MOLECULAR AND CELLULAR BIOLOGY, Dec. 1992, p. 5640–5651
0270-7306/92/125640-12$02.00/0

Vol. 12, No. 12

Cloning and Characterization of *SRP1*, a Suppressor of Temperature-Sensitive RNA Polymerase I Mutations, in *Saccharomyces cerevisiae*

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Received 15 June 1992/Returned for modification 12 August 1992/Accepted 8 September 1992

The *SRP1-1* mutation is an allele-specific dominant suppressor of temperature-sensitive mutations in the zinc-binding domain of the A190 subunit of *Saccharomyces cerevisiae* RNA polymerase I (Pol I). We found that it also suppresses temperature-sensitive mutations in the zinc-binding domain of the Pol I A135 subunit. This domain had been suggested to be in physical proximity to the A190 zinc-binding domain. We have cloned the *SRP1* gene and determined its nucleotide sequence. The gene encodes a protein of 542 amino acids consisting of three domains: the central domain, which is composed of eight (degenerate) 42-amino-acid contiguous tandem repeats, and the surrounding N-terminal and C-terminal domains, both of which contain clusters of acidic and basic amino acids and are very hydrophilic. The mutational alteration (P219Q) responsible for the suppression was found to be in the central domain. Using antibody against the SRP1 protein, we have found that SRP1 is mainly localized at the periphery of the nucleus, apparently more concentrated in certain regions, as suggested by a punctate pattern in immunofluorescence microscopy. We suggest that SRP1 is a component of a larger macromolecular complex associated with the nuclear envelope and interacts with Pol I either directly or indirectly through other components in the structure containing SRP1.

In eukaryotic cells, rRNA genes are tandemly repeated, and their transcription by RNA polymerase I (Pol I) takes place in the nucleolus. In the yeast *Saccharomyces cerevisiae*, Pol I consists of about 14 different polypeptide subunits (5, 32), and the genes for many of these subunits have been cloned and characterized. However, other components involved in rRNA gene transcription or nucleolar structure are largely unexplored.

One of the approaches that we have taken to identify such components is to isolate and study suppressors of temperature-sensitive (ts) Pol I mutants. We first isolated and characterized several ts mutants of *S. cerevisiae* which have mutational alterations in the largest subunit, A190, of Pol I (45). Starting with two ts mutants (*rpa190-1* and *rpa190-5*), both of which have mutational alterations in the zinc-binding domain located close to the N terminus of A190, we isolated extragenic suppressors and characterized some of them (21, 49). One suppressor gene (*SRP3*) was identified as the gene encoding the second-largest subunit, A135, of Pol I, and the amino acid alteration responsible for suppression was found to be in the zinc-binding domain located near the C terminus of this protein (49). This result, combined with other observations (29), suggested that the zinc-binding domain of A190 is in physical proximity to and interacts with that of the A135 subunit. In this report, we describe our work on the characterization of another suppressor gene, *SRP1*, which specifically suppresses both *rpa190-1* and *rpa190-5* but not other *rpa190* ts mutations analyzed (21). We have found that the *SRP1* suppressor also suppresses two *rpa135* ts mutations, both of which have mutational alterations in the zinc-binding domain of A135, but not other *rpa135* ts mutations. We have cloned and sequenced the *SRP1* suppressor gene, and in addition, we have found that its protein product appears to be associated with the nuclear envelope and is not a Pol I subunit. These results are discussed in connection with a possible role of the SRP1 protein in maintenance of the intact nucleolar structure and in the transcriptional activity of Pol I in vivo.

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MATERIALS AND METHODS

Media, strains, and plasmids. YEP-glucose medium contains 1% yeast extract, 2% Bacto Peptone (Difco), and 2% glucose. Synthetic glucose (SGlu) medium (2% glucose, 0.67% yeast nitrogen base [Difco], 0.5% Casamino Acids [Difco]) was supplemented with tryptophan and required bases as described by Sherman et al. (34). Synthetic galactose (SGal) medium is the same as SGlu but with 2% galactose substituted for glucose. For making solid medium, 2% agar was added.

The yeast strains and plasmids used in this study are described in Table 1. NOY476, the diploid strain in which one of the *SRP1* genes is disrupted, was constructed as follows. The 2.7-kb *Bgl*II-*Bgl*II fragment carrying the *LEU2* gene prepared from YEp13 (4) was inserted into the *Bam*HI site of pUC19. The *Pst*I-*Sma*I fragment containing *LEU2* was then excised from the resultant plasmid. Separately, the *SRP1* gene on a 2.4-kb *Hpa*I-*Bgl*II fragment was excised from pNOY134 and inserted between the *Eco*RV and *Bam*HI sites of pBluescript II KS(−) (Stratagene, La Jolla, Calif.). The middle part of the *SRP1* gene (1,172 bp between the *Pst*I and *Eco*RV sites) on this plasmid was then substituted by the aforementioned *LEU2 Pst*I-*Sma*I fragment (see Fig. 3B). The *Hin*dIII-*Sac*I fragment containing the disrupted *SRP1* gene was prepared from the resultant plasmid and then transformed into NOY397, with selection for *LEU2* transformants. One of the transformants is NOY476. The

TABLE 1. Yeast strains and plasmids used

Designation	Genotype and comments	Reference or source
Strains		
NOY259	*MATα ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1 rpa190-1*	45
NOY260	*MATα ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1*	45
NOY265	*MATα ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1 rpa190-3*	45
NOY267	*MATα ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1 rpa190-5*	45
NOY343	*MATα trp1-Δ1 rpa190-5 SRP1-1*	21
NOY397	*MATa/α ade2-1/ade2-1 ura3-1/ura3-1 his3-11/his3-11 trp1-1/trp1-1 leu2-3,112/leu2-3,112 can1-100/can1-100*	49
NOY452	*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100 Δrpa135::LEU2* pNOY114 (*rpa135-21 TRP1*)	
NOY476	*MATa/α ade2-1/ade2-1 ura3-1/ura3-1 his3-11/his3-11 trp1-1/trp1-1 leu2-3,112/leu2-3,112 can1-100/can1-100 Δsrp1::LEU2/SRP1*	
NOY483	*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100 Δrpa135::LEU2* pNOY150 (*rpa135-11 TRP1*)	
w303-1a	*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100*	41
BJ2168	*MATa prc1-407 prb1-1122 pep4-3 leu2 trp1 ura3-52*	Yeast Genetic Stock Center
Plasmids		
YCp50	*E. coli*-yeast shuttle vector carrying *CEN4 ARS1 URA3*	27
pRS314	*E. coli*-yeast shuttle vector carrying *CEN6 ARSH4 TRP1* and with multicloning site from pBluescript KS(−)	36
pRS316	*E. coli*-yeast shuttle vector carrying *CEN6 ARSH4 URA3* and with multicloning site from pBluescript KS(−)	36
pUN35	*E. coli*-yeast shuttle vector carrying *CEN4 ARS1 TRP1* and with multicloning site from pBluescript KS(−)	12
pNOY122	Derivative of YCp50 carrying *SRP1-1* on a cloned DNA fragment of about 6 kb derived from a partial *Sau*3A1 digest of NOY343 DNA; the original isolate of the *SRP1-1* gene	
pNOY114	Derivative of pRS314 carrying *rpa135-21* on a DNA fragment of about 5 kb	
pNOY132	Derivative of pUN35 carrying *SRP1-1* on the 2.4-kb *Hpa*I-*Bgl*II fragment	
pNOY133	Derivative of pRS316 carrying the 2-kb *Kpn*I-*Hpa*I fragment upstream and the 0.8-kb *Bgl*II-*Sac*I fragment downstream of *SRP1*	
pNOY134	Derivative of pRS316 carrying *SRP1* on a DNA fragment of about 5 kb; the original isolate of the *SRP1* gene	
pNOY150	Derivative of pRS314 carrying *rpa135-11* on a DNA fragment of about 5 kb	

expected structure of the disrupted chromosomal *SRP1* locus was confirmed by Southern hybridization using a suitable probe.

Cloning. Cloning of *SRP1-1* (*SRP1* carrying the original suppressor mutation) was done as described previously (49). Purified genomic DNA (200 μg) from NOY343 was partially digested by *Sau*3AI, and DNA fragments of about 5 to 15 kb were collected after centrifugation in a linear NaCl gradient. The recovered DNA was ligated into the *Bam*HI site of YCp50, a centromere plasmid; the resultant plasmids were first amplified in *Escherichia coli* DH5α (Bethesda Research Laboratories, Gaithersburg, Md.) and then transformed into NOY267 (*rpa190-5*). Temperature-resistant transformants were selected at 37°C. One of the desired plasmids carrying *SRP1-1* was designated pNOY122.

The wild-type *SRP1* gene was cloned by a gap repair method (24). The ca. 2-kb *Kpn*I-*Hpa*I fragment upstream and the 0.8-kb *Bgl*II-*Sac*I fragment downstream of *SRP1* were excised from pNOY122 and cloned into the *Kpn*I-*Eco*RV and *Bam*HI-*Sac*I sites of pRS316, respectively. The resultant plasmid (pNOY133) was cleaved at the *Sma*I site and transformed into NOY260 to repair the gap between *Hpa*I and *Bgl*II by recombination with the chromosomal *SRP1* region. The plasmid (pNOY134) recovered from transformants contained the wild-type allele of *SRP1*.

Sequencing. Suitable subfragments of the 2.4-kb *Hpa*I-*Bgl*II fragment carrying the cloned *SRP1-1* allele were first cloned into M13mp18 and/or M13mp19. Several series of deletion subclones were then constructed with the Cyclone I Biosystem (IBI, New Haven, Conn.), and their DNA sequences were determined on both strands by the dideoxy method with T7 DNA polymerase (40). The 550-bp *Xba*I-*Eco*RI fragment of the wild-type gene was sequenced in the same way.

The mutational alteration responsible for suppression was identified as follows. A series of chimeric genes was constructed from the wild-type and mutant genes. These chimeric genes were introduced into NOY267 (*rpa190-5*) and examined for the ability to suppress the *rpa190-5* mutation. The wild-type segment, which was responsible for the absence of suppression, was then sequenced on both strands, and its sequence was compared with the nucleotide sequence of the mutant gene.

Antibodies. The rabbit antibody against the Pol I A190 subunit was prepared in this laboratory (46). The rabbit antibodies against A135 and SSB1 were provided by M. Riva and A. Sentenac and by J. Broach, respectively. The mouse monoclonal antibodies (MAbs) against nuclear pore proteins, MAb 306 and MAb 414, were provided by J. P. Aris and G. Blobel. Their properties were described previously (2, 9). The mouse MAb against yeast fibrillarin, MAb A66, was provided by J. P. Aris and G. Blobel. The rabbit polyclonal antibody against the yeast mitochondrial malate dehydrogenase was provided by L. McAlister-Henn. The antibody against SRP1 was made as follows. A plasmid carrying a *lacZ-SRP1* fusion gene placed under the control of

the T7 RNA polymerase promoter was constructed. For this purpose, the *Sma*I-*Sal*I fragment carrying most of *lacZ* but missing the N-terminal portion was excised from pMC1871 (33) and inserted between *Eco*RI and *Sal*I sites of pJES307, which is a derivative of pT7-7 (39) and is the same as pJES311 (44) except that the *ntrC* gene is not present, yielding pNO3096. The *Dra*I-*Bgl*II fragment, which contains DNA coding for the C-terminal one-sixth of SRP1 protein (from lysine 457 to the end; see Fig. 3), was inserted into the *Eco*RI site within the *lacZ* in pNO3096. The resultant plasmid, pNOY3145, was transformed into JM105 (48), and synthesis of the fusion protein was induced by adding 1 mM isopropylthiogalactopyranoside (IPTG) following an infection with phage mGP1-2, which carries the gene for T7 RNA polymerase (39). The fusion protein was separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels, eluted from the gels, concentrated, and injected into sheep (and also rabbits). The anti-SRP1 antibody was affinity purified from the sheep antiserum as described by Sambrook et al. (28), using a nitrocellulose membrane (BA85; Schleicher & Schuell, Keene, N.H.) containing the purified fusion protein.

Immunoblot analysis. Western immunoblot experiments were done as previously described (21). Yeast cell extracts were subjected to SDS-polyacrylamide gel electrophoresis and transferred onto BA85 or Immobilon-P (Millipore, Bedford, Mass.) membranes. The membranes were treated with the antibodies and stained by using an immunoassay kit (Bio-Rad Laboratories, Richmond, Calif.). Depending on primary antibodies, either goat anti-rabbit immunoglobulin G (IgG) alkaline phosphatase conjugate (Bio-Rad) or donkey anti-sheep IgG-alkaline phosphatase conjugate (Sigma, St. Louis, Mo.) was used as a second antibody.

Purification and fractionation of nuclei. Nuclei were purified by the method of Aris and Blobel (3). Strain BJ2168 was grown in YEP-glucose. Cells were collected, and cell walls were digested by yeast lytic enzyme (ICN Biochemicals, Costa Mesa, Calif.) in spheroplasting medium (1 M sorbitol, 1% glucose, 0.2% yeast nitrogen base, 0.2% Casamino Acids, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], 50 mM Tris base, 2 mM dithiothreitol). The spheroplasts were washed several times with spheroplast medium and with wash buffer (1.2 M sorbitol, 2% Ficoll 400, 25 mM morpholine ethanesulfonic acid [MES]-Tris [pH 6.5]) and then broken in lysis buffer (20% Ficoll 400, 20 mM potassium phosphate [pH 6.5], 1 mM $MgCl_2$), using a Dounce homogenizer. The lysate was centrifuged for 5 min at 13,400 × *g* in a Sorvall HB-4 rotor. The supernatant was transferred to another tube and centrifuged as before for 10 min. The supernatant was then transferred to SW28 ultraclear tubes (Beckman Instruments, Palo Alto, Calif.) filled with a three-step gradient of 30, 40, and 50% Ficoll 400 and centrifuged for 1 h at 18,000 rpm. After centrifugation, the layers of 30 and 40% Ficoll 400 were collected as the purified nuclei fraction, while a top layer of white film, the 20% Ficoll layer, the 20 to 30% interface, and the 50% layer were collected as low-density membrane, soluble, high-density membrane, and pellet fractions, respectively. The positions of these four fractions are indicated as 2, 3, 4, and 6, and that of the nuclei fraction is indicated as 5, on the diagram in Fig. 7A.

The purified nuclei preparation was further fractionated by the method of Allen and Douglas (1). The purified nuclei were suspended in TP buffer (10 mM Tris-HCl, 10 mM Na_2HPO_4 [pH 8.0]) at a DNA concentration of 200 μg/ml. Heparin (sodium salt, grade I; Sigma) was added to make 300 μg/ml, and the solution was incubated at 22°C for 5 min and then centrifugated for 10 min at 5,000 × *g*. The supernatant was kept as the heparin-soluble protein fraction. The pellet was washed twice with 0.25 M-STKMC buffer (0.25 M sucrose, 50 mM Tris-HCl [pH 7.4], 25 mM KCl, 5 mM $MgCl_2$, 3.3 mM $CaCl_2$) and resuspended in detergent buffer (0.3 M sucrose, 20 mM HEPES [pH 7.4], 0.5 mM $MgCl_2$, 2% Triton X-100). This sample was centrifuged for 10 min at 3,000 × *g* after 10 min of incubation on ice. The pellet was resuspended in high-salt buffer (0.3 M sucrose, 20 mM HEPES [pH 7.4], 0.5 mM $MgCl_2$, 1 M NaCl) and incubated for 10 min on ice. After centrifugation for 10 min at 1,000 × *g*, the pellet was kept as the nuclear matrix-lamina-pore complex fraction. Protein concentrations of each fraction were determined by using MicroBCA protein assay reagent (Pierce, Rockford, Ill.), and suitable aliquots containing equal amounts of protein were used for Western immunoblot analysis.

Glycerol gradient sedimentation analysis of SRP1 and Pol I in crude extracts. Cells were grown in YEP-glucose, collected, washed, and broken by shaking with glass beads, using the buffer conditions specified by Riva et al. (25). The soluble fraction was first obtained after the removal of insoluble membrane fragments and unbroken cells by centrifugation (15,000 × *g* for 5 min in a Brinkman model 5412 microcentrifuge). Aliquots were then subjected to glycerol gradient centrifugation essentially as described by Riva et al. (10 to 30% [vol/vol] glycerol gradient for 17 h at 40,000 rpm at 2°C in a Beckman SW40 rotor; for buffer conditions, see reference 25). After centrifugation, 0.5-ml fractions were collected and protein was precipitated with trichloroacetic acid. The precipitate was dissolved in 200 μl of SDS sample buffer, and 10 μl was analyzed by SDS-gel electrophoresis followed by immunoblotting with antibodies against the A135 subunit of Pol I, SRP1, and yeast mitochondrial malate dehydrogenase. The pellet at the bottom of the centrifuge tubes was also dissolved in SDS sample buffer and analyzed in the same way. Yeast mitochondrial malate dehydrogenase is a dimer of the subunit (M_r 33,000) at neutral pH (38), and its molecular weight (66,000) is close to that of SRP1 (60,403 from the deduced amino acid sequence or 67,000 from SDS-gel electrophoretic analysis; this work); hence, sedimentation of this enzyme was analyzed as a size reference marker.

Immunofluorescence staining and microscopy. Cells were grown in YEP-glucose medium to a cell density of about A_{600} 0.2. One milliliter of 37% formaldehyde was added directly to 9 ml of the culture. The cells were fixed for 30 min at 30°C except when the primary antibody was MAb 306. In the latter case, the cells were prepared and immunofluorescence was performed as described by Clark and Abelson (6). The primary antibodies were sheep anti-SRP1 (undiluted) and mouse MAb 306 (diluted 1:12.5). Secondary antibodies were goat anti-sheep IgG conjugated with fluorescein isothiocyanate (diluted 1:2,000) and goat anti-mouse IgG conjugated with rhodamine (diluted 1:2,000).

Nucleotide sequence accession number. The GenBank accession number for the *SRP1* nucleotide sequence reported in this paper is M75849.

RESULTS

The *SRP1-1* mutation suppresses mutations in zinc-binding domains of both A190 and A135 subunits. *SRP1* was originally identified as the allele-specific suppressor gene which suppresses ts mutants *rpa190-1* and *rpa190-5*, both of which are amino acid substitutions in the zinc-binding domain of

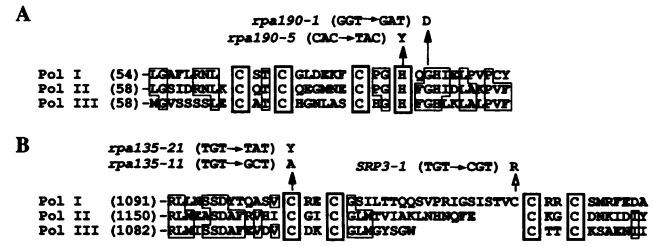

FIG. 1. Sequences of putative zinc-binding regions in the largest (A) and second-largest (B) subunits of yeast RNA polymerases. The amino acid alterations caused by ts mutations which can be suppressed by *SRP1-1* are indicated by closed arrows. The amino acid alteration caused by another mutation in *rpa135* (*SRP3-1*) which can suppress *rpa190-1* and *rpa190-5* ts mutations is shown by an open arrow. As discussed in the text (see Discussion), two ts mutations in the second-largest subunit of Pol II (G1167F and C1182A) are not suppressed by *SRP1*. The consensus putative zinc-binding motifs $CX_2CX_6CX_2H$ (A) (alternatively, the consensus might be $CX_2CX_9HX_2H$ [44a, 49]) and $CX_2CX_{8-20}CX_2C$ (B) are indicated by boxes with thick lines. Other conserved residues (identical residues found in at least two of the sequences) are indicated by boxes with thin lines. The number in parentheses at the beginning of each sequence indicates the position in the sequence of the first amino acid shown.

the largest subunit (A190) of Pol I (21) (Fig. 1A). Among 37 independently isolated suppressors starting either from *rpa190-1* or from *rpa190-5*, 27 were in this locus, and all of them suppressed these two mutations but not two other ts mutations, including *rpa190-3*, which has a different amino acid substitution located in a different region of the A190 subunit (21) (Fig. 2A). Because of evidence suggesting that the zinc-binding domain of the second-largest subunit (A135) of Pol I is in physical proximity to and interacts with the A190 zinc-binding domain (49; see introduction), we examined whether the *SRP1* suppressor (*SRP1-1*) can also suppress mutations in the zinc-binding domain of A135. One of our ts mutants, *rpa135-11*, was constructed by site-directed mutagenesis and carries a Cys-1104-to-Ala mutation in A135 (36a) (Fig. 1B). We found that introduction of the *SRP1-1* allele on a plasmid (cloned as described below) into this strain suppressed growth defects of this mutant at 37°C (Fig. 2). We then examined nine other independent *rpa135* ts mutants (23, 36a), the mutational alterations of which had not yet been identified. Only one of them (*rpa135-21*) was found to be suppressed by *SRP1-1* (Fig. 2). Remarkably, the *rpa135-21* mutation was found to be a change of the same cysteine as that affected by *rpa135-11*, Cys-1104 to Tyr (Fig. 1B). Although we have not determined mutational alterations of the other eight *rpa135* ts mutants which were not suppressed by the suppressor, it is obvious that the suppression by *SRP1-1* is highly specific. This specificity may suggest a direct interaction between the protein encoded by *SRP1* and a specific region of Pol I comprising these two zinc-binding domains, one located near the N terminus of the A190 subunit and the other located near the C terminus of the A135 subunit. However, it is also possible that SRP1 interacts with this specific region of Pol I indirectly through other proteins or other macromolecules.

Cloning and sequencing of *SRP1*. A gene bank was constructed from DNA isolated from strain NOY343, which carries *SRP1-1* as well as *rpa190-5*, and was used to transform NOY267 (*rpa190-5*). Transformants were selected for the ability to grow at 37°C, and plasmids were recovered from each of 12 such transformants. All of them contained a *Hpa*I-*Bgl*II DNA fragment of about 2.4 kb (Fig. 3A); after

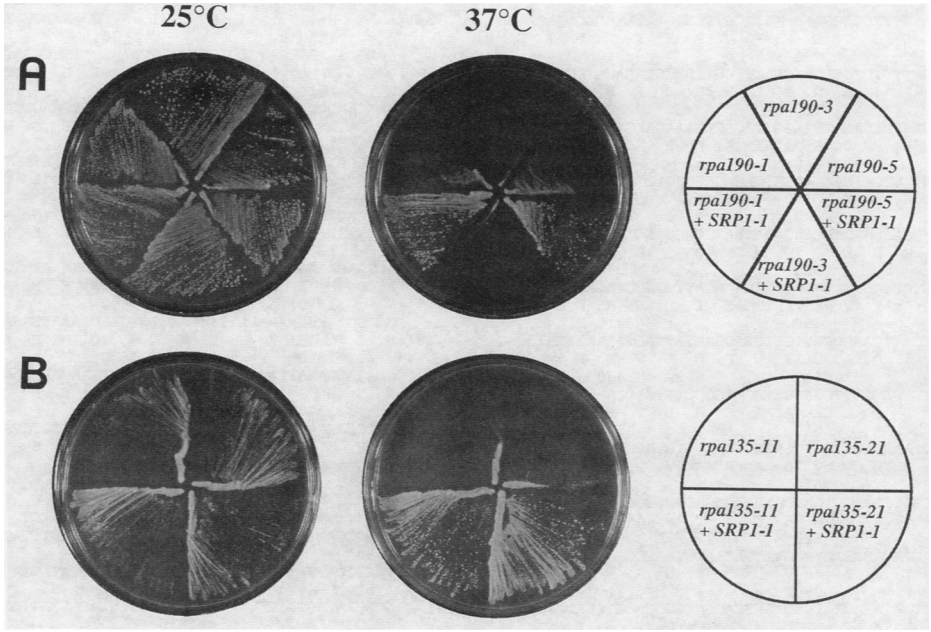

FIG. 2. Growth of Pol I ts mutants with and without the *SRP1-1* suppressor mutation. Growth of yeast strains carrying *rpa190* (A) or *rpa135* (B) ts mutations was examined by growing the strains on SGlu medium either without tryptophan (for *rpa190* mutants) or without uracil (for *rpa135* mutants) at 25 and 37°C, as indicated. The *rpa190* strains examined were NOY259, NOY265, and NOY267 carrying control vector pUN35 (*rpa190-1*, *rpa190-3*, and *rpa190-5*, respectively) and the same three strains carrying suppressor plasmid pNOY132 (*rpa190-1* + *SRP1-1*, *rpa190-3* + *SRP1-1*, and *rpa190-5* + *SRP1-1*, respectively). The *rpa135* strains examined were NOY483 and NOY452 carrying control vector YCp50 (*rpa135-11* and *rpa135-21*) and the same two strains carrying suppressor plasmid pNOY122 (*rpa135-11* + *SRP1-1* and *rpa135-21* + *SRP1-1*, respectively).

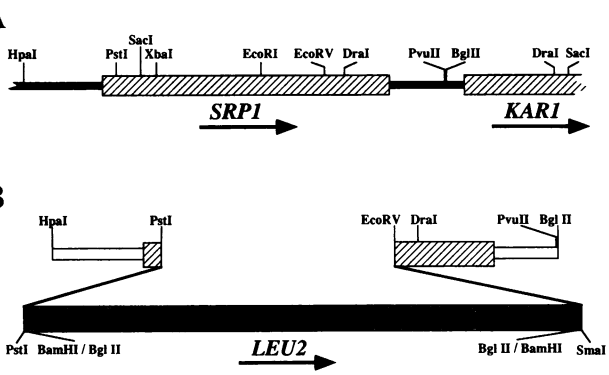

FIG. 3. (A) Restriction enzyme map of the *SRP1* region on the chromosome, including part of *KAR1*. The protein-coding regions are shown as striped boxes. Direction of transcription is shown by arrows. (B) Structure of the DNA fragment used for construction of the null allele (*srp1::LEU2*).

subcloning, this fragment was found to have the ability to suppress both *rpa190-1* and *rpa190-5*, but not *rpa190-3*, in agreement with the original genetic results obtained for *SRP1* suppressors.

The nucleotide sequence of this *Hpa*I-*Bgl*II fragment was determined and is shown in Fig. 4. (The nucleotide sequence shown in Fig. 4 is the wild-type sequence; see below for the mutational alteration.) The sequence reveals an open reading frame which would code for a protein of 542 amino acids with a calculated molecular mass of 60,403 Da. Further subcloning of various DNA fragments within the *Hpa*I-*Bgl*II region demonstrated complete correlation between the presence of this open reading frame and the ability to suppress *rpa190-1* (and *rpa190-5*), supporting the conclusion that this open reading frame represents *SRP1*. In addition, after sequencing the gene from the mutant strain, we cloned the corresponding gene from the wild-type parent strain (NOY260), using a gap repair method as described in Materials and Methods. Comparison of the wild-type sequence with the mutant sequence showed that the suppressor mutation (*SRP1-1*) involves a base substitution (C to A) at nucleotide position 656, which corresponds to an amino acid

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-450                -430                -410
GTTAACTTAATCGACCGAATTAATTCAATAGTAATCAATTCCAATGCACCGAAGAGTTTG
-390                -370                -350
CCAGTCGAACTATCTAAGTGATACTTGTCCATTTTTCCTCGAAGCGCAAAAGAGATTACA
-330                -310                -290
TCACCATTAACGAATTTTCTTCTTTTCTTTTTTCTTCAAGAGTTGATGGGGAAGGACTAG
-270                -250                -230
GAAGGAAACGGTTCAGATACGAATTTAGTGATTAATCATATTCTGCTCAAGAAGCGACTT
-210                -190                -170
GTAAGGTAAGGAAAACCCGCATTCACTGACGGAATAAGGAACAGGACCAACTATAAGCCT
-150                -130                -110
CCTTTTACACTCACATCTTCAGGCTTTATTTTGTTAAGACTGAAACATTTCCCACTTAAT
 -90                 -70                 -50
TGAAGTTACATCGTTATTTTATAGAACGATCTTTCTATTTTGTTTTTTGTTCACTTGATA
 -30                 -10    Sau3AI
TACCTTCCCTTCTACATAACAAATTAATAAAT
          10                  30                  50
ATGGATAATGGTACAGATTCTTCCACGAGCAAGTTCGTTCCCGAATATAGACGAACAAAC
 1 M  D  N  G  T  D  S  S  T  S  K  F  V  P  E  Y  R  R  T  N
          70                  90                 110
TTTAAGAATAAAGGCAGATTCTCTGCAGATGAACTTCGTCGTCGTAGAGATACACAACAG
21 F  K  N  K  G  R  F  S  A  D  E  L  R  R  R  R  D  T  Q  Q
         130                 150                 170
GTCGAATTAAGAAAAGCAAAAAGAGATGAAGCTTTGGCCAAAAGAAGAAACTTTATTCCC
41 V  E  L  R  K  A  K  R  D  E  A  L  A  K  R  R  N  F  I  P
         190                 210                 230
CCAACTGATGGCGCTGATTCTGATGAAGAAGATGAGAGCTCCGTTTCTGCGGACCAACAA
61 P  T  D  G  A  D  S  D  E  E  D  E  S  S  V  S  A  D  Q  Q
         250                 270                 290
TTTTACAGCCAGTTGCAGCAAGAACTACCACAAATGACTCAGCAACTTAACTCTGATGAT
81 F  Y  S  Q  L  Q  Q  E  L  P  Q  M  T  Q  Q  L  N  S  D  D
         310                 330                 350
ATGCAAGAGCAATTGAGTGCTACTGTTAAGTTTAGACAAATTTTGTCTAGAGAACACCGC
101 M  Q  E  Q  L  S  A  T  V  K  F  R  Q  I  L  S  R  E  H  R
         370                 390                 410
CCTCCAATTGATGTCGTCATTCAAGCCGGTGTTGTTCCAAGATTAGTAGAATTTATGCGT
121 P  P  I  D  V  V  I  Q  A  G  V  V  P  R  L  V  E  F  M  R
         430                 450                 470
GAAAACCAACCTGAAATGTTACAATTGGAGGCTGCTTGGGCTTTGACTAACATTGCATCA
141 E  N  Q  P  E  M  L  Q  L  E  A  A  W  A  L  T  N  I  A  S
         490                 510                 530
GGTACATCTGCTCAAACAAAAGTGGTTGTTGATGCTGACGCTGTACCTCTTTTCATTCAA
161 G  T  S  A  Q  T  K  V  V  V  D  A  D  A  V  P  L  F  I  Q
         550                 570                 590
CTATTATATACCGGCTCCGTTGAAGTTAAAGAACAAGCCATTTGGGCCTTAGGTAACGTT
181 L  L  Y  T  G  S  V  E  V  K  E  Q  A  I  W  A  L  G  N  V
         610                 630                 650     *
GCAGGTGATTCAACTGACTACAGAGACTACGTTTTACAATGTAATGCCATGGAGCCAATT
201 A  G  D  S  T  D  Y  R  D  Y  V  L  Q  C  N  A  M  E  P  I
         670                 690                 710
TTGGGTCTTTTTAACTCCAATAAACCATCTTTGATCAGGACCGCTACGTGGACTTTATCC
221 L  G  L  F  N  S  N  K  P  S  L  I  R  T  A  T  W  T  L  S
         730                 750                 770
AATTTATGCAGGGGTAAAAAACCACAACCAGATTGGTCAGTGGTCTCACAAGCGTTGCCA
241 N  L  C  R  G  K  K  P  Q  P  D  W  S  V  V  S  Q  A  L  P

          790                 810                 830
ACCTTAGCGAAATTAATCTATTCGATGGACACTGAAACTTTAGTTGATGCTTGTTGGGCT
261 T  L  A  K  L  I  Y  S  M  D  T  E  T  L  V  D  A  C  W  A
          850                 870                 890
ATCTCTTATCTATCTGACGGACCACAAGAAGCTATTCAAGCAGTGATCGATGTTAGAATT
281 I  S  Y  L  S  D  G  P  Q  E  A  I  Q  A  V  I  D  V  R  I
          910                 930                 950
CCTAAAAGACTTGTTGAATTACTGAGCCATGAATCGACTTTAGTCCAGACTCCTGCTTTA
301 P  K  R  L  V  E  L  L  S  H  E  S  T  L  V  Q  T  P  A  L
          970                 990                1010
AGAGCTGTAGGTAATATAGTCACTGGTAATGACTTACAGACTCAGGTCGTTATAAATGCT
321 R  A  V  G  N  I  V  T  G  N  D  L  Q  T  Q  V  V  I  N  A
         1030                1050                1070
GGTGTCTTACCTGCGTTAAGACTTCTGCTAAGCTCTCCAAAAGAAAATATCAAGAAAGAA
341 G  V  L  P  A  L  R  L  L  L  S  S  P  K  E  N  I  K  K  E
         1090                1110                1130
GCGTGTTGGACCATTTCCAATATTACGGCTGGTAATACTGAACAAATTCAAGCGGTAATT
361 A  C  W  T  I  S  N  I  T  A  G  N  T  E  Q  I  Q  A  V  I
         1150                1170                1190
GACGCGAACTTGATTCCTCCATTAGTTAAACTTTTGGAAGTTGCAGAATATAAAACTAAA
381 D  A  N  L  I  P  P  L  V  K  L  L  E  V  A  E  Y  K  T  K
         1210                1230                1250
AAAGAAGCTTGTTGGGCTATTTCCAATGCCTCTTCAGGTGGTTTACAAAGACCAGATATC
401 K  E  A  C  W  A  I  S  N  A  S  S  G  G  L  Q  R  P  D  I
         1270                1290                1310
ATAAGATATTTAGTATCTCAAGGGTGTATAAAACCATTGTGTGATTTGCTAGAAATTGCT
421 I  R  Y  L  V  S  Q  G  C  I  K  P  L  C  D  L  L  E  I  A
         1330                1350                1370
GACAACAGAATAATTGAAGTTACCTTAGATGCTCTTGAAAATATTTTAAAGATGGGTGAA
441 D  N  R  I  I  E  V  T  L  D  A  L  E  N  I  L  K  M  G  E
         1390                1410                1430
GCTGACAAAGAAGCTCGTGGTTTGAATATCAATGAAAATGCCGATTTTATCGAAAAGGCT
461 A  D  K  E  A  R  G  L  N  I  N  E  N  A  D  F  I  E  K  A
         1450                1470                1490
GGTGGTATGGAAAAGATTTTCAACTGTCAACAAAATGAAAATGACAAGATTTATGAAAAA
481 G  G  M  E  K  I  F  N  C  Q  Q  N  E  N  D  K  I  Y  E  K
         1510                1530                1550
GCATACAAAATCATTGAAACCTACTTTGGTGAAGAAGAAGACGCCGTAGACGAAACTATG
501 A  Y  K  I  I  E  T  Y  F  G  E  E  E  D  A  V  D  E  T  M
         1570                1590                1610
GCTCCACAAAATGCCGGTAATACTTTCGGCTTTGGTTCTAATGTCAACCAACAATTCAAT
521 A  P  Q  N  A  G  N  T  F  G  F  G  S  N  V  N  Q  Q  F  N

TTTAAC
541 F  N
                    1650                1670
TAACTGAATGTGAAGAAAAAGATCAACTAAATAAGTTTATTCAGATAAATCTTCATTATC
1690                1710                1730
TGATCCATTATTTTTAAGTTTTCTTTTAATGTATATAAGAAATTCTAATCTTTTAATTC
1750                1770                1790
GCACAATAGTATTTTCATGTACTAGTTCGTACAGTATTCTCTTTCTCTGGGGTTTTTCCC
1810                1830                1850
TTTTATAGAAGTTGACTTGTTCTATAGTTGTTATATATGTATTGCTGGGTTTTTTAATCT
1870                1890                1910
ATTTTGCGGAAGACGCCAAAAATAAGAAAAAAATTGGAAAGACACAAGAAAAAAGTAAGA
1930
ATATCCACAGCTGAAAGATCT
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FIG. 4. Nucleotide and predicted amino acid sequences of *SRP1*. The coding sequence starts at nucleotide 1 and ends at nucleotide 1626. The nucleotide (656) altered in *SRP1-1* (C→A) is indicated by an asterisk, and the transcription start sites (A at −205 and −204) determined by S1 mapping (data not shown) are underlined. The *Sau*3AI site (−65 to −62) used for fusion to the *GAL7* promoter is also underlined.

substitution (proline to glutamine) at amino acid position 219 (Fig. 4), demonstrating that the suppressor mutation is in fact in this open reading frame.

Comparison of the deduced amino acid sequence of *SRP1* with sequences in the GenBank and EMBL data bases by using the FASTA or TFASTA program (10) failed to reveal any known protein with significant sequence similarity. However, it was found that the nucleotide sequence of the 3'-end region (545 bp) of the *Hpa*I-*Bgl*II fragment is 95% identical to the published nucleotide sequence of a region upstream of the *KAR1* gene (26). Small differences (26 of 545 bases) observed between the sequence presented here and the published sequence could be due to the difference of strains used for cloning. We conclude that *SRP1* is located upstream of *KAR1*, the distance between the two genes (coding regions) is 420 bp, and both genes are transcribed in the same direction (Fig. 3A). By separating intact chromosomal DNA by orthogonal field alteration gel electrophoresis followed by Southern analysis, we localized *SRP1* on chromosome XIV (data not shown). This result is consistent with the conclusion presented above on the location of *SRP1*, since *KAR1* was previously mapped on chromosome XIV (26).

Regarding the deduced amino acid sequence of SRP1 protein, a computer program (Genetic Computer Group program COMPARE [10]) as well as visual inspection revealed the presence of eight (degenerate) 42-amino-acid tandem repeats in the middle of the molecule (Fig. 5). These repeats are contiguous, and only the first and last repeats (43 and 45 amino acids, respectively) differ from the consensus length of 42 amino acids. Well-conserved residues in the repeats are clustered in two regions which are relatively rich in hydrophobic amino acids (Fig. 5B; see also the hydrophobicity plot in Fig. 5C). It should be noted that the consensus sequence given in Fig. 5B has been defined in a somewhat arbitrary way (see the legend to Fig. 5) and that similarity can be recognized in other sequences among subsets of repeats. For example, the sixth and seventh repeats share the sequence KKEACW (358 to 363 and 400 to 405), which contains only two residues (A and W) listed among those in the consensus sequence. Thus, the central region consists of the eight repeated segments of almost identical size and probably similar structure. Surrounding this central region, which spans positions 123 to 462, there are several clusters of acidic or basic amino acids, making both the N- and C-terminal regions highly hydrophilic (Fig. 5C). For example, there are 16 basic amino acids, including 4 contiguous arginines (and 6 acidic amino acids) in the region covering 40 amino acids (positions 17 to 56), making this particular region highly basic. However, the molecule as a whole is quite acidic, having a calculated isoelectric point of 4.63. The proline altered by the suppressor mutation (*SRP1-1*) described above is present in the third repeat (proline with an asterisk at position 219 in Fig. 5B). Although not at exactly the same position, there is proline residue around this position in each repeat. The change from proline to glutamine is expected to cause a significant alteration in the local secondary structure, but the significance of this mutational alteration as well as that of the unique repeat structure must await further studies (see Discussion).

***SRP1* is an essential gene.** A deletion of the cloned *SRP1* gene was created by replacing the *Pst*I-*Eco*RV DNA fragment in the middle of *SRP1* with a DNA fragment carrying *LEU2* (Fig. 3B). The *Hpa*I-*Bgl*II fragment carrying *srp1*::*LEU2* (Fig. 3B) was then transformed into three different diploid strains (*SRP1 leu2/SRP1 leu2*; e.g., NOY397), and

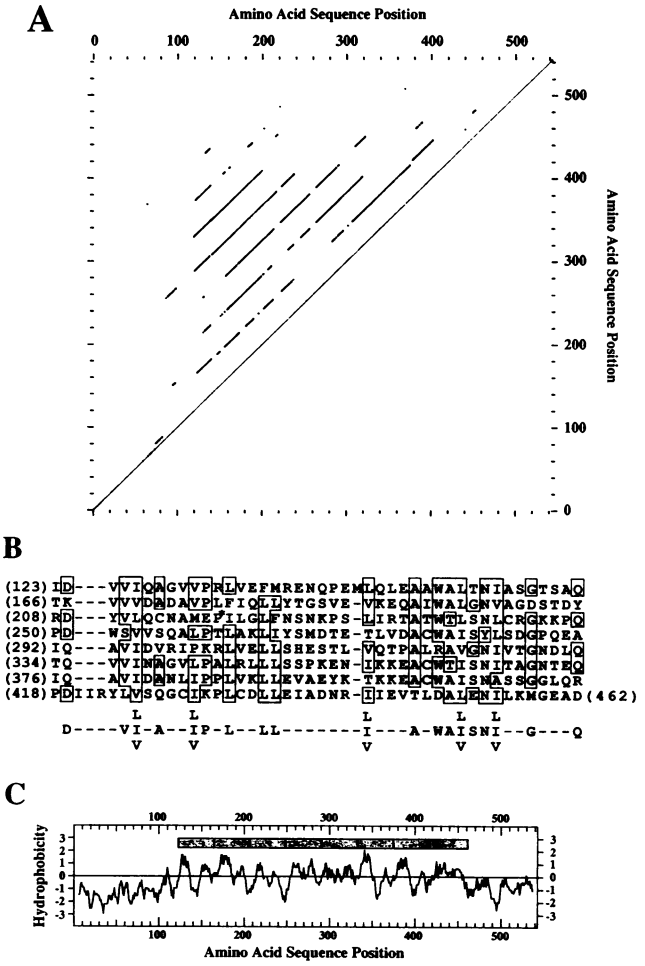

FIG. 5. Repeating amino acid sequences in SRP1. (A) Dot matrix analysis of the SRP1 sequence against the SRP1 sequence. The amino acid residue numbers are given on both vertical and horizontal axes. The dot matrix plot was prepared by using the Genetics Computer Group program COMPARE with a window of 30 and a stringency of 15. The parallel lines indicate the possible presence of eight similar tandemly repeated sequences. (B) Comparison of the aligned tandemly repeated sequences in SRP1. Any amino acid found at least four times at the same position is included in the consensus sequence shown at the bottom. In addition, any leucine, isoleucine, or valine that appears more than four times at the same position is included in the consensus sequence as LIV. The number in parentheses at the beginning of each repeat indicates the position of the first amino acid in the repeat. The position of the last amino acid in the last repeat is also indicated. (C) Hydrophobicity pattern of SRP1, analyzed as described by Kyte and Doolittle (19). The positions of the eight repeats shown in panel B are indicated as boxes.

Leu^+ transformants were selected (e.g., NOY476). These Leu^+ diploids were then sporulated, and altogether 34 tetrads were dissected and analyzed. In 30 cases, only two viable spores were recovered from each tetrad; in the remaining 4 cases, only one viable spore was recovered. All of the viable spores were Leu^-. These results show that *SRP1* is essential for germination and/or growth.

Cellular localization of *SRP1*. We constructed a *lacZ-SRP1* fusion gene, expressed it in *E. coli*, and purified the fusion protein as described in Materials and Methods. This fusion protein contains the C-terminal domain (from lysine at

position 457 to the C-terminal end) of the SRP1 protein. Specific polyclonal antibodies (anti-SRP1 antibodies) against this fusion protein were then prepared and affinity purified. We first confirmed that the antibodies thus prepared detected a single protein species with an apparent molecular weight of 67,000, which is close to the calculated molecular weight of SRP1 (60,403; see above) in Western blot analysis of crude yeast lysates, demonstrating specificity of the antibodies (data not shown; see Fig. 7 and 8). We used the antibodies to determine the cellular location of SRP1 protein in normal yeast cells by immunofluorescence microscopy. Contrary to our initial expectation from the genetic interaction of SRP1 with Pol I, the anti-SRP1 antibodies showed punctate staining at the periphery of the nucleus (Fig. 6A) rather than staining of the nucleolus where Pol I is localized (for an example of the crescent-shaped nucleolar structure, see reference 7). (Appropriate controls, such as preimmune or anti-β-galactosidase sera, did not show the staining observed with the anti-SRP1 antibodies.) Since the punctate pattern was similar to that observed with antibodies against components of the nuclear pore complex, NUP1 (9) or NSP1 (22), we compared the pattern obtained with anti-SRP1 antibodies directly with that obtained with MAb 306, which was used by Davis and Fink (9) to visualize NUP1 protein. As can be seen in Fig. 6B and C, double-labeling immunofluorescence microscopy showed that most of the dots stained with anti-SRP1 were also stained with MAb 306. It appears that SRP1 is preferentially localized at or near the nuclear pore defined by the NUP1 protein. However, we stress that this conclusion is based on low-resolution immunofluorescence microscopy, and precise localization must await immunoelectron microscopic studies. Regardless of this uncertainty, one can conclude that SRP1 is localized mainly in certain regions of the nuclear periphery, probably in close association with the nuclear envelope.

Localization of SRP1 protein was also studied by biochemical fractionation. Yeast spheroplast lysates were fractionated by Ficoll step gradient centrifugation into nuclei, the low- and high-density membrane fractions, and the soluble fraction. Analysis of these fractions by SDS-gel electrophoresis followed by immunoblotting showed that SRP1 protein is present mostly in the nuclear fraction (Fig. 7A). Purified nuclei were further fractionated as described by Allen and Douglas (1) for analysis of the protein composition of nuclear subfractions. The nuclei were extracted sequentially with heparin, nonionic detergent, and high salt. Immunoblotting showed that SRP1 was found in both heparin-soluble and residual (called matrix-lamina-pore complex by Allen and Douglas [1]) fractions (Fig. 7B). A significant enrichment of SRP1 (relative to total protein) in this residual fraction was observed with some preparations of nuclei but not with other preparations. We estimate that on average, about 20% of SRP1 was in the matrix-lamina-pore complex fraction and the remainder was mostly released by heparin (see the legend to Fig. 7). In contrast, fibrillarin, a nucleolar protein known to be complexed with nucleolar small RNA (15, 31), was almost entirely in the heparin-soluble fraction (Fig. 7B). Pol I was found both in heparin-soluble and residual fractions, but compared with SRP1, it was more abundant in the heparin-soluble fraction (Fig. 7B and data not shown). Regarding distribution of nuclear pore proteins, we carried out Western blot analysis with MAb 414 and found that, like SRP1, a majority of the nuclear pore proteins (100-kDa proteins) recognized by this antibody (2, 9) was released by heparin, but quantitative comparison of this protein and SRP1 with respect to distribution among various subnuclear fractions was difficult mainly for technical reasons. Thus, the results of biochemical fractionation confirmed that SRP1 is localized in the nucleus but failed to give a definitive answer with respect to the subnuclear localization of SRP1. Nevertheless, the results are in general consistent with the conclusion obtained from immunofluorescence microscopy, namely, that SRP1 is a component of or associated with the nuclear envelope.

We then examined whether SRP1 is complexed with Pol I when the two proteins are released from large cellular structures. In addition, we wished to determine whether SRP1 exists as a monomer with a molecular mass of 60,000 to 65,000 Da (see above) in crude extracts. Yeast cells were disrupted by shaking with glass beads and then centrifuged to yield soluble and particulate fractions. By immunoblot analysis, we found that about half of Pol I and half of SRP1 were in the soluble fraction and that the remaining halves were in the particulate fraction. Aliquots of the soluble fraction were then subjected to glycerol gradient centrifugation, and the sedimentation properties of Pol I and SRP1 were compared by immunoblotting each fraction with antibodies against Pol I and SRP1. As a size reference marker, the position of yeast mitochondrial malate dehydrogenase (M_r 66,000 [38]) was also determined by using antibodies against this enzyme. The results shown in Fig. 8 indicate that the majority of SRP1 sedimented faster than the reference malate dehydrogenase and that the SRP1-containing materials were heterogeneous in size. In fact, a major fraction of SRP1 sedimented faster than Pol I (M_r 620,000) and was found in the pellet fraction, although a considerable portion of SRP1 also sedimented more slowly than Pol I. Thus, we failed to obtain positive evidence for a complex containing both Pol I and SRP1. The striking heterogeneity of sedimentation rates of SRP1-containing materials suggests that SRP1 might be associated with structures, possibly nuclear envelope fragments, but further studies are required to identify components associated with SRP1. We also synthesized [^{35}S]methionine-labeled SRP1 by using a reticulocyte in vitro protein-synthesizing system and RNA prepared from the cloned gene as a template, mixed it with nonradioactive Pol I, and then immunoprecipitated Pol I. No coprecipitation of radioactive SRP1 was observed (data not shown). We conclude that the majority of SRP1 is in large complexes or structures and does not exist as a monomer in the extracts and that the interaction of SRP1 with Pol I, if it exists in vitro, is probably weak and requires other components normally associated with SRP1 (or Pol I or both).

Presence of proteins immunologically related to SRP1 in other organisms. As the first step to study proteins related to SRP1 in other organisms, we examined proteins from HeLa cells and the yeast *Schizosaccharomyces pombe*. Total protein samples were subjected to SDS-gel electrophoresis followed by Western blotting with anti-SRP1 antibodies directed to the C-terminal domain of SRP1 (see above). We detected a single protein species in each organism that cross-reacted with the antibodies. Appropriate control experiments showed that the proteins detected are related to SRP1 and not to *E. coli* β-galactosidase (data not shown). Thus, it is possible that proteins homologous to SRP1 exist in other eukaryotic organisms.

DISCUSSION

Suppression of Pol I ts mutations by *SRP1* mutations. We found that a single amino acid substitution in SRP1 suppresses a certain subset of Pol I ts mutations. Two of four

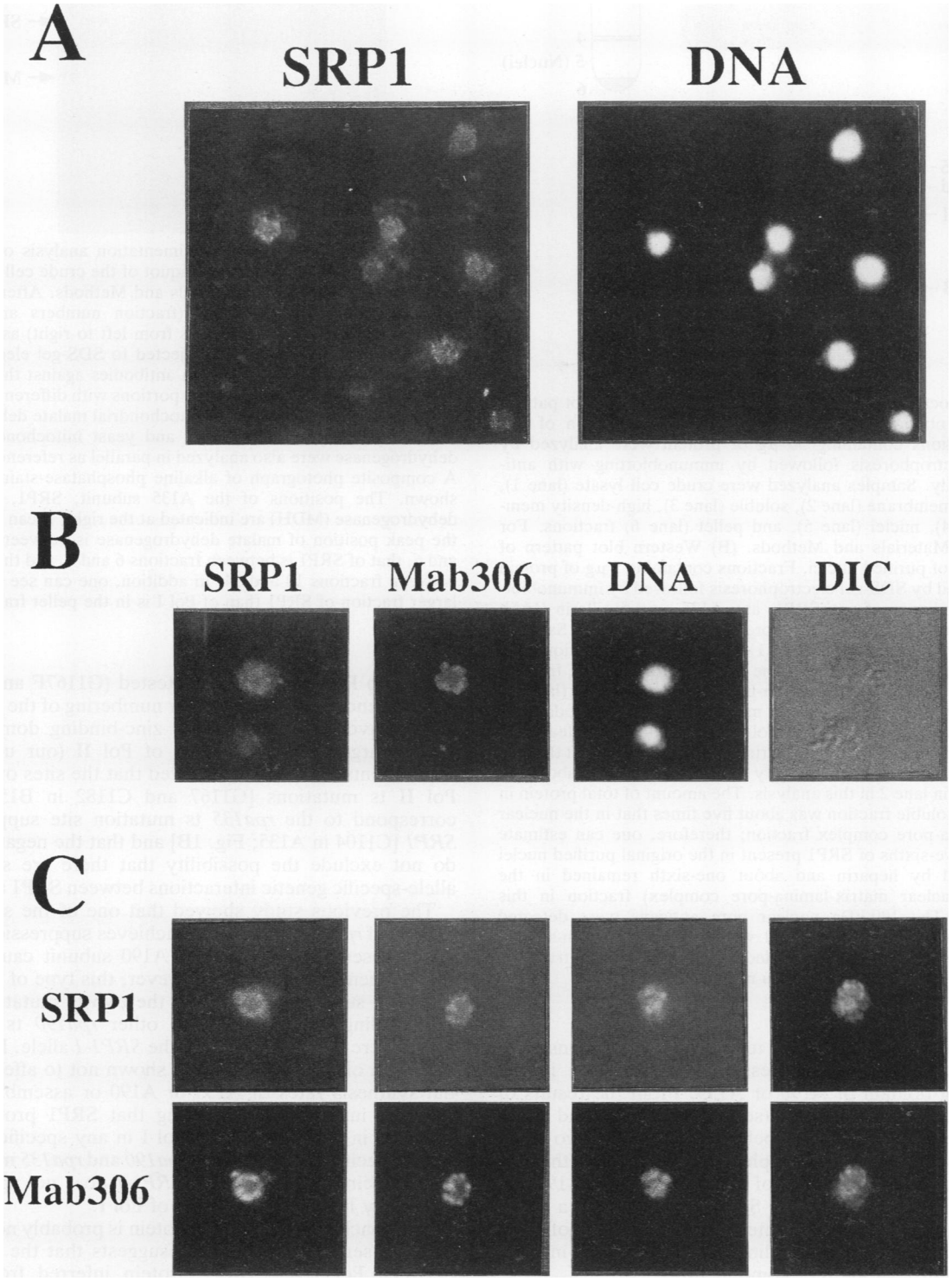

FIG. 6. Punctate staining of the nuclear envelope by immunofluorescence, using antibody against SRP1. (A) Localization of SRP1 by immunofluorescence (fluorescein) and of DNA (DAPI staining) in NOY397. (B and C) Double-label immunofluorescence of SRP1 (fluorescein) and NUP1 (MAb 306 antibody; rhodamine). In panel B, DAPI staining of DNA and differential interference contrast (DIC) pictures of the same cells are also shown. For the rightmost sample in panel C, diploid strain NOY397 was used; for all other samples, strain w303-1a was used. For more details, see text.

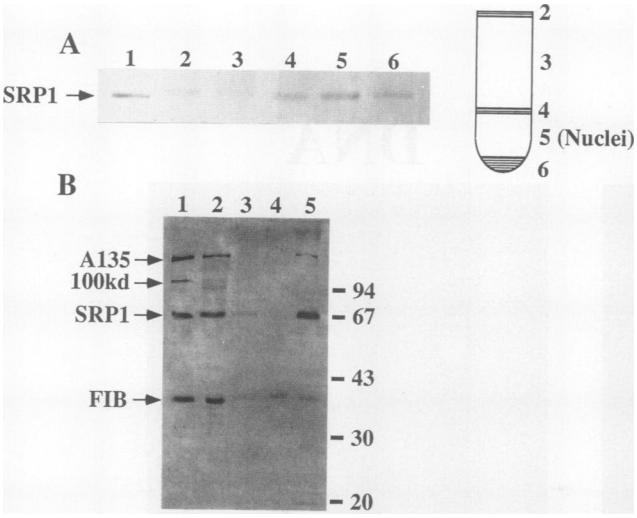

FIG. 7. Localization of SRP1 protein. (A) Western blot pattern of fractions obtained after Ficoll gradient centrifugation of cell lysate. Fractions containing 30 μg of protein were analyzed by SDS-gel electrophoresis followed by immunoblotting with anti-SRP1 antibody. Samples analyzed were crude cell lysate (lane 1), low-density membrane (lane 2), soluble (lane 3), high-density membrane (lane 4), nuclei (lane 5), and pellet (lane 6) fractions. For details, see Materials and Methods. (B) Western blot pattern of subfractions of purified nuclei. Fractions containing 5 μg of protein were analyzed by SDS-gel electrophoresis followed by immunoblotting with a mixture of anti-SRP1, anti-A135, anti-fibrillarin (MAb A66), and anti-100-kDa nuclear pore protein (MAb 414). Samples analyzed were purified nuclei (lane 1), heparin-soluble fraction (lane 2), detergent-soluble fraction (lane 3), high-salt-soluble fraction (lane 4), and nuclear matrix-lamina-pore complex fraction (lane 5). The positions of molecular weight marker proteins (in kilodaltons) are shown at right, and those of Pol I A135, 100-kDa nuclear pore antigens (100kd), SRP1, and fibrillarin (FIB) are indicated at the left. It should be noted that the intensity of SRP1 in lane 5 is about the same as that in lane 2 in this analysis. The amount of total protein in the heparin-soluble fraction was about five times that in the nuclear matrix-lamina-pore complex fraction; therefore, one can estimate that about five-sixths of SRP1 present in the original purified nuclei was released by heparin and about one-sixth remained in the insoluble (nuclear matrix-lamina-pore complex) fraction in this experiment. The 100-kDa nuclear pore antigens were detected clearly in the original nuclei and weakly in the heparin-soluble fraction. The reason why we failed to find subnuclear fractions enriched in the 100-kDa proteins is not known.

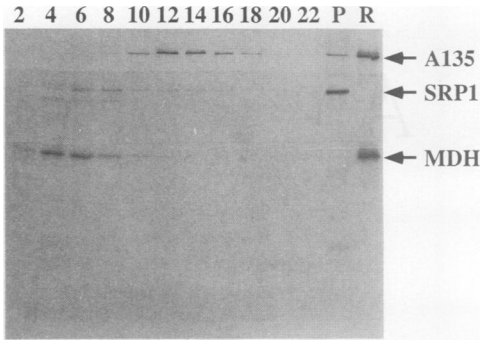

FIG. 8. Glycerol gradient sedimentation analysis of SRP1 and Pol I in crude cell extracts. An aliquot of the crude cell extract was analyzed as described in Materials and Methods. After centrifugation, even-numbered fractions (fraction numbers are indicated above the lanes; sedimentation is from left to right) as well as the pellet fraction (lane P) were subjected to SDS-gel electrophoresis followed by immunoblotting with antibodies against the A135 subunit of Pol I and SRP1 (upper two portions with different developing times) and those against yeast mitochondrial malate dehydrogenase (bottom portion). Purified Pol I and yeast mitochondrial malate dehydrogenase were also analyzed in parallel as references (lane R). A composite photograph of alkaline phosphatase-stained filters is shown. The positions of the A135 subunit, SRP1, and malate dehydrogenase (MDH) are indicated at the right. It can be seen that the peak position of malate dehydrogenase is between fractions 4 and 6, that of SRP1 is between fractions 6 and 8, and that of Pol I is between fractions 12 and 14. In addition, one can see that a much larger fraction of SRP1 than of Pol I is in the pellet fraction.

rpa190 ts mutations and two of ten *rpa135* ts mutations were suppressed; all of the suppressible mutations lie in the zinc-binding domain of A190 or A135. From the results of genetic suppression analysis (see introduction) and other considerations (29, 49), we believe that these two zinc-binding domains are in direct physical contact and that the observed specific suppression of mutations in both A190 and A135 zinc-binding domains by SRP1 reflects either a direct contact between the SRP1 protein and the regions of Pol I comprising these two zinc-binding domains or some indirect interaction through another component(s).

Although we cannot completely eliminate an (unlikely ad hoc) explanation that the SRP1 mutation somehow increases the cellular concentration of zinc, no phenotypic suppression of the two *rpa190* mutations in the A190 zinc-binding domain was observed with externally added zinc salts (unpublished data cited in reference 45). In addition, we have found that the *SRP1-1* suppressor mutation does not suppress two Pol II ts mutations tested (G1167F and C1182A; see reference 43 and Fig. 1B for numbering of the sequence), which have alterations in the zinc-binding domain of the second-largest subunit (B150) of Pol II (our unpublished experiments). (It should be noted that the sites of these two Pol II ts mutations [G1167 and C1182 in B150] do not correspond to the *rpa135* ts mutation site suppressed by *SRP1* [C1104 in A135; Fig. 1B] and that the negative results do not exclude the possibility that there are some other allele-specific genetic interactions between SRP1 and Pol II.)

The previous study showed that one of the suppressors (*SRP5*) of *rpa190* ts mutations achieves suppression because of increased synthesis of the A190 subunit caused by increased gene dosage (21). However, this type of suppressor mutation suppressed not only the two ts mutations in the zinc-binding domain but also other *rpa190* ts mutations which were not suppressed by the *SRP1-1* allele. In addition, depletion of SRP1 protein was shown not to affect differential synthesis rates of A135 or A190 or assembly of these subunits into Pol I, suggesting that SRP1 protein is not involved in the synthesis of Pol I in any specific way (50). Thus, specific suppression of *rpa190* and *rpa135* mutations in the zinc-binding domains by *SRP1-1* does not appear to be caused by increased synthesis of Pol I.

The conclusion that SRP1 protein is probably not involved in the assembly of Pol I also suggests that the interaction between Pol I and SRP1 protein inferred from genetic suppression data is probably not a transient interaction during assembly but rather an interaction which takes place after the synthesis of Pol I, either during its function or during its resting time in the pool as a nonfunctioning enzyme or both. It was previously concluded that the primary defect of *rpa190-1* and *rpa190-5* (which are suppressible by the *SRP1* suppressor mutation identified in this

study) is in assembly and/or instability of the assembled enzyme (21). Thus, it now appears that the defect in these mutants is instability of the enzyme and that the mutational alteration in SRP1, which is proposed to interact with the mutant forms of Pol I directly or indirectly, improves their stability. For example, one can imagine that Pol I interacts with a structure containing SRP1 through a region comprising the zinc-binding domains of A190 and A135, that mutations in the zinc-binding domains make the interaction weaker, rendering Pol I more susceptible to proteolytic degradation, and that the *SRP1* mutations somehow strengthen the interaction between Pol I and the structure containing SRP1.

Structural features of SRP1 protein. The amino acid sequence of SRP1 deduced from the nucleotide sequence of the gene reveals that the protein consists of three regions. Both the N- and C-terminal regions are highly hydrophilic, containing clusters of acidic and basic amino acids. The central region consists of eight 42-amino-acid tandem repeats, each containing exactly 42 amino acids except for the first and the last repeats, which contain 43 and 45 amino acids. The presence of contiguous tandem repeats is reminiscent of similar structural features recently recognized in other proteins. For example, erythrocyte ankyrin consists of three domains, and the N-terminal domain is almost entirely composed of 22 tandem repeats of exactly 33 amino acids each except for one with 29 amino acids (20). This domain binds to an integral membrane protein. It was suggested that erythrocyte ankyrin, like other members of the ankyrin family of proteins and other proteins possessing ankyrin-like repeats, mediates interaction between integral membrane components and cytoskeletal elements (reference 20 and references cited therein). Other recently discovered examples of contiguous tandem repeats employ the 34-amino-acid TPR motif which is repeated 9 times in the *CDC23* gene in *S. cerevisiae* (35) and 10 times in the *nuc2*$^+$ gene in *S. pombe* (17) and is also repeated in other proteins (reviewed in reference 13). In the case of *nuc2*$^+$, which is required for mitotic chromosome disjunction, the protein was found to be present in the nuclear scaffold/matrix-like fraction (16), and it was suggested that the TPR domains interact with each other or with other proteins to form a part of the chromosome scaffold (16, 47).

Although we do not find primary sequence similarity between the 43-amino-acid repeats in SRP1 and the 34-amino-acid TPR repeats or the ankyrin repeats, the central domain containing the repeats in SRP1 may also be involved in protein-protein interactions, as demonstrated or implicated in these previous cases. As in the case of the TPR repeats, well-conserved amino acid residues in the SRP1 repeats are apparently clustered in two regions in a repeat which are rich in hydrophobic amino acids. Thus, it is possible that these two regions participate in intra- and/or intermolecular interactions as suggested for the TPR repeat (13, 16). Although we do not know the secondary structure of each repeat and cannot make any specific suggestion about the structure of the central region, it is interesting to note that the suppressor mutation involves the change from proline to glutamine at the position adjacent to the conserved proline position in the repeats (Fig. 5B); therefore, the mutation is expected to cause a significant change in local secondary structures that may affect the suspected intra- and/or intermolecular interactions (or interactions with other proteins) of the repeated regions.

As discussed below, SRP1 is probably associated with the nuclear envelope and plays an important role in maintenance of the nucleolar structure. Thus, it is tempting to speculate that the N-terminal (and/or C-terminal) domain interacts with highly charged molecules, possibly with nucleic acids (perhaps DNA including rRNA genes), and the central domain participates in the interaction with other SRP1 protein molecules or other nuclear proteins, perhaps components of the skeleton/matrix, thus forming a large nuclear structure which is required for maintenance of the nucleolar structure and function.

Subnuclear localization of SRP1 and Pol I. Immunocytochemical studies show that SRP1 protein is mostly localized at the nuclear periphery, apparently concentrated in regions at or near the nuclear pore complex as visualized by specific antibodies that react with NUP1 protein (9), a previously characterized nuclear pore component. In contrast, a similar immunochemical method showed that Pol I is localized in the crescent nucleolar structure. Thus, the conclusion on SRP1 localization is in apparent conflict with the proposed (direct or indirect) interaction between Pol I and SRP1 discussed above. One possibility is that although SRP1 is mainly concentrated in regions associated with the nuclear envelope, SRP1 also exists in the nucleoplasm. It might be that the proposed interaction takes place in the nucleolus and that SRP1 observed at the nuclear envelope does not participate in the interaction. Alternatively, it is possible that only a portion of Pol I interacts with SRP1 at the periphery of the nucleus and that the remaining Pol I in the nucleolus is in a different state. Such a differentiation in location (and state) may reflect a differentiation in function. In this connection, it should be noted that in higher eukaryotes, Pol I has been shown by immunocytochemistry to be present more or less uniformly in the fibrillar center (30). However, the results of autoradiographic studies using [^{3}H]uridine pulse have indicated that the site of rRNA transcription is at the periphery of the fibrillar center and/or at the adjacent dense fibrillar component (for reviews, see references 14, 18, and 37). Thus, one could speculate that Pol I function takes place at the nuclear periphery in association with a structure containing SRP1 protein and that the interaction with SRP1 in this region may be important in relation to the stability of Pol I. As mentioned in Results, we have observed that a significant fraction of Pol I is in fact tightly associated with the insoluble matrix-lamina-pore complex (Fig. 7B). The presence of two different states of Pol I was also previously reported for mammalian cells by Dickinson et al.; Pol I tightly associated with the nuclear skeleton/matrix comprises most of the actively functioning Pol I, while nonfunctioning Pol I does not have such association (11; see also a review in reference 8).

Regardless of the exact location and the nature of the interaction of SRP1 with Pol I, SRP1 appears to play an important structural role in maintenance of the crescent nucleolar structure. This conclusion was obtained by using a strain in which the production of SRP1 was controlled by the *GAL7* promoter; depletion of SRP1 by transfer from galactose to glucose media caused striking morphological changes of the nucleolus as examined by 4′,6-diamidino-2-phenylindole (DAPI) staining and immunofluorescence staining with antibodies against nucleolar proteins (50). In addition, Pol I plays an important role in maintenance of the intact nucleolar structure (23a). Thus, it is possible that the proposed interaction of SRP1 with Pol I represents one of the key elements used to construct the complex nucleolar structure in the yeast *S. cerevisiae*.

In conclusion, we have cloned and sequenced the *SRP1* gene, a specific suppressor of ts mutations in the zinc-

binding domains of the A190 and A135 subunits of Pol I. The gene product, SRP1 protein, appears to have interesting structural features, perhaps reflecting its structural role as a component of a larger macromolecular complex associated with the nuclear envelope. While genetic evidence strongly suggests a specific (direct or indirect) interaction of SRP1 with Pol I, immunofluorescence microscopy does not show exact colocalization of these two proteins. This apparent contradiction is a challenge for future studies on this subject. If there is in fact an SRP1 homolog in higher eukaryotic cells as suggested from the positive immunochemical cross-reaction obtained with HeLa cell protein extracts, more detailed cytochemical studies could be carried out by using such cells with the larger nucleus to study this problem. In addition, the availability of the cloned *SRP1* gene has made it possible to isolate ts mutations in *SRP1* (our unpublished experiments) and then analyze their suppressors. Such genetic approaches combined with more direct biochemical studies of SRP1 and supramolecular complexes containing SRP1 should be useful for elucidating the nature of the proposed SRP1-Pol I interaction and the exact role of SRP1 in maintenance of the intact nucleolar structure.

ACKNOWLEDGMENTS

We thank A. Sentenac for the Pol II ts mutants used for our unpublished experiments cited in this report and for antibodies against A135, G. Blobel and J. P. Aris for MAbs 306, 414, and A66, J. Broach for antibodies against SSB1, L. McAlister-Henn and J. Steffan for antibodies against yeast mitochondrial malate dehydrogenase, and D. D. Cunningham for cultured HeLa cells. We also thank J. Keener, C. Greer, R. A. Bradshaw, L. E. Vickery, and S. Arfin for discussion and S. Pfleiger for help in preparation of the manuscript.

This work was supported by Public Health Service grant R37GM35949 from the National Institutes of Health.

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