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Appearance of a New Species of Ribonucleic Acid During Sporulation in *Saccharomyces cerevisiae*

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In the course of study on ribonucleic acid (RNA) metabolism during sporulation in *Saccharomyces cerevisiae*, a new species of RNA (20*S*) was observed in sporulating cells by polyacrylamide gel electrophoresis. The relative content of this RNA to total RNA increased linearly early in sporulation. Labeled adenine was preferentially incorporated into 20*S* RNA during the early stages of sporulation. The correlation between the physiological and genetic control of sporulation and the synthesis of 20*S* RNA are discussed.

Meiosis is a process of intracellular differentiation involving both morphological and biochemical changes. In yeast, meiosis leads to the production of an ascus containing four haploid ascospores. The process is complex; changes in ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein contents (2, 5) and in histone contents (Bhargava and Halvorson, *submitted for publication*) have been observed. Recently, temperature-sensitive sporulation mutants blocked at various stages of meiosis in *Saccharomyces cerevisiae* were isolated (4, 6).

These findings, as well as the dependence of meiosis on protein synthesis (5), raise the possibility that the transcription products produced during sporulation may differ from those synthesized during mitotic growth. The purpose of the present experiments is to examine the classes of RNA synthesized during meiosis in yeast. Evidence is presented for the appearance during meiosis of a new high molecular-weight RNA (20*S*) species.

MATERIALS AND METHODS

Organisms. *S. cerevisiae* strain Y-290 (diploid lysine auxotroph) was used for most of the experiments in this study. The sporulation frequency of this strain was 70 to 75% after 48 hr in sporulation medium.

Media. YEP medium consisted of 2% dextrose, 2% peptone (Difco), and 1% yeast extract. KAc medium consisted of 2% potassium acetate, 50 μg of L-lysine hydrochloride per ml, and 20 μg of tetracycline per ml to inhibit bacterial growth.

Sporulation. Cells were grown in YEP for 24 to 26 hr (early stationary phase), harvested, and introduced into KAc sporulation medium at 5×10^7 cells per ml as previously described (5). To determine the percentage of asci, at least 500 cells and asci were counted in a hemocytometer. Buds also were counted as a single cell.

Cell breakage and RNA extraction. Cells were harvested by centrifugation at 1,000 × *g* for 5 min, washed once with cold distilled water, and suspended in four volumes of 1 M sorbitol solution containing 20% glycerol and 5% polyvinylpyrrolidone. The cell suspension was placed in a French pressure cell, frozen in a dry ice-alcohol bath for 7 min, and then passed through the orifice at a pressure of 8,000 psi. Immediately after thawing, 0.1 volume of 10% sodium dodecyl sulfate (SDS) solution and one volume of water-saturated phenol were added. Delay in addition of these reagents resulted in RNA degradation, especially in the case of the homogenate of sporulating cells. Phenol extraction of RNA was carried out three times at 5 C. RNA was precipitated by addition of two volumes of alcohol and stored at −20 C. To avoid RNA degradation, nuclease-free glassware and reagents were used throughout the extraction procedure and electrophoresis.

Polyacrylamide gel electrophoresis of RNA. Polyacrylamide gel electrophoresis was carried out by the method of Peacock and Dingman (11) with a slight modification. The buffer (PD) contained 108 g of tris (hydroxymethyl)aminomethane, 9.3 g of Na_2 ethylenediaminetetraacetic acid, and 55 g of boric acid (*p*H 8.3) in 1 liter of water. The stock solution of acrylamide contained 10% acrylamide and 0.5% *N*,*N*'-methylene bis-acrylamide and was stored at 5 C.

To make 12 gels the following were used: 10 ml of 10% acrylamide solution, 2 ml of PD buffer, 0.4 ml of 10% SDS, 27.2 ml of distilled water, 0.04 ml of 0.5% *N*,*N*,*N*',*N*'-tetramethylethylethylenediamine, and 0.4 ml of 10% ammonium persulfate (freshly prepared). The solution was poured into glass tubes (6 by 8 to 12 cm) and overlaid carefully with water. The buffer was 0.075 × PD. A $\frac{1}{100}$ volume of 10% SDS was added to the lower reservoir buffer to prevent RNA degradation. The RNA samples were dissolved in 1/20 PD buffer containing 5% sucrose and 0.1% SDS and centrifuged; the clear supernatant fluid was used to estimate RNA concentration and to overlay on the gels. For optimum separation, 25 μg (5 to 50 μliters) of RNA per ml was layered over the gels.

Currents of 1 ma/tube were used until the samples had entered the gels, and 5 ma/tube was used thereafter. Electrophoresis was carried out at room temperature at 5 ma/tube for 2 to 2.5 hr. The gels were stained with 0.1% Toluidine Blue O in 1% acetic acid for 1 hr, destained by soaking in 1% acetic acid for 18 to 20 hr, and scanned at 580 nm in a Gilford spectrophotometer.

Isotope experiment. Cells were inoculated into YEP medium containing 5 μCi of adenine-8-^3H per ml at a density of 10^4 cells/ml and incubated at 30 C for 30 hr. The cells were harvested and introduced into KAc sporulation medium containing 0.66 μCi of adenine-8-^{14}C per ml as previously described (5). After 6 and 12 hr of incubation at 30 C, the cells were harvested, washed, and suspended in 1 M sorbitol containing 20% glycerol and 5% polyvinylpyrrolidone. Cell breakage with French press and subsequent extraction of RNA were carried out as described above. After electrophoresis of the radioactive RNA, the gel was fractionated by an Auto-gel-divider with a Unifract collector (Savant Instrument, Inc., Hickville, N.Y.). Fractions were collected directly into scintillation vials containing 0.5 ml of 5 mM ethylenediaminetetraacetic acid. After extraction of RNA with gentle shaking at room temperature for 18 to 20 hr, 19 ml of scintillation liquid [7 g of 2,5-diphenyloxazole, 0.3 g of dimethyl 1,4-bis-2-(5-phenyloxazolyl), and 100 g of naphthalene in 1 liter of dioxane] was added to each vial, and ^{14}C and ^3H were determined in a Packard liquid scintillation spectrometer.

Material. Adenine-8-^{14}C (5.5 Ci/mole) was obtained from New England Nuclear Corp., Boston, Mass., and adenine-8-^3H (22.6 Ci/mmole) was from Schwarz BioResearch Inc., Orangeburg, N.Y. The other chemicals used in this study were analytical reagent grade.

RESULTS

Changes in the macromolecule contents during the early stage of sporulation are summarized in Fig. 1. RNA content linearly increases to a maximum level at T_{12} and then decreases gradually to the initial level. Protein content rises over the first 6 hr and then declines over the remainder of sporulation. DNA increases between T_6 and T_{12} as previously reported by Esposito et al. (5) and Sando (*personal communication*). Croes (3, 4), however, reported a slight decrease in RNA and protein content during sporulation.

To determine if the changes in RNA levels during meiosis reflect differential transcription, RNA was extracted from the cells at the different stages of sporulation and analyzed by polyacrylamide gel electrophoresis (Fig. 2). There are two major bands and two minor bands in a gel of RNA from T_0 cells and from vegetative cells. In sporulating cells (T_6 to T_{24}), a new band appears which migrates between the 18*S* and 26*S* ribosomal RNA (rRNA) species. From the migration rate of this unique RNA, we estimate its size to be approximately 20*S* (1, 8). This band disappears after ribonuclease treatment. As shown in

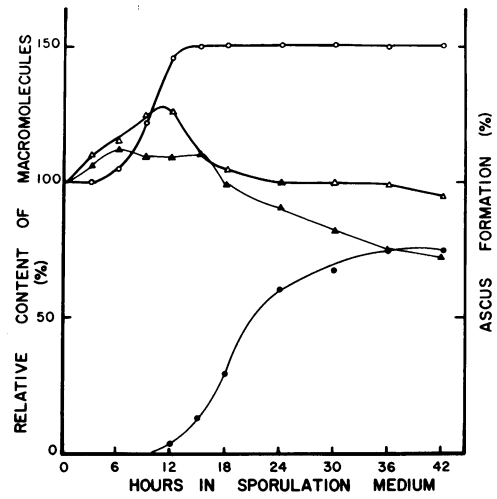

FIG. 1. *Changes in RNA, DNA, and protein during sporulation. Cells of Y-290 were grown in YEP medium, transferred to KAc medium, and incubated at 30 C with vigorous aeration. Samples of culture medium were removed at intervals, and DNA, RNA, and protein contents as well as the number of asci were determined. Values for macromolecules are given as per cent of T_0 values; DNA (○), RNA (△), protein (▲), ascus (●).*

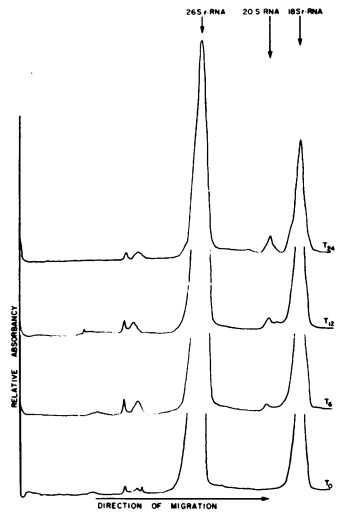

FIG. 2. *Polyacrylamide gel analysis of RNA from sporulating cells. RNA was carefully extracted from the cells (Y-290) at the different stages of sporulation and separated by electrophoresis on polyacrylamide gels (25 μg of RNA per 20 μliters per tube). Electrophoresis was for 2 hr at 5 ma/tube. Gels stained with Toluidine Blue were scanned at a wavelength of 580 nm with a Gilford spectrophotometer. Under these conditions, 4S and 5S RNA migrated to the far right side of the figure and are not illustrated.*

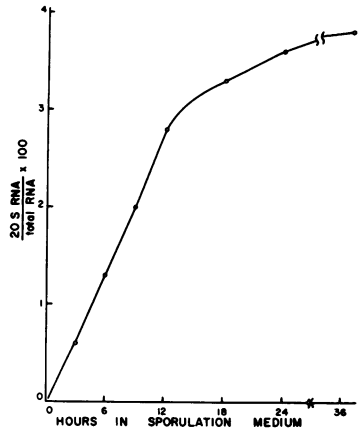

FIG. 3. *Kinetics of 20S RNA synthesis during sporulation. RNA was isolated at various stages of sporulation and separated by gel electrophoresis as described in the legend in Fig. 2. The area under each peak was measured and, from this, the 20S RNA/total RNA × 100 was determined and plotted as a function of hours during sporulation.*

Fig. 3, the relative content of this new species of RNA increases linearly during the course of sporulation until T_{12}, the time at which DNA and RNA reach maximum levels.

One possible origin of this RNA is that it is a degraded product of the increased RNA turnover during sporulation (6). To examine this possibility, a double labeling experiment was performed. Cells were grown during vegetative growth in medium containing ^{3}H-adenine. At T_0, the cells were transferred to KAC containing ^{14}C-adenine. The radioactive RNA extracted from the cells at T_6 and T_{12} was separated by polyacrylamide gel electrophoresis. After fractionation of the gel, distributions of radioactivity were determined. As shown in Fig. 4, the radioactivity profiles are very similar to the RNA profiles (Fig. 2). However, the ^{14}C/^{3}H ratio varies significantly throughout the gel. The highest ratio is found in the region between the two cytoplasmic rRNA species. A significant increase in the ^{14}C/^{3}H ratio also occurs in the region of the mitochondrial rRNA species (tubes 21 to 25; 46 to 49; H. Morimoto, and H. O. Halvorson, Proc. Nat. Acad. Sci. U.S.A., *in press*). The lowest ratios were observed in the two cytoplasmic rRNA regions of the gel. These findings demonstrate not only that various RNA classes are synthesized during sporulation but that some classes (e.g., 20S RNA) are preferentially synthesized at T_6 (Fig. 4A) and T_{12} (Fig. 4B).

Table 1 shows the relation between the physiological state of cells and the occurrence of 20S RNA. This species of RNA is detectable only in the sporulating cells (see control 24 hr after

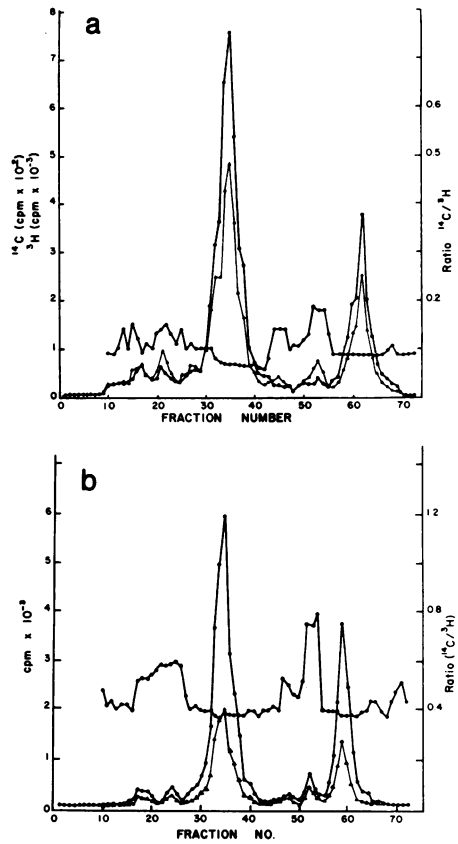

FIG. 4. *Polyacrylamide gel analysis of RNA labeled during sporulation. Cells (Y-290) grown for 30 hr at 30 C in YEP medium containing ^{3}H-adenine were harvested by centrifugation, washed with sterile water, and transferred to KAc medium containing ^{14}C-adenine. RNA was extracted from the cells at T_6 (Fig. 4A) and T_{12} (Fig. 4B) and separated by electrophoresis (25 μg of RNA/tube). The gels were fractionated with the autogel-slicer, the RNA was extracted with ethylenediaminetetraacetic acid solution for 20 hr, and the radioactivities in each fraction were determined in a liquid scintillation counter: ^{3}H (○), ^{14}C (▲), ratio of ^{14}C/^{3}H (●).*

transfer to 1% KAc) or after prolonged incubation in stationary-phase cells (72 hr). In the latter case, the early steps of the sporulating process might have occurred.

Table 2 summarizes a survey of the appearance of 20S RNA in various haploid, disomic, and polyploid species of yeast placed under conditions of sporulation. The 20S RNA was detected only in sporulating a/α diploid strains or in strains which were disomic for chromosome III *and* carried both a and α alleles. Only traces of 20S RNA could be detected in the sporulating diploid Y-85. This RNA was not observed in poorly sporulating cultures of a triploid (Y-192) or penta-

TABLE 1. *Relation between growth conditions and occurrence of the new species of RNA*[a]

Medium	Growth condition	Incubation time (hr)	20S RNA	Per cent ascus formed
5% Glucose in YEP	Anaerobic	120	–	0
2% Glucose in YEP	Anaerobic	120	–	0
2% Glucose	Aerobic	12	–	0
		24	–	0
		72	+	0
2% Galactose in YEP	Aerobic	12	–	0
		24	–	0
		72	+	0
Early stationary-phase cells transferred to 1% KAc	Aerobic	24	+	59

[a] RNA was extracted from the cells (Y-290) grown under various conditions, separated, and scanned as described in the legend in Fig. 2.

TABLE 2. *Occurrence of 20S RNA in the different strains in Saccharomyces cerevisiae*[a]

Strain	Ascus (%)	20S RNA
Y-165 Haploid a hi_4 try_5 ad_6 ur_1	0	–
Y-166 Haploid α hi_4 try_5 ad_6	0	–
Y-204 Haploid a ad_5	0	–
Y-209 Haploid α ad_5	0	–
Y-245 Haploid α/α Disomic III ad_2	0	–
Y-246 Haploid a/a Disomic III ad_1 ad_2 gal lys hi	0	–
Y-333 Haploid a/α Disomic III ad_1 lys_2	+[b]	+
Y-334 Haploid a/α Disomic III ad_2 lys_2 ur_1	+[b]	+
Y-335 Haploid a/α Disomic III ad_2 hi_7	+[b]	+
Y-336 Haploid a/α Disomic III ad_1 ad_2 hi_7 ly_2 ur_1	+[b]	+
Y-337 Haploid a/α Disomic III ad_1 ly_2	+[b]	+
Y-85 Diploid Wild type (Winge)	72	±
Y-142 Diploid Phenotype Su^+ ma^- Mg^+ (Hawthorne)	81	+
Y-163 Diploid Wild type (Williamson)	66	+
Y-185 Diploid su^- (Pretlow)	52	+
Y-191 Diploid Wild type (Mortimer)	18	+
Y-289 Diploid D ar^-	65	+
Y-290 Diploid D ly^-	70	+
Y-291 Diploid D ar^-	66	+
Y-292 Diploid D ly^-	68	+
Y-293 Diploid D ar^-	58	+
Y-316 Diploid	48	+
Y-331 Diploid Y-204 X Y-245	54	+
Y-332 Diploid Y-209 X Y-246	56	+
Y-192 Triploid	3	–
Y-193 Pentaploid	2	–

[a] Cells of various strains were subjected to the same growth condition as described in the legend for Fig. 1. RNA was extracted, separated, and scanned as in Fig. 2.

[b] Immature asci were observed in these strains (*see* Fig. 5).

ploid (Y-193) strains.

It has long been recognized that sporulation in *S. cerevisiae* is under the control of the a and α mating type alleles on chromosome III. Diploid strains heterozygous for these alleles (a/α) sporulate, whereas diploid strains homozygous for either (a/a or α/α) are unable to sporulate (12). Roth and Lusnak (13) recently showed that four such homozygous diploids were incapable of supporting DNA synthesis even after prolonged incubation in sporulation medium. Since meiotic DNA synthesis occurs early in sporulation (Fig. 1), both a and α alleles are required for some early event(s) in the sporulation cycle. Yeast strains disomic for chromosome III provide a further approach to examine the stages in meiosis. When disomic III strains which carry homologous mating type alleles (a/a or α/α) are placed under conditions of sporulation, none of the typical features of meiosis are observed. In contrast, when a/α disomic strains are transferred to sporulation medium, they show a number of changes associated with normal sporulation. In addition to 20S RNA (Table 2), after 3 days of aeration in sporulation medium, these disomic strains form spore septa and four immature asci (Fig. 5B). These strains can apparently enter but not complete meiosis. Figure 5A shows the completion of meiosis in a diploid wild-type strain.

DISCUSSION

Meiosis and sporulation in yeast are processes of intracellular differentiation which occur in eucaryotes. They are complex processes that require biochemical changes in the cells. Meiosis in yeast is more sensitive to a number of inhibitors (9), as well as to gamma radiation (10), than is mitosis. Esposito et al. (5) reported that RNA content increased slightly at the early stage of sporulation but decreased at the latter stage, whereas pulse-labeling experiments with the radioactive precursor showed two periods of the high RNA synthetic activity during sporulation in yeast (6). These findings suggest significant RNA turnover in sporulating cells.

In this paper, evidence was presented for the appearance of a new RNA (20S) during sporulation in *S. cerevisiae*. The 20S RNA was detected only in sporulating cells, and its content increased over the first 12 hr of sporulation. From labeled adenine incorporation, it was found that 20S RNA was preferentially synthesized in comparison with rRNA during this period. Only cells

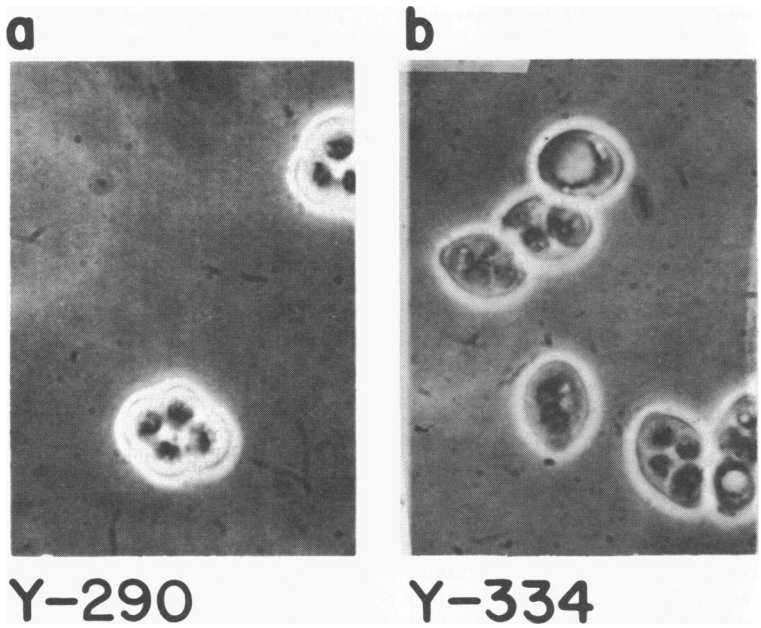

FIG. 5. *Mature and immature asci. Cells of Y-290 (A) and Y-334 (B) were incubated in KAc medium at 30 C for 3 days. Magnification ×1,000.*

capable of sporulating, or at least completing the early stages of meiosis, have the potential to produce 20*S* RNA when placed under the conditions of sporulation. The function of this RNA is as yet unknown. Biochemical properties of this RNA are reported in the accompanying paper (7).

The data in Table 2 lead to the conclusion that both the a and α alleles on chromosome III are required for the synthesis of 20*S* RNA as well as regulating the ability to undergo meiosis. Disomic III strains containing a/a or α/α neither enter meiosis nor produce 20*S* RNA, whereas a/α disomic III strains are able to synthesize 20*S* RNA and produce immature asci containing spore septa after a long period of incubation under sporulating conditions. These disomic strains and the conditional meiotic mutants (4 to 6) provide useful tools for investigating the biochemical events associated with meiosis.

ACKNOWLEDGMENTS

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