reaction to amplify from genomic DNA a 690 bp fragment corresponding to the M domain (amino acids 300–522) of fission yeast Srp54p (20). Taq DNA polymerase and other PCR reagents were from United States Biochemicals. The oligonucleotide primers employed, synthesized at the University of Illinois Biotechnology Center, were SP54M1 (5′GGTGATCTAGAAGGTCTGATGG3′) and SP54M2 (5′GAGGGAAGCTTACTAACGTCTTCGA3′). The *Hind*III and *Xba*I restriction sites (underlined) incorporated into the primers allowed the amplified fragment to be subcloned in frame with TrpE in the plasmid pATH2 (42; provided by P. Novick). Restriction enzymes and T4 DNA ligase used for this purpose were purchased from BRL. After overexpressing the 60 kDa fusion protein as described previously (43), inclusion bodies were prepared according to Harlow and Lane (44) and solubilized proteins (~2.5 mg) resolved on a 10% SDS-polyacrylamide gel. The polypeptide of interest was excised, electroeluted, and precipitated with cold acetone. Mouse polyclonal ascites fluid was produced by the method of Lacy and Voss (45) using an initial injection of 50 μg of the purified TrpE–Srp54p fusion protein. Clarified ascites fluid was treated with 0.2% dextran sulfate and 0.9% calcium chloride to remove lipoproteins and a 50% ammonium sulfate cut was prepared. After resuspension in PBS (44), the antiserum was dialyzed against Tris-buffered saline (TBS; 50 mM Tris–HCl (pH 7.6), 150 mM NaCl) overnight at 4°C. Alternatively, polyclonal antibodies were raised in rabbits by injection of homogenized gel slices containing aproximately 150 μg of fusion protein at three week intervals. These antisera were prepared by Cocalico Biochemicals, Inc.

A gene fusion in which most of the Srp54p coding sequence was inserted downstream from glutathione-S-transferase (46) was constructed by inserting a DNA fragment extending from the first *Ssp*I restriction site within the fission yeast *srp54* gene (derived by partial digestion) to the *Eco*RI site just downstream from the stop codon (20) into the same sites of the pGEX-3X vector (Pharmacia). The GST–Srp54 fusion protein was overexpressed in *E.coli* and antibodies were prepared and affinity purified as described previously (47).

Western blotting

Proteins were transferred electrophoretically from an SDS-polyacrylamide gel to an Immobilon-P membrane (Millipore) at 250 mA overnight at 4°C. The membrane was blocked in TS with 5% bovine serum albumin for 1 hr followed by three 5 min washes in TS. The ascites fluid was diluted 1:500 in TS containing 0.1% Tween-20 and 1% BSA (TSTB). The blot was incubated in this solution for 45−60 min at room temperature. After three washes in TS, the filter was incubated with a 1:7500 dilution of an alkaline phosphatase anti-mouse IgG conjugate in TSTB for 30 min at room temperature. Finally, the blot was washed three times in TSTB and the bands were visualized using the AP Western blot detection kit (Promega).

Immunoprecipitation of SRP RNA under native conditions

Immunoprecipitation and extraction of RNA from cell fractions enriched in SRP were performed as described previously (35). Briefly, an aliquot of a post-mitochondrial supernatant prepared from cells disrupted mechanically was precipitated with antibodies directed against the Srp54p−TrpE fusion protein. RNA was then extracted from these fractions and electrophoresed over a 6% polyacrylamide/8 M urea gel.

Generation and phenotypic analysis of SRP RNA mutants

A deletion of domain III was constructed starting with a plasmid in which sites for the restriction enzymes *Xho*I and *Dra*III had been introduced within the SRP RNA coding sequence by mutating positions 88, 133, and 135 as described previously (28). This DNA was cleaved with both enzymes and the over-hanging ends blunted with T4 DNA polymerase, followed by ligation (41). A double mutant containing both the G4C and A154U substitutions was generated by site-directed mutagenesis using a kit (Amersham) based on the method of Taylor *et al.* (48). A single-stranded template was prepared from the G4C mutant described previously (28) and mutagenized with the oligonucleotide A154U (5′GTTTCCAACCTCCATCGGTAC3′; the position mutated is underlined). The viability of strains harboring each of these mutant alleles was determined using the complementation assay described earlier (36).

DEAE-Sepharose retention assay for analysis of Srp54p binding to SRP RNAs

To make a construct for *in vitro* transcripton and translation of intact *S.pombe* Srp54 protein, the intron was first deleted from the cloned *srp54* gene carried on the plasmid pSP54-U (2) by site-specific mutagenesis (49) using the oligonucleotide 54-ΔI (5′CTTCAGTGAATGAAGAGCTTGTCGATACTCTGC3′). Restriction enzyme recognition sequences were introduced at the 5′ and 3′ termini of the coding sequence, respectively, using the oligonucleotides 54-Nde5′ (5′TTAATCTGACCATATGGTTTTTG3′; site underlined) and 54-Bam3′-1 (5′GAAGACGTTAGGATCCTTCCCTCGTTA3′; site underlined). The *Nde*I−*Bam*HI fragment containing the intronless gene was inserted into the plasmid pET11d (50) to create p54T7. A plasmid for *in vitro* transcripton and translation of the *S.pombe* Srp54p M domain was constructed by PCR amplification using the primers 54M-NcoI (5′CTCAAAGCCCATGGGACTCGGTG3′; site underlined) and 54-Bam3′-2 (5′TATGGGATCCGTGAGTACATTAT3′; site underlined), followed by subcloning of the resulting fragment into the *Nco*I site of pAR3039 (50) to create p54MT7. For *in vitro* transcription, p54T7 or p54MT7 DNA (1 μg) was cleaved with *Bam*HI and incubated with T7 RNA polymerase under standard conditions (51). Transcripts (1 μg) were translated in the presence of 300 μCi of [^{35}S]-methionine (Amersham) in a wheat germ extract (Promega) according to the manufacturer's instructions, in a 50 μl reaction volume.

A construct for T7 transcription of fission yeast SRP RNA was prepared by first amplifying the coding sequence using PCR primed with the oligonucleotides 7SPCR-5′ (5′AATGGCTTGGTCGAAGTGTT3′) and 7SPCR-3′ (5′CCGGATCCAACGACCGTTGTGTTTATAC3′). After filling in with Klenow fragment to complete partially synthesized strands and cleaving at the 3′ end with *Bam*HI (site underlined), the fragment was subcloned into the plasmid pDW19 (provided by N. Pace) to create p7ST7. To generate a transcript lacking domain III, we used the same PCR oligos to amplify DNA from the deletion mutant described above. After cleavage of each plasmid with *Bam*HI, T7 transcription was carried out as for the Srp54p DNAs; transcripts derived from this construct are identical to native fission yeast SRP RNA with the exception of 5 extra nucleotides at the 3′ end and two missing nucleotides and one mutation at the 5′ end. *E.coli* 4.5S RNA (a kind gift of M. J. Fournier) was purified from total RNA by gel electrophoresis.

T7-transcribed fission yeast SRP RNA and *E.coli* 4.5S RNA (1 μg) were tested for binding to *in vitro*-translated intact Srp54p or to the M domain by incubating with an aliquot (10 μl) of the relevant translation reaction in the presence of 1 mg/ml poly(U) for 20 min at 25°C. After adjusting the salt to 0.5 M potassium acetate, RNA−protein complexes were recovered by binding to DEAE-Sepharose as described earlier (11) and proteins were analyzed by SDS-PAGE and visualized by fluorography.

Immunoprecipitation of SRP RNA from cells labeled *in vivo* with [^{32}P]-orthophosphate

Liquid cultures (100 ml) of mutant haploid strains were grown to an OD_{600} of 3.0 at 30°C, pelleted by centrifugation, and shifted to non-permissive conditions in prewarmed media for 2 hr. The wild-type control culture was grown to an OD_{600} of 1.5. All cultures were labeled with 2 mCi of $H_3{}^{32}PO_4$ for 3 hr. An SRP-enriched fraction was prepared by a modification of procedures previously described (35, 52). Briefly, after harvesting by centrifugation, the cell pellets (0.5−0.9 g) were resuspended in 0.5 ml of lysis buffer (35) containing 0.05% Nikkol and disrupted by five one-minute pulses in a micro bead-beater (Biospec Products). The lysate was layered over a 0.5 ml cushion containing 0.5 M sucrose in 2.2 ml Beckman Ultraclear centrifuge tubes and spun at 12,000 rpm for 10 min in a Beckman TS-55 bench top ultracentrifuge rotor. The supernatant was layered over another cushion of identical composition and spun at 64,000 rpm for 1 hr in a TLA 100.2 rotor, and the pellet was resuspended in 0.5 ml of lysis buffer containing 500 mM potassium acetate, layered over a third 0.5 ml cushion to which potassium acetate was added (final concentration 500 mM), and spun at 64,000 rpm for 1 hr. Native immunoprecipitation of the [^{32}P]-labeled fractions was performed as described above for non-radioactive RNA. Quantitation of the gel was performed using a Molecular Dynamics PhosphorImager.

Testing SRP RNA mutants for suppression by multicopy *srp54*

Haploid strains in which the *srp7::ura4* gene disruption (24) was complemented by either the G4C, A154U, or TS-point mutant

SRP RNA gene (28, 35) on a multicopy plasmid were transformed with pSP54-A, which was derived by cloning a *BglII* fragment containing the *srp54* gene into the vector pAD3, which carries an *ade6* selectable marker (40). Seven transformants of each strain carrying markers for both plasmids and the chromosomal gene disruption were tested for growth defects as described earlier (36). Cells from the colonies that grew under normally non-permissive conditions were grown in the absence of selection for pSP54-A under permissive conditions and plated for single colonies. At least four colonies that had lost the *srp54* plasmid were then tested for reappearance of the growth defect characteristic of that strain. To determine generation times, each suppressed mutant, as well as a control wild-type strain was grown to early log phase under standard conditions (30°C, Edinburgh minimal media (EMM; 53) with the necessary supplements) and then shifted to the non-permissive temperature using pre-warmed media of the appropriate osmotic strength (see footnotes 3 and 4, Table 2). Growth was monitored by OD_{600} and the data plotted using CricketGraph software for the Macintosh.

Northern blot analysis of cell fractions from SRP RNA mutants

Cell fractionation of mutant strains was performed as described earlier (35), RNA was extracted from equal volumes of each fraction, and the resulting samples were electrophoresed over a 6% polyacrylamide/8M urea gel in a Mini-Protean II apparatus (BioRad). The gel was stained with ethidium bromide, photographed, soaked for 2×20 minutes in 50 mM sodium acetate and the RNA transferred at 0.8 mA for 45 minutes onto Hybond membrane (Amersham) in a Mini-Protean II electroblotting apparatus (BioRad). The blot was UV-crosslinked for 2 min and prehybridized in 5×SSC, 10×Denhardt's solution and 0.2% SDS for 2 hr at 37°C. Hybridization was performed at 40°C for 2 hr using the same solution, to which was added a [^{32}P]-labeled oligonucleotide probe specific for *S.pombe* SRP RNA (7S-Tx; 25). The blot was washed for 2×5 min and 1×15 min in 5×SSC, 0.5% SDS at room temperature. For quantitation, the blot was scanned using a Molecular Dynamics phosphorimager.

Immunoprecipitation of [^{35}S]-labeled Srp54p and SRP proteins

S.pombe strain TM011 (*h*$^-$, *leu1.32*, protease deficient; a kind gift of M. Yanagida) was labeled with $Na_2{}^{35}SO_4$ by a modification of the procedure described by Harlow and Lane (44). Cells were grown at 31°C to an OD_{600} of 1.2 in 500 ml of EMM minimal medium (53) containing leucine at 100 μg/ml, harvested by centrifugation at 5,000×g for 10 min at room temperature, and washed with 100 ml of PBS. The washed cells were resuspended in 25 ml of sulfate-free minimal medium (44) supplemented with leucine, transferred to 500 ml of the same medium, and incubated for 30 min at 31°C. The culture was then labeled with 10 mCi of $Na_2{}^{35}SO_4$ (ICN), which was diluted into 250 μl of medium before addition. After 3 hr, the efficiency of incorporation was about 60%.

The labeled cells were used to prepare a salt wash fraction enriched in fission yeast SRP by scaling down procedures described earlier (35, 52). Briefly, spheroplasts from a 500 ml culture were lysed in 10 ml of lysis buffer. The low-salt pellet from the post-mitochondrial supernatant was homogenized in 5 ml of lysis buffer adjusted to 0.5 M potassium acetate, layered over a sucrose cushion and re-centrifuged as described above. The fraction above the cushion was retained for immunoprecipitation. Scintillation counting showed that the salt wash fraction (4×10^3 cpm/μl) contained less than 5% of the radioactivity originally present in the post-mitochondrial supernatant ($>10^6$ cpm/μl).

For each immunoprecipitation, 300 μl of the labeled salt wash was diluted with 400 μl of IP buffer (10 mM Tris−HCl [pH 8.0], 150 mM NaCl, 0.5% Tween-20, 0.1 mM EDTA) and the samples were pre-cleared by adding 75 μl of a 1:1 suspension of Protein A-Sepharose (Pharmacia) and incubating at 4°C for 10 min before spinning for 10 sec in a microfuge. The supernatants were then transferred to a fresh tube and 30 μl of pre-immune, immune, or affinity-purified rabbit sera raised against the GST−Srp54p fusion protein was added. Samples were incubated at 4°C with rocking for several hours before adding 65 μl of a 1:1 suspension of Protein A-Sepharose beads and incubating for an additional hour with rocking at 4°C. Beads were collected by a 5 sec spin in the microfuge and then washed 5 times with 1 ml of NET-2 (35). To assay proteins present in the immunoprecipitate, the beads were resuspended after the final

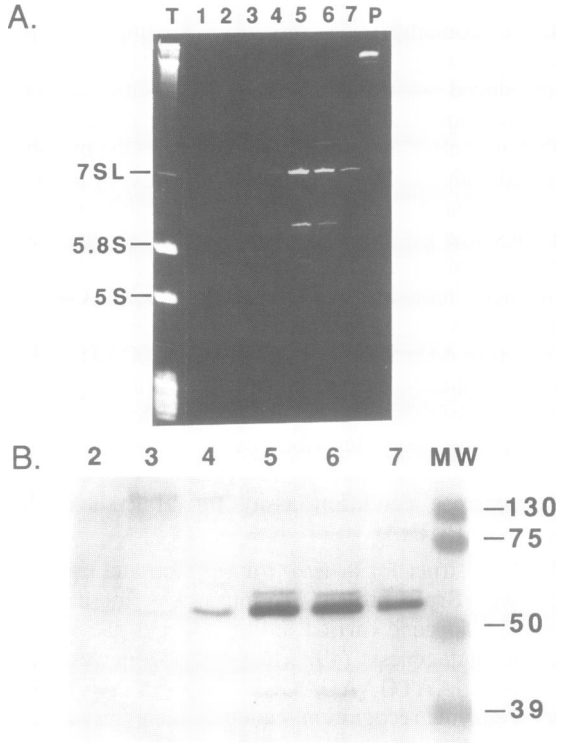

Figure 1. Analysis of the sedimentation of fission yeast 7SL RNA and Srp54p on a sucrose velocity gradient. A salt wash fraction enriched in fission yeast SRP was concentrated on DEAE-Sepharose and loaded on a 5 to 20% sucrose gradient as previously described (24). After centrifugation, the gradient was divided into ten fractions (numbered from the top) in addition to the pellet (P). Panel **A** shows an ethidium bromide-stained 6% polyacrylamide-7M urea gel to display the RNAs present in each fraction. Total fission yeast RNA (lane T) is run as a size marker and the position of 7SL RNA, as well as 5.8S and 5S rRNAs, are indicated. Panel **B** is a Western blot in which proteins from the gradient fractions, resolved by SDS-PAGE, were transferred to a nylon membrane and probed with polyclonal ascites fluid from mice immunized with the TrpE−Srp54p fusion protein. The primary antibodies were visualized with alkaline phosphatase-conjugated anti-mouse IgG. MW: molecular weight markers.

wash in 75 μl of SDS gel loading buffer. Samples were boiled for 2–3 min, centrifuged briefly, and 10 μl of the supernatant electrophoresed through either a 10 or 15% SDS-polyacrylamide gel.

RESULTS

S.pombe Srp54p and 7SL RNA co-purify and are complexed *in vivo*

In earlier work, we found that application of a classical biochemical fractionation procedure similar to the one employed for purification of signal recognition particle from canine pancreas (54) to a fission yeast extract produces a sample highly enriched in 7SL RNA and 9–11 polypeptides (26). Among the proteins that co-purify with SRP RNA is a species that migrates on SDS-PAGE at 57 kDa, the predicted size of the polypeptide encoded by the cloned *srp54* gene (20). To confirm this identification, we carried out velocity sedimentation of a fission yeast lysate followed by RNA and protein analysis of the resulting fractions. The extract was concentrated on DEAE-Sepharose and loaded onto a 5 to 20% sucrose gradient as described previously (24), which was centrifuged for a time sufficient to pellet ribosomes. RNA was extracted from a portion of each fraction that showed significant absorbance at 260 nm and analyzed by electrophoresis on a denaturing polyacrylamide gel (Figure 1A). Since these samples were prepared from a whole cell extract rather than an SRP-enriched fraction, several small RNA species other than 7SL RNA (most likely of nuclear origin) are also present. The same fractions were analyzed for the presence of Srp54p on a Western blot probed with antibodies directed against the TrpE–Srp54p fusion protein (Figure 1B). Both 7SL and Srp54p peak in fraction 5 of this gradient, with a shoulder of each trailing into fractions 6 and 7, suggesting that they are components of the same ribonucleoprotein particle.

To test directly whether *S.pombe* Srp54p is complexed with 7SL RNA, we performed immunoprecipitation under native conditions using antibodies directed against both fusion proteins generated in this study (see Materials and Methods). Figure 2, lane 1 shows an ethidium bromide-stained gel of RNA immunoprecipitated from an SRP-enriched fraction with mouse polyclonal antibodies directed against the TrpE–Srp54p fusion protein. A single RNA species identical in size to *S.pombe* 7SL RNA (24–26) is observed in this lane, but not in the sample immunoprecipitated with the negative control serum produced against an unrelated TrpE fusion protein (lane 2). Rabbit polyclonal antibodies directed against the GST–Srp54p fusion protein also precipitate a single discrete RNA species which, moreover, hybridizes on northern blots with the cloned gene encoding 7SL RNA (data not shown). We therefore conclude that Srp54p and 7SL RNA are components of the same stable RNP complex in *S.pombe* cells.

S.pombe Srp54p binds to *E.coli* 4.5S but not to its cognate RNA *in vitro*

Having shown that fission yeast Srp54p is complexed with 7SL RNA *in vivo*, we wanted to examine the requirements for binding using a direct biochemical assay. Since DEAE-Sepharose retention of T7 RNA polymerase transcripts had been used in other laboratories to examine protein binding to SRP RNA (11, 32, 38, 55), we employed a similar protocol for our experiments. As shown in Figure 3, when a T7 transcript of fission yeast SRP RNA is incubated with [^{35}S]-labeled Srp54 protein translated in a wheat germ extract, binding is virtually undetectable above background (compare lanes 3 and 4 with lanes 5 and 6). This result was not unanticipated, since the original footprinting experiments with SRP components purified from canine pancreas showed that, while the Srp9/14p and Srp68/72p heterodimers and the Srp19p monomer could associate independently with SRP RNA, Srp54p bound only in the presence of Srp19p (27, 29). DEAE-Sepharose retention assays with T7-transcribed human 7SL and *in vitro*-translated mammalian Srp54p (32, 38) also demonstrated that these components assemble only when Srp19p

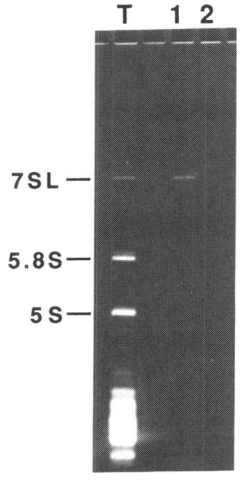

Figure 2. Immunoprecipitation of 7SL RNA with α-Srp54p. An SRP-enriched fraction prepared as described in Materials and Methods was immunoprecipitated under native conditions with either mouse polyclonal ascites fluid against the TrpE–Srp54p fusion protein (lane 1) or control ascites fluid against an unrelated TrpE fusion protein (lane 2). RNA extracted from the immunoprecipitates was electrophoresed through a 6% polyacrylamide/7 M urea gel and visualized by staining with ethidium bromide. Total *S.pombe* RNA was run as a marker (lane T).

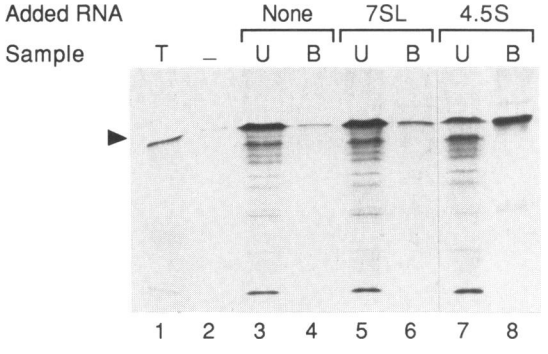

Figure 3. *In vitro* binding of Srp54p to SRP RNAs. A cloned version of the *srp54* gene lacking the intron was transcribed *in vitro* with T7 RNA polymerase and translated in a wheat germ extract in the presence of [^{35}S]-methionine (see Materials and Methods for details). An aliquot of the translation mixture was incubated for 20 min at 25°C with the indicated RNA in the presence of poly(U), followed by recovery of RNA–protein complexes using a DEAE-Sepharose retention assay (11). Proteins from each fraction were analyzed by SDS-PAGE and visualized by fluorography. The total (T) translation mixture (10% of the amount included in other samples) and a mock incubation (None) including all components except the added RNA were run as controls. 7SL = *S.pombe* SRP RNA; 4.5S = *E.coli* SRP RNA (a kind gift of M. Fournier); unbound (U) = DEAE-Sepharose flowthrough; bound (B) = protein retained on DEAE-Sepharose.

is present. In contrast, mammalian Srp54p is retained on DEAE-Sepharose under the same conditions by *E.coli* 4.5S RNA (32, 38), a homolog of SRP RNA consisting essentially of domain IV (see Figure 4). We therefore tested whether *S.pombe* Srp54p could likewise bind to 4.5S. Our data (Figure 3, lanes 7 and 8) demonstrate significant retention of the fission yeast protein on DEAE-Sepharose by the bacterial RNA, providing biochemical evidence that fission yeast Srp54p contacts the RNA in domain IV, as we had concluded earlier based on the results of assaying mutant cell lysates by native immunoprecipitation (35). Control experiments show that this interaction is specific, since no retention is observed in the sample containing poly(U) only (Figure 3, lanes 3 and 4), nor with *S.pombe* U6 snRNA or a 65 nucleotide polylinker transcript (data not shown). Because the M domain of mammalian Srp54p has been shown to contain the determinants for RNA binding (32, 38), we carried out the same series of assays with protein translated from a transcript of our construct containing only the M domain of fission yeast Srp54p. As expected, this domain binds to 4.5S RNA but not detectably to 7SL RNA (data not shown); retention was consistently less efficient than with the intact protein, perhaps indicating a role for the G domain in modulating the conformation of the M domain.

Domain III is required for SRP RNA function *in vivo*

Based on the results of *in vitro* assays on SRP RNAs lacking individual helices, it was concluded that, although Srp19p produces RNase footprints on both domains III and IV, its major binding determinant lies in the former (30). Because this domain is invariably absent from bacterial SRP RNAs (reviewed in 1), and no role other than facilitating Srp54p binding had been ascribed to the Srp19 protein, we decided to test whether an RNA in which this helix is deleted (see Figure 4) could function in *S.pombe*. The results of a complementation assay (Table 1, line 1) indicate that deletion of domain III is lethal, since this allele cannot support growth as the sole source of SRP RNA in the cell. One possible explanation for the inability of Srp54p to bind on its own to full-length SRP RNAs, whether of mammalian or fission yeast origin, is that the Srp19 protein stabilizes a productive conformation of SRP RNA; the notion that this RNA can adopt different structures is consistent with the observation that, under the low salt conditions employed to assay GTPase activity, mammalian Srp54p apparently interacts with its cognate RNA even in the absence of Srp19p (56). As an initial test of the hypothesis that SRP RNA folds in high salt into a conformation in which domain III masks the recognition site for

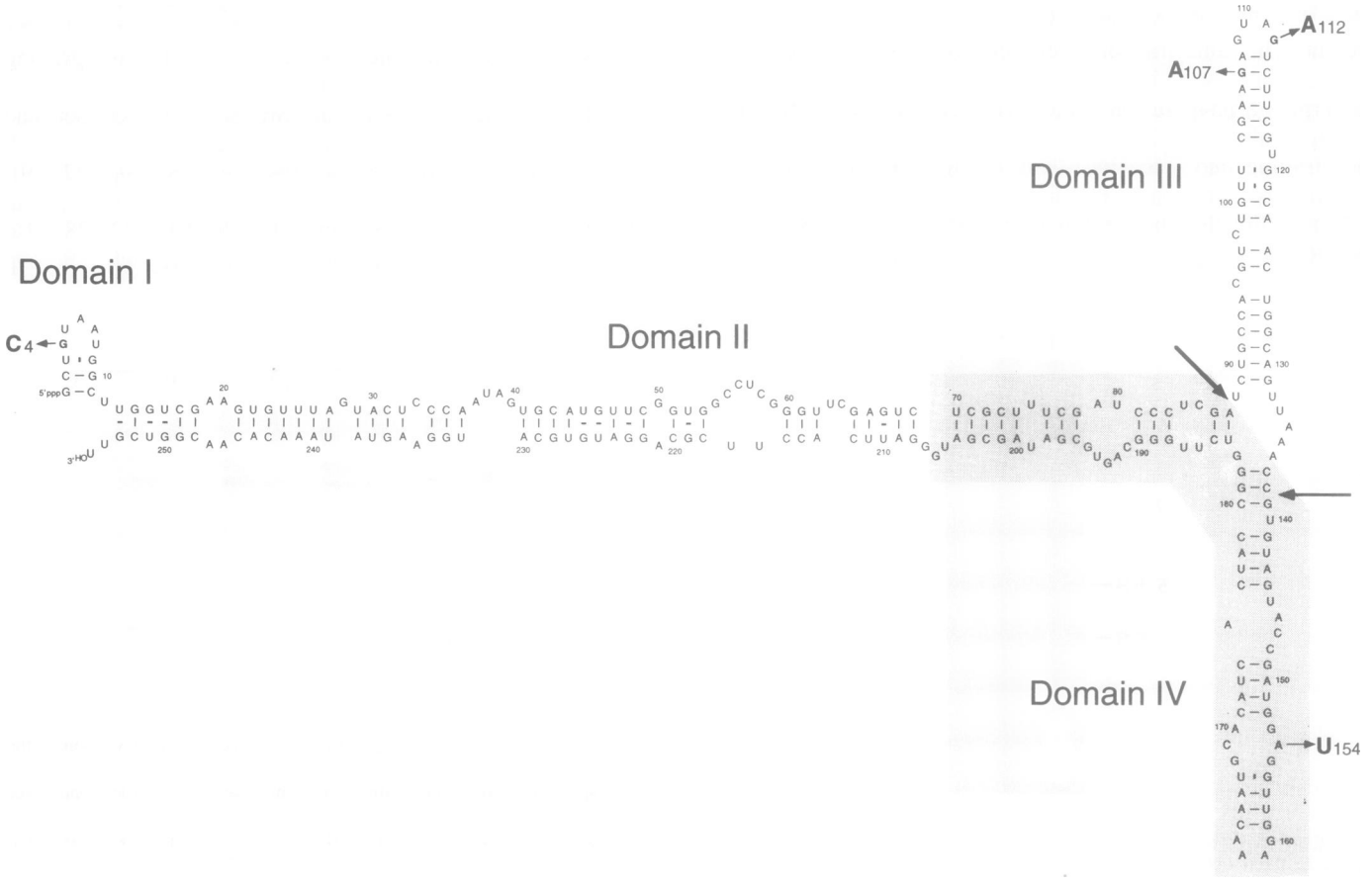

Figure 4. Secondary structure of *S.pombe* SRP RNA. The secondary structure shown is taken from Liao *et al.* (28) and the four domains are designated according to the nomenclature of Poritz *et al.* (34). Point mutations relevant to the present work are indicated by small arrows and the end-points of the domain III deletion are indicated by large arrows. The portion of the molecule corresponding to sequences present in *E.coli* 4.5S RNA is enclosed within a shaded box.

Srp54p in domain IV, we assayed a mutant transcript lacking this helix using the DEAE-Sepharose assay described in the preceding section. No retention of the protein was detected (data not shown), although it remains possible that the lack of binding is due to aberrant folding (see Discussion).

Mutations throughout SRP RNA reduce binding to Srp54p *in vivo*

To explore further the requirements for binding of Srp54p to SRP RNA, we turned to an *in vivo* binding assay that we had previously used to examine a variety of *srp7* domain IV mutants, including lethal, conditional and phenotypically silent alleles, for co-immunoprecipitation with α-Srp54p (35). One of the mutants most dramatically affected was A154U (see Figure 4), a likely base-specific contact for Srp54p not only in *S.pombe* but in *E.coli* (55). Since the earlier experiments were carried out on diploid cells grown under conditions that would permit growth of the A154U mutant even in the absence of a wild-type allele, we wanted to determine the effects of the mutation on binding to Srp54p in haploid cells after shifting to the non-permissive condition. An A154U strain, as well as two other mutants and an isogenic wild-type haploid, were each labeled with [^{32}P]-orthophosphate under the appropriate restrictive condition (see the legend to Figure 5) followed by cell fractionation and native immunoprecipitation with antibodies directed against the TrpE−Srp54p fusion protein. The immunoprecipitated RNAs, as well as RNA extracted from the supernatants, were resolved by gel electrophoresis. The results, shown in Figure 5, indicate that the A154U mutant is severely impaired in binding to Srp54p under conditions that do not permit growth: even after a 15-fold longer exposure of the gel (not shown), no 7SL band is visible in the immunoprecipitate, and quantitation indicates that ≤ 0.1% of the mutant RNA is present in this lane. These results are consistent with our earlier report (35) that, following a similar cell fractionation procedure, immunoprecipitation of the A154U mutant RNA by α-Srp54p was reduced to below the limits of detection by ethidium bromide staining even under permissive conditions.

The second RNA examined using this assay is TS-point, a mutant that is temperature-sensitive for growth as a consequence of two mutations (G107A,G112A) in and near the tetranucleotide loop capping the domain III hairpin (28; see Figure 4). Although these mutations lie in a region that is absent from *E.coli* 4.5S RNA, which binds avidly to *S.pombe* Srp54p, we anticipated that they might reduce association of the RNA with Srp54p based on the failure of the domain III deletion mutant to associate with this protein. As expected, TS-point RNA exhibits diminished association with Srp54p *in vivo* (Figure 5). However, although no band is visible in the lane containing immunoprecipitated RNA even after a 15-fold longer exposure of the gel (not shown), quantitation reveals that the binding defect is less severe than for A154U, since in this case, ca. 1.5% of the mutant RNA is brought down by α-Srp54p.

To further explore SRP RNA−Srp54p interactions *in vivo*, we used this assay to test whether binding is affected by the G4C mutation, which is is located in domain I (28), at the end opposite from the Srp54p binding site in the secondary structure of SRP RNA (see Figure 4). Unexpectedly, our results demonstrate that

Table 1. Phenotypic analysis of SRP RNA mutants. The diploid strain RM2a (24; genotype *srp7::ura4/srp7, leu1-32/leu1/32, ade6$^-$/ade6$^-$, ura4$^-$/ura4$^-$*) was transformed with a *LEU2* plasmid containing the indicated SRP RNA mutant allele and a sporulation-competent derivative identified. After sporulation, haploids were tested for growth on selective medium (36)

Allele	# of colonies analyzed[1]	# of Leu$^+$ colonies[2]	# of Leu$^+$, Ura$^+$ colonies[3]
ΔdomIII	94	94	0
A154U, G4C	70	70	0

[1]Spores were plated first on rich (YEA) medium (53) to allow growth of all viable haploids.
[2]Haploid cells were replicated to EMM minimal medium (53) supplemented with adenine + uracil, each at 100 mg/l. All cells harboring the plasmid (which carries a functional *LEU2* gene) will grow on these plates.
[3]Haploid cells were replicated to minimal medium supplemented with adenine. Cells must carry both the disrupted allele and the plasmid to grow.

Table 2. Suppression of SRP RNA mutants by overexpression of Srp54p

Allele	Phenotype *in vivo*	Predicted protein binding defect[1]	Suppression by multicopy *srp54*[2]
A154U[3]	Severe OTS[3]	Srp54p	Complete
TS-point[4]	TS[4]	Srp19p	Complete
G4C[4]	Severe OTS[4]	Srp9/14p	Partial

[1]A model for the region of SRP RNA bound by each fission yeast SRP protein is presented in Liao *et al.* (28).
[2]Haploid strains containing a disrupted copy of the SRP RNA gene complemented by a plasmid carrying a mutant copy of SRP RNA were transformed with a multicopy vector containing the *S.pombe srp54* gene, followed by testing for suppression of the growth defect conferred by the mutant allele (see Materials and Methods for details). The growth rate of each mutant strain was then determined as described in Materials and Methods.
[3]Described by Selinger *et al.* (35). Severe OTS: unable to grow on media containing 20% glycerol at 37°C.
[4]Described by Liao *et al.* (28). TS, unable to grow at 37°C on standard media.

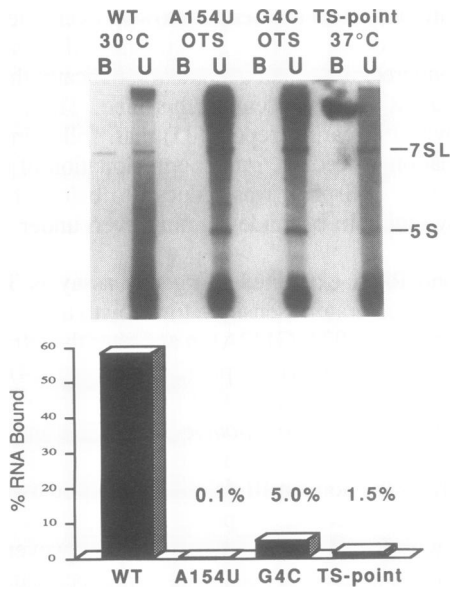

Figure 5. Immunoprecipitation of [^{32}P]-labeled SRP RNAs from conditional mutants with antibodies directed against the Srp54 protein. After growth under standard conditions, cells of the indicated haploids harboring conditional alleles of SRP RNA and an isogenic wild-type strain were shifted to non-permissive conditions and labeled with $H_3{}^{32}PO_4$ (see Materials and Methods for details). A154U and G4C are sensitive to the combination of high temperature and high osmotic strength (designated OTS), and TS-point is sensitive to high temperature (37°C) alone. After 2 hr, an SRP-enriched fraction was prepared from each strain and subjected to immunoprecipitation under native conditions as described in Materials and Methods. (Top) RNA extracted from the immunoprecipitate (B) or from the unbound material remaining in the supernatant after spinning out the protein A-Sepharose beads (U) was electrophoresed over a 6% polyacrylamide/8M urea gel and visualized by autoradiography. (Bottom) The amount of SRP RNA in each lane was determined by phosphorimager analysis and a bar graph generated to illustrate the percentage immunoprecipitated from each strain with α-Srp54p, calculated as [(7SL radioactivity in lane B) divided by (7SL radioactivity in lane U + 7SL radioactivity in lane B)] ×100.

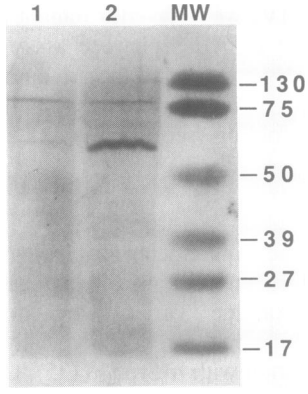

Figure 6. Western blot of fission yeast microsomes probed with α-Srp54p. Aliquots (20 μl) of rough microsomes (RM; lane 2) and salt-washed rough microsomes (KRM; lane 1), prepared from the same lysate and resuspended at equal concentrations based on A_{280} (52), were boiled in 100 μl of sample buffer and electrophoresed through a 10% polyacrylamide gel containing SDS. Spr54p was detected as described in the legend to Figure 1. MW: molecular weight markers.

the G4C allele exhibits an approximately ten-fold reduction in association with the protein relative to wild-type SRP RNA (Figure 5). This observation was surprising because the altered nucleotide in this allele lies in the binding site of the mammalian Srp9/14p heterodimer (29), for which no evidence existed to suggest an interaction with the Srp54p binding domain (see Discussion), in contrast to Srp19p. The conclusion that domains I and IV, or the proteins to which they are bound, can somehow communicate is reinforced by the finding that, while the G4C and A154U mutants are viable under normal growth conditions (28, 35), an allele containing both substitutions cannot complement the *srp7::ura4* gene disruption (Table 1, line 2); synergistic lethality between conditionally growth-defective mutants implies at least an indirect interaction between the affected components.

Mutations throughout SRP RNA can be phenotypically suppressed by overexpression of the Srp54 protein

In an earlier study, we showed that the growth defects of mutants with substitutions in the domain IV tetranucleotide loop and closing base pair of fission yeast SRP RNA are ameliorated by expression of Srp54p from a multicopy plasmid (35). Since the immunoprecipitation experiments presented in the last section demonstrate that all three alleles examined exhibit reduced binding to Srp54p under non-permissive conditions, we tested whether and to what extent overexpression of the protein could suppress their growth defects, with the results shown in Table 2. Haploid cells harboring either the A154U, TS-point, or G4C SRP RNA mutants were transformed with a multicopy plasmid expressing the *S.pombe srp54* gene under its own promoter, and transformants carrying the correct markers were tested for viability under conditions that normally do not permit growth. Upon re-streaking, all seven plasmid-bearing transformants tested for each mutant formed colonies under restrictive conditions. Moreover, when these cells were cured of the plasmid and re-tested for growth defects, they displayed the phenotype characteristic of the allele that they carried, demonstrating that the suppression is dependent on the presence of excess copies of the *srp54* gene. To gain further insight into the assembly of SRP *in vivo*, we determined growth rates in liquid media for the A154U, TS-point and G4C mutants transformed with the multicopy *srp54* plasmid (data not shown). The generation time of the A154U transformants at high temperature in media containing an osmotic perturbant is indistinguishable from that of an isogenic wild-type strain. The complete suppression of this allele was anticipated, since it affects a likely sequence-specific contact for Srp54p (35, 55). The presence of the plasmid also restores growth at high temperature for the TS-point mutant to wild-type levels, indicating that its defects are likewise fully compensated. The growth defect of the G4C mutant, on the other hand, is only partially overcome by overexpression of Srp54p, since its growth rate remains less than that of an isogenic wild type strain even in the presence of the *srp54* plasmid. Thus, as might be expected, suppression correlates in an inverse fashion with the level of Srp54p binding under non-permissive conditions to each mutant RNA.

Fission yeast Srp54p associates with microsomes in a salt-labile interaction

As a consequence of its role as an adaptor between the cytoplasmic protein synthetic machinery and the translocation apparatus of the endoplasmic reticulum, mammalian SRP is associated with membranes in a salt-labile interaction (1, 57). To determine whether this is also true of the fission yeast Srp54

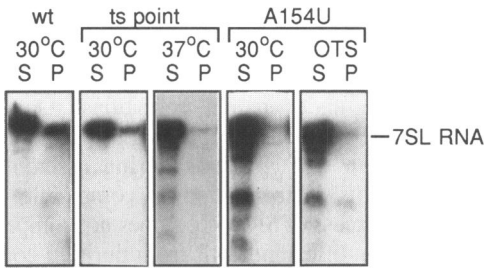

Figure 7. Northern blot analysis of SRP RNA in subcellular fractions of strains carrying SRP RNA mutant alleles. RNA was extracted from the supernatant (S) and pellet (P) fractions of a cytoplasmic fraction derived from the indicated strain after a high speed spin under low salt conditions (24). The RNA was electrophoresed through a 6% polyacrylamide/8 M urea gel, blotted and probed with an oligonucleotide specific for SRP RNA (see Materials and Methods for details).

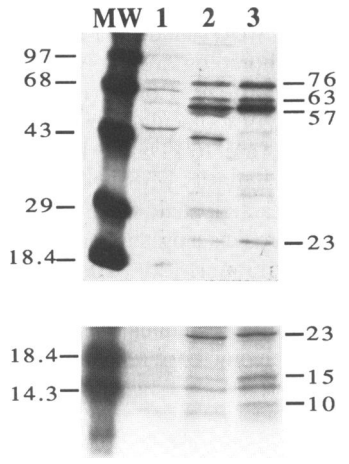

Figure 8. Immunoprecipitation of fission yeast SRP proteins with α-Srp54p. An SRP-enriched fraction was prepared from [^{35}S]-labeled cells as described in Materials and Methods. A. Native immunoprecipitation with sera from a rabbit injected with the GST−Srp54p fusion protein. The immunoprecipitated proteins, resolved by SDS-PAGE on a 10% (top) and a 15% (bottom) polyacrylamide gel to separate both the high and low molecular weight components, were visualized by autoradiography. MW: molecular weight markers; lane 1: pre-immune serum; lane 2: immune serum; lane 3: affinity-purified immune serum.

protein, we examined the distribution of the protein in membrane fractions prepared in either high or low salt buffers. Figure 6 shows a Western blot of fission yeast microsomal proteins probed with antibodies directed against the TrpE−Srp54p fusion protein. These microsomes are largely devoid of polysomes and were shown in other experiments to support the translocation of both homologous and heterologous proteins (52). Equal amounts of protein were loaded in each lane, and the blot was probed in antibody excess. In lane 2, which contains rough microsomes prepared in low salt, a prominent band migrating at the position of fission yeast Srp54p (57 kDa) is observed. This protein is specific to the Srp54p antigen, since it was not observed in a parallel blot probed with antibodies raised against an unrelated TrpE fusion protein (data not shown). In lane 1, which contains proteins prepared from microsomes depleted of loosely bound material by washing with high salt, there is no detectable signal in this size range, demonstrating that the interaction of Srp54 protein with the microsomal pellet is disrupted by 0.5 M potassium acetate. This behavior is similar to that of both fission yeast 7SL RNA (24) and mammalian SRP components (57).

Mutations in SRP RNA alter its subcellular distribution

In addition to fractionating with ER-derived membranes, SRP exhibits a low affinity association with ribosomes and a high affinity binding to polysomes translating proteins bearing ER-specific signal sequences (58). Since mammalian Srp54p can be cross-linked to signal sequences (32, 39) and is almost certainly the subunit that contacts the SRP receptor in the ER membrane (9, 56, 59), association of SRP with these organelles is likely to depend on the presence of this protein. We therefore performed the cell fractionation experiment shown in Figure 7 to determine whether SRP RNAs harboring the TS-point and A154U mutations are defective in binding to particulate components of the cytoplasm. When TS-point cells were fractionated and SRP RNA in the pellet vs. supernatant quantitated by northern blotting, we found that, after growth under permissive conditions, its fractionation was similar to the wild-type RNA (Figure 7). After growth of the mutant strain at high temperature, the amount of RNA in the pellet fraction was greatly reduced, indicating that growth parallels association of the particle with particulate material. The association of SRP RNA containing the A154U mutation with larger cytoplasmic structures was also reduced; however, a diminution was observed even when cells were propagated under permissive conditions (Figure 7). This observation suggests that mutating a likely base-specific contact for Srp54p (35, 55) so dramatically destabilizes the association of SRP RNA with the protein that the particle is unable to withstand the high salt (0.5 M) conditions required for cell fractionation.

Fission yeast Srp54p co-immunoprecipitates with five other proteins

The foregoing experiments were described with the implicit assumption that fission yeast SRP contains a homolog of each protein found in the mammalian particle. With the exception of Srp54p, however, this supposition is based on inference and analogy (see Introduction) rather than on direct evidence. Because the particle containing *S.pombe* 7SL RNA has a mobility on a sucrose velocity gradient nearly identical to its mammalian counterpart, approximately 11S (24), it certainly must contain components in addition to the RNA and Srp54p. To identify the remaining proteins that make up SRP in fission yeast, we performed immunoprecipitation under native conditions on [^{35}S]-labeled fission yeast lysates using antibodies directed against the GST−Srp54p fusion protein. We previously showed that 50−70% of the total fission yeast 7SL RNA is present in a high speed cytoplasmic supernatant prepared in high salt (23, 26). Thus, to improve the signal-to-noise ratio in these experiments, we exploited the salt-extractability of the *S.pombe* particle to obtain a fraction highly enriched in SRP and containing less than 5% of the [^{35}S] originally present in the post-mitochondrial supernatant (see Materials and Methods). The proteins present in samples immunoprecipitated with two antibody preparations of different purity, together with a control immunoprecipitate, are shown in Figure 8A. After discounting bands that are brought down by pre-immune serum (lane 1) and comparing the relative intensities of the signals in the samples

precipitated with immune serum (lane 2) and affinity-purified immune serum (lane 3), we conclude that five proteins in addition to the 57 kDa Srp54p homolog are likely to be components of this ribonucleoprotein particle. Comparison of their mobilities on SDS-PAGE to those of molecular weight standards indicates that their apparent sizes are 76 kDa, 63 kDa, 23 kDa, 15 kDa, and 10 kDa. The high intensity of Srp54p in lane 3 relative to the other proteins was anticipated, since the DNA sequence of the cloned *srp54* gene predicts a protein that, like other homologs, is unusually rich in methionine (20). The two smallest proteins that co-immunoprecipitate with α-Srp54p (10 kDa and 15 kDa), although the faintest of the six bands, were consistently present at a similar relative stoichiometry in repetitions of this experiment.

DISCUSSION

We have employed a combined genetic and biochemical approach to further characterize a previously identified ribonucleoprotein from fission yeast with striking similarities to mammalian signal recognition particle. The data presented in this manuscript extend the parallels to *in vitro* assembly properties, since fission yeast Srp54p and, more specifically, its M domain, bind to *E.coli* 4.5S RNA but not to *S.pombe* 7SL RNA under stringent high salt conditions. While RNA species homologous to the 7SL subunit of signal recognition particle have been isolated from bacteria, these molecules invariably lack a counterpart to domain III and are generally missing domain I as well, implying that the protein components associated with these structures may be absent or not directly associated with the RNA (discussed further in Ref. 34). Unexpectedly, a specific deletion of domain III of the fission yeast RNA to create a transcript with a predicted secondary structure analogous to SRP RNA from the bacterium *Bacillus subtilis* (60), which can functionally substitute for 4.5S RNA *in vivo* (61, 62), did not rescue binding. This observation implies that the differences between bacterial and eukaryotic SRP RNAs cannot be reconciled by considering secondary structure features alone; perhaps the elucidation of tertiary interactions in diverse SRP-like RNAs will reveal a common folding pattern. The inability of the domain III deletion to support growth as the sole copy of SRP RNA in *S.pombe* suggests further that this region, and/or the Srp19 protein to which it is bound, plays a critical role in eukaryotic SRP RNA. The synergy between mutations in domains I and IV reinforces the conclusion that the function of eukaryotic signal recognition particle encompasses aspects that are dispensable or relegated to other components in bacteria.

A second novel facet of our work is that it provides evidence for cooperativity in the *in vivo* assembly of SRP. Based on the observation that the *srp54* gene on a multicopy plasmid suppressed the growth defects conferred by mutations in one of the two regions of *S.pombe* SRP RNA protected from RNase digestion by canine Srp19p, we previously proposed that the assembly of SRP *in vivo* is ordered, with Srp19p binding to SRP RNA preceding and facilitating Srp54p binding (35). The model predicts that non-null mutations in the domain III tetraloop, the other region protected by Srp19p, will also be suppressed by overexpression of Srp54p, an expectation confirmed by the ability of a multicopy *srp54* plasmid to fully compensate for the defects conferred by the TS-point allele. We did not, however, anticipate the suppression of the G4C mutant, which lies in the Srp9/14p binding site (28), since not only was there no direct evidence that this protein influences Srp54p binding, but fluorescence studies had shown that its stable association with the RNA exhibits no detectable cooperativity with the Srp68/72p heterodimer (63). In addition to the observed suppression, the G4C mutation reduces association of the RNA with Srp54p as judged by the results of immunoprecipitation under native conditions. One possible explanation for the diminished binding is that it reflects cooperativity between SRP proteins during assembly of the particle *in vivo*. This interpretation is compatible with the observation that an excess of SRP RNA does not compete during *in vitro* reconstitution of canine SRP from purified components (5). *In vivo*, the assembly of eukaryotic ribonucleoprotein particles generally takes place in a different compartment from the one in which they function: ribosomes are formed in the nucleus, while spliceosomal snRNPs are assembled, at least in part, in the cytoplasm. It will thus be of interest to determine whether some or all of the SRP proteins are transported to the nucleus for assembly with the RNA. The results presented in this report, in combination with previously published data, would point to Srp19p as a good candidate for a nucleation factor to initiate assembly of eukaryotic SRP, and to Srp54p as the most likely component to lock the particle into a stable conformation. A second, not exclusive, explanation for the effect of domain I mutations on Srp54p association with SRP RNA is that it reflects functional aspects of the SRP cycle. Signal recognition particles lacking the Srp9/14p heterodimer, which binds to domain I, do not mediate translational pausing but are competent to promote translocation of presecretory proteins *in vitro* (3, 64), while particles reconstituted with alkylated Srp54p lack both functions (65). These observations, in combination with our data, may imply that signal sequence binding by the Srp54 protein triggers a conformational change in the particle that affects interactions between domains I and IV.

Perhaps the most significant result of the experiments presented here is the demonstration that six polypeptides, as well as 7SL RNA, are immunoprecipitated under native conditions with α-Srp54p. Assuming a single copy of each subunit, the predicted molecular weight of the fission yeast particle is 328 kDa, as compared to 335 kDa for mammalian SRP. These figures are in good agreement with both the relative and absolute mobilities of the two ribonucleoproteins on a sucrose velocity gradient (24). The observation that each putative subunit of fission yeast SRP is similar in size to a component of mammalian signal recognition particle provides the first evidence that that the overall subunit structure of SRP is conserved between two widely divergent eukaryotes. The *S.pombe* Srp19 protein candidate, at 23 kDa, shows the greatest molecular weight discrepancy. However, an even larger homolog of this protein, 31.5 kDa, is encoded by the recently identified *S.cerevisiae SEC65* gene (16). Although proof of equivalence will require cloning and sequence analysis of the genes encoding the five remaining polypeptides, the most straightforward interpretation of the co-immunoprecipitation experiment is that these species represent subunits of *S.pombe* SRP. In combination with earlier biochemical and genetic data evidence that the two ribonucleoprotein particles are organized similarly (24, 28), the conservation of both the number and approximate sizes of SRP proteins between mammals and fission yeast suggests a one-to-one functional correspondence.

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