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7SL RNA from *Schizosaccharomyces pombe* is encoded by a single copy essential gene

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We have identified an abundant ribonucleoprotein particle from *Schizosaccharomyces pombe* with properties related to those of the vertebrate signal recognition particle (SRP), including cytoplasmic localization, association with microsomes and ribosomes at low, but not high, salt concentrations and high resistance to micrococcal nuclease. The 256-nucleotide RNA component carries a 5'-triphosphate group and shows close secondary structure, and limited primary sequence homology to vertebrate 7SL RNA. 7SL-like RNAs were also detected in a number of other fungi. The single copy gene (*SRP7*) encoding *S.pombe* 7SL was disrupted by insertion of a transposon carrying the selective marker *LEU2*, and the disrupted gene was used to replace one chromosomal *SRP7* gene in a diploid strain. Haploid *srp7*$^-$ strains fail to germinate.

Key words: ribonucleoprotein/*Schizosaccharomyces pombe*/signal recognition particle/*SRP7*/*LEU2*

Introduction

Signal recognition particle (SRP) is an abundant cytoplasmic ribonucleoprotein particle containing six proteins, together with an RNA component, 7SL RNA, which is essential for function *in vitro* (Walter and Blobel, 1980, 1982; Gundelfinger *et al.*, 1983).

In *in vitro* systems, SRP is required for the recognition of signal sequences in proteins destined for translocation into the lumen, or insertion into the membrane of the endoplasmic reticulum (ER). In heterologous systems using mammalian SRP and wheat germ ribosomes, SRP arrests the translation of the nascent polypeptide when the signal sequence emerges from the ribosome (Walter *et al.*, 1981; Meyer *et al.*, 1982); this arrest is released by interaction of the SRP complex with an ER membrane protein designated docking protein (Meyer *et al.*, 1982) or SRP receptor protein (Gilmore *et al.*, 1982a, b). Translation then resumes accompanied by translocation or membrane insertion. However, the phenomenon of translational arrest has not yet been detected in homologous systems prepared from mammalian cells (Meyer, 1985) or wheat germ (Prehn *et al.*, 1987).

To determine whether SRP plays the same role *in vivo*, genetic techniques are of great importance. To this end we sought SRP in the fission yeast *Schizosaccharomyces pombe*, for which techniques exist allowing the direct replacement of chromosomal genes by copies manipulated *in vitro*.

We have identified a particle from *S.pombe* with features characteristic of SRP and cloned the RNA component. This shows close secondary structure homology to vertebrate 7SL RNA and is essential for viability, opening the way for a detailed genetic analysis of the role of 7SL RNA in SRP function *in vivo*.

Results

Identification of SRP

S.pombe contains numerous RNA species of approximately 7S, including RNase P RNAs (Krupp *et al.*, 1986), snRNAs (Tollervey, 1987) and a 7S pre-rRNA (D.Tollervey, unpublished). To distinguish 7SL from these, an enrichment scheme for SRP was devised, based on the properties of vertebrate SRP. A cytoplasmic fraction was obtained from a lysate of spheroplasts of *S.pombe* by centrifugation at low speed to discard nuclei and cell debris. The supernatant was centrifuged at high speed to pellet microsomes and ribosomes which were then resuspended in low-salt buffer and again pelleted. This pellet was resuspended in high-salt buffer to release SRP and again centrifuged. In the supernatant an RNA species of ~260 nucleotides is greatly enriched, and is the most abundant RNA present (Figure 1, lane 4). The high-salt supernatant was further fractionated on a sucrose gradient; in fractions of approximately 10S, only one RNA species is detectable. The sedimentation velocity of this RNA is reduced by phenol extraction prior to centrifugation (data not shown) and it is therefore likely to be in a ribonucleoprotein particle. To distinguish between association of *S. pombe* SRP with microsomes and ribosomes, these were also prepared separately (data not shown). Recovery of SRP from the microsomal and ribosomal fractions was approximately equal. Relative staining by ethidium bromide indicates that the copy number *S.pombe* 7SL is ~20- to 30-fold less than that of rRNA, corresponding to around 10 000 copies/cell. Following fractionation of a lysate of *Aspergillus nidulans* in a manner identical to that shown for *S.pombe* in Figure 1, a very prominent 7S-sized RNA is visible.

Cloned *S.pombe SRP7* (see below) was transformed into *Saccharomyces cerevisiae* on the replicating plasmid pDB262 (Wright *et al.*, 1986). The gene is expressed in *S.cerevisiae*, although the steady-state level of the RNA is lower than in *S.pombe*. The transcripts are slightly longer than in *S.pombe* and heterogeneous, with three major species being produced (Figure 2). It seems possible that *S.cerevisiae* correctly recognizes the A and B promoter elements and the termination site (see below), but cannot correctly identify signals in the 5' flanking region, which are found for other 7SL RNA genes (Ullu and Weiner, 1985). In cell fractionation experiments the RNA synthesized in *S.cerevisiae* behaves identically to free cytoplasmic RNA (e.g. tRNA) and is therefore unlikely to be packaged into a SRP-like particle (Figure 2). Moreover, comparison of sucrose gradient mobilities of native and phenol-extracted lysates indicates that

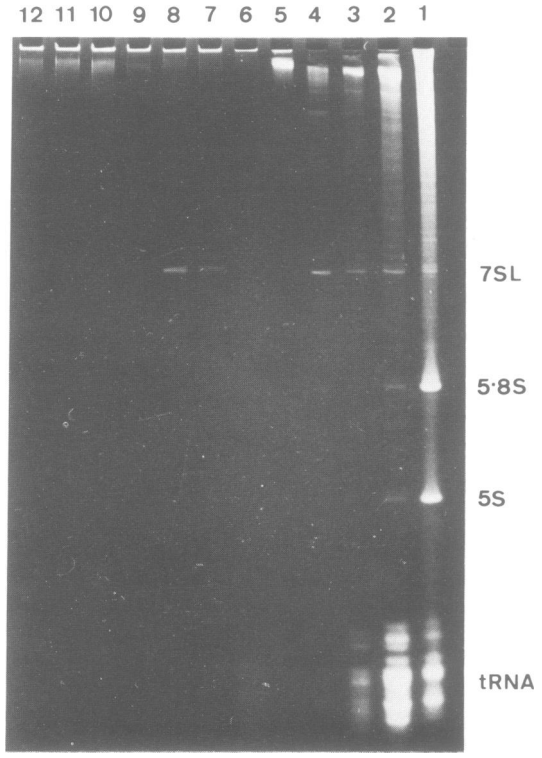

Fig. 1. Enrichment of 7SL RNA from *S.pombe*. SRP was enriched as described in the text. At each stage RNA was prepared from an equivalent fraction of the preparation and separated on a polyacrylamide gel stained with ethidium bromide. **Lane 1**, total RNA; **lane 2**, first high-speed supernatant (10 mM KAc); **lane 3**, second high-speed supernatant (10 mm KAc); **lane 4**, third high-speed supernatant (500 mM KAc); **lane 5**, sucrose gradient fraction 2; **lane 6**, fraction 4; **lane 7**, fraction 6; **lane 8**, fraction 8; **lane 9**, fraction 10; **lane 10**, fraction 12; **lane 11**, fraction 14; **lane 12**, fraction 16. The positions of 7SL, 5.8S, 5S and tRNA are indicated.

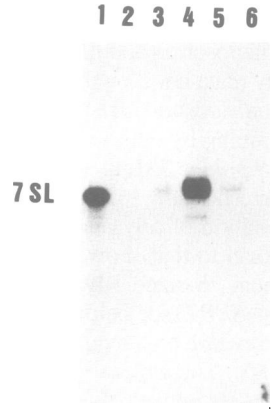

Fig. 2. Expression of *S.pombe SRP7* in *S.cerevisiae*. A strain of *S.cerevisiae* carrying the *SRP7* gene on a replicating plasmid was lysed and fractionated exactly as shown for *S.pombe* in Figure 1. RNA prepared from each fraction was separated on a polyacrylamide gel. Northern hybridization with *SRP7* was used to determine the distribution of 7SL RNA. **Lane 1**, total RNA from *S.pombe*; **lane 2**, total RNA from an untransformed strain of *S.cerevisiae*; **lane 3**, nuclear pellet from transformed strain; **lane 4**, first high-speed supernatant (10 mM KAc); **lane 5**, second high-speed supernatant (10 mM KAc); **lane 6**, third high-speed supernatant (500 mM KAc). Lanes 3–6 contain the same proportion of the preparation and ~4-fold more cell-equivalents than the total RNA lanes. The position of *S.pombe* 7SL RNA is indicated.

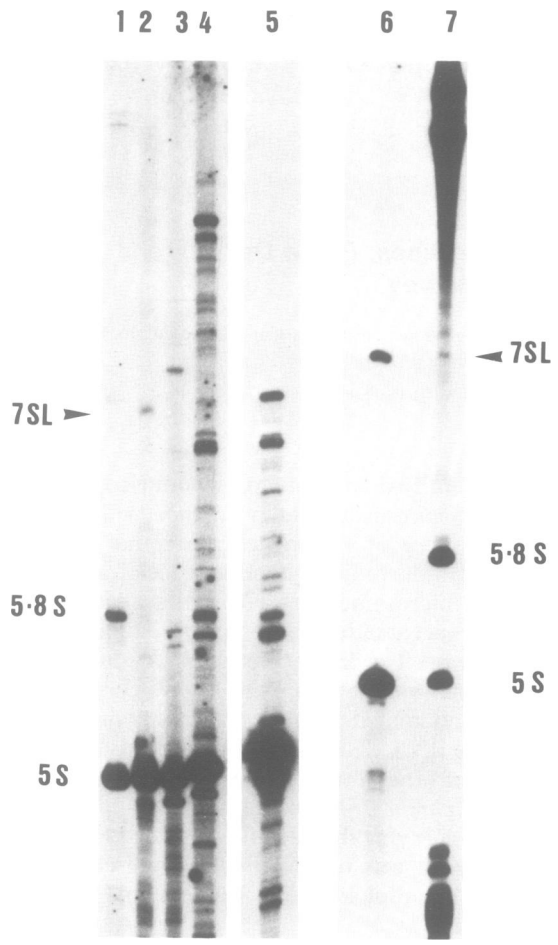

Fig. 3. Specific labelling of RNA species carrying 5′-triphosphate groups. **Lane 1**, total RNA from *S.pombe* 3′ end-labelled; **lane 2**, labelling of RNA with 5′-triphosphate groups from *S.pombe*; **lane 3**, labelling of RNA with 5′-triphosphate groups fron *N.crassa*; **lane 4**, labelling of RNA with 5′-triphosphate groups from *S.diastaticus*; **lane 5**, labelling of RNA with 5′-triphosphate groups from *Y.lipolytica*; **lane 6**, labelling of RNA with 5′-triphosphate groups from *A.nidulans*; **lane 7**, total RNA from *A.nidulans* labelled *in vivo*. RNAs are separated on 6% acrylamide gels containing 7 M urea. The positions of *S.pombe* 5S, 5.8S and 7SL are indicated.

this RNA is not present as a ribonucleoprotein particle (data not shown).

RNA species with 5′-triphosphate termini were identified by specific labelling with vaccinia guanylyltransferase. Two major RNA species are labelled in total RNA from *S.pombe* (Figure 3, lane 2); one of these is 5S rRNA and the other co-migrates with the 7SL-like RNA described above. An additional RNA species is detected, which is ~400 nucleotides in length and 10-fold less highly labelled than 7SL RNA. In some preparations, a band similar in size to 5.8S rRNA is also seen. In total RNA from *A.nidulans* (Figure 3, lane 6) or *Neurospora crassa* (Figure 3, lane 3), 7S-sized RNAs are also very prominently labelled. The RNA labelled from *A.nidulans* co-migrates with the RNA enriched by cell fractionation as described above. From the budding yeasts, *S.cerevisiae*, *S.diastaticus* (Figure 3, lane 4) or *Yarrovia lipolytica* (Figure 3, lane 5), much more complex patterns of labelling are observed. Several bands appear to be common between these species; however, two-dimensional gel analysis (data not shown) reveals that none of the prominent

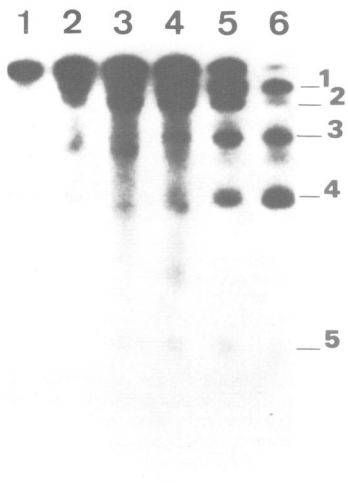

Fig. 4. Micrococcal nuclease digestion of SRP from *S.pombe*. SRP was digested for 30 min at 37°C in buffer containing 400 mM KAc. **Lane 1**, total RNA from *S.pombe*; **lane 2**, without added nuclease; **lane 3**, 1000 U/ml nuclease; **lane 4**, 3000 U/ml nuclease; **lane 5**, 10 000 U/ml nuclease; **lane 6**, 30 000 U/ml nuclease. Lanes 1–6 are probed with the entire *SRP7* gene, the intensity of hybridization of smaller fragments therefore underrepresents their relative abundances. Fragments 1–5 are indicated; estimated sizes are: 1, 215 nucleotides; 2, 190 nucleotides; 3, 155 nucleotides; 4, 115 nucleotides; 5, 65 nucleotides. A *Hin*dIII–*Sau*3A probe, specific for the 5′ 77 nucleotides of the RNA, hybridizes to fragment 2 and, more weakly, to fragments 1 and 3; a *Hpa*II–*Mst*II probe, specific for the 3′ 75 nucleotides of the RNA hybridizes to fragments 1 and 5.

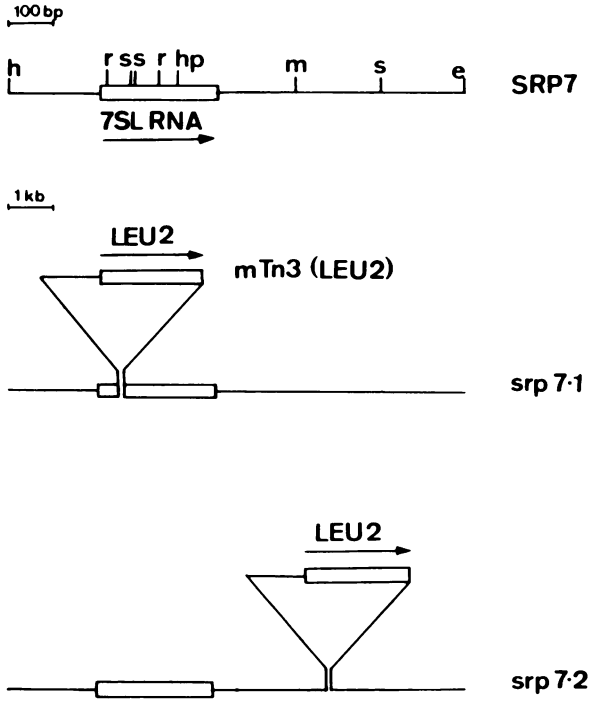

Fig. 5. Insertions made in *SRP7*. *SRP7*: region containing *SRP7* which was subcloned into pHSS6, the target plasmid for transposon mutagenesis (Seifert *et al.*, 1986). *srp7.1* and *srp7.2*: insertion sites of m-Tn*3* (*LEU2*) in the respective alleles. Restriction sites are: h, *Hin*dIII; r, *Rsa*I; s, *Sau*3A; hp, *Hpa*II; m, *Mst*II; e, *Eco*RI.

7S-sized species from *S.cerevisiae* correspond in mobility to RNAs 3′ end-labelled by RNA ligase, and they therefore probably lack the 3′-hydroxyl group characteristic of all stable RNAs. It is possible that these species are derived from killer, a group of dsRNA viruses with 5′-triphosphate groups. From *Y.lipolytica* (Figure 3, lane 5) an additional 7S-sized RNA is prominently labelled. From *S.cerevisiae* or *S.diastaticus* a substantially larger RNA species is prominently labelled; the possible relationship of this RNA to 7SL is currently being investigated (V.Ribes and D.Tollervey, unpublished).

To analyse further its structure, SRP from *S.pombe* was subjected to micrococcal nuclease digestion. The 7SL RNA is very resistant to digestion within the particle. At high levels of enzyme a set of specific fragments is generated (Figure 4). Northern hybridization with the complete gene, or probes specific for the 5′ or 3′ regions of the gene, indicate that the highly resistant fragments 3 and 4 are derived from the central region of the molecule, homologous to the equally resistant S region of vertebrate 7SL (Gundelfinger *et al.*, 1983).

Cloning of SRP7

RNA prepared from sucrose gradient fractions was separated on a polyacrylamide gel and the 7S RNA was eluted. cDNA probe was used to screen a *Hin*dIII genomic bank of *S.pombe* DNA in pDB262 (Wright *et al.*, 1986). Positive clones contain a 3.6-kb *Hin*dIII fragment; that used for further study is designated pCOL2. The approximate location of the gene (Figure 5) was determined by restriction mapping and Southern hybridization. The sequence of this region was determined by dideoxynucleotide sequencing (Sanger *et al.*, 1977) of fragments subcloned in M13 (Messing *et al.*, 1981). The end points of the coding region were identified by comparison with the 5′ and 3′ end sequences of the RNA (Figure 6). From this we calculate the RNA to be of 256 nucleotides.

SRP7 shows features typical of polymerase III transcribed genes of the tRNA type (Hofstetter *et al.*, 1981; Sharp *et al.*, 1981), including termination in a polypyrimidine tract and sequences with high homology to the A and B promoter elements, at positions +8 to +16 and +59 to +70 respectively (Figure 6). The position of the A block relative to the 5′ end of the RNA and the spacing between the blocks is similar to that found for tRNAs. 'TATA' sequences occur in the 5′ flanking sequence at positions −8 to −11 and −26 to −31; however, this entire region is very A+T-rich and the significance of this is unclear. No homology to the 5′-flanking promoter element of human 7SL RNA genes is detected (Ullu and Weiner, 1985).

The primary sequence and predicted secondary structure of *S.pombe* 7SL RNA were compared with those of mammalian 7SL (Figures 7 and 8). The sequence of the *S.pombe* RNA is 44 nucleotides shorter than human 7SL and thus a gap must appear in the alignment. Comparison of the predicted secondary structures strongly indicates that the 5′ structural domain of metazoan 7SL has been precisely deleted in *S.pombe*. The predicted secondary structure of the remaining region of *S.pombe* 7SL is very similar to the structure of human 7SL (Zwieb, 1985) and regions of primary and secondary structural homology are closely aligned (Figure 7). The structures of both major loops and the central rod are well conserved. The region of highest primary sequence homology corresponds to loop 200 of human 7SL and loop 2 of *S.pombe* 7SL and the structure of this stem loop is also highly conserved.

The clearest difference between the structures of the central

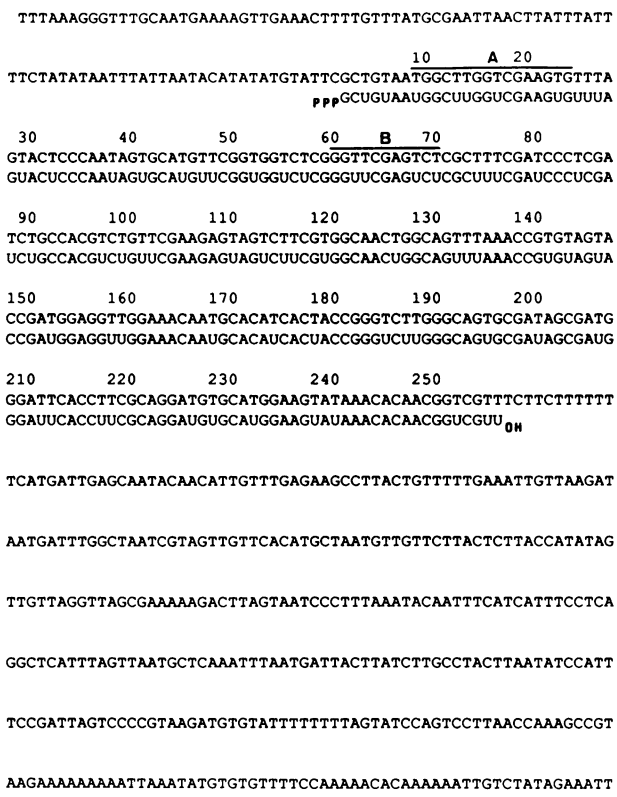

Fig. 6. DNA and RNA sequence of *SRP7*. The upper line shows the sequence of the non-coding strand of DNA, beginning 93 nucleotides before the 5′ end of 7SL and extending 377 nucleotides beyond the 3′ end. The lower line shows the sequence of the RNA together with its 5′-triphosphate group. Overlined sequences show homology to class I polymerase III promoter elements A and B (Hofstetter *et al.*, 1981; Sharp *et al.*, 1981). The overlined A block corresponds to the extended region of homology proposed by Sharp *et al.* (1981).

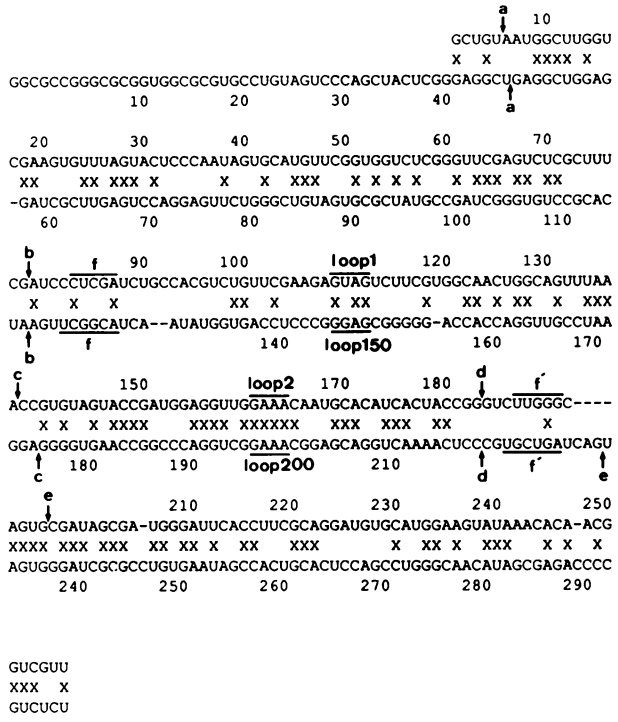

Fig. 7. Primary sequence alignment of 7SL RNA from *S.pombe* and human. The sequences of 7SL RNA from *S.pombe* (upper line) and human (lower line) are aligned and matched bases are indicated. Features of the secondary structure are indicated on the primary structure alignment: a, 5′ end of 5′ side of central rod; b, 3′ end of 5′ side of central rod; c, 5′ end of loop 2/loop 200 stem; d, 3′ end of loop 2/loop 200 stem; e, 5′ end of 3′ side of central rod; f/f′, regions base paired in alternative conformation with extended central rod (see text and Figure 8B); loops, single-stranded loop regions. These sites are also shown in Figure 8A and B.

'S' regions is the absence of a stem at the base of the loop 1 stem in *S.pombe* which is present in the stem of loop 150 of human 7SL. However, Zwieb (1985) reports that in 7SL RNAs from humans, *Xenopus* and *Drosophila*, this region can be drawn in an alternative configuration, supported by compensatory base changes, in which the central rod is extended by an additional stem between nucleotides 120−125 and 221−226. A similar structure can be constructed for this region of *S.pombe* 7SL (Figures 7 and 8B). More complex alternative structures in dynamic equilibrium are proposed for human 7SL by Zwieb and Ullu (1986). In these the paired regions at the bases of the loop 150 and loop 200 stems are destabilized by base pairing with other regions of the molecule. Similar alternative structures can be drawn for *S.pombe* 7SL in which nucleotides 89−93 at the base of the loop 1 stem are paired with nucleotides 190−194, 3′ to the loop 2 stem, and nucleotides 172−182 at the base of the loop 2 stem are paired with nucleotides 197−207 in the central rod (data not shown). In the structure shown in Figure 8, nucleotides 146−175 are drawn to resemble the open structure of nucleotides 181−215 of human 7SL reported by Zwieb (1985). For both organisms additional base pairing could be drawn in this region.

The structure of the central rod is similar between *S.pombe* and metazoans, and the position and composition of several bulged loops and nucleotides are conserved. The clearest difference is the reduction of the loop around positions 66/280 to a single bulged G in *S.pombe*. This loop is a major site of micrococcal nuclease cleavage and its replacement by a G residue, at which the nuclease cannot cut, must alter the cleavage pattern (Figure 4). Other alterations may be due to differences in the precise nucleotide sequences of the bulged loops, since the nuclease exhibits some sequence specificity.

Disruption of SRP7

To determine the number of copies of *SRP7* in the genome, the 357-bp *Sau*3A−*Mst*II fragment was purified and hybridized to genomic DNA cut with a number of restriction enzymes. In each case a single band is visible, and the intensity agrees well with that of the plasmid diluted to the abundance of a single copy genomic fragment (Figure 9).

To disrupt the *SRP7* gene, the transposon m-Tn*3* (*LEU2*) (Seifert *et al.*, 1986) was allowed to insert itself into the coding sequence in *Escherichia coli* (Figure 5) and these constructs were used as linear fragments to replace one copy of the *SRP7* gene in an h^+/h^+ diploid strain of *S.pombe*. The disrupted allele used is designated *srp7.1* and carries the transposon ~150 nucleotides from the 5′ end of the gene (Figure 5). As a control another allele, *srp7.2*, in which the transposon is inserted outside the coding region, was also tested (Figure 5). Diploid transformants carrying the *srp7.1* construction correctly inserted at the *SRP7* locus were ident-

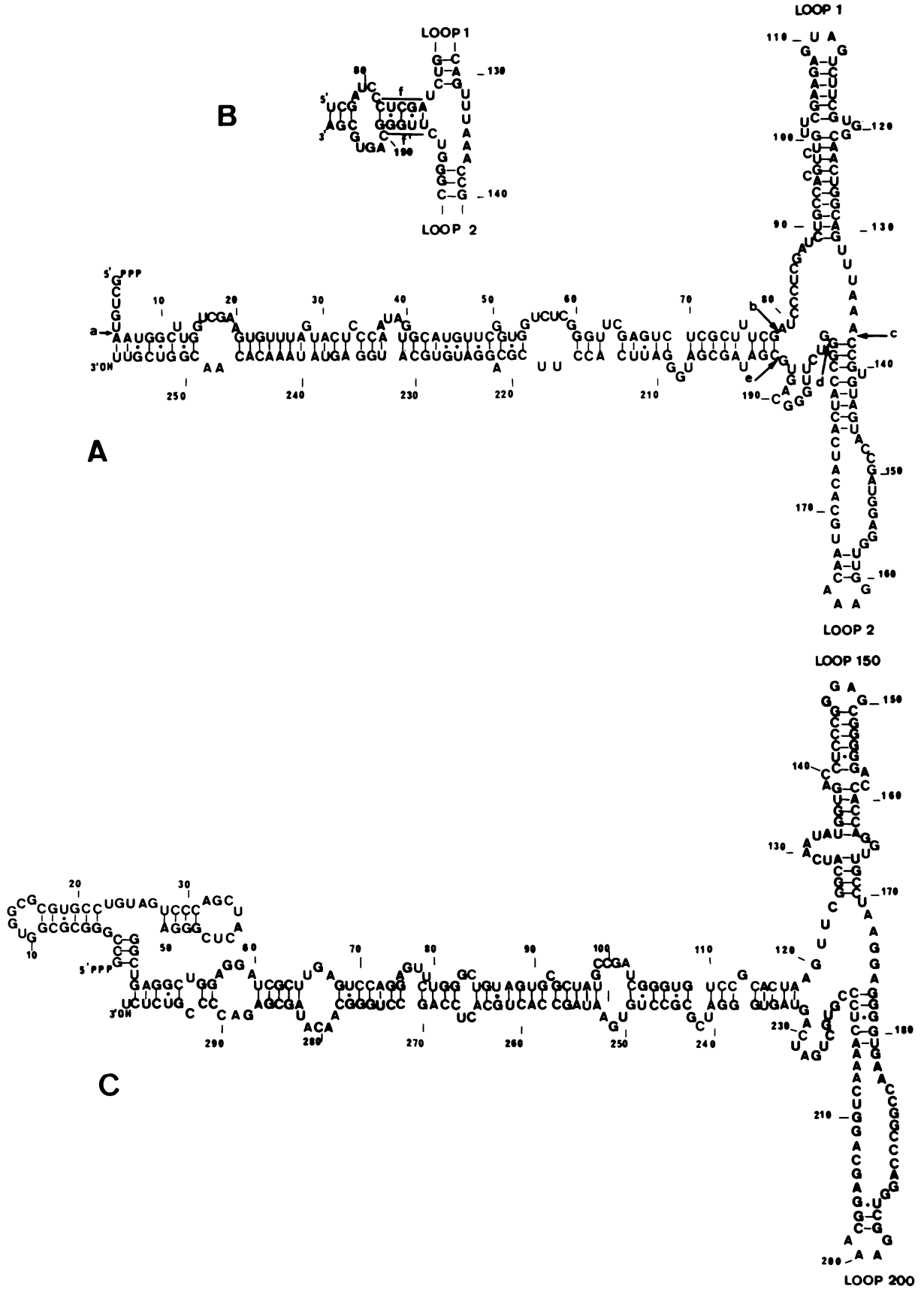

Fig. 8. Predicted secondary structure of 7SL RNA from *S.pombe*. **Panel A** shows the structure predicted by the computer program of Zuker and Steigler (1981), except that the central region of the loop 2 stem has been opened and nucleotides 158−160 and 165−167 have been base paired. In addition, the predicted structure shows the central rod extended by an additional stem, as shown in **panel B. Panel C** shows the structure of human 7SL RNA redrawn from Zwieb (1985). Sites a−f shown in the primary alignment (Figure 7) are also marked in panels A and B.

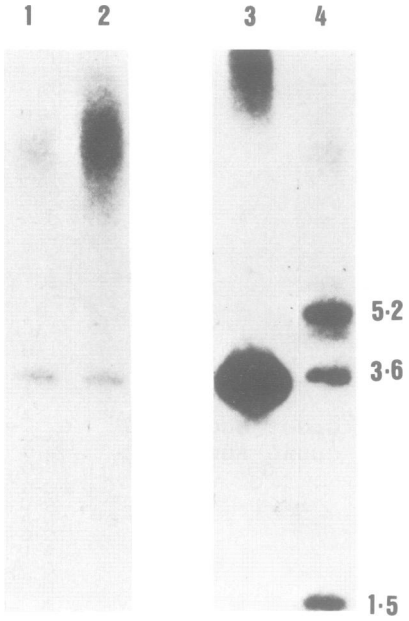

Fig. 9. Southern hybridization with cloned *SRP7*. **Lane 1**, genomic DNA from untransformed strain; **lanes 2** and **3**, plasmid pCOL2 (SRP7), lane 2 is a dilution to an abundance equivalent to that of a single copy genomic fragment; **lane 4**, genomic DNA from a transformed diploid strain. Each DNA is cut with *Hin*dIII and the probe is a *Sau*3A−*Mst*II fragment carrying *SRP7* (see Figure 5). The 3.6-kb band is the intact copy of *SRP7*, 5.2- and 1.5-kb bands are expected in the transformed strain due to cutting within the inserted transposon.

ified by Southern hybridization (Figure 9). Diploid *SRP7/srp7.1* strains are unimpaired in growth, although the level of 7SL RNA is detectably reduced compared with *SRP7/SRP7* strains (data not shown). Rare h^+/h^{90} spontaneous mutants, which can undergo sporulation, were identified and both tetrads and random spores from these were analysed. Amongst 450 random spores from the *SRP7/srp7.1* diploid, none were found to carry the *LEU2* marker inserted in *srp7.1*. Each of 10 tetrads analysed yielded only two viable spores and a further four tetrads yielded one viable spore, none of which carry the inserted $LEU2^+$ marker. In such tetrads the *srp7.1* spores are visibly smaller than *SRP7* sister spores. Spores carrying *srp7.1* fail to germinate.

Amongst the random spores analysed from the control *SRP7/srp7.2* diploid, 50% were found to carry the $LEU2^+$ marker associated with *srp7.2*. The growth of *srp7.2* haploid strains is identical to that of *SRP7* strains.

Discussion

The particle we have identified from *S.pombe* shows numerous similarities to vertebrate SRP; it is an abundant, cytoplasmic, ribonucleoprotein particle, highly resistant to micrococcal nuclease digestion, which is associated with ribosomes and microsomes at low, but not high salt concentrations. In addition, the RNA component is the only appropriately sized RNA which we have detected as possessing the 5′-triphosphate group characteristic of 7SL RNA and other polymerase III primary transcripts.

The alignment between the primary and secondary structures of *S.pombe* 7SL RNA and that of vertebrates is complicated because the *S.pombe* RNA, at 256 nucleotides, is ~44 nucleotides shorter than vertebrate 7SL. Comparison of the predicted secondary structures strongly indicates that this is due to the precise deletion of the small 'tRNA-like' domain at the 5′ end of vertebrate 7SL. The remainder of the molecule can be folded into a structure very similar to that described by Zwieb (1985) for vertebrate 7SL. Moreover, the *S.pombe* RNA can also be folded into structures closely resembling alternative conformations of human 7SL (Zwieb, 1985; Zwieb and Ullu, 1986) some of which are proposed to be in dynamic equilibrium, providing evolutionary support for both the existence and functional importance of dynamic conformational changes in the structure of 7SL RNA. Consistent with the similarities in secondary structure, micrococcal nuclease digestion of SRP from either *S.pombe* or dog (Gundelfinger *et al.*, 1983) produces a distinct set of highly resistant fragments; the most resistant fragments being derived from the central 'S' region of mammlian 7SL and from the equivalent region of *S.pombe* 7SL.

Vertebrate 7SL consists of two major functional domains. One contains the central 'S' region of 7SL RNA and the 19K, 54K, 68K and 72K proteins, and is required for protein translocation *in vitro* (Siegel and Walter, 1986). The other contains the 5′ and 3′ 'Alu-like' regions of 7SL and the 9K and 14K proteins and is implicated in the arrest of translation of nascent polypeptides, possibly via a direct interaction between the 'tRNA-like' 5′ region of 7SL and the ribosome (Zwieb, 1985). However, translational arrest is not strictly required for translocation *in vitro* (Siegel and Walter, 1985) and has not been detected in some *in vitro* systems (Meyer, 1985; Prehn *et al.*, 1987). The apparent absence of the 5′ region of 7SL RNA in *S.pombe* suggests that translational arrest may not be significant in this organism.

Almost all experiments on protein translocation in eukaryotes have utilized *in vitro* systems. However, the demonstration that SRP is required for this process *in vitro* cannot be taken as proof that this is also the case in the more optimal conditions *in vivo*. The disruption of the gene *SRP7* encoding *S.pombe* 7SL RNA is therefore an important test for the essentiality of SRP *in vivo*. 7SL is required for viability in *S.pombe* since haploid $srp7^-$ spores are unable to germinate. This is not of course a demonstration that $srp7^-$ cells are actually deficient in protein translocation or secretion; proof of this must await the generation of conditional mutants. However, the reduced size of $srp7^-$ spores is at least consistent with this since spore wall synthesis might be expected to impose an increased requirement for protein export and SRP, which cannot be met in the mutants.

Materials and methods

Strains and media

Growth and handling of *S.pombe* was described by Gutz *et al.* (1974). The diploid strain used for gene disruption carries h^+, *leu1.32*/h^+, *leu1.32*; *ura4-Δ18/ura4-Δ18*; *ade6.704/ade6.704* (P.Nurse, personal communication).

RNA extraction

RNA was prepared by the hot phenol/guanidinium method (Maniatis *et al.*, 1982) as modified by Tollervey and Mattaj (1987).

Isolation of SRP and 7SL RNA

S.pombe was grown in YEPD liquid medium to OD_{600} 1.0, harvested, washed and spheroplasted at 30°C in buffer containing 1 M sorbitol, 10 mM DTT and 20 mM $NaPO_4$, pH 6.5, with 0.5 mg/g zymolyase 60 000 and 1 mg/g novozyme 234. Spheroplasts were harvested by centrifugation and

lysed in a Dounce homogenizer in buffer A (10 mM KAc, 20 mM Tris Ac, pH 7.5, 5 mM MgAc, 1 mM DTT, 5 mM VRC, 0.2 mM PMSF, 0.01% Triton X-100).

The lysate was cleared of nuclei and cell debris by centrifugation in an Eppendorf centrifuge. The supernatant was centrifuged for 1 h at 35 000 r.p.m. in a 60 Ti rotor to pellet microsomes and ribosomes. The pellet was resuspended in buffer A and centrifuged as before. The pellet was resuspended in buffer B (500 mM KAc, 50 mM Tris Ac, pH 7.5, 10 mM MgAc, 1 mM DTT, 0.2 mM PMSF, 0.01% Triton X-100) to release SRP and centrifuged as before. Supernatant (1 ml) was loaded onto a 5–30% sucrose gradient in buffer B and spun for 16 h at 30 000 r.p.m. in an SW41 rotor. Samples of 400 μl were taken, 400 μl of phenol and EDTA to 20 mM were added and tubes were shaken at 65°C for 10 min. Chloroform (400 μl) was added and the aqueous phase was recovered by centrifugation. Following extractions with phenol/chloroform and chloroform/isoamylalcohol, RNA was recovered by ethanol precipitation and separated on 8% polyacrylamide gels containing 8.3 M urea.

For separation of microsomes and ribosomes the technique of Prehn *et al.* (1987) was slightly modified. Spheroplasts were lysed in a Dounce homogenizer in buffer containing 0.25 M sucrose, 40 mM KAc, 50 mM Tris Ac, pH 7.5, 2 mM MgAc, 5 mM DTT and 10 μg/ml PMSF. Cell debris was pelleted by centrifugation at 3000 r.p.m. Microsomes were then pelleted by centrifugation at 20 000 r.p.m. for 40 min in a SS34 rotor. SRP was released from the microsomal pellet by resuspension in the above buffer containing 500 mM KAc (final concentration) and the stripped microsomes were pelleted as before. Ribosomes in the first microsomal supernatant were pelleted by centrifugation at 40 000 r.p.m. for 2.5 h in a 60 Ti rotor. The pellet was then washed and SRP eluted as described above.

Micrococcal nuclease digestion

The high-salt supernatant containing SRP was diluted to give a final concentration of 400 mM KAc and 1 mM $CaCl_2$, and the quantities of micrococcal nuclease indicated were added. The reaction was incubated for 30 min at 37°C and terminated by addition of EDTA to 15 mM and phenol extraction at 65°C.

RNA labelling and sequencing

Radiolabelled cDNA probe for colony and Southern hybridization was prepared by annealing gel-purified 7SL RNA to 6-nucleotide random primers (Pharmacia) at 100 ng/μl and extending these with reverse transcriptase (Life Science) in the presence of [α-^{32}P]dCTP. For labelling of RNA species carrying 5′-triphosphate groups, total RNA prepared as above was further treated with proteinase K as described by Maniatis *et al.* (1982) and labelled with vaccinia guanylyltransferase (BRL) in the presence of [α-^{32}P]GTP. RNA sequences were determined enzymatically using gel-purified RNA labelled at the 5′ or 3′ end.

Genomic DNA preparation

DNA was prepared as described by Holm *et al.* (1986).

Transposon mutagenesis and transformation

The method of transposon mutagenesis was used exactly as described for *S.cerevisiae* by Seifert *et al.* (1986). The transposon inserted, m-Tn*3* (*LEU2*) carries the *S.cerevisiae* selective marker *LEU2* which complements *leu1*$^-$ of *S.pombe* (Beach and Nurse, 1981). *S.pombe* was transformed using the protocol described for *S.cerevisiae* by Ito *et al.* (1983).

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