Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11131–11135, December 1992
Biochemistry

A putative ATP-dependent RNA helicase involved in *Saccharomyces cerevisiae* ribosome assembly

(cold-sensitive mutation/assembly defect/rRNA processing)

TRACY L. RIPMASTER, GEORGE P. VAUGHN, AND JOHN L. WOOLFORD, JR.

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213

Communicated by Mary Edmonds, August 17, 1992

ABSTRACT **We have isolated a cold-sensitive mutant of *Saccharomyces cerevisiae* in which there is a deficit of 60S ribosomal subunits. Cold sensitivity and the assembly defect are recessive and cosegregate, defining a single essential gene that we designate *DRS1* (deficiency of ribosomal subunits). The wild-type *DRS1* gene was cloned by complementation of the cold-sensitive phenotype of *drs1*. Sequence analysis reveals a high degree of similarity to a family of proteins that are thought to function as ATP-dependent RNA helicases. Pulse–chase analysis of ribosomal RNA synthesis and processing indicates that the *drs1* mutant accumulates the 27S precursor of the mature 25S rRNA. These results suggest that, as in pre-mRNA splicing, RNA helicase activities are involved in ribosomal RNA processing.**

Details of the pathway by which the 70 to 80 different ribosomal proteins and 3 or 4 rRNA molecules are assembled into functional eukaryotic 40S and 60S ribosomal subunits remain largely undefined. Ribosome biogenesis occurs primarily in the nucleolus of eukaryotes, where rRNA is transcribed, processed, and associated with ribosomal proteins imported from the cytoplasm (reviewed in refs. 1–3). Nucleolar preribosomal particles can be detected that contain rRNA precursors, ribosomal proteins, and nonribosomal proteins (4). Final steps of maturation of ribosomal subunits take place in the cytoplasm (5).

Much insight has been gained into the nature of ribosome assembly in *Escherichia coli* by reconstitution of purified rRNA and ribosomal proteins *in vitro* into functional 30S and 50S subunits (6, 7). The success of this technique led to detailed assembly maps (8, 9) and more recently to elucidation of rRNA secondary structural changes during the process of 30S subunit assembly (10). The lack of a cell-free system to reconstitute eukaryotic ribosomes from separate components has prompted a genetic approach to study eukaryotic ribosome biogenesis.

To explore the nature of ribosome biogenesis in the genetically tractable organism *Saccharomyces cerevisiae*, mutants unable to synthesize rRNAs or ribosomal proteins or blocked in rRNA processing have been obtained (reviewed in refs. 2 and 3). Depletion or inactivation of rRNA or individual ribosomal proteins blocks proper assembly of the subunit containing that protein or rRNA and leads to rapid turnover of the other RNAs and proteins in that subunit. Unlike the case in *E. coli*, however, none of these yeast mutants has allowed identification of assembly intermediates whose protein content could be studied biochemically.

Several yeast small nucleolar RNAs (snoRNAs) or nucleolar proteins have been identified that affect rRNA processing. Depletion of the U3 or U14 snoRNAs or of the nucleolar proteins Nop1p or Gar1p causes defects in 35S pre-rRNA processing and dramatic reductions in the amount of 18S rRNA (11–14). Slow-growing strains of yeast bearing a disruption of the *NSR1* gene show a similar phenotype, as well as changes in the steady-state levels of ribosomal subunits (15).

Cold-sensitive bacterial strains have been a rich source of mutants defective in ribosome subunit assembly (reviewed in ref. 2). A few of these mutants were shown to contain defective ribosomal proteins, but in most cases the molecular basis of the assembly defect was not determined. With the subsequent development of sophisticated molecular techniques in yeast, we reasoned that a screen of cold-sensitive yeast mutants could be exploited to identify and characterize gene products necessary for ribosome assembly, including nonribosomal as well as ribosomal molecules. We describe one such mutant, defective in ribosome synthesis, *drs1*, and the cloning and sequencing* of the *DRS1* gene. The predicted amino acid sequence of the Drs1 protein (Drs1p) has striking similarity to that of a family of putative ATP-dependent RNA helicases.

MATERIALS AND METHODS

Strains and Media. *S. cerevisiae* strain DS94 (*MAT***a**, *ura3-52, trp1Δ101, leu2-3,112, his3-11,15, lys2-801*), obtained from Elizabeth Craig (University of Wisconsin, Madison), was used as the wild-type parent from which cold-sensitive mutant strains were derived by mutagenesis with ethyl methanesulfonate. DBY1034 (*MAT***a**, *ura3-52, his4-51, lys2-801*) was obtained from David Botstein (Stanford Medical School, Stanford, CA) and used for outcrosses. JWY2165 (*MAT***a**, *ura3-52, his3-11,15, leu2-3,112, drs1-1*) was the outcrossed cold-sensitive spore used to isolate *DRS1* by transformation with yeast genomic DNA. JWY749 (*MAT***a**/α, *ura3-52/ura3-52, trp1-Δ101/trp1-Δ101, lys2-801/lys2-801, leu2-Δ1/leu2-Δ1, his3-Δ200/his3-Δ200*) was the diploid strain used to disrupt the *DRS1* gene. Standard yeast genetic methods were employed (16). Yeast cells were grown in either YEPD or defined synthetic media supplemented with 2% dextrose as a carbon source (17). Yeast were transformed by the lithium acetate method (18).

Materials. Restriction endonucleases and T4 DNA ligase were obtained from Boehringer Mannheim. *E. coli* DNA polymerase holoenzyme was a gift of William E. Brown (Carnegie Mellon University). [α-^{32}P]dATP and [α-[^{35}S]thio]-dATP were obtained from Amersham. [*methyl*-^{3}H]Methionine was obtained from Dupont/NEN.

Fractionation and Characterization of Polyribosomes. Yeast strains were grown to a density of 4.0–6.0 × 10^7 cells per ml in 200 ml of YEPD at 30°C and shifted to 13°C for 2 hr. Polyribosomes were extracted and analyzed on 7–47% su-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: snoRNA, small nucleolar RNA; Drs1p, Drs1 protein; HA, hemagglutinin.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L00683).

crose gradients, using an ISCO density gradient fractionator, model 640, as described by Baim *et al.* (19).

DNA Manipulations and Southern and Northern Analyses. Recombinant DNA techniques, gel electrophoresis, Southern blotting, and hybridization of DNA were performed as described in Sambrook *et al.* (20). Electrophoresis, blotting, and hybridization of RNA were performed as described by Larkin and Woolford (21). Nytran membrane (Schleicher & Schuell) was used for both DNA and RNA blotting. Subclones designed to be used in both *E. coli* and yeast were inserted into the pRS series of vectors (22). The λ phage and cosmid library of overlapping yeast genomic fragments was probed as described by Riles and Olson (23).

DNA Sequence Determination and Analysis. Restriction fragments from pDRS1 were subcloned in pBluescript II KS(+) and SK(+) (Stratagene). The sequences of both strands of pDRS1 were determined by the dideoxy chain-termination method (24), using Sequenase Version 2.0 (United States Biochemical). Gaps of sequence in either strand were completed by using synthetic oligonucleotide primers synthesized by Operon Technologies (Alameda, CA). Sequence analysis and data base searches for sequence similarities were performed with computer programs developed by the Genetics Computer Group's Sequence Analysis Software Package (25).

Pulse-Chase Analysis of rRNA Synthesis. The synthesis, processing, and accumulation of large rRNAs were assayed by pulse-chase analysis and gel electrophoresis exactly as described by Moritz *et al.* (26).

Epitope Addition, Biochemical Fractionation, and Immunoblot Analysis. The sequence encoding the nine-amino acid influenza virus hemagglutinin (HA) epitope was inserted into *DRS1* by site-directed mutagenesis (27). Yeast cells were fractionated as described by Aris and Blobel (28). Proteins from cytoplasmic and nuclear fractions were subjected to PAGE, electrophoretically transferred to nitrocellulose, and assayed by immunoblot analysis using the Protoblot Immunoscreening System (Promega). Monoclonal antibody 12CA5, generated against the influenza virus HA epitope (29), was a generous gift of Peter Kolodzeij (University of California, San Francisco). Antibodies against yeast Nop1p were a kind gift of Robert Hamatake (National Institute of Environmental Health Sciences).

RESULTS

Identification of the Cold-Sensitive *drs1* Mutant Deficient in 60S Ribosomal Subunits. We identified the *S. cerevisiae drs1* mutant by screening a collection of cold-sensitive mutants for those defective in the assembly or function of ribosomes (unpublished results). Sucrose gradient analysis of extracts prepared from *drs1* cells grown at the permissive temperature of 30°C and shifted to the nonpermissive temperature of 13°C revealed that the *drs1* mutation results in a deficit of 60S ribosomal subunits relative to 40S subunits and in decreased levels of 80S monosomes and polyribosomes (Fig. 1). As a result of the shortage of 60S subunits, half-mer polyribosomes, which contain 43S initiation complexes stalled at the initiator AUG (26), were formed. When the *drs1* mutant was crossed to wild-type strain DBY1034, the 60S subunit deficit and cold sensitivity both segregated 2:2 and cosegregated with each other, indicating that both phenotypes are due to a single mutation in a nuclear gene. The heterozygous *drs1*/*DRS1* diploid was not cold sensitive and contained wild-type levels of ribosomal subunits; thus *drs1* is a recessive mutation.

Isolation of the *DRS1* Gene. To isolate the *DRS1* gene, a cold-sensitive haploid (JWY2165) derived from the outcross to wild-type strain DBY1034 was transformed with a library of yeast genomic DNA cloned in YCp50 (30). One plasmid,

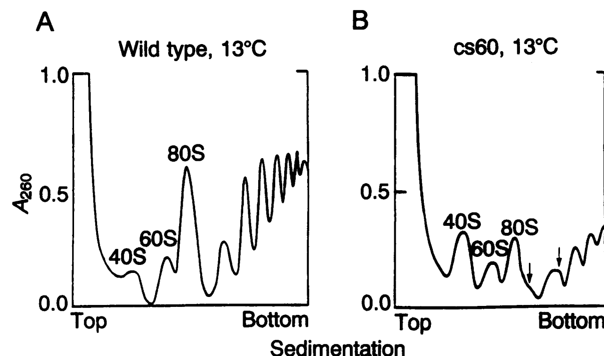

FIG. 1. The cold-sensitive *drs1* mutant cs60 is deficient in 60S ribosomal subunits at 13°C. Mutant cells grown at 30°C to 4.0×10^7 cells per ml were shifted to 13°C for 2 hr. Cell lysates containing ribosomal subunits and polyribosomes were separated by centrifugation on 7–47% sucrose velocity gradients. Peaks representing free 40S and 60S subunits and 80S monosomes are labeled. Fractions containing half-mer polyribosomes are indicated by arrows.

pDRS1, was recovered that complemented the cold-sensitive phenotype. The restriction map of the 6.5-kilobase (kb) insert of yeast genomic DNA in pDRS1 is shown in Fig. 2. To confirm that pDRS1 contains *DRS1*, we demonstrated that a restriction fragment from pDRS1 recombined with the *drs1* genomic locus. A 2.0-kb *Bam*HI–*Sal* I fragment containing the 3′ 275 codons of *DRS1* was subcloned in integrating plasmid YIp5 containing *URA3*. This plasmid was linearized with *Bgl* II and used to transform JWY2165. Selecting for uracil-independent (Ura^+) transformants yielded strain JWY2166. The *URA3* marker is flanked by one full-length copy of *drs1* that contains the cold-sensitive mutation and by one truncated copy of *DRS1*. The integrity of the recombination event was confirmed by genomic Southern blotting (data not shown). The cold-sensitive (Cs^-) transformant JWY2166 was crossed to wild-type strain DBY1034, diploids were sporulated, and tetrads were dissected. Seventeen tetrads segregated 2 Ura^+Cs^- to 2 Ura^-Cs^+, indicating that the plasmid had integrated at or near the *drs1* locus and therefore that plasmid pDRS1 contains the *DRS1* gene.

Localization of the *DRS1* Gene and Identification of the *DRS1* Transcript. Deletion of genomic sequences 5′ of the

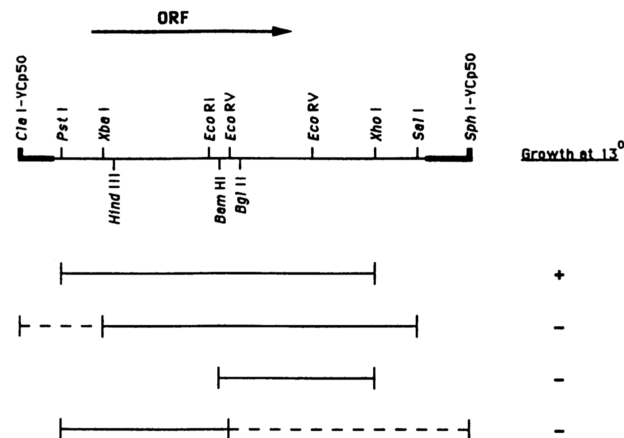

FIG. 2. Restriction map of yeast genomic DNA containing the *DRS1* gene. Only the cloned yeast DNA segments are indicated. Insert DNA from the original complementing plasmid pDRS1 is shown at the top. YCp50 DNA is indicated by the bold lines. Broken lines indicate DNA deleted from pDRS1. The ability of various subclones or deletion plasmids derived from pDRS1 to complement the cold sensitivity of *drs1* was tested by transformation into the *drs1* mutant strain. The open reading frame (ORF) determined by sequencing pDRS1 is indicated by the horizontal arrow.

Xba I site or 3′ of the *Bam*HI site destroyed the ability of pDRS1 to complement the cold-sensitive phenotype of *drs1*, indicating that *DRS1* spans these two sites (Fig. 2). The *Pst* I–*Xho* I fragment subcloned in the centromere-based plasmid pRS315 was able to complement the cold sensitivity of JWY2165. DNA and RNA blot hybridization with the *Pst* I–*Xho* I fragment demonstrated that *DRS1* is a single-copy gene that encodes a 2.3-kb poly(A)$^+$ mRNA (data not shown). *DRS1* was localized to the left arm of chromosome XII, centromere-proximal to *ppr1*, by hybridization of *DRS1* DNA to a blot of separated yeast chromosomes and then by probing an ordered set of λ and cosmid clones that covers most of the yeast genome (23) (data not shown).

***DRS1* Encodes a Putative ATP-Dependent RNA Helicase.** The complete nucleotide sequence of *DRS1* and the predicted amino acid sequence of Drs1p are shown in Fig. 3. Comparison of the inferred amino acid sequence of Drs1p with proteins in current data bases indicated that the sequence of Drs1p shares extensive identity to a family of proteins thought to function as ATP-dependent RNA helicases. Fig. 4 aligns the predicted amino acid sequence of Drs1p with the sequences of three other proteins known or postulated to function as ATP-dependent RNA helicases: eIF-4A, SrmB, and Spb4p. eIF-4A is the founding member of the "DEAD-box" RNA helicase family and is thought to function in unwinding mRNA during translation initiation. The *srmB* gene was isolated as a high-copy suppressor of a mutation in ribosomal protein L24 in *E. coli* (31), and Spb4p is involved in maturation of yeast rRNA (32).

The region extending from amino acid residue 238 to residue 582 of Drs1p includes motifs similar to the seven conserved motifs common to the RNA helicase family of proteins, including domains I and III, which are postulated to bind and hydrolyze nucleoside triphosphates. These are underlined in bold in Fig. 3. Also underlined in Fig. 3, located amino-terminal to the central helicase motif, are the sequences "DEAK" and "SAT", which are related to domains III and IV. The amino-terminal 172 amino acid residues are acidic in nature, containing 34% glutamic and aspartic residues, including several poly(glutamic acid) stretches. The carboxy-terminal domain of Drs1p is also highly charged, consisting of 43% glutamic, aspartic, lysine, and arginine residues.

***DRS1* Is an Essential Gene.** A *drs1* null allele was created by replacing the *Bam*HI–*Bgl* II fragment within the *DRS1* coding sequence with the *HIS3* gene. A *Pst* I–*Xho* I fragment

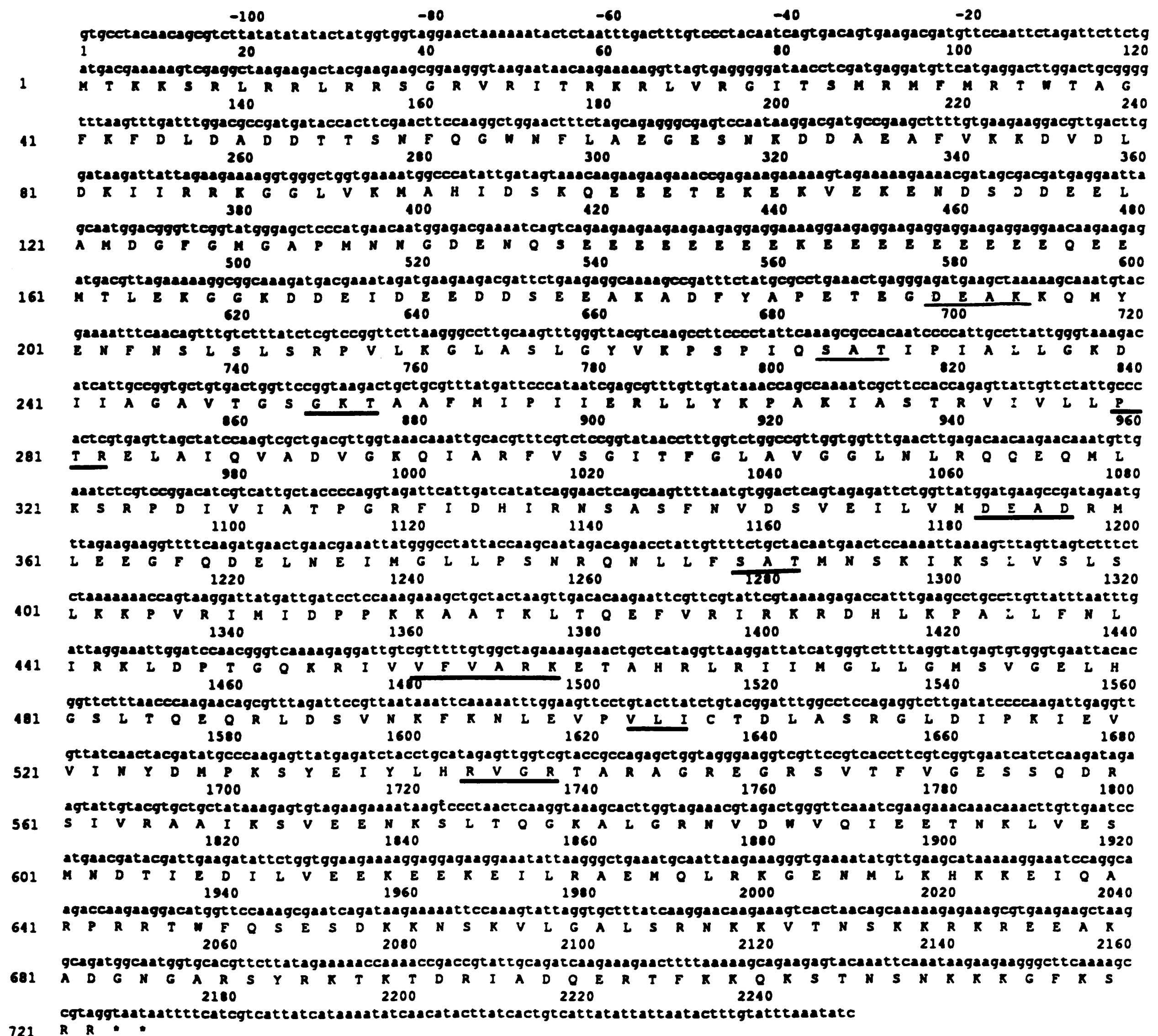

FIG. 3. Nucleotide sequence of DRS1 and predicted amino acid sequence of the Drs1p. Predicted amino acid sequences within Drs1p that are found to be conserved among a superfamily of putative ATP-dependent RNA helicases are underlined. Nucleotides are numbered above the sequence and amino acids are numbered to the left of each line.

```
          241       Domain I                                 Domain II
eIF-4A    VIAQAQSGTG KTATFAISIL QQI...EL.. ...DLKATQA LVLAPTRELA QQIQKVVMAL GDYMGASCHA
SrmB      VLGSAPTGTG KTAAYLLPAL QHLLDFPR.. ..KKSGPPRI LILTPTRELA MQVSDHAREL AKHTHLD...
Spb4      VVVDSVTGSG KTAAFVIPVL EKVVKEEANT SKFKKAHFHS LIIAPTRELS RQIESVVLSF LEHYPSDLFP
Drs1      IIAGAVTGSG KTAAFMIPII ERLLYKPA.. ...KIASTRV IVLLPTRELA IQVADVGKQI ARFVSGITFG
          306                                                  Domain III
eIF-4A    .......CIG GTNVRAEVQK LQMEAPHIIV GTPGRVFDML .NRRYLSPKY IKMFVLDEAD EMLSRGFKDQ
SrmB      .IATITGGVA YMNHA....E VFSENQDIVV ATTGRLLQYI KE.ENFDCRA VETLILDEAD RMLDMGFAQD
Spb4      IKCQLLVGTN EATVRDDVSN FLRNRPQILI GTPGRVLDFL .QMPAVKTSA CSMVVMDEAD RLLDMSFIKD
Drs1      ......LAVG GLNLRQQEQM LK.SRPDIVI ATPGRFIDHI RNSASFNVDS VEILVMDEAD RMLEEGFQDE
          369            Domain IV
eIF-4A    IYDIFQKLNS NTQVVLLSAT MPSD.VLEVT KKFMRDPIRI LVKKEELTLE GIRQFYINV. EREEWKLDTL
SrmB      IEHIAGETRW RKQTLLFSAT LEGDAIQDFA ERLLEDPVEV SANPSTRERK KIHQWYYRAD DLEH.KTALL
Spb4      TEKILRLLPK QRRTGLFSAT MRSAG.SDIF KTGLRNPVRI TVNSKNQAPS SLKLNYCVVN PAE..KLQLL
Drs1      LNEIMGLLPS NRQNLLFSAT MNSK.IKSLV SLSLKKPVRI MIDPPKKAAT KLTQEFVRIR KRDHLKPALL
          438           Domain V
eIF-4A    CDLYETLTIT ...QAVIFI. NTRRKVDWLT EKMHA..... ..RDFTVSAM HGDMDQKERD VIMREFRSG.
SrmB      VHLLKQPEAT ...RSIVFVR NRKRERVHEL ANWLREA... ...GINNCYL EGEMVQGKRN EAIKRLTEG.
Spb4      VSILNNYKF. ..KKCIVYFP TCVSVSYFYS FIQYLGKRNI LVNEVEIFSL HGKLQTSART KTLTAFTDSL
Drs1      FNLIRKLDPT GQKRIVVFV. ARKETAHRLR IIMGL..... ..LGMSVGEL HGSLTQEQRL DSVNKFKNL.
          499      Domain VI                  Domain VII
eIF-4A    SSRVLITTDL LARGIDVQQV SLVINYDLPT NRENYIHRIG RGGRFGRKGV AINMVTE... ..........
SrmB      RVNVLVATDV AARGIDIPDV SHVFNFDMPR SGDTYLHRIG RTARAGRKGT AISLVEA..H DHLLLGKVGR
Spb4      SNSVLFTTDV AARGIDIPDV DLVIQLDPPT NTDMFMHRCG RTGRANRVGK AITFLNEGRE EDFIPFMQVK
Drs1      EVPVLICTDL ASRGLDIPKI EVVINYDMPK SYEIYLHRVG RTARAGREGR SVTFVGESSQ DRSIVRAAIK
```

FIG. 4. Alignment of the predicted amino acid sequences of Drs1p, eIF-4A, SrmB, and Spb4 protein. Shaded areas indicate the high degree of identity among these proteins within the seven conserved sequence motifs found in RNA helicases. The numbering above each row of sequences corresponds to the amino acid sequence of Drs1p.

containing this *drs1*Δ::*HIS3* disruption was used to transform diploid JM749, which is homozygous for *his3*-Δ200, and His^+ colonies were selected. The expected transplacement was confirmed by genomic Southern blotting (data not shown). Diploids were sporulated and tetrads were dissected. In 45 of 45 tetrads, only two viable spores were recovered, all of which were His^-. To rule out the possibility that *DRS1* is essential only for spore germination, pDRS1 bearing *DRS1* and *URA3* was used to transform the *drs1*::*HIS3*/*DRS1* diploid and the transformant was subsequently sporulated. Upon dissection of tetrads, His^+ spores were recovered, all of which were Ura^+, demonstrating that Drs1p is also essential for mitotic growth.

Formation of 25S rRNA Is Impaired in the *drs1* Mutant. Processing and stability of rRNAs were assayed in the *drs1* mutant by pulse–chase analysis. Cells were grown at the permissive temperature of 30°C to 4.0×10^7 cells per ml. Half of the culture was maintained at 30°C and half was shifted to the nonpermissive temperature of 13°C for 2 hr. Cells were labeled for 10 min with [*methyl*-^{3}H]methionine and then incubated with excess unlabeled methionine (1 mg/ml) for 15 or 30 min. Total RNA was extracted and subjected to denaturing gel electrophoresis to resolve 18S and 25S rRNA as well as any rRNA processing intermediates. As shown in Fig. 5, synthesis and processing of rRNAs were normal in *drs1* at 30°C as evidenced by the accumulation of mature 18S and 25S rRNAs at a 1:1 molar ratio. Processing of 20S rRNA to 18S rRNA was normal at 13°C, but 25S rRNA did not accumulate in significant levels, due to either abnormal processing or degradation. Although amounts of a species migrating at the position of 27S pre-rRNA were similar in *drs1* and wild-type cells at 13°C, our analysis might not distinguish normal from abnormal processing intermediates. The deficit of 25S rRNA relative to 18S rRNA that accumulated in *drs1* at 13°C is consistent with the observed depletion of 60S subunits in *drs1* at 13°C. Synthesis and processing of rRNAs extracted from the wild-type parent strain were identical to those observed in *drs1* at 30°C (data not shown). As in the *drs1* strain, labeling of rRNAs was less efficient in the wild-type strain at 13°C than at 30°C. However, all of the 27S rRNA precursor labeled in the pulse at 13°C was processed to 25S rRNA by the 15-min chase. Moreover, 18S and 25S rRNA accumulated at a 1:1 molar ratio at all times tested.

Biochemical Fractionation Indicates That Drs1p Is Located in the Nucleus. To determine the intracellular location of Drs1p, it was tagged at amino acid 689 with a nine-amino acid influenza virus HA epitope (29) by oligonucleotide-directed mutagenesis of *DRS1*. The HA-tagged allele of *DRS1* was shown to be functional by transforming a *drs1*Δ::*HIS3* strain containing plasmid-borne *DRS1* and *URA3* with a plasmid containing *HA*:*DRS1* and *LEU2*. Upon plating Leu^+ Ura^+His^+ transformants on 5-fluoroorotic acid to select for loss of the *URA3* plasmid (34), viable His^+Leu^+ colonies were recovered. Biochemical fractionation and immunoblot analysis using monoclonal antibodies directed against the HA epitope indicated that Drs1p is found predominantly in the nucleus (Fig. 6*A*). The small amount of signal detected in the cytoplasm could be a result of leakage from the nuclei during the purification procedure.

DISCUSSION

DRS1 was cloned by its ability to complement a cold-sensitive mutation causing a deficit of 60S ribosomal subunits. The predicted amino acid sequence of Drs1p contains identity to seven motifs found among a family of proteins referred to as "DEAD-box" helicases, all of which are postulated to function in unwinding RNA duplexes. Pulse–chase analysis of the *drs1* mutant showed a defect in processing of the 27S precursor to the mature 25S rRNA.

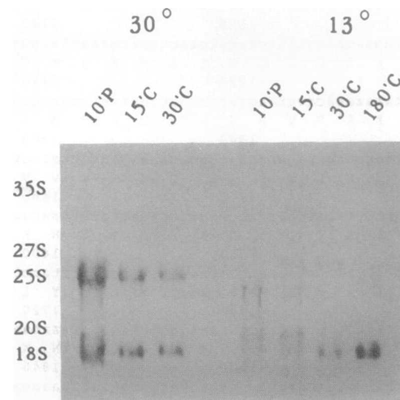

FIG. 5. Synthesis and accumulation of rRNA in the *drs1* mutant. RNA was extracted from cells labeled with [*methyl*-^{3}H]methionine for 10 min and "chased" for 15, 30, or 180 min with excess unlabeled methionine, at the permissive temperature of 30°C and after a 2-hr shift to the nonpermissive temperature of 13°C. RNA was resolved by denaturing gel electrophoresis, blotted to nylon membranes, sprayed with EN^{3}HANCE (New England Nuclear), and exposed to x-ray film at −70°C. The positions of mature 18S and 25S rRNAs and the 27S precursor of 25S rRNA are shown. 10'P, 10-min pulse; 15'C, 15-min chase; etc.

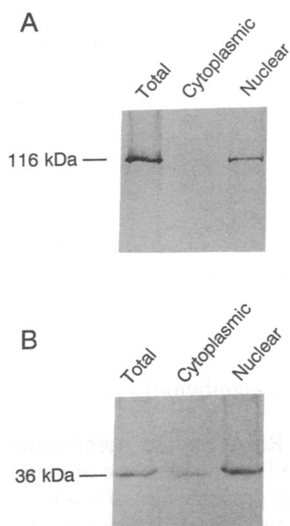

FIG. 6. Drs1p is predominantly localized in the nucleus. A *drs1Δ::HIS3* strain carrying the HA:*DRS1* allele was partitioned into cytoplasmic and nuclear fractions. Equal microgram amounts of protein from each fraction were subjected to PAGE, blotted, and probed with anti-HA antibodies to detect tagged Drs1p (*A*) or with antibodies against Nop1p (*B*). Nop1p is a yeast nucleolar protein (28) and served as a control for the fractionation procedure.

Western immunoblot analysis of cellular fractions indicated that the majority of Drs1p is nuclear, consistent with its involvement in rRNA processing.

Three other helicases that affect ribosome assembly have been identified. The *E. coli srmB* gene functions as a gene dosage suppressor of a ribosomal protein mutation causing a defect in 50S subunit assembly (31). Similarly, the *E. coli deaD* gene was identified as a multicopy suppressor of a mutation in the ribosomal protein S4 (34). The *S. cerevisiae spb4* mutation was identified by its ability to suppress a temperature-sensitive mutation in *PAB1*, encoding poly(A)-binding protein (35) and was subsequently shown to affect the processing step that converts 27S rRNA to the mature 25S species (32). It is noteworthy that the carboxyl termini following the helicase domains of Drs1p, SrmB, and Spb4p contain large proportions of charged amino acid residues. These charged carboxyl-terminal domains have also been found in other proteins known to bind RNA such as U1 70K (36). Perhaps this domain in Drs1p interacts directly with rRNA or with ribosomal subunits.

Both the *spb4* and *drs1* mutants affect maturation of the 27S rRNA to mature 25S rRNA, indicating that more than one helicase may be involved in this process. Both *SPB4* and *DRS1* are required for mitotic growth, suggesting that the functions of their proteins are not redundant. One other mutant, *rrp1*, has been identified and shown to be defective specifically in the processing of 27S rRNA in yeast (37), but the nature of the *RRP1* gene product has not been determined. The *rrp1* defect has been mapped to the right arm of chromosome IV, linked to *mak21* (38), indicating that *rrp1* and *drs1* are not allelic.

ATP-dependent RNA helicases have been implicated in many cellular processes, including translation initiation (reviewed in ref. 39), and pre-mRNA splicing (40). Five additional putative RNA helicase genes were identified in yeast by using polymerase chain reaction technology, some of which have not been assigned functions (41). eIF-4A and SrmB mentioned previously, and human p68, another member of the "DEAD-box" family, all have been shown to hydrolyze ATP and unwind RNA duplexes *in vitro* (31, 42, 43).

Small nucleolar ribonucleoprotein particles (snoRNPs) have been shown to be involved in rRNA processing (reviewed in ref. 3). By analogy to the role of small nuclear ribonucleoprotein particles (snRNPs) in pre-mRNA splicing, these snoRNPs are thought to form complexes with rRNA to facilitate processing events. RNA helicases involved in rRNA processing could function in the intramolecular unwinding of rRNA secondary structure to promote rRNA–snoRNA interactions or to expose binding sites for ribosomal or nonribosomal proteins required for processing. Destabilization of snoRNA–rRNA and snoRNA–snoRNA interactions formed during the processing events might also require helicase activity. Additional as-yet-unidentified ATPases or helicases may also be required for rRNA processing and ribosome assembly in yeast.

We thank Drs. Tien-Hsien Chang (Ohio State University) and William McClure (Carnegie Mellon University) for critical reading of the manuscript. This work was supported by National Institutes of Health Grant GM28301. G.P.V. was a recipient of National Science Foundation Research Experience for Undergraduates Award BBS8712919.

1. Hadjilov, A. A. (1985) *The Nucleolus and Ribosome Biogenesis* (Springer, New York).
2. Woolford, J. L., Jr. (1991) *Adv. Genet.* **29**, 63–118.
3. Woolford, J. L., Jr., & Warner, J. R. (1991) *Molecular and Cellular Biology of the Yeast Saccharomyces* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 587–626.
4. Trapman, J., Retel, J. & Planta, R. J. (1975) *Exp. Cell Res.* **90**, 95–104.
5. Udem, S. A. & Warner, J. R. (1973) *J. Biol. Chem.* **248**, 1412–1416.
6. Traub, P. & Nomura, M. (1968) *Proc. Natl. Acad. Sci. USA* **59**, 777–784.
7. Nierhaus, K. H. & Dohme, F. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4713–4717.
8. Mizushima, S. & Nomura, M. (1970) *Nature (London)* **226**, 1214–1218.
9. Held, W. A., Ballou, B., Mizushima, S. & Nomura, M. (1974) *J. Biol. Chem.* **249**, 3103–3111.
10. Stern, S., Powers, T., Changchien, L.-M. & Noller, H. (1989) *Science* **244**, 783–790.
11. Li, H. V., Zagorski, J. & Fournier, M. J. (1990) *Mol. Cell. Biol.* **10**, 1145–1153.
12. Girard, J.-P., Lehtonen, H., Caizergues-Ferrer, M., Amalric, F., Tollervey, D. & Lapeyre, B. (1991) *EMBO J.* **11**, 673–682.
13. Hughes, J. M. X. & Ares, M., Jr. (1991) *EMBO J.* **10**, 4231–4239.
14. Tollervey, D., Lehtonen, H., Carmo-Fonseca, M. & Hurt, E. C. (1991) *EMBO J.* **10**, 573–583.
15. Lee, W. C., Zabetakis, D. & Mélèse, T. *Mol. Cell. Biol.* **12**, 3865–3871.
16. Mortimer, R. K. & Hawthorne, D. C. (1966) *Genetics* **53**, 165–173.
17. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 166–167.
18. Ito, H., Fukuda, Y., Murata, K. & Kimara, A. (1983) *J. Bacteriol.* **153**, 163–168.
19. Baim, S. D., Pietras, D. C., Eustice, D. C. & Sherman, F. (1985) *Mol. Cell. Biol.* **5**, 1839–1846.
20. Saunbrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
21. Larkin, J. C. & Woolford, J. L., Jr. (1983) *Nucleic Acids Res.* **11**, 403–420.
22. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19–27.
23. Riles, L. & Olson, M. V. (1992) *Genetics*, in press.
24. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
25. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
26. Moritz, M., Pulaski, B. & Woolford, J. L., Jr. (1991) *Mol. Cell. Biol.* **11**, 5681–5692.
27. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
28. Aris, J. P. & Blobel, G. (1988) *J. Cell Biol.* **107**, 17–31.
29. Wilson, I. A., Niman, H. L., Houghten, R. A., Cherenson, A. R., Connoly, M. L. & Lerner, R. A. (1984) *Cell* **37**, 767–778.
30. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. & Fink, G. R. (1987) *Gene* **60**, 237–243.
31. Nishi, K., Morel-Deville, F., Hershey, J. W. B., Leighton, T. & Schnier, J. (1988) *Nature (London)* **336**, 496–498.
32. Sachs, A. B. & Davis, R. W. (1990) *Science* **247**, 1077–1079.
33. Boeke, J. D., LaCroute, F. & Fink, G. R. (1984) *Mol. Gen. Genet.* **197**, 345–356.
34. Toone, W. M., Rudd, K. E. & Friesen, J. D. (1991) *J. Bacteriol.* **173**, 3291–3302.
35. Sachs, A. B. & Davis, R. W. (1989) *Cell* **58**, 857–867.
36. Query, C. C., Bentley, R. C. & Keene, J. D. (1989) *Cell* **57**, 89–101.
37. Andrew, C., Hopper, A. K. & Hall, B. D. (1976) *Mol. Gen. Genet.* **144**, 29–37.
38. Fabian, G. R. & Hopper, A. K. (1987) *J. Bacteriol.* **169**, 1571–1578.
39. Pain, V. M. (1986) *Biochem. J.* **235**, 625–637.
40. Wassarman, D. A. & Steitz, J. A. (1991) *Nature (London)* **349**, 463–464.
41. Chang, T.-H., Arenas, J. & Abelson, J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1571–1575.
42. Rozen, F., Edery, I., Meerovitch, K., Dever, T. E., Merrick, W. C. & Sonenberg, N. (1990) *Mol. Cell. Biol.* **10**, 1134–1144.
43. Hirling, H., Scheffner, M., Restle, T. & Stahl, H. (1989) *Nature (London)* **339**, 562–564.