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

Byron C. Hann, Mark A. Poritz, and Peter Walter

Department of Biochemistry and Biophysics, University of California Medical School, San Francisco, California 94143-0448

Abstract. We have isolated and sequenced genes from *Saccharomyces cerevisiae* (*SRP54*^{sc}) and *Schizosaccharomyces pombe* (*SRP54*^{sp}) encoding proteins homologous to both the 54-kD protein subunit (SRP54^{mam}) of the mammalian signal recognition particle (SRP) and the product of a gene of unknown function in *Escherichia coli*, ffh (Römisch, K., J. Webb, J. Herz, S. Prehn, R. Frank, M. Vingron, and B. Dobberstein. 1989. *Nature (Lond.).* 340:478–482; Bernstein H. D., M. A. Poritz, K. Strub, P. J. Hoben, S. Brenner, P. Walter. 1989. *Nature (Lond.).* 340:482–486). To accomplish this we took advantage of short stretches of conserved sequence between ffh and SRP54^{mam} and used the polymerase chain reaction (PCR) to amplify fragments of the homologous yeast genes. The DNA sequences predict proteins for SRP54^{sc} and SRP54^{sp} that are 47% and 52% identical to SRP54^{mam}, respectively. Like SRP54^{mam} and ffh, both predicted yeast proteins contain a GTP binding consensus sequence in their NH_2-terminal half (G-domain), and methionine-rich sequences in their COOH-terminal half (M-domain). In contrast to SRP54^{mam} and ffh the yeast proteins contain additional Met-rich sequences inserted at the COOH-terminal portion of the M-domain. *SRP54*^{sp} contains a 480-nucleotide intron located 78 nucleotides from the 5′ end of the open reading frame. Although the function of the yeast homologues is unknown, gene disruption experiments in *S. cerevisiae* show that the gene is essential for growth. The identification of *SRP54*^{sc} and *SRP54*^{sp} provides the first evidence for SRP related proteins in yeast.

THE signal recognition particle (SRP)[1] was originally defined by in vitro assays as a soluble factor present in mammalian cells that is required for the targeting of nascent secretory proteins to the endoplasmic reticulum (Walter and Blobel, 1980). SRP, purified on the basis of this assay, is a small ribonucleoprotein containing six polypeptides and one RNA (7SL RNA) (Walter and Blobel, 1982). It functions as an adapter between secretory protein translation and secretory protein translocation across the membrane. Although protein translocation can occur after termination of protein synthesis in certain systems, SRP-promoted translocation is obligatorily cotranslational (Garcia and Walter, 1988).

Several groups have reconstituted translation and translocation of yeast proteins in vitro using cell extracts from the yeast *S. cerevisiae* (Hansen et al., 1986; Rothblatt and Meyer, 1986; Waters and Blobel, 1986). As yet there is no evidence for a component of this system that has the physical or mechanistic properties indicative of SRP, nor have attempts to reconstitute the yeast system with canine SRP proven fruitful. The strongest indication that an SRP-related machinery exists in yeast comes from the identification of RNAs in two species of yeast, *Yarrowia lipolytica* and *S. pombe*, which share strong secondary structure homology with higher eukaryotic 7SL RNA (Brennwald et al., 1988; Poritz et al., 1988; Ribes et al., 1988). However, no convincingly homologous RNA has been described in *S. cerevisiae*. While the gene for the *S. pombe* RNA (*SRP7*) is essential (Brennwald et al., 1988; Ribes et al., 1988), its function remains unknown.

Photochemical cross-linking experiments have shown that the 54-kD protein subunit of SRP (SRP54) binds to the signal sequence of nascent secretory proteins during their synthesis (Krieg et al., 1986; Kurzchalia et al., 1986). A cDNA clone for this protein has recently been isolated (Bernstein et al., 1989; Römisch et al., 1989). Its predicted amino acid sequence contains a putative GTP-binding site in the NH_2-terminal half (G-domain) and an unusually methionine-rich COOH-terminal half (M-domain). The G-domain shares homology with the α-subunit of the SRP receptor (SRα, a known GTP binding protein [Connolly and Gilmore, 1989]) as well as with two previously uncharacterized *E. coli* pro-

1. *Abbreviations used in this paper*: SRP, signal recognition particle; PCR, polymerase chain reaction.

teins: the gene products of the ffh and ftsY genes (Byström et al., 1983; Gill et al., 1986). Ffh is highly similar over its entire length to SRP54^{mam} while ftsY is more similar to SRα.

The nature of these similarities has led to a model in which GTP hydrolysis is involved in regulating sequential steps of the targeting pathway (Bernstein et al., 1989; Römisch et al., 1989). GTP could be used to give unidirectionality to certain steps in signal recognition or targeting and/or to improve the fidelity of the reaction. We have proposed that the M-domain is involved in signal sequence binding (Bernstein et al., 1989). This hypothesis is based on the conserved abundance of Met residues in mouse SRP54 and ffh and the fact that many of the methionine residues are found on one face of predicted α-helices. According to this hypothesis, the flexible methionine side chains form or contribute to a hydrophobic pocket and provide the necessary plasticity to accommodate different signal sequences. Thus, specific binding of signal sequences could occur despite their lack of primary sequence conservation.

One of the recent applications of the polymerase chain reaction (PCR) is to use degenerate oligonucleotides coding for conserved regions of proteins to identify homologous genes in organisms in which the gene has not previously been described (Kamb et al., 1989). We have used this technique to isolate SRP54 homologues in *S. pombe* and *S. cerevisiae*. These genes represent a second entry point, in addition to the 7SL RNA in *S. pombe* and *Y. lipolytica*, into the molecular genetics of SRP. Furthermore the yeast protein sequences allow a phylogenetic analysis of the structure of the G- and M-domains.

Materials and Methods

Identification of SRP54^{sc} and SRP54^{sp}

PCR was performed using either genomic DNA or cDNA as previously described (Kamb et al., 1989; Saiki et al., 1988). Reactions (20 μl) containing 10 ng of yeast genomic DNA isolated according to Davis et al. (1980) were primed with degenerate synthetic oligonucleotides (synthesized by the Biomolecular Resource Center, University of California, San Francisco) (20mers, 10 μM) (see Fig. 1) and 0.5 U Taq polymerase (Cetus Corp., Emeryville, CA). Canine cDNA prepared from polyA$^+$ RNA as described by Frohman et al. (1988) was used in the reaction and was a gift of Harris Bernstein (University of California, San Francisco, CA). After 40 cycles of denaturation, (94°C, 1 min), annealing (45°C, 1 min), and extension (55°C, 3 min), (thermocycler, Perkin-Elmer Corp., Norwalk, CT, and Cetus Corp.), the reaction products were separated by electrophoresis on 6% polyacrylamide gels and visualized by ethidium bromide staining. Yeast PCR products that comigrated with the PCR product from the canine cDNA were eluted from the gel and sequenced directly as follows. Single stranded templates were generated by an additional forty cycles of PCR (conditions as above), using half of the eluted DNA and in the presence of only one of the primers used during the initial amplification. Reactions were extracted with phenol, precipitated twice with ethanol in the presence of 2 M NH_4OAc to remove unincorporated dNTPs and one third of the sample was used for sequencing. The opposite primer was end-labeled with [^{32}P]γ-ATP and used for sequencing with Sequenase (United States Biochemical Corp.) according to manufacturer's instructions but omitting the "labeling" step (J. LaBaer, personal communication).

To isolate genomic clones of the *S. cerevisiae* and *S. pombe* SRP54 homologues the respective PCR fragments were radiolabeled by primer extension (Maniatis et al., 1982) and used to screen genomic libraries. The SRP54^{sp} gene was isolated from a plasmid library of *S. pombe* strain sp972h- (provided by M. Yanagida, Kyoto University, Japan). Based on restriction mapping and Southern analysis a 3-kb Sac I fragment was subcloned into the Sac I site of pBluescript II SK+ (Stratagene, La Jolla, CA) to generate the plasmid pSP54-01. The SRP54^{sc} gene was isolated from a plasmid library of *S. cerevisiae* strain S288C (Carlson and Botstein, 1982). A 2.3-kb Spe I–Hind III partial digest fragment was subcloned into the equivalent sites in pBluescript II SK$^+$ generating the plasmid pSC54-01.

Both genes were sequenced by the dideoxy method (Sanger et al., 1977) using Sequenase. Internal oligonucleotide primers were used as necessary to facilitate sequencing. To confirm the putative splice site in SRP54^{sp}, a PCR reaction was performed using *S. pombe* cDNA prepared from total RNA as described above and the oligonucleotides 5′-ACTCTGCGTTAGGGGAC-3′ (sense, bases 31–47) and 5′-TGTTTCCAAAAGTGCCGTAC-3′ (antisense, bases 606–587) as primers. The major amplified band of 95 nucleotides was sequenced directly as described above.

Disruption of SRP54^{sc}

A 4.8-kb Hind III–Eco RI fragment containing the LYS2 gene from pBR328 (Barnes and Thorner, 1986) was inserted between the Hind III and Eco RI sites of the SRP54^{sc} coding sequence in pSC54-01, thereby deleting 258 bp of coding sequence from SRP54^{sc} (see Fig. 4A). The resulting plasmid (pSC54-L2) was cut with Xba I and Cla I to generate an 8-kb fragment containing SRP54^{sc}::LYS2 with 0.6-kb 5′ and 1.4-kb 3′ of *S. cerevisiae* DNA flanking the LYS2 gene. This fragment was introduced into a lys2$^-$ diploid *S. cerevisiae* strain TR1 (a/α, trp1/trp1, lys2/lys2, his3/his3, ura3/ura3, ade2/ade2; obtained from E. Schuster and C. Guthrie, University of California, San Francisco [Parker et al., 1988]) by one-step gene replacement (Orr-Weaver et al., 1981) using the LiOAc transformation method (Ito et al., 1983). After selecting for growth on Lys$^-$ plates surviving colonies were sporulated. Tetrad dissection and genetic analysis were performed by standard procedures (Sherman et al., 1974).

Southern analysis was performed as follows. DNA was prepared from the parent and transformant (Davis et al., 1980) and digested with either Ase I or Nsi I. The resulting fragments were separated by electrophoresis in 0.8% agarose then transfered to Gene Screen (New England Nuclear, Boston, MA). A ^{32}P-labeled probe was prepared covering a region of the *SRP54^{sc}* from bases 584–1,288, roughly corresponding to the M-domain. Hybridization was performed as described (Church and Gilbert, 1984) but at moderate stringency (42°C in 30% formamide, 7% SDS, 200 mM $NaPO_4$, pH 7.5, 300 mM NaCl, 1 mM EDTA).

Results

To isolate yeast genes encoding homologues of SRP54^{mam}, we took advantage of regions within the G-domains that are highly conserved between SRP54^{mam} and ffh. (We refer to the mammalian proteins as SRP54^{mam} since canine and mouse SRP54 sequences differ in only 3 out of 504 amino acids.) Specifically, we chose two short, closely spaced sequence stretches, the first including part of the first consensus sequence (box I) characteristic of GTP binding proteins (Dever et al., 1987); the second, a highly conserved motif between consensus boxes I and II. Degenerate oligonucleotides encoding these amino acids sequences ("A," Fig. 1 *A*) or their antisense ("B," Fig. 1 *A*) were synthesized and used in PCR to amplify DNA sequences using canine cDNA or *S. pombe* genomic DNA as template. The data presented in Fig. 1 *B* (lane *2*) show that amplification of *S. pombe* DNA–generated multiple products. However, a major band comigrated with the amplification product of the canine cDNA template (Fig. 1 *B*, lanes *1* and *2*, *arrow*). These products were in the expected size range of 104–107 nucleotides, predicted on the basis of the conserved spacing between the two primers in SRP54^{mam} and ffh. The analogous PCR reaction using genomic DNA from *S. cerevisiae*, however, resulted in a complex banding pattern in the relevant size range (data not shown). Many of these products were likely to be unrelated to the desired product since they were also generated if only oligonucleotide "B" was present in the reactions.

The 104 nucleotide PCR product of *S. pombe* genomic DNA was eluted from the gel and sequenced directly (see Materials and Methods). We found an open reading frame

A

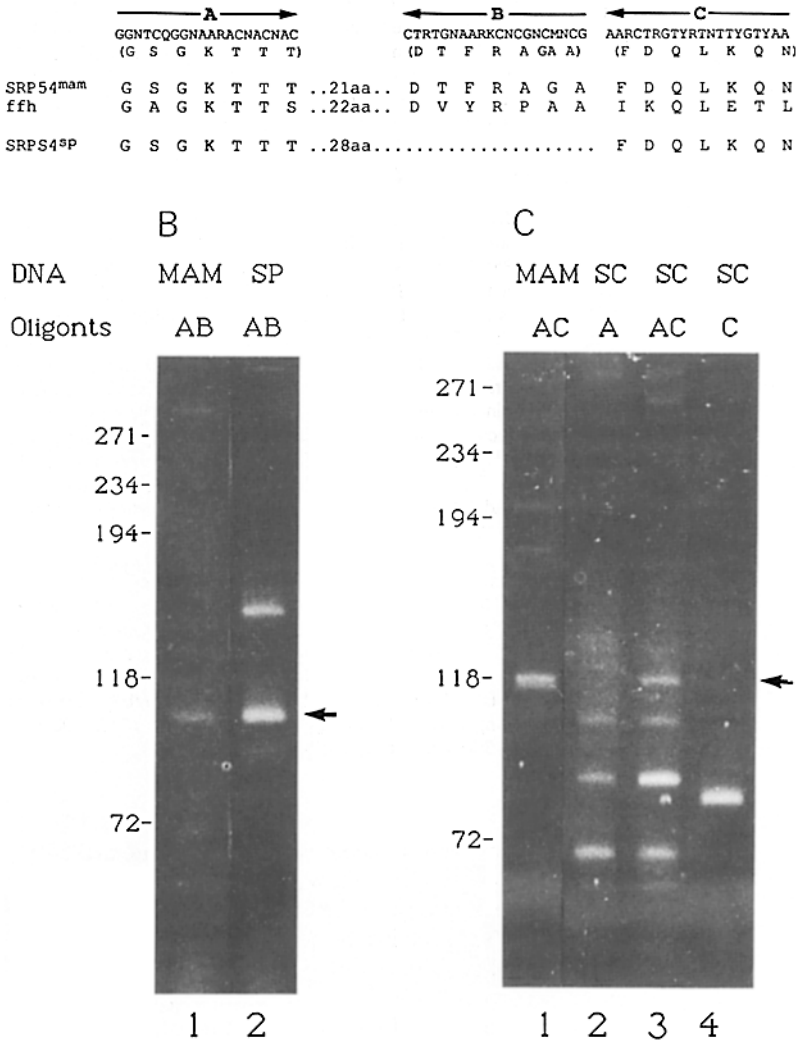


Figure 1. PCR strategy and reaction products. *A*, The PCR strategy is schematized showing the amino acid sequences (one-letter code) from SRP54^{mam}, ffh, and SRP54^{sp} which were used to generate synthetic oligonucleotides "A" (1,536-fold degenerate), "B" (4,096-fold degenerate), and "C" (64-fold degenerate). The mixed bases are represented as follows: $R = A$ or G; $K = G$ or T; $Y = C$ or T; $M = C$ or G; $Q = A$, T, or C; and $N = G$, A, T, or C. The arrows point 5′ to 3′. Amino acids encoded by the oligonucleotides ("A") or their reverse complement ("B" and "C") are indicated in brackets. *B*, PCR products from reactions using oligonucleotides "A" and "B" to amplify mammalian (canine) cDNA (lane *1*) or *S. pombe* (lane *2*) genomic DNA. The arrow marks the predicted size product, present in both lanes. *C*, PCR products using oligonucleotides "A" and "C" to amplify *S. cerevisiae* (lane *3*) or mammalian (lane *1*) template DNA are shown. Lanes *2* and *4* show the products of the control reactions using *S. cerevisiae* DNA and oligonucleotides "A" or "C" alone. The arrow shows the predicted size product, present in both lanes *1* and *3*.

that predicted an amino acid sequence with 66% identity with SRP54^{mam} over a stretch of 18 amino acids. These results indicated that we had indeed amplified DNA from a *S. pombe* gene homologous to SRP54^{mam}. No conclusive sequence data, however, could be gathered from the various products generated from *S. cerevisiae* template DNA. Using the sequenced *S. pombe* PCR product as a probe, we isolated clones containing genomic DNA fragments from a plasmid library. Sequence analysis revealed an open reading frame (Fig. 2 *A*) with extensive homology to SRP54^{mam} (Fig. 3 *A*). Towards the amino terminus, the reading frame lacked an initiating methionine; however, the presence of consensus sequences for 5′ and 3′ splice sites as well as for a splice branch point (boxed in Fig. 2 *A*) (Mertins and Gallwitz, 1987) predicted the existence of a 480-nucleotide intron that separates an exon encoding the NH_2-terminus from the rest of the coding sequence. The 26 amino acids encoded by this exon were homologous to the NH_2-terminus of SRP54^{mam} (Fig. 3 *A*). Two oligonucleotides flanking the putative splice site were used in PCR to amplify cDNA prepared from *S. pombe* RNA (see Materials and Methods). The major product was sequenced directly and confirmed the use of the proposed splice site in vivo (data not shown). The predicted translation product (Fig. 2 *A*) contains 522 amino acids with a predicted molecular mass of 57-kD and a pI of 9.9. Henceforth we refer to this gene as *SRP54^{sp}*.

The alignment of SRP54^{sp} with SRP54^{mam} revealed additional regions of identity that are not present between SRP54^{mam} and ffh. Under the assumption that these new identities are characteristic of eukaryotic SRP54, we designed an additional oligonucleotide ("C," Fig. 1 *A*) to amplify a homologous gene fragment from *S. cerevisiae* DNA. The results of PCR using oligonucleotides A and C are shown in Fig. 1 *C*. A major product of the anticipated length of 125 nucleotides was obtained (Fig. 1 *C*, lane *3*), comigrating precisely with a product obtained using mammalian cDNA as template (Fig. 1 *C*, lane *1*). Direct sequencing confirmed its identity as an SRP54 homologue. We proceeded to isolate and sequence a genomic clone containing the complete gene. The primary sequence, depicted in Fig. 2 *B*,

A

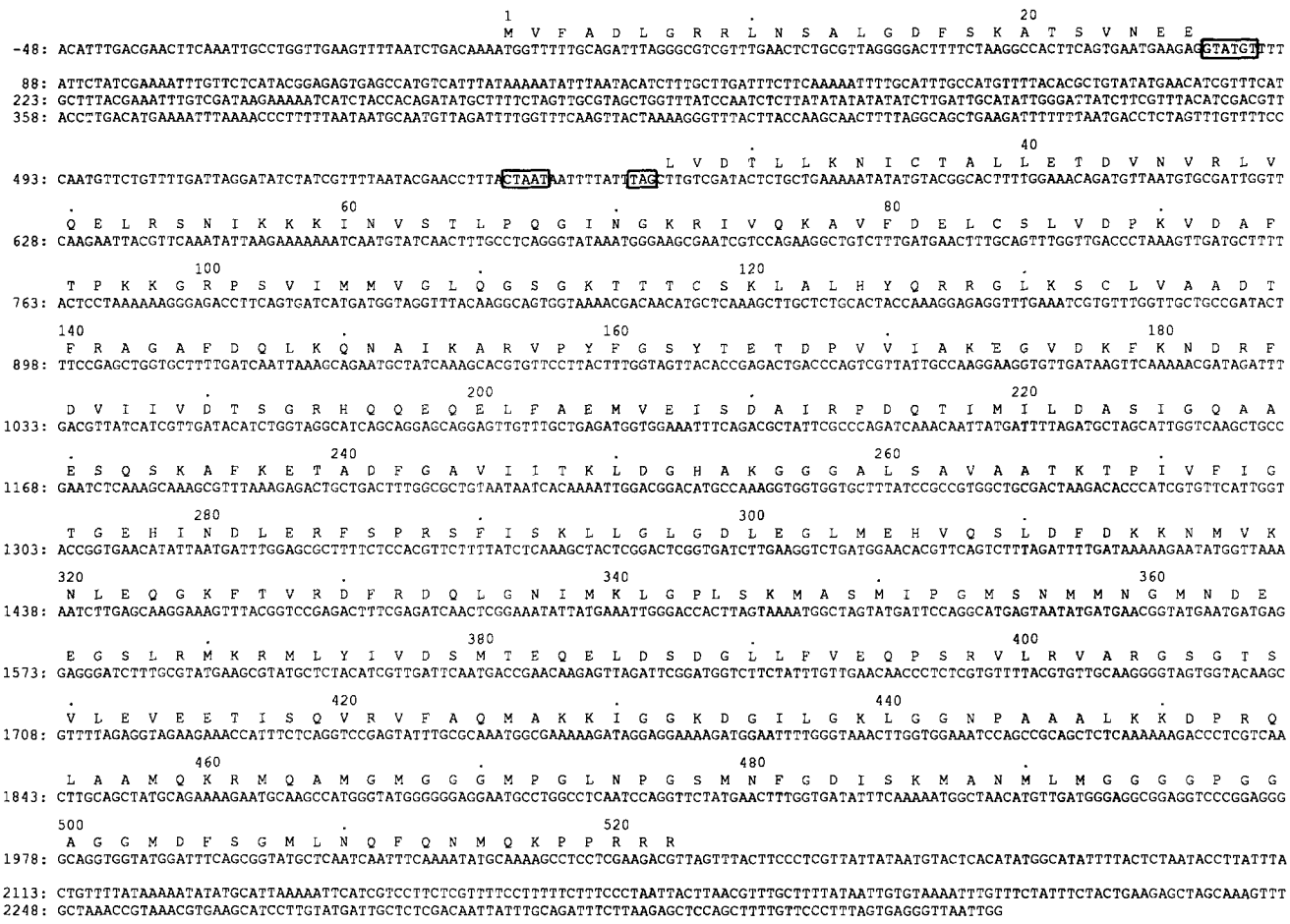


Figure 2. Nucleotide sequence and deduced amino acid sequence of *SRP54^{sp}* and *SRP54^{sc}*. *A*, The nucleotide and the deduced amino acid sequence of SRP54^{sp} are shown. Bases are numbered starting at the "A" of the initiating ATG. Regions matching the consensus sequences (Mertins and Gallwitz, 1987) for the splice branch point and 5′ and 3′ splice sites are boxed. *B*, The nucleotide and the deduced amino acid sequence of SRP54^{sc} are shown. Bases are numbered starting at the "A" of the initiating ATG. A characteristic tripeptide repeat, discussed in the text, is underlined.

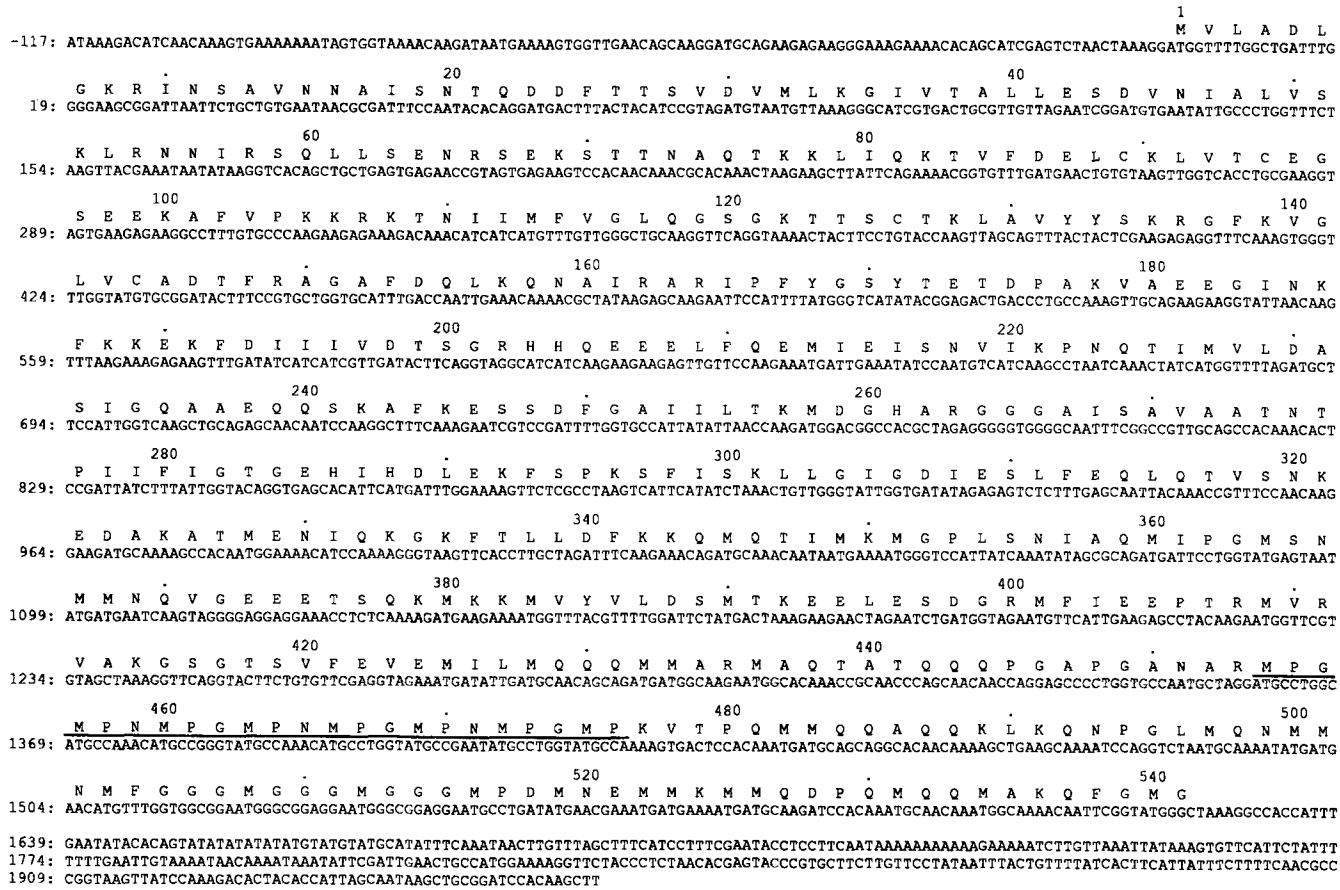
encodes a basic protein (pI = 9.5) of 541 amino acids and a predicted molecular mass of 60 kD. We refer to this gene as *SRP54^{sc}*.

Fig. 3 *A* shows the alignment of SRP54^{mam}, SRP54^{sp}, SRP54^{sc}, and ffh. The overall sequence similarity between the four gene products is notable. The conservation is most striking between the three eukaryotes and especially in the G-domain (SRP54^{mam} residues 1–295). In this region, the pairwise sequence identity among the eukaryotes varies from 59–63% and pairwise sequence similarity ranges from 78–85% (based on the rules given in Fig. 3 *A*). Each of the four sequences contains the GTP-binding site consensus (indicated in Fig. 3 *A*).

As a first step towards characterizing the function of SRP54^{sc}, we determined whether the gene is essential for viability. We replaced one chromosomal copy of the wild-type SRP54^{sc} gene in a diploid cell with a disrupted copy in which 258 nucleotides of coding sequence was replaced by a DNA fragment encoding the LYS2 gene (Fig. 4 *A*). We used Southern analysis (Fig. 4 *B*) to confirm that the disrupted copy had replaced one of the wild-type alleles. DNA was prepared from the parent (Fig. 4 *B*, lanes *1* and *3*) and transformant (Fig. 4 *B*, lanes *2* and *4*) and digested with two different restriction enzymes that cut at sites flanking the chromosomal locus. With each enzyme digest, hybridization of the parent strain with a probe specific to the COOH-terminal region of SRP54^{sc} revealed a single band corresponding to the intact gene (Fig. 4 *B*, lanes *1* and *3, asterisks*). In the transformant, an additional band was present, indicative of the disrupted gene (Fig. 4 *B*, lanes *2* and *4, arrows*), and, in each case, was of the expected size.

After sporulation of the heterozygous diploid, tetrads were dissected and the haploid segregants were scored for viability. Out of nine tetrads, eight gave rise to a viable to nonviable spore ratio of 2:2. In one case, only a single spore was viable. Additional evidence that the disrupted copy of the SRP54^{sc} gene cosegregated with nonviability came from the observation that none of the surviving daughter cells were able to grow on lys$^-$ media. Furthermore, Southern analysis of DNA from the viable segregants resulted in the hybridization pattern of the wild-type gene (data not shown). Taken together, these results indicate that the SRP54^{sc} gene is

B



present in single copy in the haploid genome and is essential for growth.

Discussion

We have isolated genes encoding proteins homologous to SRP54^{mam} from both the budding yeast *S. cerevisiae* and the fission yeast *S. pombe*. The cloning was accomplished using regions of homology between SRP54^{mam} and ffh as a starting point for PCR. The yeast protein sequences reveal extended regions of conservation between one another and with SRP54^{mam} and ffh that cover the previously described G- and M-domains. We have also shown that *SRP54^{sc}* is an essential gene. This together with the fact that SRP54 homologues have been found in evolutionarily distant species suggests that these proteins are likely to fulfill similar and essential function(s) in all cells. In this regard, the nomenclature "SRP54^{sc}" and "SRP54^{sp}" reflects our conjecture that, based on the structural information we have obtained, the function of these gene products will be related to that characterized for SRP54^{mam}.

This conjecture is further supported by the fact that homology in the G-domain is not limited to the sequences directly involved in GTP binding. This is consistent with the idea that the entire domain is under evolutionary pressure to maintain interactions with other components of the cell, again suggesting a very similar function for the protein in different species. As noted before, SRP54 is a member of a new subfamily of GTP binding proteins that also includes SRα and its putative *E. coli* homologue ftsY (Bernstein et al., 1989; Römisch et al., 1989). For all proteins of this subfamily, the third of three sequence motifs defining GTP binding proteins, box III, deviates from the consensus at a single amino acid (TKXD rather than NKXD).

Within this subfamily, SRP54 homologues are distinguished from SRα and ftsY by the amino acids comprising GTP-binding consensus box I. Among the SRP54 homologues there is almost no variation between species (GLQGSGKT in eukaryotic SRP54 and GLQGAGKT in ffh; consensus: GXXXXGKS/T). SRα and ftsY also share homology in this consensus box but with a different sequence in the four nonconserved positions (GVNGVGKS/T). In the crystal structure of EF-Tu and ras this region forms a loop over the β-γ phosphate bond of bound GTP (Jurnak, 1985; LaCour et al., 1985; Pai et al., 1989; Tong et al., 1989). The position of these residues with respect to the bound GTP and the phenotype of point mutations of these amino acids (Gibbs et al., 1984; McGrath et al., 1984) implicate their involvement in the regulation of the rate of catalysis (i.e., GTP hydrolysis). In addition, the rate of GTP hydrolysis is known to be an important aspect of the function of GTP binding proteins as molecular clocks. The G-domains of SRP54 and SRP receptor homologues may, therefore, be distinct in this regard.

The M-domains of both yeast proteins are characterized by

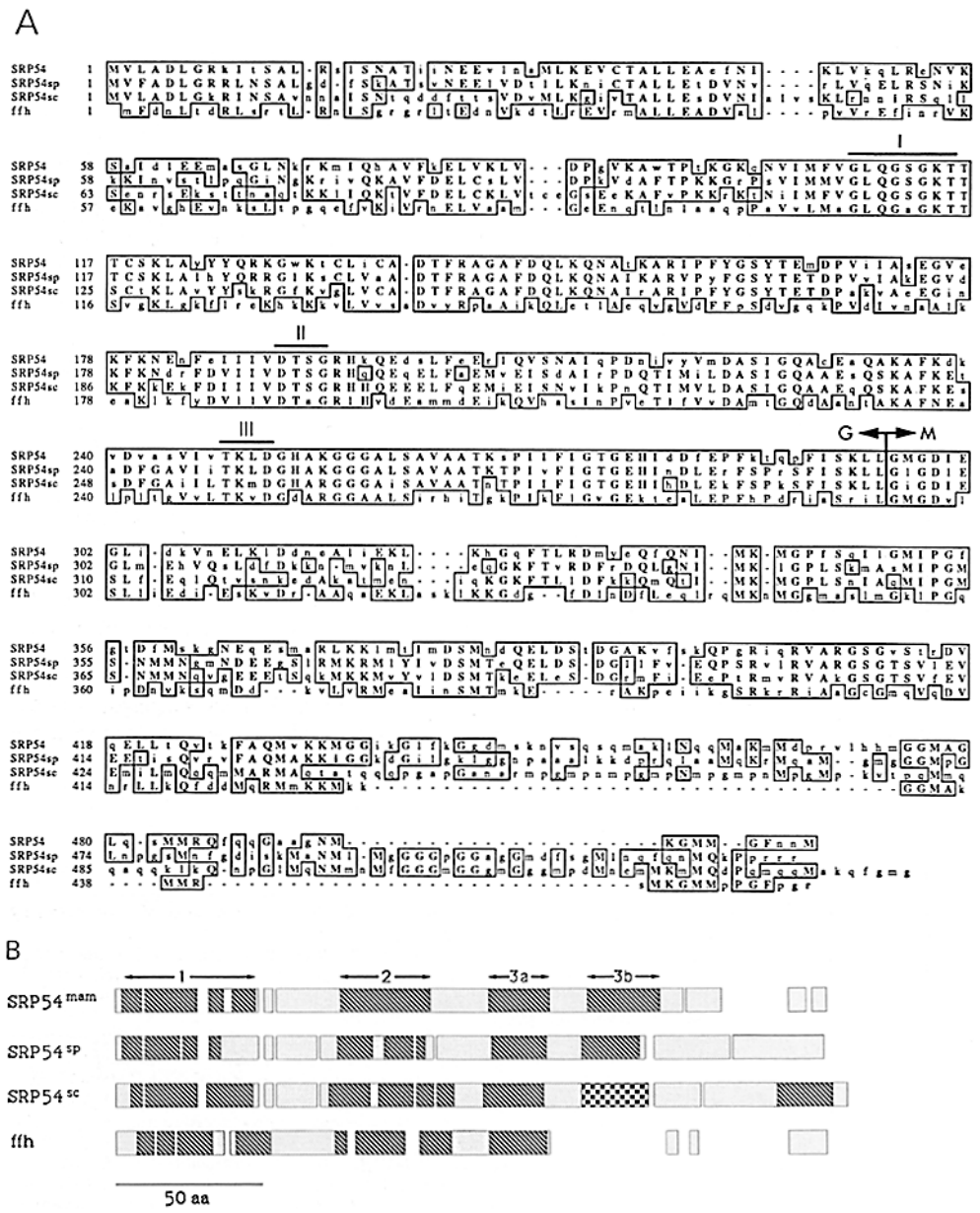


Figure 3. Alignment of SRP54 homologues. *A*, The deduced amino acid sequences (one-letter code) are aligned for SRP54^{mam} (mouse) (Bernstein et al., 1989), SRP54^{sp}, SRP54^{sc}, and ffh (Byström et al., 1983). (In the canine SRP54 R[205], S[209] and P[344] are replaced by M[205], A[209], and L[344], respectively [Römisch et al., 1989].) Two or more identical amino acids in one position are indicated by capital letters. Amino acids of similar chemical properties are boxed, using the following similarity rules: $L = I = M = V = F = W$; $K = R = H$; $D = E = Q = N$; $G = A = S$; $T = V$; $A = V$; $F = Y = H = W$; $T = S$ (Dayhoff et al., 1972). Note that some positions are boxed because of two independent pairwise similarities. Gaps are indicated by dashes. The positions of the regions matching the GTP-binding consensus sequences are indicated above the alignment. The consensus sequences are box I: GXXXXGKS/T; box II; DXXG; box III: NKXD (Dever et al., 1987). The division between the G- and M-domains (as defined from the alignment with SRα and ftsY by Bernstein et al., 1989), is indicated. *B*, Aligned as in *A*, the M-domains of the four proteins are shown schematically. A secondary structure prediction was performed according to established methods (Garnier et al., 1978). Regions that are very likely to form α-helices are shaded in dark. The position MPG/N tripeptide repeat in SRP54^{sc} is checkered. Above the alignment putative helices previously designated by Bernstein et al. (1989) are indicated.

an abundance of Met residues: 8% SRP54^{sp} and 18% for SRP54^{sc} (13% for ffh and 11% for SRP54^{mam}). We have proposed that this domain contains a flexible signal sequence binding pocket composed, in part, of a number of amphipathic helices that bear clusters of methionines on one face (Bernstein et al., 1989). Secondary structure predictions of the M-domains of the yeast proteins suggest that helices of comparable length are likely to form in corresponding positions (see Fig. 3 *B*) (Finer-Moore and Stroud, 1984; Garnier et al., 1978). Although the putative helices in yeast are less amphipathic in character than their mammalian or bacterial counterparts, many of the Met residues are found clustered on one face of the predicted helices 2 and 3a (not shown). COOH terminal to helix 3a, the primary structures are more divergent from one another. Nevertheless, the structural motif is conserved in the case of SRP54^{sp} (helix 3b in Fig. 3 *B*). Curiously, in the corresponding position, SRP54^{sc} contains a tripeptide (MPG/N) repeated eight times (underlined in Fig. 2 *B*) in which Gly and Asn are found in an alternating pattern. Because of its Pro and Gly content, this sequence is unlikely to form a stable α-helix. The tripeptide repeats, however, resemble sequences found in collagen and related proteins where they form tight left-handed helices known as collagen helices (Traub and Piez, 1971). Since collagen helices have three residues per turn, the eight methionine residues would be found clustered on one face of the helix. While such helices are normally found oligomerized in triple-stranded helices, it is conceivable that this secondary structure element is present in SRP54^{sc} as a single helix stabilized by other features in the M-domain. Structural analy-

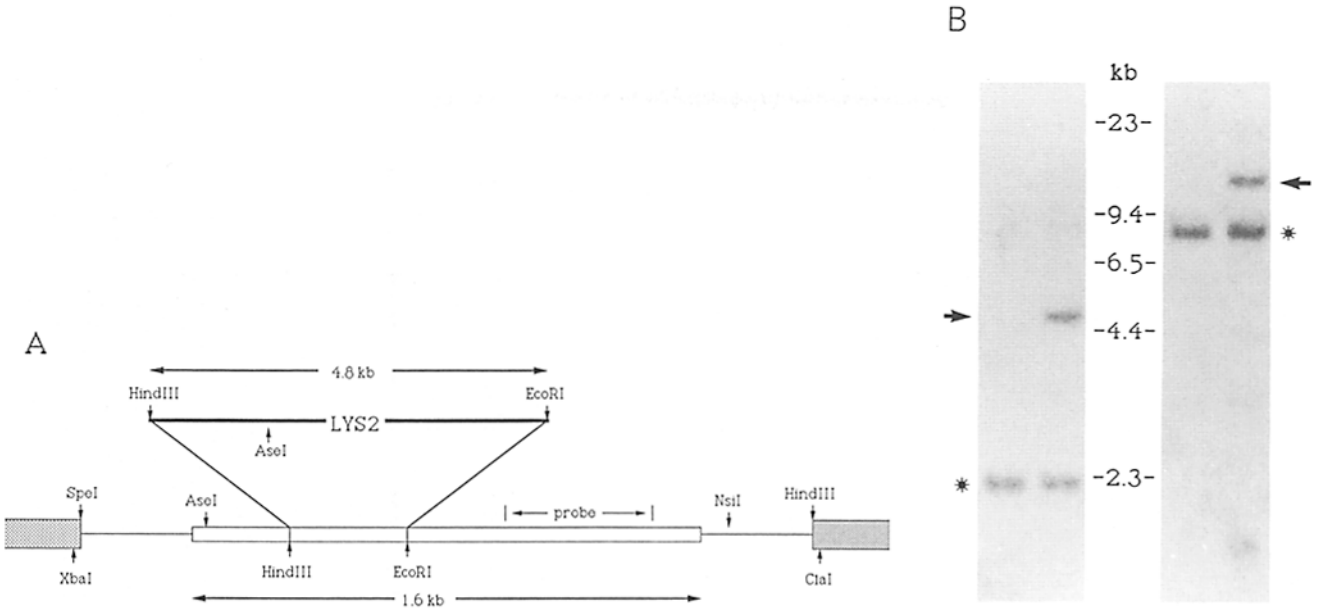


Figure 4. Gene disruption of *SRP54^{sc}*. *A*, The construction of plasmid pSC54-L2 containing *SRP54^{sc}::LYS2* is shown schematically. The open box indicates the coding sequence of *SRP54^{sc}* reading from left to right; the shaded boxes correspond to vector sequences. Restriction sites referred to in the text are shown. The position of the probe used for the Southern analysis shown in panel B is indicated. Note that the fragment containing the LYS2 gene is depicted in a different scale. *B*, Southern blot analyses showing genomic DNA from the parent (lanes *1* and *3*) or transformants containing the *SRP54^{sc}::LYS2* disruption (lanes *2* and *4*) are shown. DNA was digested with Ase I (lanes *1* and *2*) or Nsi I (lanes *3* and *4*). Fragments corresponding to the intact gene are marked by asterisks; fragments corresponding to the disrupted gene are marked by arrows.

ses will be required to elucidate the organization of the M-domain, however, the phylogenetic evidence has already suggested that an abundance of Met residues is important.

The isolation of yeast homologues to SRP54 will allow detailed analyses of the function of this protein in vivo. Major questions remain to be answered. For example, is yeast SRP54 part of a ribonucleoprotein with similar properties to that of mammalian SRP, and, if so, does its RNA component contain the conserved motifs found in other SRP RNAs (Poritz et al., 1988; Struck et al., 1988)? In particular, does SRP54^{sp} associate with the previously described SRP7 RNA? Most importantly, it remains to be determined that functional features of an SRP-dependent pathway are conserved in yeast. If an SRP-dependent, strictly co-translational targeting pathway exists in parallel to the posttranslational mode of translocation, it is unclear how different proteins would choose which route to follow. A distinction may be required between signal sequences that at present are considered to be more or less interchangeable. Alternatively, yeast SRP54 could function either alone or as part of a ribonucleoprotein in a posttranslational pathway to help maintain preproteins in a translocation competent state after they have been released from the ribosome.

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