Suppressor Analysis of Temperature-Sensitive Mutations of the Largest Subunit of RNA Polymerase I in *Saccharomyces cerevisiae*: a Suppressor Gene Encodes the Second-Largest Subunit of RNA Polymerase I

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The *SRP3-1* mutation is an allele-specific suppressor of temperature-sensitive mutations in the largest subunit (A190) of RNA polymerase I from *Saccharomyces cerevisiae*. Two mutations known to be suppressed by *SRP3-1* are in the putative zinc-binding domain of A190. We have cloned the *SRP3* gene by using its suppressor activity and determined its complete nucleotide sequence. We conclude from the following evidence that the *SRP3* gene encodes the second-largest subunit (A135) of RNA polymerase I. First, the deduced amino acid sequence of the gene product contains several regions with high homology to the corresponding regions of the second-largest subunits of RNA polymerases of various origins, including those of RNA polymerase II and III from *S. cerevisiae*. Second, the deduced amino acid sequence contains known amino acid sequences of two tryptic peptides from the A135 subunit of RNA polymerase I purified from *S. cerevisiae*. Finally, a strain was constructed in which transcription of the *SRP3* gene was controlled by the inducible *GAL7* promoter. When this strain, which can grow on galactose but not on glucose, was shifted from galactose medium to glucose medium, a large decrease in the cellular concentration of A135 was observed by Western blot analysis. We have also identified the specific amino acid alteration responsible for suppression by *SRP3-1* and found that it is located within the putative zinc-binding domain conserved among the second-largest subunits of eucaryotic RNA polymerases. From these results, it is suggested that this putative zinc-binding domain is in physical proximity to and interacts with the putative zinc-binding domain of the A190 subunit.

Each of the three nuclear eucaryotic RNA polymerases, RNA polymerases I, II, and III (or A, B, and C), is a complex, multisubunit enzyme consisting of 9 to 14 polypeptide subunits (for a review, see reference 35). In contrast, procaryotic RNA polymerases have a more simple structure consisting of three core subunits (β′, β, and α) and a specific initiation factor (σ). The intricate structure of the eucaryotic enzymes may reflect the complex requirements for their biosynthesis (including transport to the nucleus or nucleolus), as well as the regulation of their transcriptional activities.

Despite this difference in subunit complexity, significant structural and functional similarity exists between the procaryotic and eucaryotic polymerases. In particular, the three procaryotic core subunits appear to have homologs among subunits of eucaryotic enzymes. Significant amino acid sequence homology was found between the *Escherichia coli* β′ subunit and the largest subunits of eucaryotic RNA polymerase I (15, 25, 38, 46), II (2, 19, 39), and III (2, 38), based on sequence studies of the cloned genes encoding these subunit proteins from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Trypanosoma brucei*, and *Drosophila melanogaster*. The genes for the second-largest subunit of eucaryotic RNA polymerase II were also cloned from *S. cerevisiae* (40, 47) and *D. melanogaster* (11), and extensive amino acid sequence homology to the *E. coli* β subunit was demonstrated (11, 40). Since immunological studies of yeast RNA polymerases had indicated structural similarities among the second-largest subunits of RNA polymerases I, II, and III as well as among their largest

subunits (16), the second-largest subunits of RNA polymerases I and III are also expected to have homology to the *E. coli* β subunit. This expectation was recently confirmed for RNA polymerase III by James et al., who have cloned and sequenced the gene for the second-largest subunit of the polymerase from *S. cerevisiae* (16a). Finally, from limited amino acid sequence similarity, the third-largest subunit (RPB3) of *S. cerevisiae* RNA polymerase II has been suggested to be a homolog of the *E. coli* RNA polymerase α subunit (24, 45). On the basis of the comparison between the eucaryotic and procaryotic enzymes, it is expected that the major catalytic functions of the eucaryotic enzyme involve only a few of its many polypeptide subunits (see, e.g., the discussion on RNA polymerase II in references 40 and 45). Specifically, on the basis of extensive studies of the *E. coli* enzyme (for reviews, see references 9 and 48) and limited studies of eucaryotic polymerases (see, e.g., reference 31), it is almost certain that the two large subunits, the homologs of the β′ and β subunits, play the major role in the catalytic function of these polymerases, including binding to DNA templates, substrate binding, polymerization activity, and termination.

To study RNA polymerase I and its role in the regulation of rRNA synthesis, we have taken a genetic approach with *S. cerevisiae* as an experimental organism (24a, 43). Starting with temperature-sensitive *rpa190* mutants of *S. cerevisiae* carrying mutations in the gene (*RPA190*) for the largest subunit (A190) of RNA polymerase I (43), we have isolated extragenic suppressors (*SRP*) of these mutations (24a). In this paper, we describe the cloning and characterization of one of these suppressors, *SRP3*. We have found that the suppressor gene is a mutated form of the gene encoding the

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TABLE 1. Strains and plasmids used in this study

Yeast strain or plasmid	Description
Strains	
NOY259..................	*MATα rpa190-1 ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1*
NOY260..................	*MATα RPA190 ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1*
NOY265..................	*MATα rpa190-3 ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1*
NOY267..................	*MATα rpa190-5 ura3-52 his4-Δ401 leu2-3,112 trp1-Δ1*
NOY345..................	*MATα rpa190-5 ura3-52 SRP3-1*
NOY397..................	*MATa/MATα 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*
NOY398..................	*MATa/MATα 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 rpa135::LEU2/RPA135*
NOY399..................	*MATa ade2-1 ura3-11 trp1-1 can1-100 leu2-3,112 rpa135::LEU2* pNOY83 (*pGAL7-RPA135 URA3*)
NOY400..................	*MATa ade2-1 ura3-1 his3-11 trp1-1 can1-100 leu2-3,112 rpa135::LEU2* pNOY80 (*RPA135 URA3*)
w303-1a	*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100*
Plasmids	
YCp50..................	*E. coli*-yeast shuttle vector carrying *URA3* (32)
pRS316..................	*E. coli*-yeast shuttle vector carrying *URA3* and with the multicloning site from pBluescript KS(−) (37)
pNOY79	Derivative of YCp50 carrying *SRP3-1* on a cloned DNA fragment of about 9 kb derived from a partial *Sau*3AI digest of NOY345 DNA
pNOY80	Derivative of pRS316 carrying *srp3⁺* (*RPA135*) on a DNA fragment of about 7 kb (Fig. 4A)
pNOY83	Derivative of pRS316 carrying the *pGAL7-RPA135* fusion gene (Fig. 4B)
pNOY86	Derivative of pRS316 carrying the 1.8-kb *Eco*RI-*Xho*I fragment which is upstream from RPA135 and the ca. 0.7-kb *Nsi*I-*Eco*RV fragment which is downstream from RPA135 (Fig. 1C; see Materials and Methods)

second-largest subunit (A135) of RNA polymerase I. As expected, we found significant homology between A135 and the β subunit of *E. coli* RNA polymerase, as well as the second-largest subunits of eucaryotic RNA polymerases II and III. We have also identified the mutational alteration responsible for suppression of the *rpa190* mutations. The alteration was found to be in a putative zinc-binding domain close to the carboxyl terminus of the protein. This result suggests that in the holoenzyme this region of the A135 subunit is physically close to the site of the original *rpa190* mutations, which are also in a putative zinc-binding domain of the A190 subunit.

MATERIALS AND METHODS

Strains and plasmids. The strains used in this study are listed in Table 1. Three temperature-sensitive strains, NOY259 (*rpa190-1*), NOY265 (*rpa190-3*), and NOY267 (*rpa190-5*), as well as their isogenic control strain, NOY260 (*RPA190*), were described previously (43). Diploid strain NOY397 was constructed from w303-1a (obtained from Rolf Sternglanz [5]) by transformation with plasmid 43A (*HO LEU2*) (13). The diploidy of Leu⁺ transformants was confirmed by their inability to mate as well as by their ability to sporulate. One of these diploid transformants was grown in the presence of leucine, and a Leu⁻ segregant which lost the plasmid was retained (NOY397).

NOY398 is a derivative of NOY397 in which one of the *srp3⁺* (*RPA135*) genes has been disrupted by a *LEU2* insertion (Fig. 1B). This disruption was carried out as follows. The *Bgl*II-*Bgl*II 2.7-kb fragment of *LEU2* prepared from YEp13 (6) was ligated into the *Bam*HI site of plasmid pUC19. The resultant plasmid was digested by *Xho*I, treated with the DNA polymerase Klenow fragment, and religated, abolishing the *Xho*I site present in the cloned fragment. The *Pst*I-*Sma*I fragment containing the *LEU2* gene was then cut out and used for disruption. The *Xho*I-*Eco*RV 5-kb fragment of *RPA135* was first cloned into pBluescript II KS(−) (Stratagene, La Jolla, Calif.) by using the *Xho*I and *Sma*I sites of this vector. The *Pst*I-*Nru*I fragment in *RPA135* on this plasmid was then replaced by the *Pst*I-*Sma*I fragment containing *LEU2*. Finally, the *Xho*I-*Bam*HI fragment which

carries the disrupted *RPA135* was cut out and introduced into NOY397, and Leu⁺ transformants were selected, yielding NOY398.

NOY399 is a strain in which the transcription of *RPA135* is under the control of the *GAL7* promoter. To construct this strain, the *Hin*dIII-*Bgl*II 0.6-kb fragment including the *GAL7* promoter region (from −274 to +3) was cut out from pAA7 (1) and cloned into pUC19 by using the *Hin*dIII and *Bam*HI sites. The *Hin*dIII-*Sma*I fragment containing the *GAL7* promoter was then cut out and inserted into pRS316 by using the *Hin*dIII and *Sma*I sites of this vector. The resultant plasmid was then cut with *Sma*I and *Sac*I, and the 4.6-kb *Pvu*II-*Sac*I fragment carrying the *RPA135* coding region prepared from pNOY80 was inserted into this gap, completing the fusion of *RPA135* to the *GAL7* promoter (see Fig. 4B). The plasmid constructed in this way was named pNOY83. Strain NOY399 was then constructed by transformation of NOY398 with pNOY83 followed by sporulation and tetrad dissection. A Leu⁺ segregant was retained, and its dependence on galactose for growth was confirmed. NOY400 was constructed in a similar way from NOY398 by using pNOY80 instead of pNOY83.

Media. YEPD and synthetic dextrose (glucose) medium (SD) were described previously (36, 43).

Cloning. Genomic DNA was isolated from NOY345 as described by Johnston (17). Purified DNA (200 μg) was partially digested by *Sau*3A1 and fractionated in an 11-ml linear NaCl gradient (5 to 25% NaCl in 3 mM EDTA [pH 8.0]). After centrifugation for 4 h at 37,000 rpm in a SW40TI Beckman rotor at 22°C, fractions containing DNA fragments of about 5 to 15 kb in size were pooled, and the recovered DNA was ligated into the *Bam*HI site of YCp50. These plasmids were transformed into *E. coli* DH5 (Bethesda Research Laboratories, Gaithersburg, Md.), and plasmid DNA was prepared from the pooled transformants. Plasmid DNA was then introduced into NOY267 (*rpa190-5*), and temperature-resistant (Ts⁺) transformants were selected on plates (SD containing 0.5% Casamino Acids and 40 μg of tryptophan per ml) after incubation for 4 to 5 days at 37°C. Plasmids isolated from these transformants were introduced into NOY267, and the Ts⁺ phenotype was confirmed.
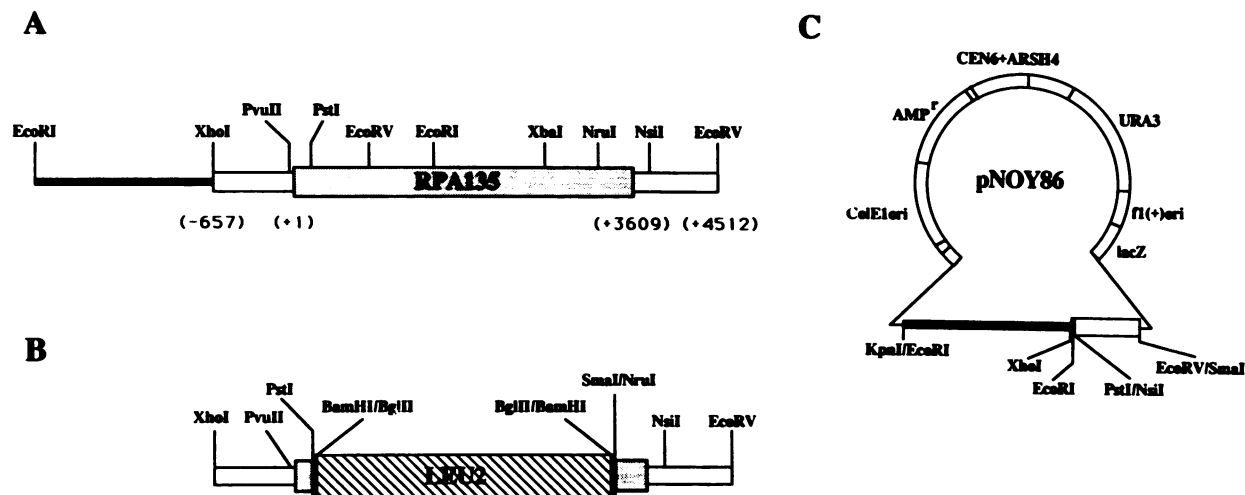
A

B

C

FIG. 1. Restriction enzyme map of the *RPA135* (*SRP3*) region and structures of DNA fragments derived from this region. (A) Diagram of the chromosomal segment of yeast DNA containing the *RPA135* (*SRP3*) gene. Nucleotide numbers given in parentheses correspond to those in Fig. 2. The protein-coding region (+1 to +3609) is shown as a stippled box. (B) DNA fragment used for construction of the null allele (*SRP3::LEU2* or *rpa135::LEU2*). (C) Structure of plasmid pNOY86 used for cloning of the wild type allele (*srp3*⁺) of *SRP3*. Note that the plasmid carries the upstream (*Eco*RI-*Xho*I) and the downstream (*Nsi*I-*Eco*RV) segments flanking the *RPA135* (*SRP3*) coding region shown in panel (A), but does not carry the central part containing *RPA135* (*SRP3*).

The wild-type *srp3*⁺ (*RPA135*) gene was cloned by a gap repair method. The 1.8-kb *Eco*RI-*Xho*I fragment which is upstream of *RPA135* and the 685-bp *Nsi*I-*Eco*RV downstream fragment (Fig. 1A) were inserted into the multiple cloning site of pRS316 by using *Kpn*I and *Xho*I sites for the former and *Pst*I and *Sma*I sites for the latter (Fig. 1C). The resultant plasmid (pNOY86) was cleaved at *Xho*I and *Eco*RI sites and transformed into NOY260 to repair the gap between *Xho*I and *Nsi*I by copying the chromosomal *RPA135* region.

Sequencing. Several DNA fragments (1 to 3 kb) were first subcloned from the 5-kb *Xho*I-*Eco*RV fragment of the cloned *SRP3* gene into M13mp18 and/or M13mp19. Several series of deletion subclones were then constructed by using the CYCLONE I BIOSYSTEM (IBI, New Haven, Conn.), and their DNA sequences were determined by the dideoxy method with T7 DNA polymerase (41). The 1-kb *Xba*I-*Nsi*I fragment of the wild-type gene was cloned into M13mp18 and M13mp19 and sequenced in the same way.

Mapping. Analysis of chromosomal DNA from NOY260 by orthogonal field alternation gel electrophoresis (OFAGE) were carried out as described by Carle and Olson (7). After electrophoresis, chromosomal DNA was blotted onto a nitrocellulose membrane (BA85; Schleicher & Schuell, Keene, N.H.) and hybridized with [³²P]DNA probes containing genes including *RPA135*, *RPA190*, *URA3*, *GAL4*, and *GAL80*. Uniformly labeled DNA probes were synthesized by using random oligonucleotide primers by the method of Feinberg and Vogelstein (12). The templates used were the 1.6-kb *Xho*I-*Eco*RV fragment containing part of *RPA135* (Fig. 1A), the 1.8-kb *Xba*I-*Xba*I fragment containing part of *RPA190* (25), the 1.2-kb *Hin*dIII-*Hin*dIII fragment containing *URA3* prepared from YEp24 (4), the 3.2-kb *Hin*dIII-*Bam*HI fragment containing *GAL4* (14), and the 2.3-kb *Hin*dIII-*Sma*I fragment containing *GAL80* (28).

Primer extension analysis. Start sites for *RPA135* mRNA transcripts were determined by a primer extension method. mRNA was isolated from NOY260 as described by Carlson

and Botstein (8). Poly(A)⁺ RNA was prepared on an oligo(dT)-cellulose column and used for primer extension analysis. The sequence of the oligonucleotide primer used is TTAATCACTTTGCTCATCTCACCAAATGCG, which is complementary to the mRNA sequence covering the translation initiation site. The primer extention reactions were carried out as described in reference 33, and the products were analyzed by electrophoresis through a 6% polyacrylamide–8 M urea sequencing gel followed by autoradiography.

Immunoblot analysis. Samples containing 60 μg of total protein were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then transferred to a BA85 nitrocellulose membrane. Antibody against the A135 subunit was provided by M. Riva and A. Sentenac, and bound antibody was visualized by using an immunoassay kit (Bio-Rad Laboratories, Richmond, Calif.) as described previously (24a, 44).

Nucleotide sequence accession number. The sequence of *RPA135* (see Fig. 2) has been submitted to the GenBank data base under accession number M3605.

RESULTS

Cloning of the *SRP3* gene. As described in the preceding paper (24a), strains carrying the *rpa190-5* (or *rpa190-1*) mutation fail to grow at 37°C. The presence of a suppressor mutation, *SRP3*, enables these strains to grow at 37°C. We used DNA from NOY345 (*rpa190-5 SRP3-1*) to clone the *SRP3* gene. A gene bank was prepared from this strain as described in Materials and Methods and was used to transform a temperature-sensitive strain, NOY267 (*rpa190-5*). Candidates were then selected from among transformants by their ability to form colonies at 37°C. Plasmids were isolated from each of 25 candidates, and physical structures of the cloned fragments were analyzed by digestion with several restriction enzymes. A DNA segment of about 9 kb was found to be shared by all of them. After further subcloning,

we found that the 5-kb XhoI-EcoRV fragment (Fig. 1A) was sufficient to suppress the rpa190-5 mutation.

The plasmid carrying this 5-kb XhoI-EcoRV fragment was introduced into two other temperature-sensitive rpa190 mutants, NOY259, which carries rpa190-1, and NOY265, which carries rpa190-3. Suppression was observed for the former, but not the latter. Thus, the allele specificity of suppression by the cloned gene is the same as that observed for SRP3-1 in the original genetic experiments (24a), suggesting that the cloned gene on the 5-kb XhoI-EcoRV fragment is in fact the suppressor SRP3.

Nucleotide sequence of the SRP3 gene. The nucleotide sequence of the XhoI-EcoRV fragment was determined as described in Materials and Methods, and the sequence is shown in Fig. 2. (The nucleotide sequence shown in Fig. 2 is actually the wild-type sequence [see the legend to Fig. 2 and below for the mutational alteration of the sequence].) The longest open reading frame found is 3,609 bp and would code for a protein of 1,203 amino acids with a calculated molecular mass of 136 kDa.

The deduced amino acid sequence of the open reading frame was compared with sequences in the GenBank and EMBL DNA data base by using a University of Wisconsin Genetics Computer Group computer program, TFASTA (30). Highly significant homology was found between the deduced amino acid sequence and the sequences of the second-largest subunits of RNA polymerases of various origins including RNA polymerase II from S. cerevisiae and E. coli RNA polymerase (Fig. 3A and C). The gene (RPD2) for the second-largest subunit of S. cerevisiae RNA polymerase II was previously cloned by Young and co-workers (40, 47), and the presence of about 10 regions showing highly significant amino acid sequence homology to the E. coli RNA polymerase β subunit was recognized in this RNA polymerase II subunit (40). Most of these regions also show homology to the deduced amino acid sequence of the cloned SRP3 gene. These observations, combined with the ability of this gene to suppress the rpa190-1 (and rpa190-5) mutation, strongly suggested that the protein encoded by this open reading frame is the second-largest subunit (called A135 by Sentenac and co-workers; see reference 35) of RNA polymerase I. This inference was supported by comparison of the deduced amino acid sequence of the protein encoded by the cloned SRP3 gene with two peptide sequences supplied by M. Riva, C. Carles, and A. Sentenac (30a). These workers determined amino-terminal sequences of two tryptic peptides derived from the A135 subunit of purified S. cerevisiae RNA polymerase I. These sequences are AGAL?GIAQD STP?IFNED and AGYNY?GNEPMYSGATGE (where ? indicates unidentified amino acid residues). We find that the first sequence starts at deduced amino acid position 971 (through 989) and is preceded by lysine 970, and the second sequence starts at position 1003 (through 1020) and is preceded by lysine 1002. No discrepancy was observed between their peptide sequences and our deduced amino acid sequence. The experiments described below give further and more direct evidence for the conclusion that the SRP3 gene represents a mutated form of the gene coding for the A135 subunit of RNA polymerase I.

Cloning of the wild-type allele of the SRP3 gene and identification of the mutational alteration. If the cloned gene encoding A135 is in fact the SRP3 gene, we should be able to find a difference in the nucleotide sequence (causing a difference in the amino acid sequence) between the cloned gene and the corresponding gene (srp3+) in strains which do not carry the suppressor gene SRP3. We cloned the gene

from NOY260 (RPA190 srp3+) by using a gap repair method. A linear DNA lacking the A135 coding region but retaining its flanking sequences was prepared starting from plasmid pNOY86 (see Fig. 1C and its legend) and introduced into strain NOY260 by transformation. Ura+ transformants were selected, and a plasmid containing the A135 region (pNOY80) was recovered. In contrast to the original plasmid (pNOY79), the plasmid (pNOY80) recovered from NOY260 did not have the ability to suppress the rpa190-5 mutation, indicating that the repaired segment contains the wild-type sequence and is different from that derived from the SRP3 strain.

To identify the location of the SRP3 mutation, a series of chimeric genes was constructed from the wild-type and mutant genes and introduced into NOY267 as pRS316-derived plasmids. We examined their ability to suppress the rpa190-5 mutation and found that the mutation responsible for suppression is within the 1-kb XbaI-NsiI fragment (Fig. 1A). The nucleotide sequence of this fragment derived from the wild-type gene was then determined. Comparison of this sequence with the sequence of the mutant gene demonstrated that the SRP3-1 mutation represents a cysteine 1127-to-arginine change which is caused by base substitution of C for T at nucleotide 4037 (Fig. 2; see also Fig. 7B). As discussed below, this mutational alteration is within the putative zinc-binding region (with the $CX_2CX_nCX_2C$ motif) near the carboxy-terminal end of the protein, which is apparently conserved among the second-largest subunits of RNA polymerase II and III analyzed so far.

Chromosomal location and the transcription start site of the SRP3 gene. Intact chromosomal DNAs from NOY260 were separated by orthogonal field alternation gel electrophoresis and transferred to a nitrocellulose membrane, and the SRP3 gene was localized by using the 1.6-kb XhoI-EcoRV fragment (Fig. 1A) as a radioactive probe. Hybridization results demonstrated that the gene is on chromosome XVI (data not shown). Identification of the chromosome was confirmed by parallel hybridization of the gels by using a probe for the GAL4 gene, which is known to be on chromosome XVI, as well as several other probes for genes of known chromosomal locations. Thus, the chromosomal location of the SRP3 gene is different from that of RPA190, which is localized on chromosome XV (24a).

The transcription start site was determined by primer extension with a radioactive oligonucleotide corresponding to nucleotide positions −13 to +17 in Fig. 2. The results indicated that transcription most probably starts at the A, 57 nucleotides upstream from the AUG translation initiation codon (data not shown). We note that there are no obvious sequences corresponding to the consensus TATA box element (TATATAA or TATAAA) in the region upstream from the coding region that we have sequenced. Failure to recognize the TATA box element was also encountered for several other yeast genes including RPA190 (25).

Disruption of the chromosomal SRP3 gene and construction of a conditional expression system. Using a diploid strain, NOY397, we first deleted most of the coding region in one of the two copies of the srp3+ gene. Disruption was carried out with the LEU2 fragment as described in Materials and Methods. The resultant strain (NOY398 [Table 1; Fig. 1B]) was sporulated, and 11 tetrads were dissected. The spore viability and leucine requirements of viable spores were examined.

None of the tetrads produced more than two viable spores, and all the viable spores were leucine auxotrophs (Leu−) (Table 2). Most of the control tetrads obtained from

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                                              -600                                                          -540
CTCGAGGACAAATCAGTTTCAATGACGATAGAGGTTAGCAAAATTACTATATTAGTGAATTAGTAACGGAGACAAAAAGATTTGTTTAACGGCTTCTTTGTAGTCATCCTGCTGTTGTTT
                                         -480                                                          -420
TTGTATGTTTAAGCTTCTTCCTCCGATTAAGTTTTGTACATTTACTTCTGTAAGTTAAAGGGCTCTTTTCTCATTATATTTCAAATGCTAAATCATGCCATGAAAACTTCAAAAAAGCAT
                                    -360                                                          -300
CAAATGAAGGAAGACGCTGATGACATCGAGTGATGAAATTATTCATAAATCTGCATCAGAGTGTTATATCTACAGAAGCAGACATGCTACAAGAGTTCAAAGCACTTAATGGCATTTCTA
                               -240                                                          -180
ATAGATTCTCAGCGCCCTTTTGAGTCTCTGCACTACCACTTAATTATCATACCGCAGAAGCGCCTCTCGGCCTCCCTTCATGCTATAATCGCTAATGACGGAAATTATTATTGAGATTAC
                          -120                                                          -60
TCTTGCGAGTGTTTTTTTTTTTTTTTTTTTTTTTTCGTATGAAAAATTTTTCAAGCGCATCTTAGAGACTCATCTCATCTCTTAATTATTCAAACTTACTICAGCTGTCTCGTAATATTC
                          -1                                                          PvuII
AAGCAAGTCTTCCTCAAGAATATTGACAAATAAACGTAGAATTACGCATTTGGTGAG
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       10              30              50              70              90             110
ATGAGCAAAGTGATTAAGCCGCCTGGCCAAGCCAGAACTGCTGATTTCCGTACCTTAGAACGTGAATCTAGGTTCATCAATCCACCTAAGGATAAATCAGCATTTCCACTATTACAGGAA
  1 M  S  K  U  I  K  P  P  G  Q  A  R  T  A  D  F  R  T  L  E  R  E  S  R  F  I  N  P  P  K  D  K  S  A  F  P  L  L  Q  E
      130             150             170             190             210             230
GCTGTTCAACCTCATATTGGGTCTTTTAACGCATTAACAGAAGGTCCGGATGGAGGTTTGTTAAATCTTGGTGTTAAAGACATCGGTGAAAAGTTATATTTGATGGAAAACCACTCAAT
 41 A  U  Q  P  H  I  G  S  F  N  A  L  T  E  G  P  D  G  G  L  L  N  L  G  U  K  D  I  G  E  K  U  I  F  D  G  K  P  L  N
      250             270             290             310             330             350
TCTGAAGACGAAATATCCAACAGCGGATACCTCGGGAATAAGCTTTCTGTTAGTGTGGAACAAGTGTCTATTGCGAAGCCAATGTCTAACGATGGTGTTTCTTCTGCAGTAGAGAGAAAA
 81 S  E  D  E  I  S  N  S  G  Y  L  G  N  K  L  S  U  S  U  E  Q  U  S  I  A  K  P  N  S  N  D  G  U  S  S  A  U  E  R  K
      370             390             410             430             450             470
GTTTACCCAAGTGAGTCAAGACAAAGGCTTACTTCTTACAGAGGTAAATTACTTTTAAAGTTGAAGTGGTCCGTGAACAATGGCGAGGAGAACTTATTTGAAGTAAGAGATTGTGGTGGT
121 U  Y  P  S  E  S  R  Q  R  L  T  S  Y  R  G  K  L  L  L  L  K  L  K  U  S  U  N  N  G  E  E  N  L  F  E  U  R  D  C  G  G
      490             510             530             550             570             590
CTACCAGTTATGCTACAGAGTAACAGATGTCACTTGAACAAGATGTCACCATATGAACTGGTACAACACAAGGAGAATCCGATGAAATAGGTGGTTATTTCATTGTTAACGGTATAGAA
161 L  P  U  N  L  Q  S  N  R  C  H  L  N  K  N  S  P  Y  E  L  U  Q  H  K  E  E  S  D  E  I  G  G  Y  F  I  U  N  G  I  E
      610             630             650             670             690             710
AAGCTTATTAGAATGCTGATTGTGCAACGTAGAAATCATCCTATGGCTATCATCAGACCTTCCTTCGCTAACAGGGGTGCATCCTATTCTCATTACGGTATTCAAATTAGATCTGTCAGG
201 K  L  I  R  N  L  I  U  Q  R  R  N  H  P  N  A  I  I  R  P  S  F  A  N  R  G  A  S  Y  S  H  Y  G  I  Q  I  R  S  U  R
      730             750             770             790             810             830
CCGGATCAAACTTCCCAAACTAATGTTTTGCATTACTTAAATGACGGTCAAGTTACATTTAGATTTTCTTGGAGAAAAAATGAATATTTAGTTCCTGTTGTTATGATTTTAAAAGCCTTG
241 P  D  Q  T  S  Q  T  N  U  L  H  Y  L  N  D  G  Q  U  T  F  R  F  S  U  R  K  N  E  Y  L  U  P  U  U  N  I  L  K  A  L
      850             870             890             910             930             950
TGTCATACCAGCGATAGGGAAATTTTTGATGGTATTATTGGTAATGACGTTAAGGATTCCTTTTTGACCGATCGTTTAGAATTACTGTTACGTGGTTTTAAGAAAAGATATCCTCATTTA
281 C  H  T  S  D  R  E  I  F  D  G  I  I  G  N  D  U  K  D  S  F  L  T  D  R  L  E  L  L  L  A  G  F  K  K  R  Y  P  H  L
      970             990             1010            1030            1050            1070
CAAAATCGCACACAAGTCTTGCAATATTTAGGAGACAAATTTCGTGTTGTCTTTCAAGCTTCCCCTGATCAATCTGATTTAGAAGTCGGACAAGAAGTCCTTGATCGCATCGTTCTCGTG
321 Q  N  R  T  Q  U  L  Q  Y  L  G  D  K  F  R  U  U  F  Q  R  S  P  D  Q  S  D  L  E  U  G  Q  E  U  L  D  R  I  U  L  U
      1090            1110            1130            1150            1170            1190
CATTTGGGTAAGGATGGCAGCCAGGATAAAATTCAGAATGTTATTGTTCATGATCAGAAAGTTGTATTCCTTGGTTGCAGGCGAATGTTCTCCAGATAACCCAGATGCTACGCAACATCAA
361 H  L  G  K  D  G  S  Q  D  K  F  R  N  L  L  F  N  I  R  K  L  Y  S  L  U  R  G  E  C  S  P  D  N  P  D  R  T  Q  H  Q
      1210            1230            1250            1270            1290            1310
GAAGTTCTTTTGGGTGGTTTCTTATATGGTATGATCCTAAAAGAAAAGATTGATGAGTACTTACAGAATATTATTGCTCAAGTCAGGATGGATATTAACCGTGGTATGGCCATTAATTTC
401 E  U  L  L  G  G  F  L  Y  G  N  I  L  K  E  K  I  D  E  Y  L  Q  N  I  I  R  Q  U  R  N  D  I  N  R  G  N  A  I  N  F
      1330            1350            1370            1390            1410            1430
AAAGACAAAAGGTACATGTCAAGAGTTTTAATGAGGGTTAATGAAAACATTGGTTCCAAAATGCAATACTTTTTATCAACTGGTAACCTGGTGTCCCAGTCTGGGTTAGATTTACAACAA
441 K  D  K  R  Y  N  S  R  U  L  N  R  U  N  E  N  I  G  S  K  N  Q  Y  F  L  S  T  G  N  L  U  S  Q  S  G  L  D  L  Q  Q
      1450            1470            1490            1510            1530            1550
GTTTCTGGTTATACTGTGGTTGCAGAAAAAATTAACTTCTACCGTTTTATCTCTCATTTTAGAATGGTTCATAGAGGCTCCTTTTTTGCGCAATTGAAAACTACCACAGTCAGAAAGTTG
481 U  S  G  Y  T  U  U  R  E  K  I  N  F  Y  R  F  I  S  H  F  R  N  U  H  R  G  S  F  F  R  Q  L  K  T  T  U  R  K  L
      1570            1590            1610            1630            1650            1670
CTACCTGAGTCGTGGGGATTCCTGTGCCCTGTTCATACACCAGATGGTTCTCCTTGTGGTTTATTAAACCATTTTGCGCACAAGTGTCGTATATCAACTCAGCAATCTGACGTCTCAAGA
521 L  P  E  S  U  G  F  L  C  P  U  H  T  P  D  G  S  P  C  G  L  L  N  H  F  R  H  K  C  R  I  S  T  Q  Q  S  D  U  S  R
      1690            1710            1730            1750            1770            1790
ATTCCTTCTATATTATACTCACTTGGTGTTGCTCCGGCATCTCACACCTTTGCTGCTGGTCCTTCTCGTGTTGTGTTCAAATTGATGGTAAAATTATTGGCTGGGTCTCTCATGAACAA
561 I  P  S  I  L  Y  S  L  G  U  R  P  R  S  H  T  F  R  R  G  P  S  L  C  C  U  Q  I  D  G  K  I  I  G  U  U  S  H  E  Q
      1810            1830            1850            1870            1890            1910
GGTAAGATCATTGCGGATACATTGAGATATTGGAAAGTTGAAGGCAAGACGCCTGGGTTACCGATTGATCTAGAAAATTGGATATGTTCCTCCCTCAACTCGTGGTCAATATCCAGGTCTC
601 G  K  I  I  R  D  T  L  R  Y  U  K  U  E  G  K  T  P  G  L  P  I  D  L  E  I  G  Y  U  P  S  T  R  G  Q  Y  P  G  L
      1930            1950            1970            1990            2010            2030
TACCTATTTGGTGGTCATTCTAGGATGCTGCGTCCTGTTCGTTACCTGCCTTTGGATAAAGAAGACATTGTTGGACCTTTTGAGCAAGTGTACATGAACATTGCTGTCACTCCTCAAGAA
641 Y  L  F  G  G  H  S  R  N  L  R  P  U  R  Y  L  P  L  D  K  E  D  I  U  G  P  F  E  Q  U  Y  N  N  I  R  U  T  P  Q  E
      2050            2070            2090            2110            2130            2150
ATTCAAAATAACGTTCATACACACGTAGAATTTACGCCCACAAATATTCTTTCTATTTTG GCTAATTTGACTCCATTTTCTGATTTCAACCAGTCTCCAAGAAACATGTATCAATGTCAG
681 I  Q  N  N  U  H  T  H  U  E  F  T  P  T  N  I  L  S  I  L  R  N  L  T  P  F  S  D  F  N  Q  S  P  R  N  'N  Y  Q  C  Q
```

the diploid NOY397 produced four viable spores. These results demonstrate that one of the *srp3*+ genes in NOY398 is disrupted by the *LEU2* insertion and that the *srp3*+ gene is essential for cell viability.

To directly demonstrate that the cloned *SRP3* (and *srp3*+) gene codes for the A135 protein, we constructed a system in which expression of this gene is achieved entirely through a galactose-driven promoter, as was done for the *RPA190* gene
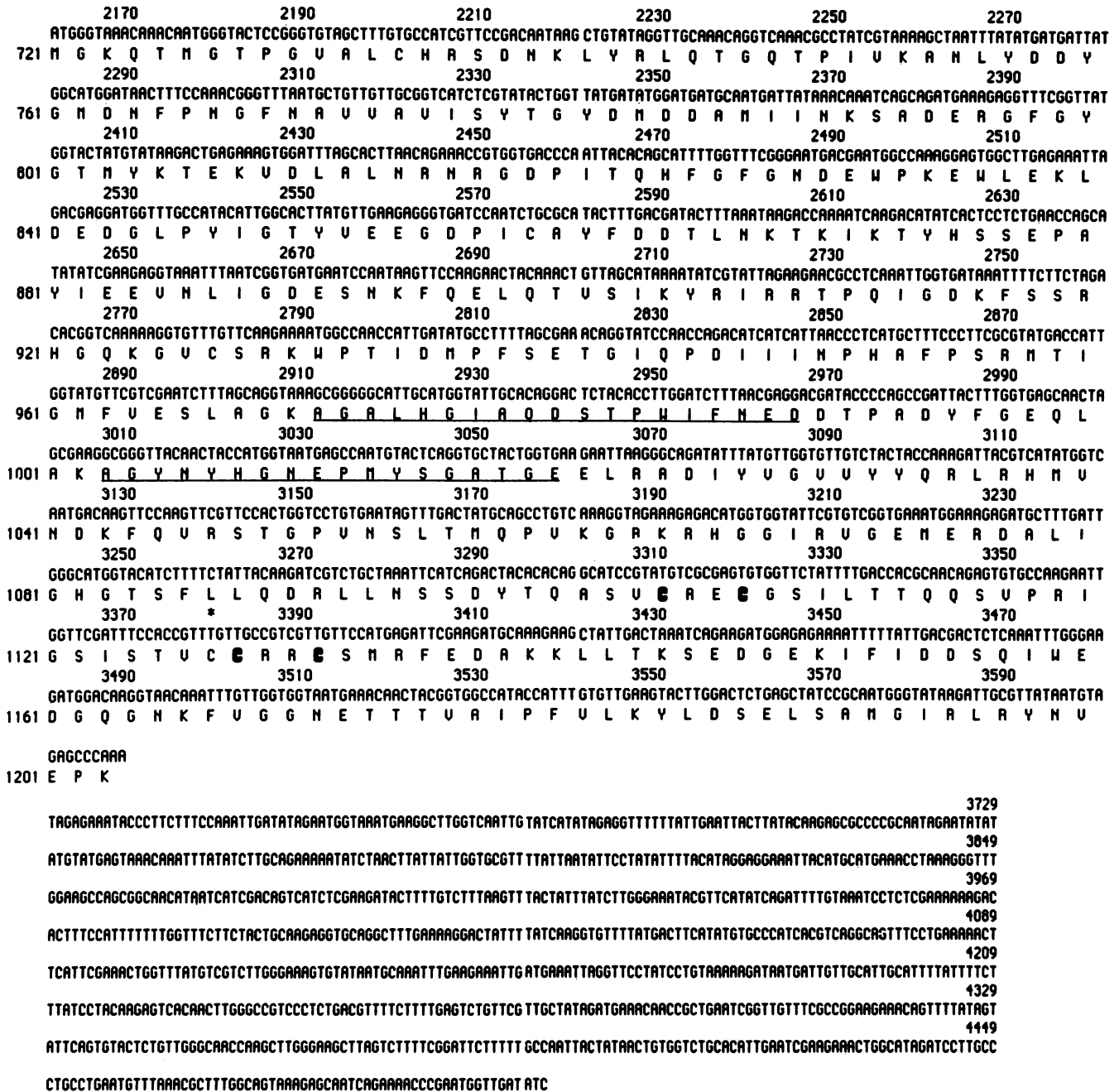
```
         2170           2190           2210           2230           2250           2270
     ATGGGTAAACAAACAATGGGTACTCCGGGTGTAGCTTTGTGCCATCGTTCCGACAATAAG CTGTATAGGTTGCAAACAGGTCAAACGCCTATCGTAAAAGCTAATTTATATGATGATTAT
721  M  G  K  Q  T  M  G  T  P  G  U  A  L  C  H  A  S  D  N  K  L  Y  A  L  Q  T  G  Q  T  P  I  U  K  A  N  L  Y  D  D  Y
         2290           2310           2330           2350           2370           2390
     GGCATGGATAACTTTCCAAACGGGTTTAATGCTGTTGTTGCGGTCATCTCGTATACTGGT TATGATATGGATGATGCAATGATTATAAACAAATCAGCAGATGAAAGAGGTTTCGGTTAT
761  G  M  D  N  F  P  N  G  F  N  A  U  U  A  U  I  S  Y  T  G  Y  D  M  D  D  A  M  I  I  N  K  S  A  D  E  R  G  F  G  Y
         2410           2430           2450           2470           2490           2510
     GGTACTATGTATAAGACTGAGAAAGTGGATTTAGCACTTAACAGAAACCGTGGTGACCCA ATTACACAGCATTTTGGTTTCGGGAATGACGAATGGCCAAAGGAGTGGCTTGAGAAATTA
801  G  T  M  Y  K  T  E  K  U  D  L  A  L  N  R  N  R  G  D  P  I  T  Q  H  F  G  F  G  N  D  E  U  P  K  E  U  L  E  K  L
         2530           2550           2570           2590           2610           2630
     GACGAGGATGGTTTGCCATACATTGGCACTTATGTTGAAGAGGGTGATCCAATCTGCGCA TACTTTGACGATACTTTAAATAAGACCAAAATCAAGACATATCACTCCTCTGAACCAGCA
841  D  E  D  G  L  P  Y  I  G  T  Y  U  E  E  G  D  P  I  C  A  Y  F  D  D  T  L  N  K  T  K  I  K  T  Y  H  S  S  E  P  A
         2650           2670           2690           2710           2730           2750
     TATATCGAAGAGGTAAAATTTAATCGGTGATGAATCCAATAAGTTCCAAGAACTACAAACT GTTAGCATAAAATATCGTATTAGAAGAACGCCTCAAATTGGTGATAAATTTTCTTCTAGA
881  Y  I  E  E  U  N  L  I  G  D  E  S  N  K  F  Q  E  L  Q  T  U  S  I  K  Y  R  I  R  R  T  P  Q  I  G  D  K  F  S  S  R
         2770           2790           2810           2830           2850           2870
     CACGGTCAAAAAGGTGTTTGTTCAAGAAAATGGCCAACCATTGATATGCCTTTTAGCGAA ACAGGTATCCAACCAGACATCATCATTAACCCTCATGCTTTCCCTTCGCGTATGACCATT
921  H  G  Q  K  G  U  C  S  R  K  U  P  T  I  D  M  P  F  S  E  T  G  I  Q  P  D  I  I  I  N  P  H  A  F  P  S  R  M  T  I
         2890           2910           2930           2950           2970           2990
     GGTATGTTCGTCGAATCTTTAGCAGGTAAAGCGGGGGCATTGCATGGTATTGCACAGGAC TCTACACCTTGGATCTTTAACGAGGACGATACCCCAGCCGATTACTTTGGTGAGCAACTA
961  G  M  F  U  E  S  L  A  G  K  A̲  G̲  A̲  L̲  H̲  G̲  I̲  A̲  Q̲  D̲  S̲  T̲  P̲  U̲  I̲  F̲  N̲  E̲  D̲  D  T  P  A  D  Y  F  G  E  Q  L
         3010           3030           3050           3070           3090           3110
     GCGAAGGCGGGTTACAACTACCATGGTAATGAGCCAATGTACTCAGGTGCTACTGGTGAA GAATTAAGGGCAGATATTTATGTTGGTGTTGTCTACTACCAAAGATTACGTCATATGGTC
1001 A  K  A̲  G̲  Y̲  N̲  Y̲  H̲  G̲  N̲  E̲  P̲  M̲  Y̲  S̲  G̲  A̲  T̲  G̲  E̲  E  L  A  A  D  I  Y  U  G  U  U  Y  Y  Q  R  L  A  H  M  U
         3130           3150           3170           3190           3210           3230
     AATGACAAGTTCCAAGTTCGTTCCACTGGTCCTGTGAATAGTTTGACTATGCAGCCTGTC AAAGGTAGAAAGAGACATGGTGGTATTCGTGTCGGTGAAATGGAAAGAGATGCTTTGATT
1041 N  D  K  F  Q  U  R  S  T  G  P  U  N  S  L  T  M  Q  P  U  K  G  R  K  R  H  G  G  I  R  U  G  E  M  E  R  D  A  L  I
         3250           3270           3290           3310           3330           3350
     GGGCATGGTACATCTTTTCTATTACAAGATCGTCTGCTAAATTCATCAGACTACACACAG GCATCCGTATGTCGCGAGTGTGGTTCTATTTTGACCACGCAACAGAGTGTGCCAAGAATT
1081 G  H  G  T  S  F  L  L  Q  D  R  L  L  N  S  S  D  Y  T  Q  A  S  U  E  R  E  E  G  S  I  L  T  T  Q  Q  S  U  P  R  I
         3370            *            3390           3410           3430           3450           3470
     GGTTCGATTTCCACCGTTTGTTGCCGTCGTTGTTCCATGAGATTCGAAGATGCAAAGAAG CTATTGACTAAATCAGAAGATGGGAGAGAAAATTTTTATTGACGACTCTCAAATTTGGGAA
1121 G  S  I  S  T  U  C  E  R  R  E  S  M  R  F  E  D  A  K  K  L  L  T  K  S  E  D  G  E  K  I  F  I  D  D  S  Q  I  U  E
         3490           3510           3530           3550           3570           3590
     GATGGACAAGGTAACAAATTTGTTGGTGGTAATGAAACAACTACGGTGGCCATACCATTT GTGTTGAAGTACTTGGACTCTGAGCTATCCGCAATGGGTATAAGATTGCGTTATAATGTA
1161 D  G  Q  G  N  K  F  U  G  G  N  E  T  T  T  U  A  I  P  F  U  L  K  Y  L  D  S  E  L  S  A  M  G  I  R  L  A  Y  N  U

     GAGCCCAAA
1201 E  P  K
```

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                                                                                           3729
     TAGAGAAATACCCTTCTTTCCAAATTGATATAGAATGGTAAATGAAGGCTTGGTCAATTG TATCATATAGAGGTTTTTTATTGAATTACTTATACAAGAGCGCCCCGCAATAGAATATAT
                                                                                           3849
     ATGTATGAGTAAACAAATTTATATCTTGCAGAAAAATATCTAACTTATTATTGGTGCGTT TTATTAATATTCCTATATTTTACATAGGAGGAAATTACATGCATGAAACCTAAAGGGTTT
                                                                                           3969
     GGAAGCCAGCGGCAACATAATCATCGACAGTCATCTCGAAGATACTTTTGTCTTTAAGTT TACTATTTATCTTGGGAAATACGTTCATATCAGATTTTGTAAATCCTCTCGAAAAAAGAC
                                                                                           4089
     ACTTTCCATTTTTTTGGTTTCTTCTACTGCAAGAGGTGCAGGCTTTGAAAAGGACTATTT TATCAAGGTGTTTTATGACTTCATATGTGCCCATCACGTCAGGCAGTTTCCTGAAAAACT
                                                                                           4209
     TCATTCGAAACTGGTTTATGTCGTCTTGGGAAAGTGTATAATGCAAATTTGAAGAAATTG ATGAAATTAGGTTCCTATCCTGTAAAAAGATAATGATTGTTGCATTGCATTTTATTTTCT
                                                                                           4329
     TTATCCTACAAGAGTCACAACTTGGGCCGTCCCTCTGACGTTTTCTTTTGAGTCTGTTCG TTGCTATAGATGAAACAACCGCTGAATCGGTTGTTTCGCCGGAAGAAACAGTTTTATAGT
                                                                                           4449
     ATTCAGTGTACTCTGTTGGGCAACCAAGCTTGGGAAGCTTAGTCTTTTCGGATTCTTTTT GCCAATTACTATAACTGTGGTCTGCACATTGAATCGAAGAAACTGGCATAGATCCTTGCC

     CTGCCTGAATGTTTAAACGCTTTGGCAGTAAAGAGCAATCAGAAAACCCGAATGGTTGAT ATC
```

FIG. 2. Nucleotide and predicted amino acid sequences of *RPA135* (*srp3*$^+$). The coding sequence starts at nucleotide 1 and ends at nucleotide 3609. Amino acid sequences corresponding to the peptide sequences determined by Sentenac and co-workers (30a) are underlined. The putative zinc-binding region with the motif CX$_2$CX$_n$CX$_2$C is indicated by outlining C at each of the four cysteine residues (amino acid positions 1104, 1107, 1128, and 1131). The nucleotide (3379) altered in *SRP3-1* (T → C) is indicated by an asterisk, and the transcription start site (A at −57) is overlined. The *Pvu*II site (−76 to −71) used for fusion to the *GAL7* promoter is also indicated.

in our previous work (44). A DNA fragment containing the *GAL7* promoter ("*pGAL7*") covering the region from −274 to +3 (with respect to the *GAL7* transcription start site) was fused to the *srp3*$^+$ gene at position −73 (16 bp upstream from the transcription start site of the *SRP3*$^+$ gene). This *pGAL7-srp3*$^+$ fusion gene was placed in centromere vector pRS316, and the resultant plasmid (pNOY83; Fig. 4) was introduced into the diploid strain NOY398, which has one of the *srp3*$^+$ genes deleted and replaced by *LEU2*. After sporulation,

tetrads were dissected on galactose plates and growth of segregants on glucose was examined. Two of the four segregants, which were Leu$^+$, grew on galactose but failed to grow on glucose. The results confirm that the *srp3*$^+$ gene is an essential gene and demonstrate that the gene present on plasmid pNOY83 is in fact transcribed from the *GAL7* promoter. One of these galactose-dependent haploid segregants, NOY399, was used in the following experiments.

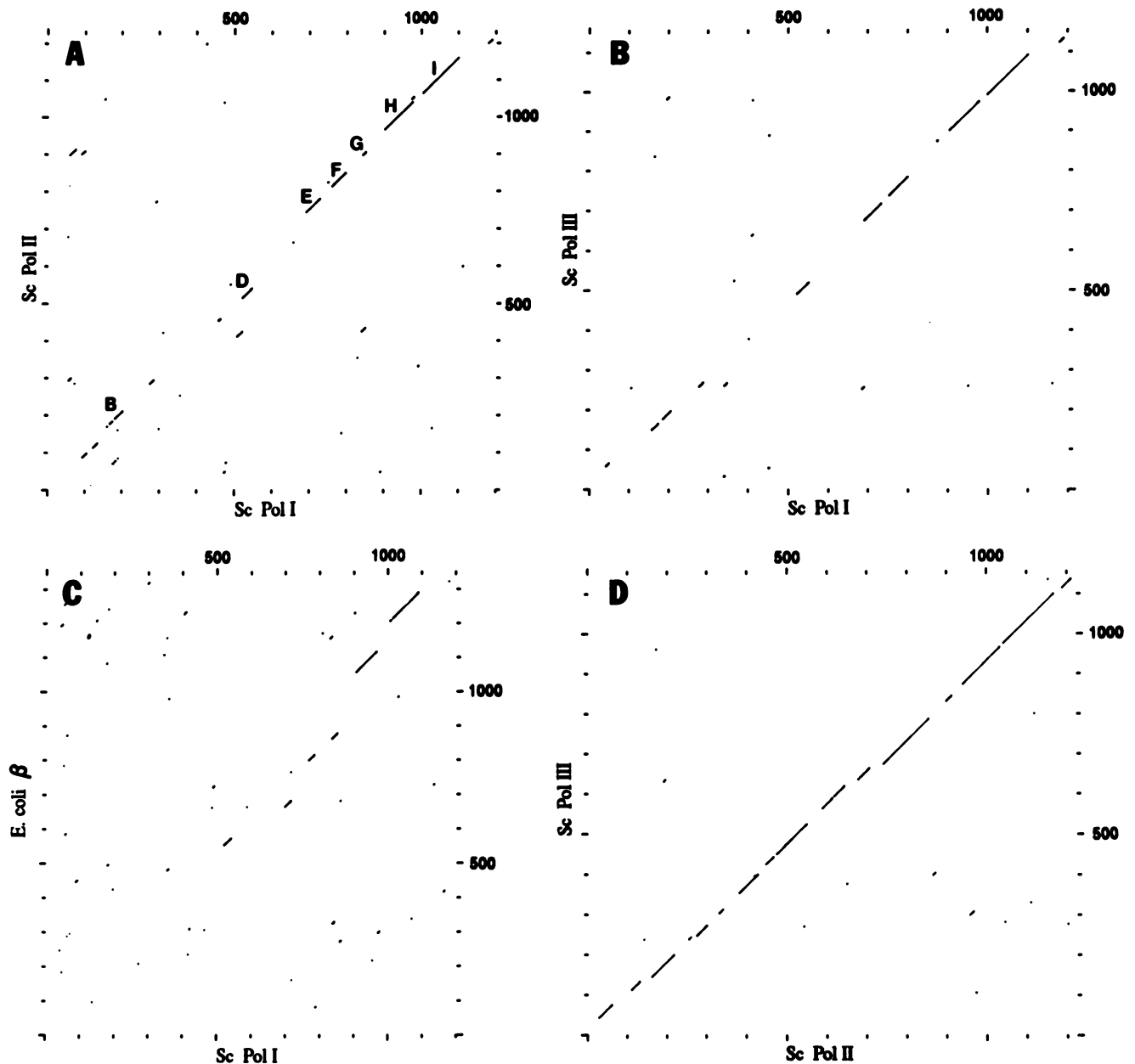NOY399 was first grown in galactose medium to the

FIG. 3. Comparison of the amino acid sequences of the second-largest subunit of *S. cerevisiae* RNA polymerase I (A135), II (B150 or RPB2), and III (C130) and the *E. coli* RNA polymerase β subunit. A dot matrix analysis of amino acid sequence similarity is shown (University of Wisconsin Genetics Computer Group program COMPARE [10]; window, 25; stringency, 15). (A) A135 versus B150. (B) A135 versus C130. (C) A135 versus *E. coli* β. (D) B150 versus C130. The seven regions conserved among the second-largest subunits of RNA polymerases are indicated in panel A; they correspond to the regions B, D, E, F, G, H, and I found by Sweetser et al. in the comparison of B150 and the *E. coli* β subunit (40).

mid-log phase. The culture was divided into two parts. One part was transferred to glucose medium, and the other part was kept in galactose medium. Cell growth was monitored. The results are shown in Fig. 5. Upon transfer to glucose medium, the growth rate of NOY399 increased initially. After about 4 h, the growth rate started to decrease, and by 8 h after the shift the decrease became very significant, approaching cessation of cellular growth. In contrast, the control strain (NOY400) carrying $srp3^+$ with its native promoter did not show such growth defects (Fig. 5). These

results show that although transcription of the $srp3^+$ gene from the *GAL7* promoter is quickly repressed upon transfer (for a review, see reference 18), the effects on growth become manifested only gradually. This may be because preexisting RNA polymerase I continues to transcribe rRNA genes, and hence the synthesis of ribosomes also continues, to some extent, after the transfer. The situation is similar to that which we observed previously for cessation of transcription of the *RPA190* gene (44). (We note that although NOY399 grows in galactose medium and forms colonies on

TABLE 2. Tetrad analysis of diploid cells with a copy of the RPA135 (SRP3) genes disrupted by LEU2

Disruption[a]	No. of tetrads with following no. of viable spores per tetrad:				No. of tetrads with following no. of Leu+ spores per tetrad:		
	4	3	2	1	2	1	0
+	0	0	8	3	0	0	11
−	9	1	0	0	−[b]	−	−

[a] +, Diploid strain NOY398 (RPA135/rpa135::LEU2); −, control diploid strain NOY397 (RPA135/RPA135).

[b] NOY397 is leu2/leu2, and so the leucine phenotype was not analyzed.

galactose plates, its growth rate in galactose is lower than that of NOY400. We have not investigated the basis of this observation.)

We measured the amounts of A135 protein after transfer to glucose in this conditional expression system. The amounts of A135 were estimated by Western immunoblot analysis with a specific antiserum against A135 protein. The cellular concentration of A135 decreased with time and became roughly 10% of that of control at 8 h after the transfer (Fig. 6). These results, combined with those described above, directly demonstrate that the cloned srp3+ gene codes for the second-largest subunit (A135) of RNA polymerase I. Following the nomenclature adopted by Mémet et al. for other RNA polymerase I genes (25), we now call the gene RPA135.

DISCUSSION

Genes for the second-largest subunit of RNA polymerases were previously cloned and sequenced for RNA polymerase II from S. cerevisiae as well as that from D. melanogaster, and the presence of significant homology to the E. coli RNA polymerase β subunit was recognized in the deduced amino acid sequences (11, 40). In addition, James et al. (16a) have recently cloned and sequenced the gene for the second-

largest subunit (C130) of RNA polymerase III from S. cerevisiae, and we have now demonstrated that the gene we have cloned as a suppressor of rpa190-1 (and rpa190-5) codes for the second-largest subunit (A135) of RNA polymerase I. Therefore, we can now compare amino acid sequences of the second-largest subunits in the three different types of RNA polymerases, i.e., RNA polymerases I, II, and III, from S. cerevisiae. The results of such an analysis are shown in Fig. 3. It can be seen that the subunits of polymerases II and III are more closely related to each other than to the corresponding subunit of polymerase I.

Amino acid sequences of the largest subunits (A190, B220, and C160) of the three forms of RNA polymerases from S. cerevisiae were previously compared by Mémet et al. (26), and the same relationship was observed; that is, B220 and C160 were found to have greater sequence similarity to each other than to A190. These authors have suggested the existence of a transient ancestor to eucaryotic RNA polymerases II and III. The present observations give further support to this suggestion.

RNA polymerases from eucaryotic as well as procaryotic organisms are zinc metalloenzymes (22). Although the exact stoichiometry of zinc has not been established, at least two zinc atoms are present per enzyme molecule (see, e.g., reference 3 for a discussion of yeast RNA polymerase I and reference 22 for a review). Consistent with these observations, previous studies on the sequences of the cloned genes revealed the presence of zinc-binding motifs in both the largest and the second-largest subunits of RNA polymerases. In the former case, the putative zinc-binding region is present near the amino terminus of the proteins and the consensus sequence is $CX_2CX_{6-12}CX_2H$ (Fig. 7A). (Although the consensus zinc-binding motif in the largest subunit was previously thought to be $CX_2CX_{9-15}HX_2H$ [21, 25, 43], $CX_2CX_{6-12}CX_2H$ seems to be more likely in view of the sequences of trypanosome RNA polymerases I and II.) This motif is found in yeast RNA polymerases I, II, and III, other eucaryotic RNA polymerases, and Halobacterium halobium polymerase (i.e., an archaebacterium polymerase), but only
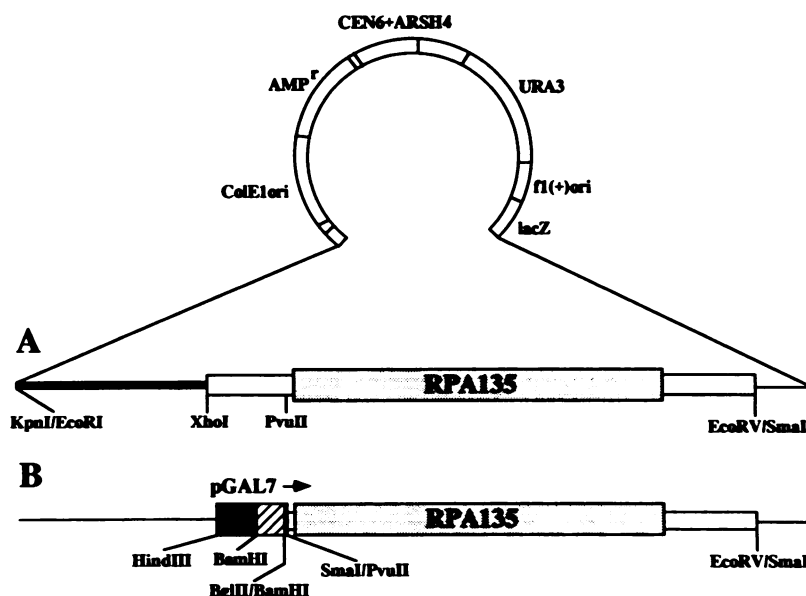

FIG. 4. Plasmids used for conditional expression of the RPA135 (SRP3) gene. (A) Control plasmid pNOY80. (B) pNOY83 carrying the RPA135 gene fused to the GAL7 promoter. The portion of this plasmid derived from vector pRS316 is shown only in panel A.
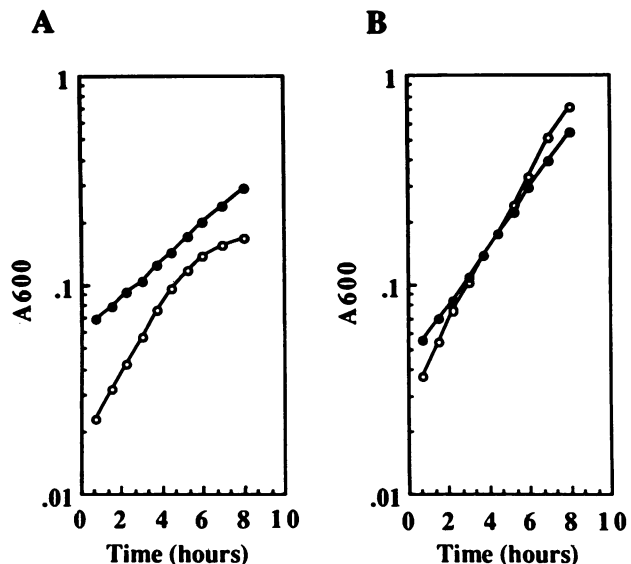
A   **B**

FIG. 5. Growth of NOY399 (A) and NOY400 (B). Cells were first grown in galactose medium. Portions were taken and resuspended in glucose medium (time zero), and growth was monitored by measuring the A_{600} of the cultures (O). The growth of portions maintained in galactose medium (●) was also monitored by measuring the A_{600}.

in an incomplete form in the *E. coli* polymerase β' subunit (21). The zinc-binding motif in the second-largest subunits was first found for yeast RNA polymerase II near the carboxyl terminus of the protein (40). Homologous sequences were subsequently found in the *S. cerevisiae* RNA polymerase III (16a), as well as in other RNA polymerases, including the *H. halobium* polymerase (21) but not the *E. coli* polymerase β subunit. We now find that *S. cerevisiae* RNA polymerase I also carries a similar sequence near the carboxyl terminus (Fig. 7B). Thus, all of the three forms of eucaryotic RNA polymerases, polymerases I, II, and III, possess this putative zinc-binding domain ($CX_2CX_{8-25}CX_2C$) in the second-largest subunit.

We have found that the mutational alteration in the *SRP3* gene responsible for suppression of *rpa190-5* (and *rpa190-1*) is located within the putative zinc-binding region (Fig. 7B). Both the *rpa190-5* and *rpa190-1* mutations, which can be suppressed by *SRP3*, are also located in the putative zinc-binding region of the largest subunit (A190) of the enzyme (43) (Fig. 7A). It should be noted that the *SRP3* suppressor fails to suppress other *rpa190* mutations, including *rpa190-3*, which is an amino acid substitution near the middle of A190
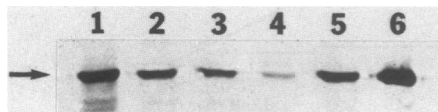
FIG. 6. Western immunoblot analysis of A135. Samples were prepared from NOY399 at various times after the shift from galactose to glucose medium (lanes 1 to 4 corresponding to 0, 2, 4, and 8 h, respectively, after shift). A control sample was prepared from NOY400 at 8 h after the shift from galactose to glucose (lane 5). Samples contained equal amounts of protein. Purified RNA polymerase I was also analyzed as a reference (lane 6) to indicate the position of A135 (arrow). A photograph of an alkaline phosphatase-stained filter is shown.

(43); that is, the suppressor is allele specific. Thus, the present results suggest that these two putative zinc-binding regions may interact during assembly and/or perhaps in the assembled enzyme and that this may be the basis for suppression by *SRP3*. In the preceding paper (24a), we have discussed evidence indicating that the putative zinc-binding domain in the A190 subunit functions to maintain a correct local conformation of the protein that is essential for proper interaction with another subunit during assembly and/or proper interaction with another subunit required for the stability of the enzyme. The present results support this conclusion.

Martin et al. (23) carried out suppressor analysis to obtain information on interactions between specific segments of the largest (B220 or RPB1) and the second-largest (B150 or RPB2) subunits of *S. cerevisiae* RNA polymerase II. They used two temperature-sensitive mutations, one located near the carboxyl terminus of B220 (in region H) and the other located near the carboxyl terminus of B150 (in region I). From the results of the suppressor analysis, they have suggested an interaction between region H of B220 and region I of B150. The sites in region I of B150 used in their experiments are separated from the putative zinc-binding consensus sequence of B150 by 17 to 37 amino acid residues (toward the amino terminus). Perhaps the simplest interpretation of their results and the present results is that both amino-terminal and carboxyl-terminal sequences of the largest subunit interact directly with a single region near the carboxyl terminus of the second-largest subunit. In addition, as also discussed by Martin et al. (23), there remains the possibility that the genetic suppression observed in our work as well as theirs is a consequence of indirect interaction of the two regions at a distance. Therefore, their results are not necessarily in conflict with ours.

Although we do not have direct evidence, we think that direct interaction of the two putative zinc-binding domains is an attractive hypothesis. In this regard, we note that a yeast killer plasmid carries an open reading frame, a presumed RNA polymerase gene, encoding a single 100-kDa polypeptide which has strong homology to several conserved regions in the two large subunits of eucaryotic as well as *E. coli* RNA polymerases (42). Inspection of the linear arrangement of these conserved regions suggests a physical proximity of the region near the carboxyl terminus of the second-largest subunit to the region near the amino terminus of the largest subunit in eucaryotic (as well as *E. coli*) RNA polymerases. In fact, on the basis of this information, Sawadogo and Sentenac (34) suggested that the two putative zinc-binding domains may be physically in close proximity, in agreement with our own suggestion based on the present work.

Analysis of extragenic suppressors of conditionally lethal mutations is a genetic approach to identification of protein components interacting with the protein encoded by a mutated gene. This method has been used in many systems including *S. cerevisiae* RNA polymerase II (23, 29) and *D. melanogaster* RNA polymerase II (27). Using this approach, we have identified the gene for A135 as a suppressor of mutations in the putative zinc-binding domain of A190. This result has confirmed our original expectation and encourages us to use the same approach to identify genes for other subunits of RNA polymerase I as well as additional components, such as specific transcription factors, involved in the transcription of rRNA genes.
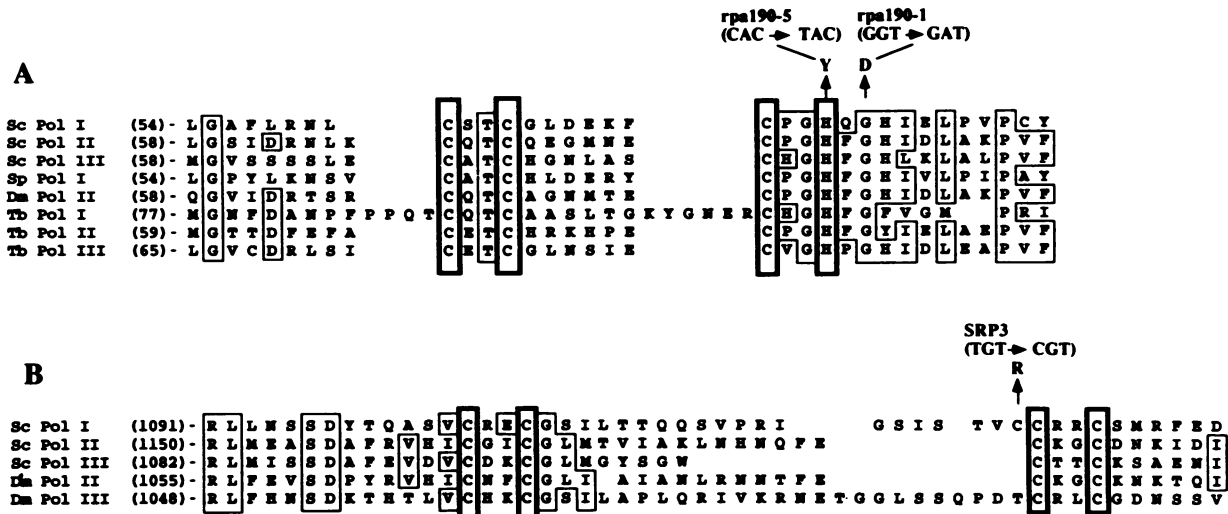
FIG. 7. Putative zinc-binding regions of the largest subunits (A) and the second-largest subunits (B) of several eucaryotic RNA polymerases. The amino acid alteration caused in A135 of *S. cerevisiae* by *SRP3-1*, as well as the amino acid alterations caused by two mutations (*rpa190-1* and *rpa190-5*), which can be suppressed by *SRP3-1*, are indicated. Consensus putative zinc-binding motifs $CX_2CX_{6-12}CX_2H$ (in panel A) and $CX_2CX_{8-25}CX_2C$ (in panel B) are indicated by boxes with thick lines. Other conserved residues (identical residues found in at least 60% of group members) are boxed with thin lines. The numbers in parentheses near the beginning of each sequence indicate the position in the sequence of the first amino acid shown. Abbreviations and references are as follows. (A), Sc (*S. cerevisiae*) Pol I (RNA polymerase I), reference 25; Sc Pol II (RNA polymerase II), reference 2; Sc Pol III (RNA polymerase III), reference 2; Sp (*S. pombe*) Pol I, references 15 and 46; Dm (*D. melanogaster*) Pol II, reference 19; Tb (*Trypanosoma brucei*) Pol I, reference 38; Tb Pol II, reference 39; Tb Pol III, reference 38. (B), Sc Pol I, this work; Sc Pol II, reference 40; Sc Pol III, reference 16a; Dm Pol II, reference 11; Dm Pol III, reference 20. It should be noted that the published sequence for the *D. melanogaster* polymerase III second-largest subunit is more similar to the *S. cerevisiae* polymerase I second-largest subunit than to the *S. cerevisiae* polymerase III second-largest subunit, and its identity has been questioned. This point has been discussed by James et al. (16a).

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