JOURNAL OF BACTERIOLOGY, Apr. 1978, p. 246-260
0021-9193/78/0134-0246$02.00/0

Vol. 134, No. 1
Printed in U.S.A.

Ribonucleoprotein Particle Appearing During Sporulation in Yeast

PETER J. WEJKSNORA† AND JAMES E. HABER*

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154

Received for publication 20 January 1978

During sporulation of *Saccharomyces cerevisiae*, most strains accumulate an unmethylated 20S RNA. Contrary to previous reports, this sporulation 20S RNA is distinct from the short-lived methylated 20S RNA precursor of 18S rRNA. This RNA species was found in a cytoplasmic 32S ribonucleoprotein particle consisting of one single-stranded 20S RNA molecule and 18 to 20 identical protein subunits of molecular weight 23,000. The ribonucleoprotein particle was resistant to ribonuclease digestion, although purified 20S RNA was ribonuclease sensitive. Both the RNA and the protein of the 32S ribonucleoprotein particle were only synthesized under conditions that induce sporulation. The accumulation of 20S RNA depended on continued protein synthesis but was actinomycin D insensitive, despite a high guanine-plus-cytosine content. Synthesis of 20S RNA stopped when cells were removed from sporulation conditions and placed in growth medium.

When cells of most *Saccharomyces* strains are placed in sporulation medium, a novel RNA species accumulates, often accounting for as much as 15% of the stable RNA synthesized during sporulation (11, 12, 25, 31). Previous studies had suggested that this 20S RNA was an unmethylated derivative of ribosomal precursor RNA, analogous to the methylated, transient 20S RNA found in vegetative cells (25, 31). However, recent genetic studies on the control of 20S RNA synthesis (4) have shown that the control of sporulation 20S RNA is cytoplasmically inherited, and unrelated to rRNA.

In this paper we describe the cytoplasmic localization of 20S RNA in a unique 32S ribonucleoprotein (RNP) particle. The 20S RNA is associated with 18 to 20 identical protein subunits that confer nearly complete ribonuclease resistance to the RNA. The RNA and the protein of the 32S particle are synthesized only under conditions that initiate sporulation. Neither 20S RNA nor its associated protein is related to rRNA or proteins.

(Some of this work was submitted by P.J.W. as part of his Ph.D. dissertation.)

MATERIALS AND METHODS

Strains. Several strains of *Saccharomyces cerevisiae* were used in this study. Diploid strain J659 has the genotype: $\frac{\mathbf{a}}{\alpha}$ $\frac{ade1}{+}$ $\frac{ade2}{+}$ $\frac{ura1}{+}$ $\frac{gal1}{+}$ $\frac{lys2}{+}$ $\frac{tyr1}{+}$ $\frac{his7}{+}$ $\frac{+}{his8}$ $\frac{+}{met2}$ $\frac{+}{ade8}$. Strain Y55 is a homothallic wild-type diploid. Strain J98 is an actinomycin D-sensitive haploid strain constructed by K. Atkinson, C. Gorenstein, and E. Falke, who were kind enough to provide it. This strain has the genotype **a** *acd1 ade1 ade5 lys2 can*^{r} *MAL6*, and can synthesize 20S RNA under sporulation conditions (4).

Cultivation and sporulation. Cells were grown in Erlenmeyer flasks either in minimal medium or in YEPD medium (31). The volume of the flasks was always at least four times the volume of liquid. The flasks were shaken in a gyratory shaker at 30°C. Growth was followed visually or by measuring absorbance at 600 nm by using a Klett-Summerson photometric colorimeter. Cells were harvested by centrifugation.

In experiments involving sporulating cells, cells were grown vegetatively to stationary phase and then washed twice in either sterile, distilled water or sterile 1% potassium acetate, pH 7. Cells were then resuspended in sterile 1% potassium acetate, pH 7, in Erlenmeyer flasks such that the flask volume was 10 times the volume of the liquid. The flasks were shaken on gyratory shakers at 30°C to assure proper aeration. Sporulation was monitored by counting the percentage of asci among cells in the population after 48 h.

Isolation of RNA. RNA was extracted from whole cells, cell fractions, or regions of sucrose gradients. When cell fractions or sucrose gradients were examined, the RNA was either extracted directly from the solution, or first concentrated by precipitation in 67% ethanol followed by high-speed centrifugation and then extracted. When RNA was extracted from whole cells, cells were harvested by centrifugation, washed and suspended in a buffer containing 0.1 M tris(hydroxymethyl)aminomethane (Tris), pH 7.4, 0.1 M NaCl, and 0.003 M ethylenediaminetetraacetic acid and placed in a chilled Bronwill homogenizing bottle. Glass beads were added in volume equal to the cell

† Present address: Albert Einstein College of Medicine, Bronx, NY 10461.

solution, and cells were lysed by rapid shaking. The apparatus was cooled with CO_2 gas from a pressurized tank. RNA was then extracted with cold water-saturated phenol as described by Wejksnora and Haber (31). The RNA concentration was determined spectrophotometrically at 260 nm or by the orcinol technique described by Schneider (22).

LiCl precipitation of RNA. RNA was fractionated according to its solubility in 2 M LiCl essentially as described by Vodkin et al. (28). Purified RNA was resuspended in 1× SSC buffer (0.15 M NaCl–0.015 M sodium citrate), and 1 volume of 4 M LiCl in 1× SSC was added. This mixture was kept at 4°C for 2 to 20 h. The precipitate was removed by centrifugation for 15 min at 10,000 rpm in a Sorvall HB4. The RNA remaining in solution was recovered by overnight precipitation in 67% ethanol at −20°C and recovered by centrifugation for 15 min at 7,000 rpm in a Sorvall HB4 rotor.

Polyacrylamide gel electrophoresis of RNA. Polyacrylamide gels (9-mm diameter) were prepared by the method of Loening (16). In general, large rRNA species were resolved by the electrophoresis of RNA on 3% polyacrylamide gels run for 12 h at 8 mA/gel. Small species were separated for 4 h at 8 mA/gel by using bromophenol blue as a marker.

Removal of RNA from polyacrylamide gel and determination of base ratios. RNA species separated by electrophoresis through an acrylamide gel were recovered by eluting 1-mm slices in 2 volumes of TES buffer (0.1 M Tris [pH 7.4]–0.01 M NaCl–0.001 M ethylenediaminetetraacetic acid) overnight with gentle swirling at room temperature or 30°C. Acrylamide particles were removed by centrifugation at low speed, and the solution was further cleared of acrylamide by a single phenol extraction. The aqueous phase was then ether extracted, and the RNA was precipitated from 67% ethanol at −20°C. The base ratios were determined as described previously (25).

Preparation of nuclei. Sporulating cells were harvested by centrifugation and washed two times in distilled water. Cells were resuspended in 0.2 M Tris, pH 9, for 20 min at 30°C, centrifuged, and washed. Cells were resuspended in spheroplast buffer (1.5 M sorbitol–0.15% mercaptoethanol–0.1 M potassium phosphate, [pH 6.8]) containing 0.5 mg of Zymolase-5000 per ml (6). After a 1-h incubation at 30°C, the cells were centrifuged at 3,000 rpm in a Sorvall SS34 rotor for 15 min. The pellet was resuspended in lysing buffer {0.01 M NaCl–0.001 M $MgCl_2$–0.01 M dithiothreitol–0.01 M spermidine–0.001 M piperazine-*N,N'*-bis(2-ethanesulfonic acid) [pH 6.5]} to which was added Nonidet P-40 to a final concentration of 1%. This was layered over a 35-ml cushion of 2 M sucrose in lysing buffer in a SW25.2 Beckman Spinco rotor and centrifuged for 45 min at 20,000 rpm at 4°C.

The supernatant was considered the cytoplasmic fraction. The pellet, containing the nuclei, was washed by centrifugation through 0.5 M sorbitol to purify intact nuclei.

Determination of radioactivity. Tube gels containing RNA samples were sliced into 1-mm sections using a Joyce-Lobel gel slicer. Samples containing ^{14}C or ^{32}P were dried on filter paper and counted in Econofluor (New England Nuclear Corp.). Samples containing ^{3}H were solubilized in protosol solution (59.5% protosol [New England Nuclear Corp.]–38% toluene–2.5% water). Gel slices and protosol solution were placed in disposable 10-ml glass vials in a wooden tray with room for 100 vials. All of the vials in one tray were covered with a sheet of Nalgene foam 0.5 inch (ca. 1.47 cm) thick, held in place by a wooden lid attached to the base with screws. The foam cover eliminated the necessity of capping and uncapping vials. The trays were shaken slowly at room temperature or 30°C for 6 h. The solubilized gel slices were then counted by using an additional 8.5 ml of Econofluor.

Protein samples in acrylamide slab gels were cut out with either a razor blade or a cork bore. Cylindrical gels were sliced with a Joyce-Lobel gel slicer. The gel piece was mixed with 0.5 to 1 ml of 30% peroxide and shaken slowly at 60°C overnight. An 8-ml portion of Aquasol (New England Nuclear Corp.) was added, and the sample was counted in a liquid scintillation counter.

Samples from sucrose gradients containing ^{32}P were placed in disposable glass vials and counted directly by Cerenkov radiation. Aqueous samples containing ^{35}S, ^{14}C, or ^{3}H were counted in Aquasol by using 0.1 ml of sample to 10 ml of Aquasol.

Separation of RNP particles. Cells were harvested by centrifugation, washed, and resuspended in a minimal volume of buffer. Standard extraction buffer contained 0.01 M Tris, pH 7.4, 0.03 M $MgCl_2$, and 0.1 M NaCl. High-KCl buffer consisted of 0.05 M sodium cacodylate, pH 6.8, 5 mM MgCl, and 0.5 M KCl. Heparin (200 μg/ml) was added to all buffers to retard ribonuclease.

The resuspended cells were transferred to a chilled Bronwill homogenization bottle (75 ml), and an equal volume of 0.5-mm glass beads was added. Both bottle and homogenization apparatus were kept cold with gas from a CO_2 tank before and during homogenization. The sample was shaken for 25 s and removed. Several volumes of cold buffer were added, and the cell lysate was poured into a chilled, siliconized 30-ml tube. The lysate was centrifuged for 10 min at 11,000 × *g* in Sorvall HB4. The supernatant was layered atop preformed 5 to 20% sucrose gradients made with the indicated buffer. Centrifugation was carried out in a Beckman Spinco SW27 rotor at 4°C at 26,000 rpm for the times indicated.

After centrifugation, gradients were collected by lowering a thin (1-mm) glass tube to the bottom of the centrifuge tube and pumping out the liquid. A flow cell allowed monitoring at 260 nm of the gradient by using a Beckman model 25 spectrophotometer and chart recorder.

Determination of particle buoyant density. Determination of the buoyant density of RNP particles was essentially according to Baltimore and Huang (1). Solutions containing up to 0.1 mg of particle per ml were either fixed in 6% gluteraldehyde or directly layered on a preformed CsCl gradient (35 to 62%) made in 0.01 M Tris–0.0015 M $MgCl_2$ (pH 7.4)–0.01 M NaCl buffer containing 0.8% Brij-58 to prevent sticking of material to the side of the tube. Centrifugation was at 170,000 × *g* (35,000 rpm) in a Spinco SW65 TI rotor for at least 5 h at 25°C. The gradient was fractionated, and the refractive index was measured by using a Bausch and Lomb refractometer at 25°C.

Isolation of proteins. In other experiments, proteins were extracted from gradient fractions containing the 32S particle or 40S and 60S ribosomal subunits in 0.1 M $MgCl_2$ with 67% acetic acid as described by Sherton and Wool (24). Proteins from the 32S particle were sometimes extracted directly from precipitated preparations of the particle by boiling in sodium dodecyl sulfate (SDS). Protein concentration was determined by the method described by Lowry et al. (17).

Electrophoretic separation of proteins. SDS-acrylamide gels were used to separate proteins by size. Samples were boiled 90 s in buffer containing 0.06 M Tris, pH 8, 10% glycerol, 5% 2-mercaptoethanol, and 3% SDS. Separation was achieved basically by the method of Laemli (13) through a 12% acrylamide slab gel. Low pH urea-acrylamide gels were also used to fractionate proteins. Electrophoresis of proteins was performed essentially as described by Gorenstein and Warner (5).

Protein molecular weight (MW) was determined in the manner described by Weber and Osborn (30).

DNA isolation. Approximately 5×10^{10} cells of strain J659 were harvested and resuspended at a density of 5×10^8 cells per ml in 0.1 M phosphate buffer, pH 6.8, containing 1.5 M sorbitol and 0.1% mercaptoethanol. The cells were treated with the enzyme preparation Zymolase-5000 for 60 min to make spheroplasts. The spheroplasts were then harvested by centrifugation and lysed by resuspending in 1 M NaCl containing 0.1 M ethylenediaminetetraacetic acid, pH 8.0. SDS was then added to a final concentration of 0.5% (6). The DNA was then isolated essentially as described by Marmur (18). The DNA was purified by equilibrium centrifugation in CsCl, and fractions containing both nuclear and mitochondrial DNA were collected, dialyzed, and precipitated.

RNA-DNA hybridization. RNA-DNA hybridization in DNA excess was carried out as described by Bishop (2). Purified DNA and ^{32}P-labeled RNA were suspended in 0.012 M potassium phosphate buffer, pH 6.8, containing 0.5 mM ethylenediaminetetraacetic acid. The samples were heated to 100°C for 4 min before adding concentrated buffer to bring the solution to a final concentration of 0.12 M phosphate (pH 6.8) and 0.5 mM ethylenediaminetetraacetic acid. The reactions were carried out at 70°C. At intervals, 25- to 50-μl samples were removed and diluted into 0.5 ml of 1× SSC buffer. The collected samples were then treated for 30 min at 25°C with a mixture of ribonucleases A and T1 at a final concentration of 25 μg/ml and 25 U/ml, respectively. The samples were then precipitated by adding 1 ml of 1-μg/ml yeast RNA and 0.5 ml of 3% cetyltrimethyl ammonium bromide (Sigma Chemical Co.). The precipitates were collected at room temperature by filtration, dried, and counted in a liquid scintillation counter.

Radioisotopes. [8-^{14}C]Adenine (40 to 60 mCi/mmol), [*G*-^{3}H]adenine (10 to 25 Ci/mmol), L-^{14}C-labeled amino acid mixture, L-^{3}H-labeled amino acid mixture, [^{35}S]methionine, and carrier-free [^{32}P]phosphoric acid were obtained from New England Nuclear Corp.

Materials. Materials were obtained from the following sources: ribonuclease T1, heparin, cycloheximide, and size-marker proteins from Sigma Chemical Co.; cesium chloride, density gradient-grade sucrose, and ultrapure urea from Schwarz/Mann; mercaptoethanol, acrylamide, bis-acrylamide, and *N,N,N',N'*-tetramethylethylenediamine from Eastman Kodak Co.; diethylpyrocarbonate from Bayer as "Baycovin"; potassium acetate, dextrose, and ammonium persulfate from Fisher Scientific Co.; Folin phenol reagent from Harleco; and Zymolase-5000 from Kirin Breweries, Ltd., Japan.

RESULTS

Relation of sporulation 20S RNA to rRNA. The base composition of sporulation 20S RNA was compared to the composition of 18S rRNA and to the short-lived 20S ribosomal precursor RNA (Table 1). The transient vegetative 20S precursor rRNA is quite similar to 18S rRNA that is derived from it, whereas both rRNA species are very different in composition from the stable 20S RNA that accumulates during sporulation. The guanine-plus-cytosine content of sporulation 20S RNA is 56% but is only 45% for rRNA. These data make it unlikely that

TABLE 1. *Base compositions of yeast RNAs*[a]

RNA species	Cytosine	Uracil	Adenine	Guanine	Reference
20S sporulation	27.0	25.5	18.3	29.2	This work
20S precursor ribosomal	19.0	28.9	26.5	25.6	This work
18S ribosomal	19.1	29.2	25.8	25.9	This work
26S ribosomal	19.8	25.3	26.6	28.4	(22)
tRNA	24.2	26.5	18.8	30.5	(21)
24S mitochondrial	16.1	34.0	32.6	17.2	(19)
16S mitochondrial	12.9	36.4	33.3	17.4	(19)
Large killer	24.7	21.9	25.3	28.0	(26)
Medium killer	21.9	23.8	31.1	23.2	(26)

[a] RNA was labeled during sporulation with 0.5 mCi of ^{32}P per ml for 16 h and then extracted and purified as described in the text. Both sporulation 20S RNA and 18S rRNA were separated by polyacrylamide gel electrophoresis, and the portions of the gel containing these species were eluted and purified again by gel electrophoresis. The 20S ribosomal precursor RNA was purified in the same manner after labeling vegetative cells with 0.5 mCi of ^{32}P per ml for 5 min before harvesting and extracting the RNA. Base ratios were determined after exhaustive digestion with 0.1 N NaOH by paper electrophoresis as described previously (25).

sporulation 20S RNA is an unmethylated, stable form of 20S ribosomal precursor RNA.

RNA-DNA hybridization experiments confirmed that 20S RNA is not complementary to the reiterated rRNA cistrons. In these experiments ^{32}P-labeled 20S RNA or 18S rRNA was purified by polyacrylamide gel electrophoresis, as described above. The RNAs were hybridized with an excess of whole yeast DNA in 0.12 M phosphate buffer at 70°C, as described in the legend to Fig. 1. The hybridizations were carried out in DNA excess so that the presence of a small amount of rRNA in the 20S RNA preparation would not obscure the hybridization of the sporulation 20S species. In an earlier study (25), hybridizations were carried out in vast RNA excess, and the hybridization of RNA homologous to rRNA probably reflected the contamination of the 20S RNA with 18S rRNA. From the results shown in Fig. 1, it is clear that although 18S RNA hybridizes to DNA quite efficiently, 20S RNA shows little hybridization even after very long hybridization times. The small amount of hybridization may reflect the presence of some rRNA contamination in the 20S preparation. The ratio of DNA to RNA in these experiments was approximately 1,500 to 1, so that we could not have detected the hybridization of most of the 20S RNA to a single cistron per genome. Nevertheless, it is clear from these results that 20S RNA does not hybridize to reiterated sequences in yeast DNA, including the 140 cistrons for rRNA per haploid genome (23) or the approximately 100 copies of 2-μm DNA (3).

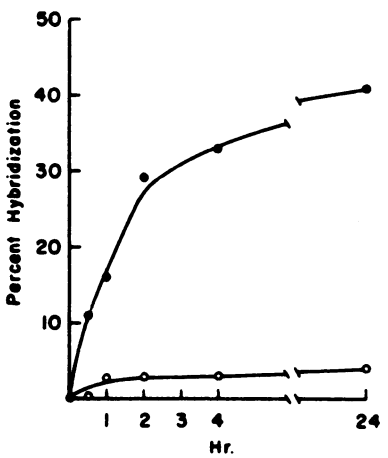

FIG. 1. *Hybridization of 18S and 20S RNA to yeast DNA. ^{32}P-labeled 18S RNA (●) (specific activity, 1.4 × 10^5 cpm/μg) or 20S RNA (○) (specific activity, 2.6 × 10^5 cpm/μg) were hybridized with whole yeast DNA, as described in the text. The final concentrations of RNA and DNA in the hybridization were 0.25 μg/ml and 375 μg/ml, respectively. At each time indicated, duplicate 25 λ samples were removed and, the fraction of ribonuclease-resistant counts was determined as described in the text. A maximum of 350 cpm were rendered ribonuclease resistant in the case of 18S RNA.*

Cytoplasmic location of 20S RNA. Examination of RNA extracted from nuclear and cytoplasmic fractions of sporulating cells (Fig. 2) shows that the 20S RNA is found in the cytoplasm along with the mature ribosomal species (18S and 26S) and that the nuclear fraction (Fig. 2B) contains only the large precursor species (35S and 27S) and minor amounts of the large mature 26S ribosomal species. This distribution of mature and precursor rRNA's is consistent with previously reported cytoplasmic and nuclear compartmentalization (6, 27).

20S RNA is found in an RNP particle. By sedimentation through sucrose gradients, we have found that 20S RNA is part of a 32S RNP particle. Sporulating cells were labeled with ^{32}P and lysed mechanically, and the subcellular components were separated on a sucrose gradient (Fig. 3). RNA extracted from the polysome and monosome regions of the gradient (Fig. 4A and B) contained primarily the ribosomal species, whereas 20S RNA was found to sediment less rapidly (Fig. 4C). To achieve better separation of the subcellular components, cells were grown to stationary phase in the presence of [^{14}C]adenine and sporulated in the presence of [^{3}H]adenine before lysis and fractionation by sucrose gradient centrifugation for 7 h (Fig. 5). The two ribosomal subunits, 40S and 60S, are clearly resolved, and a third peak (P) of 32S is seen. This 32S peak contains predominantly ^{3}H sporulation label, although both sporulation and vegetative labels are present in the ribosomal subunits. (Although some [^{14}C]adenine derived from turnover of vegetative RNA may be used during sporulation, the amount of ^{14}C label in RNA synthesized only during sporulation is very low [9, 31]. Strain J659 is prototrophic for adenine, so that when the [^{14}C] adenine is depleted from the medium during growth, the cells can apparently synthesize unlabeled adenine in the pool when cells are transferred to sporulation medium. The [^{3}H]adenine added during sporulation is incorporated preferentially to [^{14}C]adenine, much of which is excreted as [^{14}C]AMP and not reused [11].)

Recentrifugation of the 32S material (Fig. 5) through a 15 to 40% gradient containing 0.5 M KCl (high-salt buffer) resolved in a single peak (Fig. 6) containing virtually only sporulation-specific label. RNA extracted from the 32S particle consisted of 20S RNA (Fig. 7).

The 32S peak is absent in gradient centrifugations of vegetative cells. Furthermore, sporu-

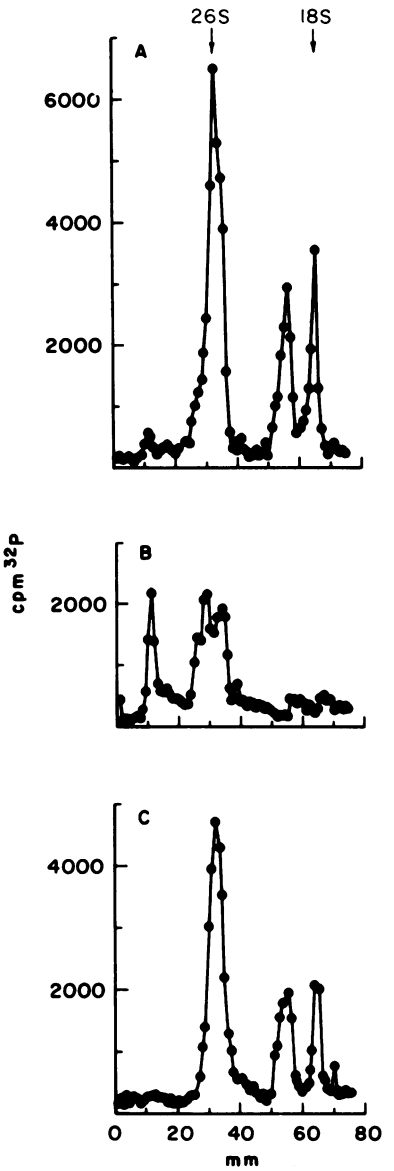

FIG. 2. *RNA of nuclear and cytoplasmic fractions of sporulation cells. Cells of strain J659 were sporulated for 4 h in the presence of 0.5 μCi of ^{32}P per ml, and spheroplasts were prepared. The spheroplasts were lysed, and nuclei were separated from the cytoplasmic fraction as described in the text. The nuclei were washed and examined microscopically, as was the cytoplasmic fraction. RNA was SDS-phenol extracted from unfractionated cells (A), and nuclear (B) and cytoplasmic (C) fractions. This RNA was displayed electrophoretically through a 3% polyacrylamide gel. Electrophoresis was carried out at 7 mA/9-mm gel for 14 h. The gels were cut into 1-mm slices, and the radioactivity in each slice was determined.*

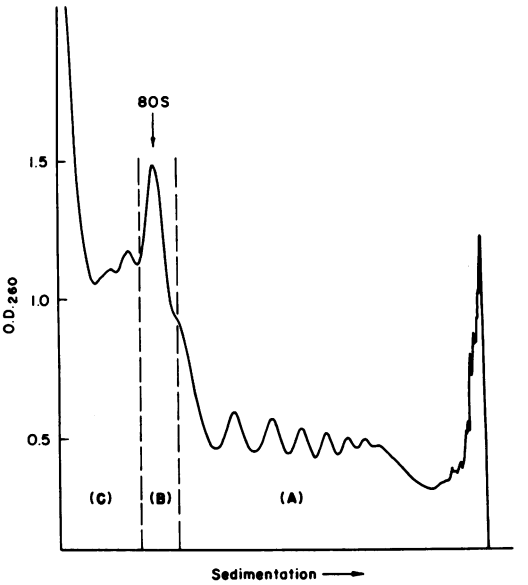

FIG. 3. *Separation of sporulating polysomes, monosomes, and subunits. Cells of strain J659 were grown to stationary phase in 20 ml of YEPD liquid and sporulated in 200 ml of 1% potassium acetate in the presence of 0.5 μCi of ^{32}P per ml for 14 h. A 200-μg portion of cycloheximide per ml was added 15 min before harvesting. The cells were harvested on ice, washed and broken in a buffer, and centrifuged through a 5 to 20% sucrose buffer gradient for 2 h at 26,000 rpm in a Spinco SW27 rotor as described in the text. The gradient was divided into polysome (A), monosome (B), and subunit (lighter than the monosome) (C) fractions. The absorbance at 260 nm was monitored with a flow cell and recorded with a chart recorder (solid line).*

lating cells of strain Y55, which fails to synthesize any 20S RNA (4), do not make a 32S particle. The particle is, however, present in cells of strain J659 sporulated for as short a period as 2 h.

The 32S particle contains protein as well as 20S RNA. As shown in Fig. 8, cells labeled during vegetative growth with ^{14}C-amino acids and during sporulation with ^{3}H-amino acids were lysed and centrifuged 7 h through a 5 to 20% sucrose gradient. The two ribosomal subunits, 40S and 60S, are labeled with both sporulation and vegetative labels, while the P peak contains predominantly the sporulation-specific ^{3}H label.

To determine the exact composition of the 32S particle and provide further evidence that it is an intact entity, particles containing ^{3}H-labeled amino acids and [^{32}P]RNA were subjected to isopycnic equilibrium centrifugation in cesium chloride. Both protein and RNA banded in the

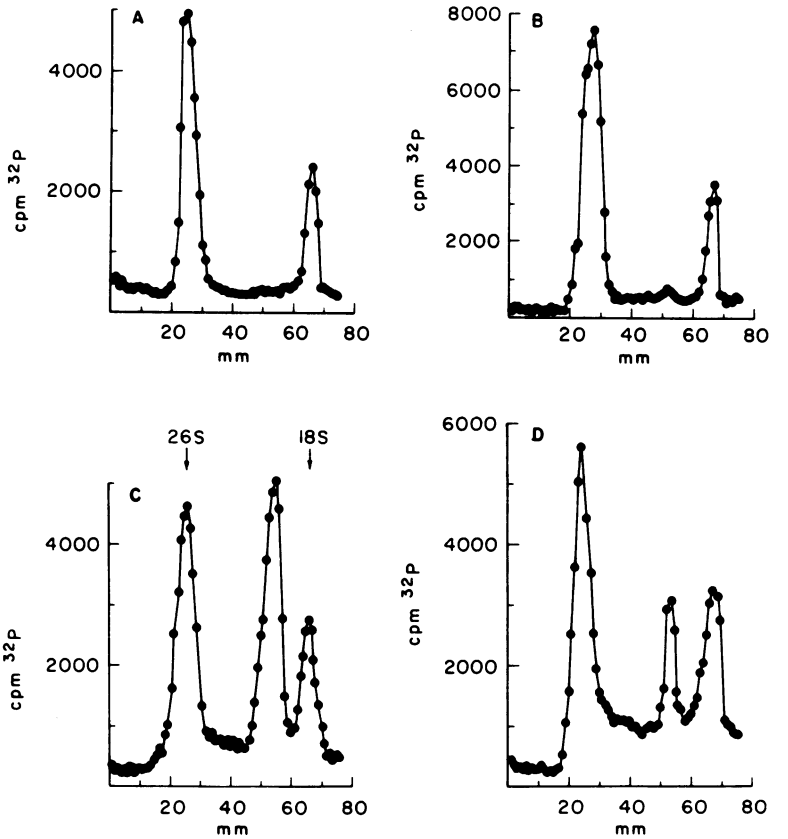

FIG. 4. *RNA of whole cells and polysome, monosome, and subunit fractions. RNA was SDS-phenol extracted from unfractionated lysate and from the polysome, monosome, and subunit fractions of the sucrose gradient (Fig. 6A, B, and C). This RNA was displayed electrophoretically across a 3% polyacrylamide gel as described in the text. (A) shows RNA extracted from the polysome region, (B) represents RNA from the monosome region, and material that sedimented in the subunit regions, or lighter than a monosome, is shown in (C). RNA extracted from the unfractionated lysate is shown in (D).*

region with a refractive index of 1.390, corresponding to a density of 1.6 g/cm^3. Based on the analytical formulas of Perry and Kelly (19), the ratio of RNA to protein in the 32S particle is 1.8.

The amounts of protein and RNA present in the P particle were also determined directly by the Lowry et al. (17) and orcinol (23) techniques, respectively, and by comparative specific activity measurements (Table 2). The ratio of RNA to protein is approximately 1.8. Thus, based on the assumption that each 32S particle contains one 20S RNA molecule of size 0.8×10^6 daltons, the particle also contains about 0.45×10^6 daltons of protein.

Properties of 20S RNA within the 32S particle. When RNA was extracted from the 32S particle isolated without the addition of the ribonuclease inhibitor diethylpyrocarbonate to the buffer, no intact 20S RNA was found. Rather, all of the RNA was found in two size classes of MW 5×10^4 and 1.6×10^5 (Fig. 9). However, the ratio of RNA to protein remained essentially unchanged, and the particle containing the cleaved RNA cosedimented in sucrose gradients with the 32S particle isolated with diethylpyrocarbonate. Furthermore, there was no change in the density of the unprotected 32S particle when measured in CsCl equilibrium sedimentation gradients. Although the 20S RNA is cleaved by ribonucleases liberated during cell breakage, the RNA apparently remains associated with the proteins of the 32S particle and little, if any, RNA is degraded to small oligonucleotides and lost.

To examine the relationship between 20S RNA and the smaller species recovered from the

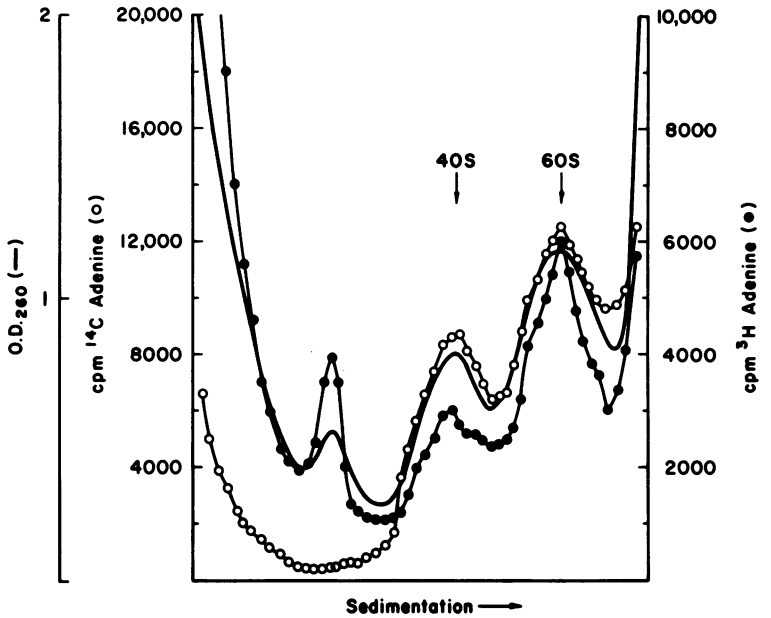

FIG. 5. *Separation of subunits. Cells of strain J659 were grown to stationary phase in 20 ml of YEPD in the presence of 0.2 μCi of [^{14}C]adenine per ml and sporulated in 200 ml of 1% potassium acetate in the presence of 0.4 μCi of [^{3}H]adenine for 14 h. Cells were broken and centrifuged through a 5 to 20% sucrose gradient for 7 h at 26,000 rpm in a Spinco SW27 rotor as described in the text. The gradient was fractionated into 0.6-ml fractions. The radioactivity of each fraction was determined by counting in Aquasol to detect ^{14}C (○) and ^{3}H (●). The counts were corrected for crossover and plotted against the optical density at 260 nm (OD_{260}) tracing (solid line).*

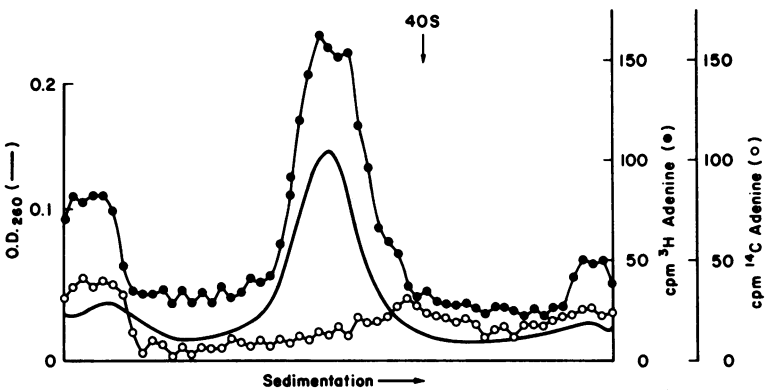

FIG. 6. *Recentrifugation of 32S region material in high-potassium chloride buffer. Material from the 32S region of Fig. 5 was layered on a 15 to 40% sucrose gradient made with buffer containing 0.5 M KCl and centrifuged 14 h at 25,000 rpm in a Spinco SW27 rotor. The gradient was fractionated, and radioactivity was determined as before. The counts were corrected for crossover and ^{14}C vegetative (○) and ^{3}H sporulating (●) were plotted against the optical density at 260 nm (OD_{260}) tracing.*

32S particle isolated without diethylpyrocarbonate, 32S particles containing intact 20S RNA were subjected to in vitro digestion with ribonuclease T1 (Fig. 10). The undigested particle contained only 20S RNA. Digestion for 10 min at 25°C in 4× SSC shows limited cleavage. However, a 10-min digestion in 1× SSC generated species of lower MWs, and a 20-min digestion in the same buffer resulted in predominately the 5 × 10^4- and 1.6 × 10^5-MW species, similar to those seen previously in isolated particles (cf. Fig. 9). The amount of RNA recovered in each

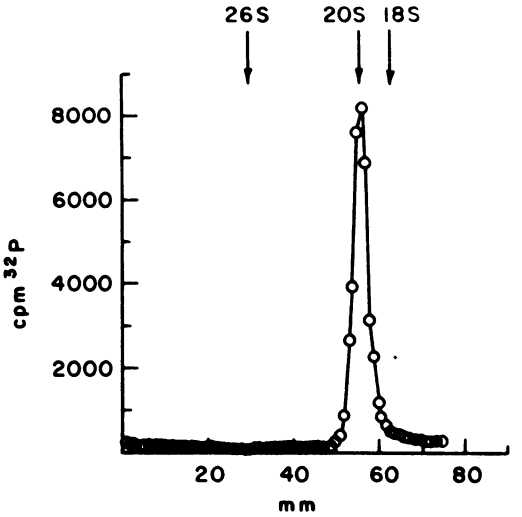

FIG. 7. *RNA of 32S particle. Sporulating cells of strain J659 were labeled 14 h with 1 μCi of ^{32}P per ml lysed as before with diethylpyrocarbonate, and 32S particles were isolated as in the legend to Fig. 6. RNA was extracted and displayed electrophoretically.*

digestion was nearly identical to the undigested sample, indicating that the cleavage products remain associated in the particle.

When 20S RNA purified from its protein was digested with ribonuclease T1 at 25°C for 15 min in 1× SSC or 4× SSC, it was degraded into pieces smaller than 4S in both high and low buffer concentrations. Thus, pure 20S RNA is quite sensitive to ribonuclease T1 unlike double-stranded killer RNA species (28).

Although 20S RNA is apparently not double stranded, it is more soluble in 2 M LiCl than rRNA. In 2 M LiCl, double-stranded RNA species remain soluble in solution, whereas single-stranded ribosomal species are precipitated (28). After 2 h (Fig. 11B) when 70% of the total RNA had precipitated, the majority of the 20S RNA remained in solution. By 8 h (Fig. 11C), 76% of the total had precipitated, and about half of the 20S RNA was seen in the precipitate. After 20 h, 80% of the total RNA had precipitated (Fig. 11D), and here the amount of 20S relative to the ribosomal species appears identical to the original input RNA (Fig. 11A).

Further evidence that this molecule is single

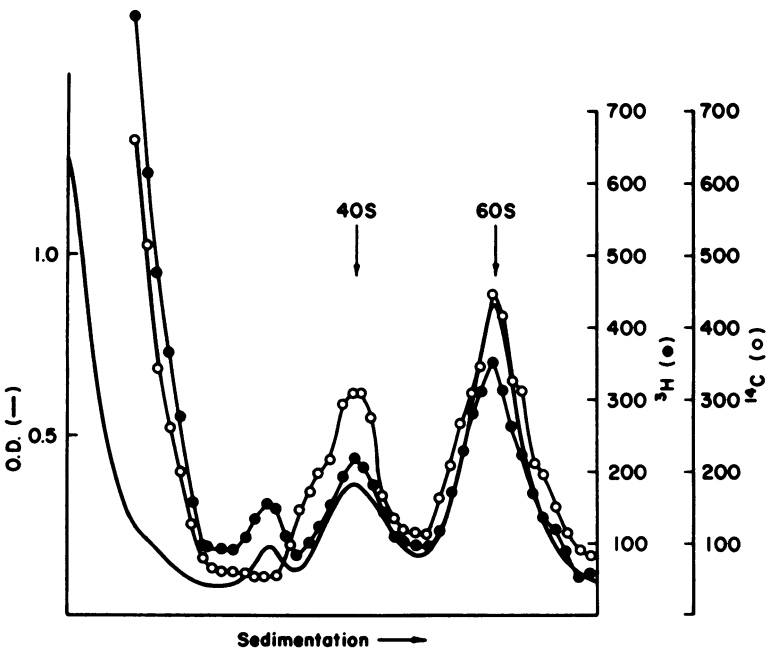

FIG. 8. *Incorporation of radioactive amino acids into RNP particles. Cells of strain J659 were grown to stationary phase in 20 ml of YEPD in the presence of 1 μCi of ^{14}C-labeled amino acid mix per ml and sporulated in 200 ml of 1% potassium acetate in the presence of 1 μCi of ^{3}H-labeled amino acid mix per ml for 14 h. Cells were broken in buffer A and centrifuged through a 5 to 20% sucrose buffer A gradient for 7 h at 26,000 rpm in a Spinco SW27 rotor as described in the text. The gradient was fractionated into 0.6-ml fractions. Absorbance at 260 nm was monitored by using a flow cell and recorded by using a chart recorder (solid line). The radioactivity of each fraction was determined by counting in Aquasol to detect ^{14}C (○) and ^{3}H (●). The counts were corrected for crossover and plotted against the optical density (OD).*

TABLE 2. *Protein and RNA content of 32S (P) particle*

Method	RNA	Protein	RNA/protein
(A)[a]			1.82 ± 0.14
Orcinol	3.1 ± 0.08 μg		
Lowry et al.		1.7 ± 0.1 μg	
(B)[b]			
Specific activity	5.2 ± 0.1 μg		1.79 ± 0.1
Lowry et al.		2.9 ± 0.1 μg	
(C)[c]			
Density (1.6003 ± 0.003 g/cm^3	64%	36%	1.78

[a] Identical volumes of solution containing P particles isolated as described previously were analyzed by the Lowry and orcinol methods described in the text. In each case, duplicate determinations were made.

[b] Independently obtained specific activity determinations were made for the RNA determination using ^{32}P-labeled particle.

[c] Percent RNA was determined as described in the text from the density of the particle. Particles made in the presence and absence of diethylpyrocarbonate have virtually identical densities.

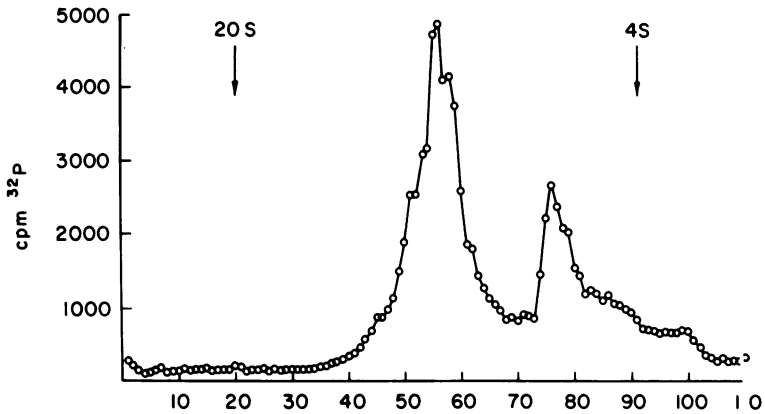

FIG. 9. *RNA isolated from 32S particle isolated without ribonuclease inhibitors. Cells of strain J659 were sporulated 14 h in the presence of 0.5 μCi of ^{32}P per ml. The cells were lysed in buffer A without ribonuclease inhibitors and isolated as shown in Fig. 5 and 6. The region of the gradient containing the 32S particle was collected into a chilled tube, and the RNA was SDS-phenol extracted. This RNA was displayed electrophoretically through a 3% acrylamide gel. Electrophoresis was conducted at 8 mA/9-mm gel for 4 h. Bromophenol blue was used as a tracking dye, and ^{3}H-labeled RNA extracted from sporulating cells was run as a size marker. The gels were sliced into 1-mm slices, and the radioactivity present in each slice was counted. Virtually all of the input radioactivity was accounted for by the radioactivity present in the sliced gel.*

stranded is its migration as a 20S RNA species through a denaturing gel containing 7 M urea, which separates double-stranded RNA species (data not shown).

No significant amount of 3'-polyadenylic acid was detected either by ribonuclease digestion with ribonuclease T1 and A or by binding labeled 20S RNA to an oligodeoxythymidylic acid column (Kraig and Haber, unpublished data).

Protein associated with 20S RNA. The 32S particle contains many copies of a single protein. The proteins of the particle were extracted by boiling the ethanol-precipitated particle in SDS buffer and displayed on a 12.5% acrylamide gel as described above. A single major band was resolved, along with two very minor bands of higher MW (Fig. 12). The amount of material present in the two minor bands varied from preparation to preparation and could be eliminated almost entirely by boiling the sample for longer than the standard 90 s. Furthermore, these larger species, if excised from the gel and reboiled in SDS, migrated with the small species, indicating that only one size species is found with the 20S RNA. This protein, when compared to the migration of proteins of known size including myoglobin, trypsin, pepsin, and catalase was found to have an MW of 23,000. The minor bands were found to be 46,000 and 69,000, further suggesting that they are aggregates of the

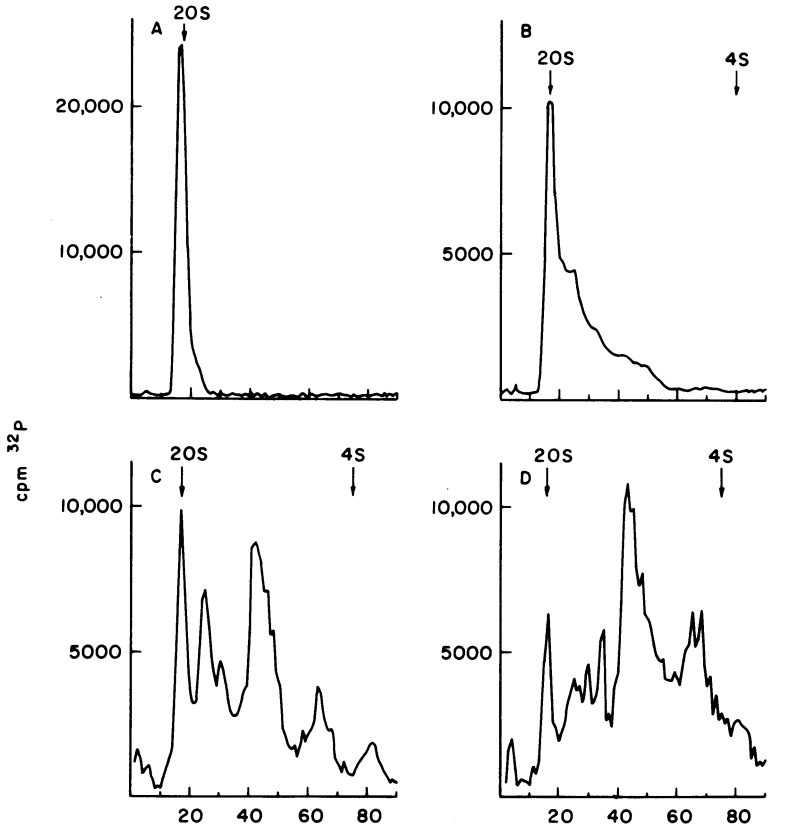

FIG. 10. *Ribonuclease resistance of isolated 32S particle. 32S particle containing intact ^{32}P-labeled 20S RNA was subjected to digestion by 100 U of T1 ribonuclease per ml. Digestions were performed at 25°C after which RNA was phenol extracted and displayed electrophoretically across a 3% acrylamide gel for 4 h at 8 mA/gel. RNAs of known sizes were also electrophoresed to provide markers. (A) shows intact RNA isolated from the particle; (B) displays RNA resulting from a 10-min digestion in 4× SSC. Digestions performed in 1× SSC for 10 min (C) and 20 min (D) are also shown.*

23,000-dalton protein. The amount of protein in the 32S particle was previously determined to be 4.5×10^5 daltons. Thus, each particle appears to contain approximately 20 copies of this 23,000-MW protein.

The 32S particle proteins, labeled as described in the legend to Fig. 8, were boiled in SDS, separated electrophoretically, and stained. The region of the gel containing the stained 23,000-MW protein was cut out, dissolved in 30% peroxide at 60°C, and counted. Essentially, only the sporulation-specific ^{3}H was found in the 23,000-MW protein (14,040 cpm of ^{3}H and 273 cpm of ^{14}C), whereas a ribosomal protein from the 25,000-MW region of the gel contained 6,164 cpm of ^{3}H and 4,206 cpm of ^{14}C. Similarly, when ^{14}C-labeled vegetative cells were mixed with ^{3}H-labeled sporulating cells before breaking the cells, only sporulation label was found in the 32S region (data not shown).

The 23,000-MW protein was not found in material sedimenting above or below the 32S region in a sucrose gradient. Furthermore, there is no detectable 23,000-MW protein when 32S regions of gradients were examined from lysed vegetative cells or from sporulating Y55 cells (that do not accumulate 20S RNA).

The 23,000-MW protein does not seem to be a ribosomal protein. Cells labeled during vegetative growth with ^{3}H-amino acids were lysed, ribosomal subunits were prepared, and proteins were extracted with acetic acid. Sporulating cells were labeled with a ^{14}C-amino acid mixture, 32S particles were isolated and precipitated with 67% ethanol, and the protein was extracted with acetic acid. Two protein samples were mixed and separated by electrophoresis through a low pH urea-acrylamide gel (Fig. 13). The ^{14}C label can be seen near the top of the gel, separate from any of the basic ribosomal proteins.

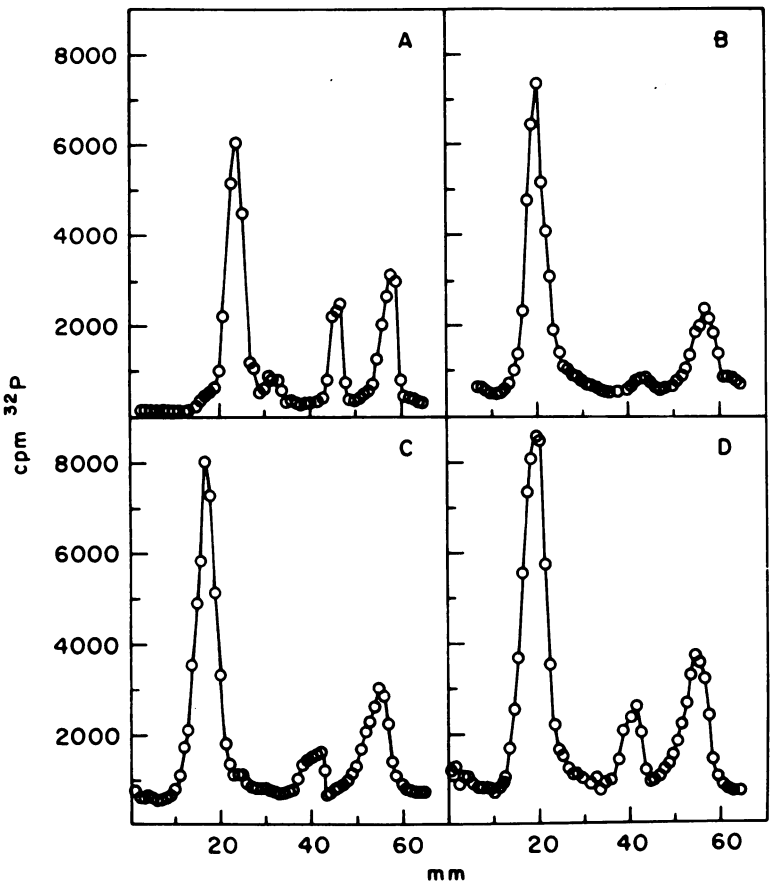

FIG. 11. *Lithium chloride precipitation of ribosomal and 20S RNA. RNA extracted from cells of strain J659 sporulated 14 h in the presence of 0.2 μCi of ^{32}P per ml was resuspended in 1× SSC. An equal volume of 4 M LiCl in 1× SSC was added, and the solution was kept at 4°C for 2, 8, and 20 h. Precipitated RNA was pelleted by centrifugation, resuspended in TES, and displayed electrophoretically through 3% acrylamide gels as described. (A) Unfractionated input RNA; (B) RNA precipitated after 2 h; (C) RNA precipitated after 8 h; and (D) RNA precipitated after 20 h.*

A sample of the 23,000-MW protein was subjected to amino acid analysis, the results of which are shown in Table 3. It should be noted that this protein contains no methionine, a finding reinforced by several unsuccessful attempts to label this protein with [^{35}S]methionine. This further distinguishes the 32S particle protein from ribosomal proteins of similar size, all of which contain methionine.

Inhibition of accumulation of 20S RNA. The dependence of 20S RNA on the continued availability of protein was examined directly by using the protein synthesis inhibitor cycloheximide. When cells of strain J659 were treated with 200 μg of cycloheximide per ml after 4 h of sporulation, both rRNA and 20S RNA accumulation were inhibited. This indicates that accumulation of the stable 20S molecule, like the ribosomal species, is dependent upon the continued synthesis of protein.

When a high concentration of actinomycin D was added to the sensitive strain J98, there was a nearly complete inhibition of rRNA synthesis. However, there was virtually no inhibition of 20S RNA synthesis (Fig. 14).

Fate of 20S RNA during germination. When spores were germinated, the 20S RNA synthesized during sporulation was not selectively degraded; however, de novo synthesis of 20S RNA stopped. Cells of strain J659 sporulated 32 h in the presence of ^{32}P were germinated in YEPD liquid in the presence of [^{14}C]adenine. RNA in spores just before introduction into YEPD (Fig. 15A) showed the characteristic sporulation pattern, the two ribosomal species, 26S and 18S, in addition to the stable 20S RNA.

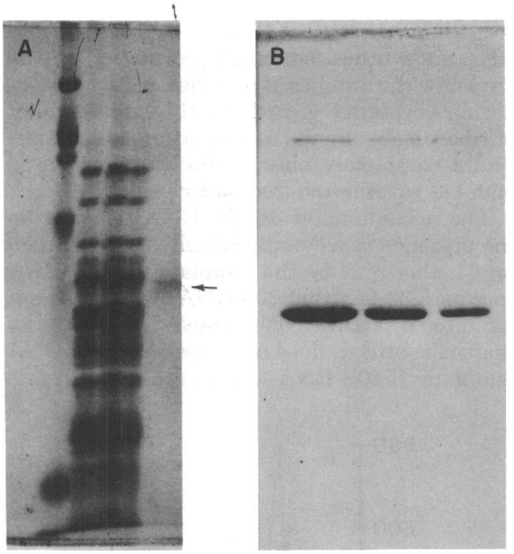

FIG. 12. *Electrophoretic separation of 32S particle proteins in SDS. The 32S particle was purified from sporulating cells of strain J659, as shown in Fig. 5 and 6. The 32S region was recovered and precipitated overnight in 67% ethanol at −20°C and then centrifuged from solution at 10,000 rpm in a Sorvall HB4 rotor for 15 min. The pellet was air dried and resuspended in sample buffer containing 3% SDS and 2-mercaptoethanol as described in the text. This solution was immersed in a boiling-water bath for 90 s and layered into preformed wells of an acrylamide slab for electrophoresis. (A) Proteins were separated by electrophoresis in a 12.5% acrylamide gel. The 32S particle contained a single protein component (arrow). The three center channels show proteins extracted from 80S ribosomes by acetic acid extraction as described in the text. The left channel shows the migration of proteins of known MW: rabbit muscle phosphorylase A (MW 92,500), bovine serum albumin (MW 67,000), beef liver glutamate dehydrogenase (MW 53,000), rabbit muscle glyceraldehyde 3-phosphate dehydrogenase (MW 36,000), and bovine hemoglobin (MW 16,000). (B) When higher concentrations of the 32S particle protein were separated electrophoretically, a major band of MW 23,000 was observed, along with a minor band at MW 69,000. The gel contained (from left to right) 16, 8, and 4 mg of protein per ml.*

After a 2-h germination (Fig. 15B), the pattern of sporulation-labeled RNA had not altered significantly. Even after 6 h (Fig. 15C), by which time the cells had doubled, sporulation-labeled 26S, 20S, and 18S RNA were still present. Only the two ribosomal species were synthesized de novo during outgrowth. After 2 h of growth (Fig. 15B), the ^{14}C label was seen only in the 26S and 18S regions, and no ^{14}C-labeled 20S RNA was seen. After 6 h of vegetative growth (Fig. 15C),

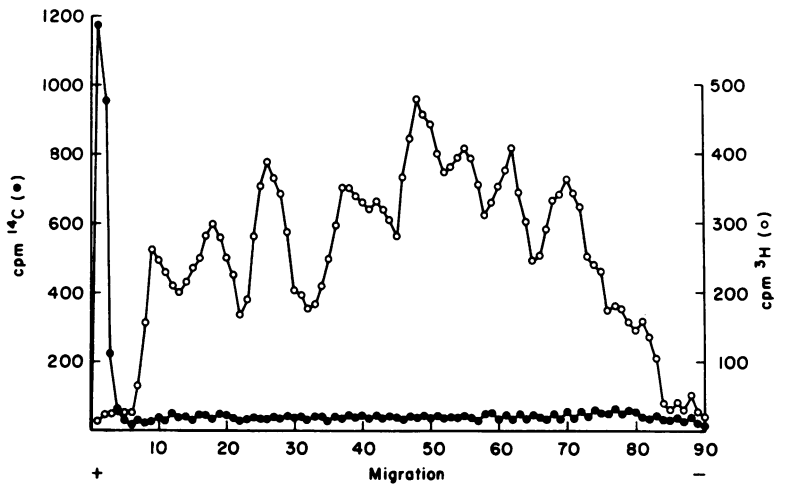

FIG. 13. *Separation of 32S particle proteins from ribosomal proteins. Proteins were extracted from ribosomal subunits labeled with ^{3}H-amino acids and from 32S particles labeled with ^{14}C-amino acids by acetic acid and dried. Samples were mixed in sample buffer containing 0.1% acetic acid, 8 M urea, 1.4 M 2-mercaptoethanol, and 10% glycerol and layered atop a tube gel (120 by 5 mm) made with 4% acrylamide–0.1% bis-acrylamide–0.057 M bis-Tris (pH 5). The upper buffer contained 0.01 bis-Tris, pH 4, and the lower buffer contained 0.178 M potassium acetate, pH 5. Basic fuchsin was used as a tracking dye, and electrophoresis was carried out toward the cathode at 150 V until the tracking dye reached the bottom of the gel. The gel was removed from the tube, frozen with Dry Ice, and sliced into 1-mm slices with a Joyce-Lobel gel slicer. The slices were dissolved overnight in 0.5 ml of 35% peroxide at 60°C. A 10-ml portion of Aquasol was added to each sample, and the radioactivity in each was determined by liquid scintillation counting. The counts present in each sample were plotted. Symbols: ^{14}C (●) and ^{3}H (○).*

TABLE 3. *Amino acid composition of the 23,000-dalton protein*[a]

Amino acid	Frequency
Aspartic acid	33
Threonine	8
Serine	20
Glutamic acid	24
Proline	12
Glycine	25
Alanine	16
Valine	15
Isoleucine	10
Leucine	17
Methionine	0
Tyrosine	3
Phenylalanine	10
Histidine	4
Lysine	14
Arginine	7

[a] The composition of the 23,000-dalton protein in the 32S RNP was determined by using protein purified as described in the legend to Fig. 7 and then extracted with acetic acid, as described in the text. Samples were run on a Durham amino acid analyzer by E. Cannon. The results are given as the number of amino acids of each sort that would be found in a protein of MW 23,000. The amount of tryptophan in the sample was not determined.

the only de novo species were the two ribosomal ones, and the only 20S RNA present was that RNA which was synthesized during sporulation and is ^{32}P labeled. Thus, no specific degradation of the sporulation made 20S RNA was seen during germination, and this species was not synthesized de novo. After 6 h of germination the 32S particle was also found (data not shown).

DISCUSSION

We have identified a cytoplasmic RNP particle containing stable, unmethylated 20S RNA. Several lines of evidence support the conclusion that 20S RNA is intimately and specifically associated with 18 to 20 copies of a 23,000-dalton protein synthesized only under sporulation conditions. The absence of vegetatively synthesized proteins in the 32S particle demonstrates that it is not a random association between 20S RNA and cellular proteins. The stability of the particle in 0.5 M KCl suggests the specificity and tenacity of the RNA-protein interactions, which are further borne out by the isopycnic banding of the particle in cesium chloride. Lastly, the stability of the particle containing cleaved 20S RNA indicates a tightly associated structure.

The synthesis of the 20S RNA-containing particle accounts for 10 to 15% of the de novo RNA synthesis during sporulation. Based on the amount of ribosomes in sporulating yeast (29), we estimate that more than 20,000 copies of 20S RNA are synthesized during sporulation. We do not know the number of particles, if any, present during vegetative growth, as the large amount of ribosomal subunits or rRNA (of similar size) would completely obscure the presence of as many as several hundred particles per cell.

The accumulation of 20S RNA depends on the presence of a cytoplasmically inherited factor (4) that may be the template for this RNA species. The fact that 20S RNA synthesis is not inhibited by actinomycin D raises the possibility that its synthesis does not depend on the DNA template. If 20S RNA were synthesized from a

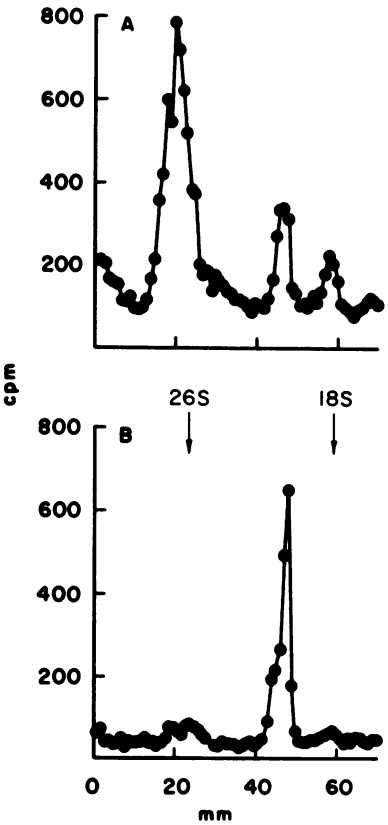

FIG. 14. *Effect of actinomycin D on synthesis of rRNA and 20S RNA. Strain J98, sensitive to actinomycin D, was grown to stationary phase in YEPD and transferred to sporulation medium at a density of* 2×10^7 *cells per ml. After 1 h, 200 μg of actinomycin D per ml was added to half of the culture, and 15 min later* ^{32}P*-labeled inorganic phosphate was added to a final activity of 1 μCi/ml. The labeling was permitted to continue for 3 h, after which cells were harvested and RNA was extracted as described in the text. (A) Accumulation of 26S, 20S, and 18S RNA in the absence of actinomycin D. (B) Synthesis of 20S RNA in the presence of actinomycin D.*

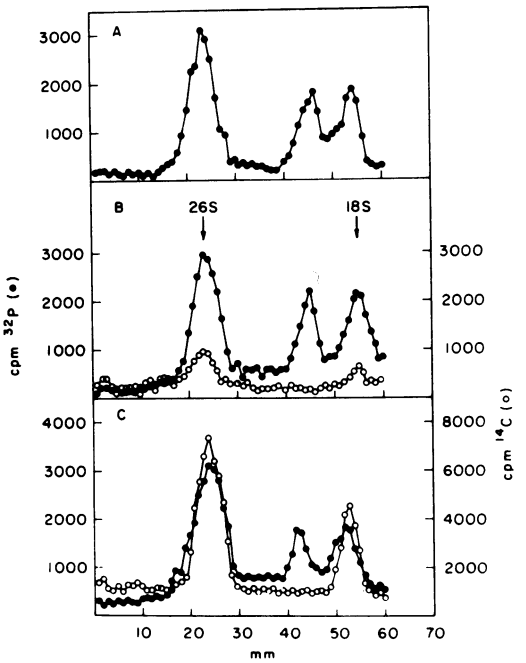

FIG. 15. *Fate of 20S during germination. Cells of strain J659 were sporulated 32 h in the presence of 0.3 μCi of ^{32}P per ml. Sporulation at this time was greater than 60%. The cells were washed twice and resuspended in YEPD medium containing 0.5 μCi of [^{14}C]adenine per ml and swirled in flasks at 30°C. Samples of cells were lysed just before introduction into YEPD and 2 and 6 h thereafter. RNA was SDS-phenol extracted from these cells and displayed electrophoretically. Electrophoresis was carried out at 7 mA/9-mm gel for 12 h. The gels were cut into 1-mm slices, and the radioactivity present in each slice was determined. Symbols: ^{32}P (●) and ^{14}C (○). (A) shows RNA isolated from cells just before germination, (B) shows RNA after 2 h of outgrowth, and RNA produced after 6 h of outgrowth is shown in (C).*

DNA template, it should have been even more sensitive to actinomycin D inhibition than rRNA, in view of the very high guanine-plus-cytosine content of that RNA and the preferential inhibition of guanine-rich sequences by the antibiotic (20). Under conditions where rRNA synthesis was nearly abolished, 20S RNA synthesis was unaffected. It is possible, therefore, that 20S RNA is transcribed from a cytoplasmic RNA template. Our RNA-DNA hybridization experiments indicate that 20S RNA is not transcribed from a reiterated DNA template, including ribosomal DNA (23) or 2-μm DNA (14).

The protein associated with 20S RNA apparently depends on cytoplasmic ribosomes for its synthesis, as cycloheximide inhibits the appearance of the 32S particle. The 20S molecule may be unable to accumulate in cells unless synthesis of the 23,000-dalton protein continues, much as ribosome biosynthesis depends on the continued synthesis of ribosomal proteins (27). We also do not know the origin of the genetic information for the particle protein. It is possible that the 20S RNA molecule carries the sequence corresponding to this protein, but two attempts to use 20S RNA as a messenger in cell-free protein synthesis have not yielded any recognizable translation products (J. Hopper, personal communication; E. Lai, personal communication). The 23,000-dalton protein found in the particle is not a ribosomal protein, as we were able to separate it from ribosomal proteins by electrophoresis. Also it is smaller than the proteins associated with the double-stranded RNA killer particle (9).

Our preliminary examinations of the 32S particle in the electron microscope revealed a roughly spherical particle about 90 angstroms (ca. 9.0 nm) in diameter. We do not yet know the arrangement of the 20S RNA and the 18 to 20 identical proteins in the particle except that the association of the protein with the RNA affords its nearly complete ribonuclease resistance. The particle containing RNA cleaved into large fragments remains intact, with a density identical to a particle with intact 20S RNA. The stability of this RNP particle is similar to a number of RNP particles containing cleaved RNA that have been described in other eucaryotes (14, 15).

Finally, we do not yet know why 20S RNA and 32S particle synthesis are induced under sporulating conditions. It is now clear, however, that there are significant differences in the control of RNA synthesis between vegetative growth and sporulation. For example, the *rna* mutants that inhibit ribosomal protein synthesis during vegetative growth fail to inhibit synthesis under sporulation conditions (7, 18a). Most recently H. J. Rhaese, R. Groscurth, and R. Scheckel (Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, I64, p. 165) have discovered that highly phosphorylated adenine compounds appear only under sporulation conditions and may regulate the number of sporulation events.

In summary, we have identified a 32S RNP particle containing single-stranded 20S RNA and about 20 copies of a single 23,000-MW protein. The particle is almost completely resistant to ribonuclease digestion. It is apparently unrelated to any of the other cytoplasmic elements in yeast cells. Its specific induction under sporulation conditions and the large amount of both RNA and protein synthesized may make it very useful in understanding changes in transcription

and translation accompanying the shift from vegetative growth to sporulation.

ACKNOWLEDGMENTS

We are extremely grateful for the generous assistance we have received from Barbara Garvik for technical assistance and a never-ending stream of good ideas, Cindy Blifeld for her help in improving our polyacrylamide gels, Court Saunders for his help with determining base ratios, Jim Hopper and Elaine Lai for both trying to translate the RNA, Edward Cannon for determining the amino acid compositions, and Paul Norton for looking at the particle in the electron microscope.

This work was supported by Public Health Service grant GM20056 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Baltimore, D., and A. Huang.** 1968. Isopycnic separation of subcellular components from poliovirus-infected and normal HeLa cells. Science **162**:572–574.
2. **Bishop, J. O.** 1972. DNA-RNA hybridization. Karolinska Symp. Res. Methods Reprod. Endocrinol. **5**:247–273.
3. **Clark-Walker, G. D., and G. L. G. Miklos.** 1974. Localization and quantification of circular DNA in yeast. Eur. J. Biochem. **41**:359–365.
4. **Garvik, B., and J. E. Haber.** 1978. New cytoplasmic genetic element that controls 20S RNA synthesis during sporulation in yeast. J. Bacteriol. **134**:261–269.
5. **Gorenstein, C., and J. Warner.** 1976. Coordinate regulation of the synthesis of eukaryotic ribosomal proteins. Proc. Natl. Acad. Sci. U.S.A. **73**:1547–1551.
6. **Groner, B., N. Hynes, and S. Phillips.** 1974. Length heterogeneity in the poly (adenylic acid) region of yeast messenger ribonucleic acid. Biochemistry **13**: 5378–5383.
7. **Haber, J. E.** 1975. Requirement for expression of genes controlling synthesis of ribosomal ribonucleic acid during sporulation of *Saccharomyces cerevisiae* cells, p. 154–164. *In* P. Gerhardt, R. Costilow, and H. Sadoff, (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
8. **Hartwell, L. H., C. S. McLaughlin, and J. R. Warner.** 1970. Identification of ten genes that control ribosome formation in yeast. Mol. Gen. Genet. **109**:42–58.
9. **Herring, A., and E. Bevan.** 1974. Virus-like particles associated with the double stranded RNA species found in killer and sensitive strains of the yeast *Saccharomyces cerevisiae*. Gen. Virol. **22**:387–394.
10. **Hopper, A., P. Magee, S. Welch, M. Friedman, and B. Hall.** 1974. Macromolecular synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:619–628.
11. **Kadowaki, K., and H. O. Halvorson.** 1971. Appearance of a new species of ribonucleic acid during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **105**:826–830.
12. **Kadowaki, K., and H. O. Halvorson.** 1971. Isolation and properties of a new species of ribonucleic acid synthesized in sporulating cells of *Saccharomyces cerevisiae*. J. Bacteriol. **105**:831–836.
13. **Laemli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Lava-Sanchez, P., and S. Puppo.** 1975. Occurrence *in vivo* of hidden breaks at specific sites of 26S ribosomal RNA of *Musca carnaria*. J. Mol. Biol. **95**:9–20.
15. **Lemke, P. A., and C. H. Nash.** 1974. Fungal viruses. Bacteriol. Rev. **38**:29–56.
16. **Loening, U. E.** 1967. Fractionation of high molecular weight ribonucleic acid by polyacrylamide gel electrophoresis. J. Biochem. **120**:251–257.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
18. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid isolated from a thermophile. Biochim. Biophys. Acta **38**:342–343.

18a. **Pearson, N. J., and J. E. Haber.** 1977. Changes in regulation of ribosome synthesis during different stages of the life cycle of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **158**:81–91.

19. **Perry, R., and D. E. Kelly.** 1966. Buoyant densities of cytoplasmic ribonucleoprotein particles of mammalian cells. J. Mol. Biol. **16**:255–268.
20. **Perry, R., and D. E. Kelly.** 1970. Inhibition of RNA synthesis by actinomycin D: characteristic dose response of different RNA species. J. Cell. Physiol. **76**:127–140.
21. **Popa, L. M., and R. Repanovivi.** 1969. The study of toluidine blue-ribosomal RNA complexes. Biochim. Biophys. Acta **182**:158–168.
22. **Schneider, W.** 1957. Determination of nucleic acids in tissues by pentose analysis. Methods Enzymol. **3**:680–684.
23. **Schweizer, E., C. MacKechnie, and H. O. Halvorson.** 1969. The redundancy of ribosomal and transfer RNA genes in *Saccharomyces cerevisiae*. J. Mol. Biol. **40**:261–277.
24. **Sherton, C., and I. Wool.** 1973. The extraction of proteins from eukaryotic ribosomes and ribosomal subunits. Mol. Gen. Genet. **135**:97–112.
25. **Sogin, S. J., J. E. Haber, and H. O. Halvorson.** 1972. Relationship between sporulation-specific 20S ribonucleic acid and ribosomal ribonucleic acid processing in *Saccharomyces cerevisiae*. J. Bacteriol. **112**:806–814.
26. **Sweeney, T. K., A. Tate, and G. R. Fink.** 1976. A study of the transmission and structure of double stranded RNAs associated with killer phenomenon in *Saccharomyces cerevisiae*. Genetics **84**:27–42.
27. **Udem, S., and J. Warner.** 1973. The cytoplasmic maturation of a ribosomal precursor ribonucleic acid in yeast. J. Biol. Chem. **248**:1412–1416.
28. **Vodkin, M., F. Katterman, and G. R. Fink.** 1974. Yeast killer mutants with altered double-stranded ribonucleic acid. J. Bacteriol. **117**:681–686.
29. **Warner, J.** 1971. The assembly of ribosomes in yeast. J. Biol. Chem. **246**:447–454.
30. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406–4412.
31. **Wejksnora, P. J., and J. E. Haber.** 1974. Methionine-dependent synthesis of ribosomal ribonucleic acid during sporulation and vegetative growth of *Saccharomyces cerevisiae*. J. Bacteriol. **120**:1344–1355.