JOURNAL OF BACTERIOLOGY, Dec. 1980, p. 1098-1112
0021-9193/80/12-1098/15$02.00/0

Vol. 144, No. 3

Messenger Ribonucleic Acid and Protein Metabolism During Sporulation of *Saccharomyces cerevisiae*

ELLEN KRAIG AND JAMES E. HABER*

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254

To investigate differences between growing yeasts and those undergoing sporulation, we compared several parameters of messenger ribonucleic acid (RNA) transcription and translation. The general properties of messenger RNA metabolism were not significantly altered by the starvation conditions accompanying sporulation. The average messenger RNA half-life, calculated from the kinetics of incorporation of [^{3}H]adenine into polyadenylic acid-containing RNA, was 20 min in both cell populations. Furthermore, 1.3 to 1.4% of the total RNA was adenylated in both growing and sporulating cells. However, the proportion of RNA that could be translated in a wheat germ system slowly decreased during sporulation. Within 8 h after the induction of sporulation, isolated RNA stimulated half as much protein synthesis as the equivalent amount of vegetative RNA. There were significant differences in protein synthesis. The percentage of ribosomes in polysomes decreased threefold as the cells entered sporulation. This decrease began within 5 min of the initiation of sporulation, and the steady-state pattern was attained within 120 min. However, the ribosomes were not irreversibly inactivated; they could be reincorporated into polysomes by returning the sporulating cells to growth medium. Though unable to sporulate, strains homozygous for mating type, *MATα/MATα*, showed a similar decrease in the number of polysomes when placed in sporulation medium. Furthermore, the same shift toward monosomes was observed during stationary phase of growth. We conclude that the redistribution of ribosomes represents a general metabolic response to starvation. Our data indicate that the loss of polysomes is most likely caused by a decrease in the initiation of translation rather than a severe limitation in the amount of messenger RNA. Furthermore, the loss of polysomes is not due to the decreased synthesis of a major class of abundant proteins. Of the 400 vegetative proteins resolved by two-dimensional gel electrophoresis, only 19 were not synthesized by sporulating cells. Approximately 10 to 20% of the cells in a sporulating culture failed to complete ascus formation. We have shown that [^{35}S]methionine is incorporated equivalently into cells committed to sporulation and cells that fail to form asci. Furthermore, the proteins synthesized by these two populations were indistinguishable on one-dimensional gels. We compared proteins labeled by various protocols, including long-term and pulse-labeling during sporulation and prelabeling during vegetative growth before transfer to sporulation medium. The resulting two-dimensional gel patterns differed significantly. Many spots labeled by the long-term techniques may have arisen by protein processing. We suggest that pulse-labeling produces the most accurate reflection of instantaneous synthesis of proteins.

Many eucaryotic microorganisms, including the slime mold *Dictyostelium discoideum* (15), the protozoan *Naegleria gruberae* (30), and the yeast *Saccharomyces cerevisiae* (9), can be induced to differentiate by selective starvation. For example, yeast cells which have been deprived of nitrogen in the presence of an oxidative carbon source will complete the vegetative mitotic cycle and then proceed through meiosis. The four haploid spores produced are contained in a protective ascus. The ability to sporulate is controlled by the information encoded at the mating type (*MAT*) locus. Only heterozygous *MAT***a**/*MATα* diploid cells can complete ascus formation. Haploid cells, *MAT***a** or *MATα*, or diploid cells homozygous for mating type are unable to sporulate.

Though yeast cells grown on acetate as a carbon source divide mitotically every 2.5 to 3 h, the completion of sporulation requires 20 to 30

h. During this time, the sporulating culture completes premeiotic DNA synthesis, chromosomal synapsis, recombination, and ascus formation in the absence of extracellular nitrogen. Both RNA and protein syntheses continue during sporulation (12). In fact, protein synthesis is required, since inhibitors of translation block ascus formation (5, 18). This macromolecular synthesis is dependent on the turnover of preexisting RNA and protein by proteolytic (16, 34) and nucleolytic (29) enzymes whose activities increase during sporulation.

The total RNA isolated from sporulating cells has twofold less polyadenylic acid [poly(A)] than that from vegetative cells (9; C. Saunders, Ph.D. thesis, Brandeis University, Waltham, Mass., 1979). This may be due either to less adenylated mRNA during sporulation or to shorter poly(A) tracts. The latter has been shown to account for most, if not all, of the decreased polyadenylation (Saunders, thesis). In view of the presumed increase in macromolecular turnover and the apparent decrease in the length of poly(A) tracts, we were interested in studying the metabolism and utilization of mRNA during sporulation in greater detail.

Furthermore, we have been examining changes in transcription and translation which accompany differentiation. There have been a number of observations to suggest that the control of macromolecular synthesis may be altered during sporulation. For example, several of the temperature-sensitive *rna* mutations which severely inhibit the synthesis of ribosomal proteins during growth at the restrictive temperature fail to effect a change during sporulation (23a). Thus, when diploid cells homozygous for *rna2* are shifted from 25 to 34°C during growth, there is a rapid decline in the relative rates of synthesis of nearly all ribosomal proteins. There is no similar inhibition of ribosomal protein synthesis when sporulating *rna2/rna2* diploids are shifted to 34°C. Furthermore, during sporulation, several newly accumulated proteins have been identified, including a cytoplasmic particle containing approximately 20 protein subunits and a 20S RNA molecule (31).

By analyzing long-term-labeled proteins on two-dimensional gels, Trew et al. (28) have identified several changes in the synthesis, accumulation, or processing of proteins during sporulation. However, asporogenous cells, homozygous for *MATα*, produced the same protein patterns. Therefore, it is likely that these proteins induced by starvation are not sufficient for sporulation or premeiotic DNA synthesis. Many differences in protein synthesis were detected in a one-dimensional gel analysis of pulse-labeled proteins (12). Once again, the changes in protein synthesis observed were also seen in *MATα/MATα* cells. We have also analyzed protein synthesis by pulse-labeling cells with [^{35}S]methionine at various points during sporulation and fractionating the products on two-dimensional gels. Even with this increased resolution, we observed no *MATa/MATα*-specific sporulation proteins.

MATERIALS AND METHODS

Strains and culture conditions. AP-1, a diploid of *S. cerevisiae*, was used throughout this work. Its genotype is:

MATa	***ade1***	***ade2***	***gal1***	***tyr1***	***lys2***	***his7***	***ura1***					
MATα	+	***ade2***	+	+	+	+	+					
					+	+	+	+	***csp***			
					ura3	***can1***	***cyh2***	***leu1***	+			

Both AP-1 and the UV-induced *MATα/MATα* derivative of it were provided by A. K. Hopper (12). Cells were grown at 30°C in AcII (1% potassium acetate, 0.6% yeast nitrogen base without amino acids, 0.5% yeast extract, 0.5% peptone, 1.02% potassium biphthalate, pH 5.5 with KOH) or YEPA (1% potassium acetate, 2% peptone, 1% yeast extract, pH 5.5) and monitored with a Klett-Summerson colorimeter equipped with a red filter. Under the conditions used, the culture doubled every 150 to 180 min. Vegetative cultures were always maintained below 5×10^7 cells per ml with maximum aeration.

For sporulation, cultures were harvested during exponential growth at a concentration of 1×10^7 to 2×10^7 cells per ml, washed, and suspended in 2 volumes of sporulation medium (1% potassium acetate, 0.2 M succinic acid, pH 5.5 with KOH) (20). The sporulation medium was buffered to pH 5.5 to increase cellular permeability (20, 21) without affecting sporulation. Sporulation was assayed microscopically after cultures were incubated for 24 to 48 h on a gyratory platform shaker and was greater than 70% in all experiments.

Determination of mRNA half-life. The procedure of mRNA half-life determination was a modification of the one used by Hynes and Phillips (14). Samples of 4 μCi of [^{3}H]adenine per ml and 10 μg of unlabeled adenine per ml were added either to the AcII vegetative culture or to a culture 4.5 h into sporulation. The addition of carrier produced linear label uptake during the 2-h experimental period. At appropriate times after label addition, a sample of cells (3 ml of the vegetative culture and 5 ml of the sporulating cells) was harvested in the cold, washed once with cold water, and fast frozen in a dry ice-ethanol bath in SDS buffer (0.1 M NaCl; 20 mM EDTA; 20 mM Tris, pH 7; 0.5% sodium dodecyl sulfate [SDS]). A constant amount of ^{14}C-labeled RNA, containing approximately 25% polyadenylated species, was added for normalization. An equal volume of cold 0.45- to 0.5-mm glass beads was added to the harvested sample, and the cells were broken by high-speed blending on a Vortex mixer. Four 20-s disruptions with 10-s intermittent cooling periods achieved at least 90% breakage. The cell lysate was resuspended in TES (0.1

M NaCl, 30 mM EDTA, 10 mM Tris, pH 7.4), brought to 0.2% SDS, and mixed with an equal volume of a 1:1 mixture of chloroform and redistilled phenol. The emulsion was shaken for 15 min at 4°C, and the phases were separated by centrifugation. Both layers were reextracted to ensure maximum recovery and purity. The RNA was then precipitated from the aqueous layer by the addition of 2 volumes of cold 95% ethanol. After 1 h at −20°C, the RNA was collected by centrifugation.

The RNA was fractionated by oligodeoxythymidylic acid [oligo(dT)]-cellulose chromatography on T3 cellulose (Collaborative Research, Inc.) as described by Hynes and Phillips (14). The column fractions were brought to 0.1 M NaCl, and, where necessary, carrier tRNA was added to a final concentration of 50 μg/ml before ethanol precipitation. The low-salt fraction, poly(A)-containing RNA, was further purified through a second column. The final precipitates were dried, resuspended, and spotted on a GF/C filter for liquid scintillation counting.

RNA isolation and translation. RNA was isolated by the method of Zitomer et al. (33) as modified by Bromley, Hereford, and Rosbash (personal communication). Briefly, cells were suspended in cold extraction buffer (0.1 M Tris, 0.1 M LiCl, 0.1 mM EDTA, pH 7.4), glass beads were added, and the mixture was blended on a Vortex mixer. SDS was added to 0.5%. An equal volume of redistilled phenol-chloroform was added, and the suspension was blended for 5 s on a Vortex mixer and then centrifuged. After reextraction, the aqueous layers were combined, brought to 0.1 M NaCl, and precipitated by the addition of ethanol. The RNA pellet was washed with 70% ethanol and suspended in 0.2 M potassium acetate for the subsequent ethanol precipitation.

The wheat germ extract was prepared as described by Hopper et al. (13). The translation reaction, containing [^{35}S]methionine, was also described previously (13). After incubation, a sample was precipitated with trichloroacetic acid and counted to determine the incorporation.

Polysome extraction. A 10- to 20-ml culture sample was harvested in the cold and suspended in Mg buffer (0.1 M NaCl, 0.01 M Tris, 30 mM $MgCl_2$, pH 7.4) plus 200 μg of cycloheximide, 0.2 μl of diethylpyrocarbonate, and 200 μg of heparin per ml of buffer. An equal volume of 0.45- to 0.5-mm glass beads was added, and the mixture was blended on a Vortex mixer twice for 20 s and then once for 10 s. Up to 1.5 ml of the sample was loaded onto a 5 to 20% sucrose gradient prepared in Mg buffer plus 200 μg of heparin per ml. The gradients were centrifuged in a Beckman SW27 rotor at 5°C for 90 min at 26,000 rpm. They were then fractionated through a flow cell attached to a Beckman spectrophotometer monitoring at a wavelength of 260 nm. Where necessary, the eluate could be collected using a fraction collector; the appropriately pooled fractions were then diluted with an equal volume of Mg buffer and precipitated with ethanol.

The fraction of ribosomes isolated as polysomes was determined directly from the optical density tracing of the gradient produced by the spectrophotometer recorder. The monosome peak and polysomal fraction were carefully cut out and then weighed on a Mettler analytical balance. The fraction of the total optical density which was in the polysomal region of the gradient was calculated.

Where radioactive polysomal RNA was required, the cells in AcII vegetative medium were labeled with [^{14}C]adenine and the sporulation culture was labeled with [^{3}H]adenine. The cells were harvested and washed, and samples from the two cultures were combined before cell disruption.

Protein isolation. A 50-μCi volume of [^{35}S]methionine was added to 5 ml of either a sporulating culture or a vegetative culture growing in a defined acetate medium (1% potassium acetate, 1.3% yeast nitrogen base without amino acids, 0.006% leucine, 0.06% threonine, 0.004% tryptophan, 0.004% histidine, 0.004% arginine, 0.006% lysine, 0.004% phenylalanine, 0.004% adenine, 0.005% uracil, and 0.005% tyrosine). After the labeling period, the cells were harvested and washed in break buffer (0.05 M Tris, pH 7.5; 0.2% SDS) plus 5% of a solution containing 3 mg pf phenylmethylsulfonyl fluoride per ml of ethanol. The pellet was suspended in 200 μl of break buffer plus phenylmethylsulfonyl fluoride, glass beads were added, and the cells were broken as described previously. The protein sample was prepared for electrophoresis by a modification of a procedure designed for spheroplasts (Blifeld, Hereford, and Rosbash, personal communication). The lysate was transferred to a microcentrifuge tube, and the buffer used to wash the beads was added to bring the volume to 0.2 ml. One-tenth volume of a solution containing 0.5 mg of micrococcal nuclease per ml, dissolved in 0.02 M $CaCl_2$ and 0.2 M Tris buffer (pH 8), was added, and the sample was incubated for 3 min at room temperature. The sample was then treated for an additional 3 min with 0.1 volume of a mixture of 1 mg of pancreatic RNase, 0.1 mg of T_1 RNase, and 1 mg of pancreatic DNase per ml in 0.01 M NaCl, 0.05 M $MgCl_2$, and 0.01 M Tris (pH 7.5). The sample was lyophilized and suspended in sample buffer (9.5 M urea, 1.6% ampholines at pH 5 to 8, 0.4% ampholines at pH 3.5 to 10, 5% mercaptoethanol, 2% Nonidet P-40) before electrophoresis.

Two-dimensional analysis of the samples was precisely as described by O'Farrell (23). The first dimension was an isoelectric focusing gel in the range of pH 5 to 7; the second dimension was a 12.5% SDS-polyacrylamide gel. Where possible, 0.2×10^6 to 1.0×10^6 trichloroacetic acid-precipitable ^{35}S cpm was loaded onto each isoelectric focusing gel. After separation in two dimensions, the gel was dried and autoradiographed for 1 to 4 days. With samples of lower specific activity, an attempt was made to load a constant number of counts to each gel, and increased efficiency was achieved by fluorography after impregnation of the gel with PPO (2,5-diphenyloxazole) (2).

Separation of cell types during sporulation. After labeling of cells with [^{35}S]methionine, the cell types were separated by a modification of the technique of Rogers, Zeesman, and Bussey (personal communication). A 5-ml sample of the sporulating culture was harvested, washed, and suspended in 1 ml of 5% sorbitol. The suspension was loaded on a linear gradient of 5 to 50% sorbitol in a 30-ml Corex tube. The gradients were centrifuged in an HB-4 rotor at 1,500 rpm for 2.5 to 3 min. They were fractionated through

a flow cell in a Beckman spectrophotometer monitoring at a wavelength of 600 nm. A 100-μl portion of each fraction was added to 5 ml of ACS liquid scintillation fluid (Amersham Corp.) and assayed in a Beckman scintillation counter.

For isolation of protein, several fractions were pooled, and cells were harvested, washed, and broken as previously described. The protein samples were diluted with equilibration buffer (10% [wt/vol] glycerol, 5% [vol/vol] mercaptoethanol, 2.3% SDS, 0.0625 M Tris, pH 6.8) and analyzed on an 11% SDS-polyacrylamide slab gel.

RESULTS

mRNA half-life. The rate of incorporation of a radioactive precursor into a macromolecular species will be linear until degradation of the labeled species begins. It is therefore possible to detemine the half-life of any relatively unstable molecule, such as mRNA, by assaying its kinetics of incorporation (8). Utilizing this technique, Hynes and Phillips (14) previously determined the half-life of mRNA to be 17 min in vegetative cultures grown on the fermentable carbon source glucose. We have measured the mRNA half-life in cells growing on an aerobic carbon source, acetate, and in sporulating cells 4.5 h after the initiation of differentiation. In both cases, [^{3}H]-adenine was added, and samples were removed from the two cultures at intervals during the 2-h experimental course. The RNA was isolated and fractionated by oligo(dT)-cellulose chromatography. The fraction not bound to the column in a high-salt wash was composed primarily of RNA lacking poly(A) sequences: rRNA, tRNA, and any mRNA with insufficiently long poly(A) tracts. As can be seen in Fig. 1, the label was incorporated linearly into the high-salt fraction, which contained predominantly RNA species stable throughout the 2-h experiment. Extrapolation of both the vegetative and sporulation high-salt curves to the abscissa revealed a 5-min lag time for equilibration of the extracellular [^{3}H]adenine with the ATP pool.

The poly(A)-containing RNA which bound to the column was eluted with low salt. Experimental points taken late in the time course showed inflated low-salt counts due to contamination by 4% of the high-salt RNA. Therefore, a second oligo(dT) column was used to further purify the low-salt fraction at each time point by removing the high-specific-activity high-salt material. As expected for an unstable species such as mRNA, the incorporation into poly(A)-containing RNA deviated from linearity during the 2-h experiment. Since the incorporation of [^{3}H]adenine at the early time points primarily reflects synthesis (rather than degradation) of mRNA, the two sets of experimental data were normalized to the same initial slope. This curve should plateau when synthesis of mRNA is equaled by its degradation. The time at which 50% of this plateau value is attained is the mRNA half-life. Since the estimation of a plateau is imprecise, we chose to use the Greenberg (8) equation for mRNA half-life to calculate the predicted curves and thus determine the best fit of the data. This equation corrects for increasing mass of the culture during the experiment. The doubling time of the vegetative culture was 150 min. Asci are first observed after 12 h, so a generation time of 720 min was assumed for the sporulation culture. The calculated theoretical curves for mRNA half-lives of 15, 20, and 25 min were normalized to the initial slopes of the experimental data. As seen in Fig. 1, the data are best described by a 20-min half-life for both vegetative and sporulating cells.

Proportion of mRNA. To measure directly the proportion of cellular RNA which contained poly(A), vegetative and sporulating cultures were labeled for 10 h with [^{14}C]adenine and [^{3}H]adenine, respectively. The RNA was isolated from each culture and combined before oligo(dT)-cellulose chromatography. After three successive purifications of the low-salt fraction, 1.3 to 1.5% of the total ^{3}H and ^{14}C counts remained in the poly(A) species (Table 1). Thus the actual proportion of poly(A) RNA was unchanged during sporulation.

However, there was a decrease in the proportion of RNA that could be translated by a wheat germ in vitro translation system. As shown in Table 1, by 4 h after the start of sporulation (T4) the stimulation of translation decreased to approximately 70% of the vegetative level, and by T8 stimulation decreased even further, to 53%. Therefore, the functional mRNA population does become more limited during sporulation.

Changes in polysome distribution. In a previous study, Mills (22) concluded that there was no change in the proportion of ribosomes in polyribosomes during sporulation. This was based on the analysis of sporulating and growing cells to which cycloheximide had been added to stabilize polysomes. We were concerned that the addition of the protein synthesis inhibitor 5 min before the cells were harvested might have resulted in the "recruitment" of monosomes into polysomes, obscuring a significant difference in the actual proportion of polysomes. We therefore developed a rapid procedure for polysome isolation, excluding the need for addition of cycloheximide to the cell culture before harvesting. To maintain the polysomes present, the drug was added to the washed cell pellet immediately before lysis. After the cell break, the lysate was fractionated on a sucrose gradient. In the com-

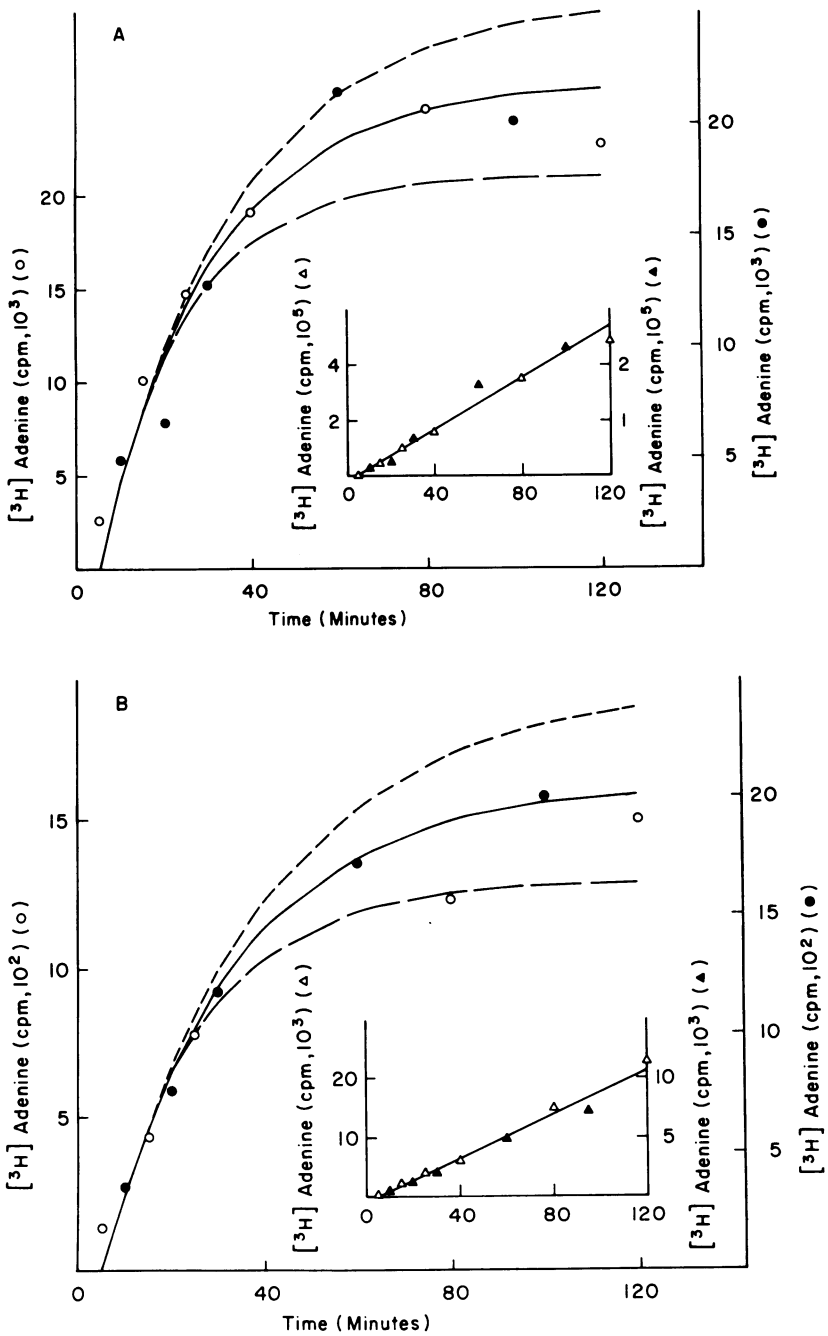

FIG. 1. *Incorporation of [^{3}H]adenine into RNA species. [^{3}H]adenine was added at 4 μCi/ml of a vegetative culture (A) and a sporulating culture (B). Samples were harvested at intervals during the following 2 h. The cells were broken, and the isolated RNA was fractionated by oligo(dT)-cellulose chromatography. Incorporation into non-adenylated RNA: rRNA, tRNA, and mRNA with insufficiently long poly(A) tracts are shown in the inset graphs. (●, ○) Incorporation onto poly(A)-containing RNA. The solid and open symbols represent separate experiments. Theoretical curves for mRNA half-lives of 15 min (——), 20 min (——), and 25 min (-----) have been drawn for comparison. The data from separate experiments and the theoretical curves were normalized to the same initial slopes.*

TABLE 1. *Proportion of poly(A)-containing RNA in vegetative and sporulating cells*

Culture	Time	Poly(A) RNA (% of total)[a]		[35S]incorporated into protein (cpm/μg of RNA)[b]	
		Expt A	Expt B	Expt A	Expt B
Vegetative	T0	1.2	1.4	1,020,000	1,210,000
Sporulating	T4	1.3	1.5	777,000	795,000
	T8	—[c]	—[c]	576,000	625,000

[a] Vegetative and sporulating cultures were long-term labeled with [14C]- and [3H]adenine, respectively. The isolated RNAs were combined and fractionated on an oligo(dT)-cellulose column. The fraction bound to the column was eluted and repurified twice. The percentage of total counts bound to the third column was then determined. A and B represent independent experiments.

[b] Isolated RNA was translated in vitro in a wheat germ system with [35S]methionine. The incorporated counts per minute were determined by trichloroacetic acid precipitation of a 2-μl sample of the reaction mixture. Endogenous incorporation was subtracted, and the incorporation per microgram of RNA was calculated for the reaction mixture. Experiment A, Translation of 1.25 μg of RNA in a 12.5-μl reaction. Experiment B, Translation of 0.625 μg of RNA in a 12.5-μl reaction.

[c] —, Not determined.

bined optical density profiles shown in Fig. 2A, there appears to be a threefold depression in polysomes during sporulation. To insure that the shift was not due to the increased RNases in sporulating cells, we labeled the vegetative culture with [14C]adenine and the sporulating culture with [3H]adenine. The cells were harvested at T4 of sporulation and combined before cell lysis. The polysomes were extracted, and the recovered optical tracing was intermediate in pattern (data not shown). As expected, the ratio of 3H to 14C counts decreased with increasing polysome size (Fig. 2B).

The percentage of ribosomes in polysomes was determined for cells during sporulation and under various growth conditions as described in Materials and Methods. The addition of the translational inhibitor cycloheximide 10 min before harvesting did not alter the recovery of polysomes from a vegetative culture. However, the percentage of polysomes did change when cycloheximide was added to a sporulating culture. Instead of the normal 25% polysomes, the 10-min drug pretreatment allowed the recovery of 57% polysomes (Table 2). Presumably, this represents recruitment of ribosomes from the monosome fraction into polysome structures.

As summarized in Table 2, the percentage of ribosomes in polysomes correlated well with the nutritional state of the culture. For example,

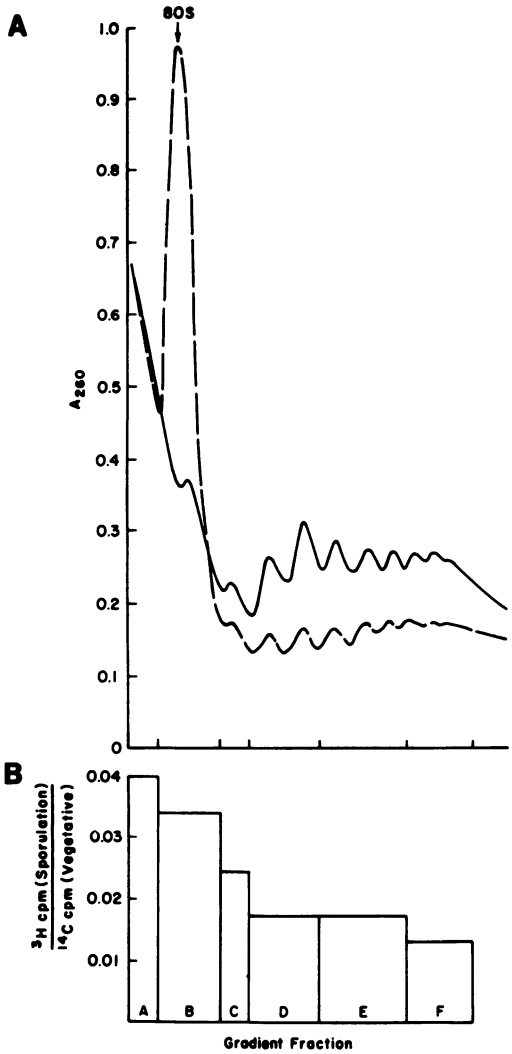

FIG. 2. *Polysome profiles from vegetative and sporulating yeast. Cells were harvested, and polysomes were fractionated on 5 to 20% sucrose gradients as described in the text. (A) The optical densities of the sporulation and vegetative profiles are combined for comparison. (B) Polysomes were coextracted from a vegetative culture labeled with [14C]adenine and a sporulating culture labeled with [3H]adenine. The fractions were pooled as shown, and the RNA was isolated. The ratio of 3H to 14C in the RNA was then determined for each of the following samples: A, material less dense than monosomes; B, monosomes; C, disomes; D, polysomes with three and four ribosomes; E, polysomes with five, six, and seven ribosomes; and F, large polysomes.*

when placed under the nitrogen starvation conditions necessary to induce sporulation, asporogenous *MATα/MATα* cells were enriched in monosomes. This shift was also observed in sta-

TABLE 2. *Percentage of ribosomes in polysomes during sporulation*

Diploid	Culture conditions[a]	% Polysomes[b]
AP-1 *MATa/MATα*	Vegetative growth	75
	Vegetative plus cycloheximide[c]	75
	Vegetative medium, stationary phase	25
	Sporulation	
	5 min	64
	10 min	56
	15 min	52
	30 min	48
	120 min	25
	240 min	25
	Sporulation (240 min) + cycloheximide[c]	57
	Sporulation (110 min) to vegetative medium (10 min)[d]	60
	Sporulation (90 min) to vegetative medium (30 min)[d]	71
AP-1 *MATα/MATα*	Vegetative growth	55
	Sporulation (120 min)	32

[a] Cells were grown in AcII medium and sporulated as described in the text.

[b] The percentage of ribosomes isolated as polysomes was determined from the optical density tracing of the gradient as described in the text.

[c] Cycloheximide was added at 200 μg/ml to culture 10 min before harvesting.

[d] Cells transferred at this time revert to vegetative growth, as they are not yet committed to sporulate.

tionary vegetative cultures of *MATa/MATα* and *MATα/MATα* cells.

Within 5 min after the initiation of starvation, a shift in ribosomes was observed. The steady-state value of 75% monosomes was attained within 2 h and remained at this level until at least 15 h into sporulation. The ribosomes in the enriched 80S peak were still potentially functional, because they began shifting to the vegetative pattern within 10 min after the cells were returned to growth medium. It is therefore likely that the shift in ribosomes is a cellular response to the starvation conditions which accompany sporulation.

To determine whether a class of mRNA was selectively localized in the monosome peak, RNA was isolated from the monosome and polysome fractions and translated in vitro. The ^{35}S-labeled proteins were fractionated on an 11% polyacrylamide gel (data not shown). Though there were differences between monosomes and polysomes, there was no evidence of sequestering of an abundant mRNA during sporulation. Differences may be detected when this analysis is extended to the higher resolution of two-dimensional gels.

Specific changes in protein synthesis. To determine whether the decreased translation is reflected in the loss of synthesis of a class of vegetative-specific proteins, we utilized two-dimensional gels. Several previous studies of protein synthesis during sporulation have been carried out using relatively long (usually 30-min to 2-h) periods of labeling with radioactive precursors (24, 28). The analysis of these gels is complicated by differences in the synthesis, processing, and turnover of particular proteins. We therefore decided to use very short pulse-labeling of cells so that we were looking almost exclusively at de novo protein synthesis. Cells were pulse-labeled for 5 min with [^{35}S]methionine during vegetative growth and during sporulation at T2, T4, T8, T12, and T20. The radioactive spots were analyzed after fractionation on two-dimensional O'Farrell gels. Two independent gel experiments were performed, and 400 resolved proteins were compared. Approximately 95% of the predominant proteins synthesized by vegetative cells (Fig. 3A) were also observed in sporulating patterns (Fig. 3B). The fluorographic exposure in Fig. 3A was chosen to demonstrate clearly the vegetative-specific spots. Figure 3B, on the other hand, is a longer exposure to illustrate the actual number of spots which may be compared without a great loss in resolution. The decreased translation in a sporulating culture appears to be an overall quantitative decrease in the synthesis of many proteins, rather than the qualitative loss of a single, significant class.

Table 3 summarizes the changes in synthesis of 33 specific proteins during sporulation. Most of these proteins are enumerated in Fig. 3A (proteins pulse-labeled during vegetative growth) and B (proteins pulse-labeled at T4 of sporulation). Many of the increases and decreases in synthesis were observed early during sporulation, though a few were not detectable until T8 or T12 and are not seen in Fig. 3. Fourteen of these proteins were synthesized to a greater extent during sporulation, and 19 were more prevalent during vegetative growth. It is clear that the changes occur at different times during sporulation.

Comparison of different procedures for labeling proteins during sporulation. We have compared our results, using 5-min pulse-labeling with [^{35}S]methionine, with two other procedures for labeling sporulating cells. Figure 4 directly compares a pulse-labeled sample (A) with one labeled for 2 h during sporulation (B). It is evident that the relative amounts of radio-

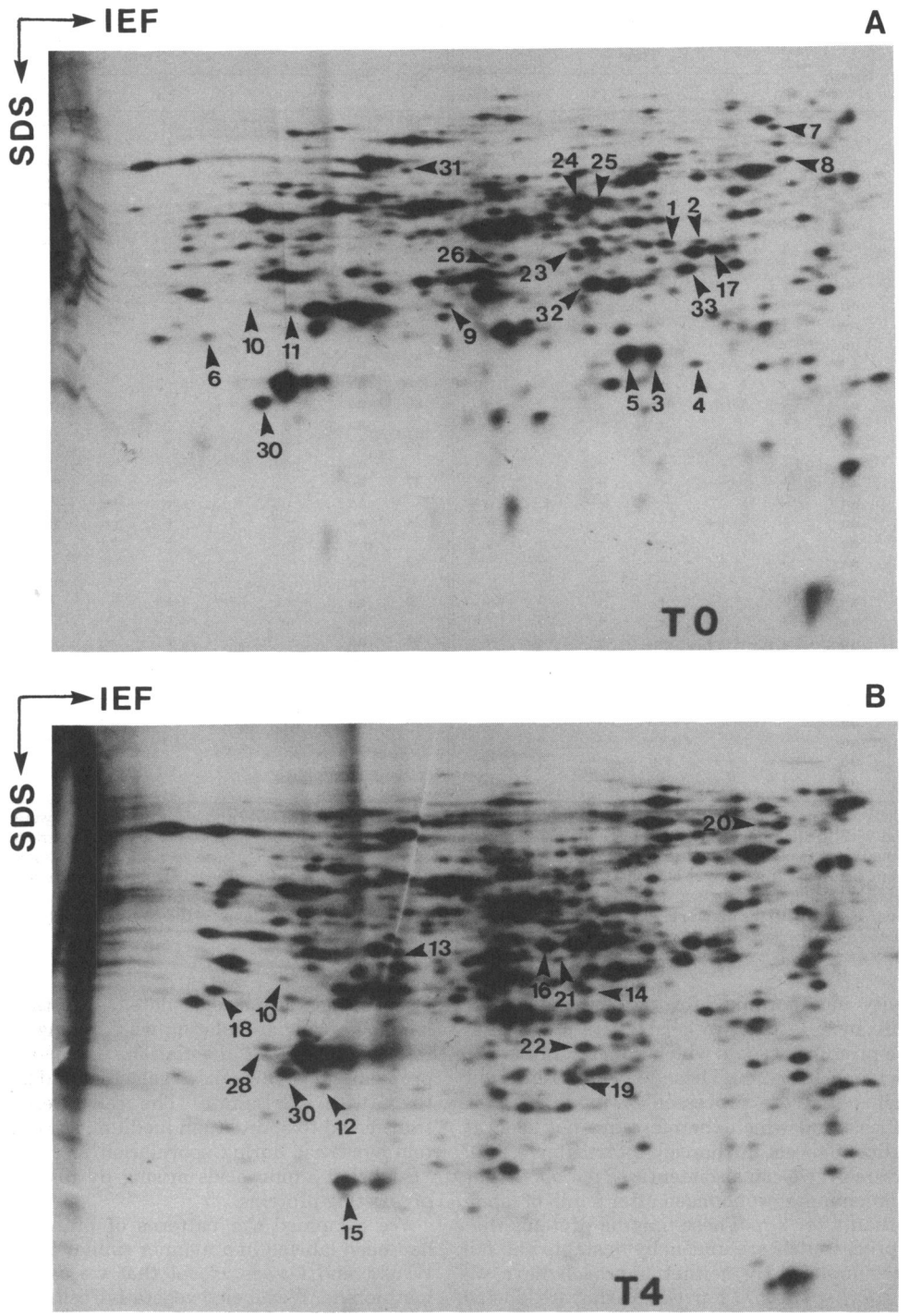

FIG. 3. *Proteins synthesized during vegetative growth (A) and at T4 during sporulation (B). Cells were pulse-labeled with [^{35}S]methionine for 5 min, and the extracted protein was fractionated on two-dimensional gels as described in the text. The gels were fluorographed, dried, and exposed to film at −70°C. The numbered spots showed significant changes in the rate of synthesis during sporulation.*

TABLE 3. *Changes in protein synthesis under sporulation conditions*[a]

Spot	Strain	Time					
		T0	T2	T4	T8	T12	T20
1	aα	++	−	−/+	−	−	−
	αα	++	−	−/+	−	−	
2	aα	+	−	−	−	−	−
	αα	+		−	−	−	
3	aα	+	−	−	−	−	−
	αα	+		−	−	−	
4	aα	+	−	−	−	−	−
	αα	+		−	−	−	
5	aα	+++	++	+	−	−	−
	αα	+++		−	−	−	
6	aα	+++	+	−	−	−	−
	αα	+++		−	−	−	
7	aα	+++	+	−	−	−	−
	αα	+		−	−	−	
8	aα	+++	++	++	+	+	−
	αα	+++		+	−	−	
9	aα	+++	++	++	+	−	−
	αα	+++		+	+	−	
10	aα	+	+++	+	+	+	−
	αα	−		++	++	++	
11	aα	+	+++	−	−	−	−
	αα	+		−	−	−	
12	aα	+	+++	+	−	−	−
	αα	+		−	−	−	
13	aα	+	+++	+	+	+	+
	αα	+		+	+	+	
14	aα	+	+++	+	+	+	+
	αα	+		+/−	+/−	+/−	
15	aα	−	+	+++	+++	+	+
	αα	−	+++	+	+		
16	aα	−	+++	+++	+++	++	+
	αα	−		+++	+	+	
17	aα	+++	++	++	++	+	−
	αα	+++		+	+	−	
18	aα	+	++	++	+	−	−
	αα	+		+	+	−	
19	aα	−	+	+	+	+	+
	αα	−		+	+	+	
20	aα	−	+	+	++	+++	+++
	αα	−		−	++	+++	
21	aα	−	+	+	+	++	+++
	αα	−		+	+	++	
22	aα	−	+	++	+	−	−
	αα	−		−	+	+	
23	aα	+	+	−	−	−	+
	αα	+		−	−	−	
24	aα	++	++	+	+	−	−
	αα	++		−	−	−	
25	aα	++	+	−	−	−	−
	αα	++		−	−	−	
26	aα	++	++	+	+	−	−
	αα	++		−	−	−	
27	aα	−		+	++	++	
	αα	−		+	++	++	
28	aα	−	+	+	++	++	++
	αα	−		+	++	++	
29	aα	−	−	−	+	++	+++
	αα	−		−	+	+++	
30	aα	+++	+++	+++	++	+	+
	αα	+++		+++	+++	+	
31	aα	+	+	+	−	−	−
	αα	+		+	−	−	
32	aα	++	++	++	+	−	−
	αα	++		+	−	−	
33	aα	++	++	++	++	+	−
	αα	++		++	+	−	

[a] Cells of strain AP-1 (*MATa/MATα*), able to sporulate, and strain AP-1 (*MATα/MATα*), unable to sporulate, were pulse-labeled with [^{35}S]methionine at the indicated times after transfer to sporulation medium. The proteins were extracted and fractionated on two-dimensional gels. Fluorographic exposures were compared visually. For each spot, the set of gels labeled at different times was compared as a group. A minus (−) indicates those times the spot was absent. Those times with detectable amounts of the spot were defined as plus (+). Greater amounts of synthesis at other times are indicated by additional pluses (++ and +++). All of the differences in protein synthesis indicated are relative to other times for the single spot and are not for comparison to other proteins.

activity incorporated into various spots differ significantly in the two gels. There are several quite prominent spots which appear only in the cells labeled for 2 h. These may well represent proteins that are processed or modified after synthesis and which therefore are not seen at significant levels in the cells pulsed for 5 min. Conversely, we can also identify a few spots that are much more pronounced after 5 min of labeling than after 2 h. These may be proteins that are processed or are unusually unstable and fail to accumulate. By neither approach have we found any spots on O'Farrell gels that are labeled in *MATa/MATα* but not in *MATα/MATα* cells.

We have also compared our results with those of Wright and Dawes (32), who used an entirely different labeling protocol. Using [^{35}S]sulfate, these investigators showed that label was preferentially incorporated into the small proportion of cells that did not sporulate, suggesting that labeling during sporulation was probing an unrepresentative cell population. To avoid this problem, they grew cells in galactose and labeled them with [^{35}S]sulfate. The cells were then transferred to sporulation medium, so that protein synthesis during sporulation incorporated ^{35}S-labeled amino acids arising by turnover of preexisting proteins.

We examined the patterns of proteins that had been labeled in a manner similar to that of Wright and Dawes except that we used [^{35}S]-methionine. We labeled vegetative cells growing in acetate overnight and transferred half of the cells to fresh growth medium and half to sporulation medium. After 4 h, both cultures were harvested, and the protein was extracted. A comparison of O'Farrell gels of proteins labeled in this fashion with the 5-min pulse-labeling at T4

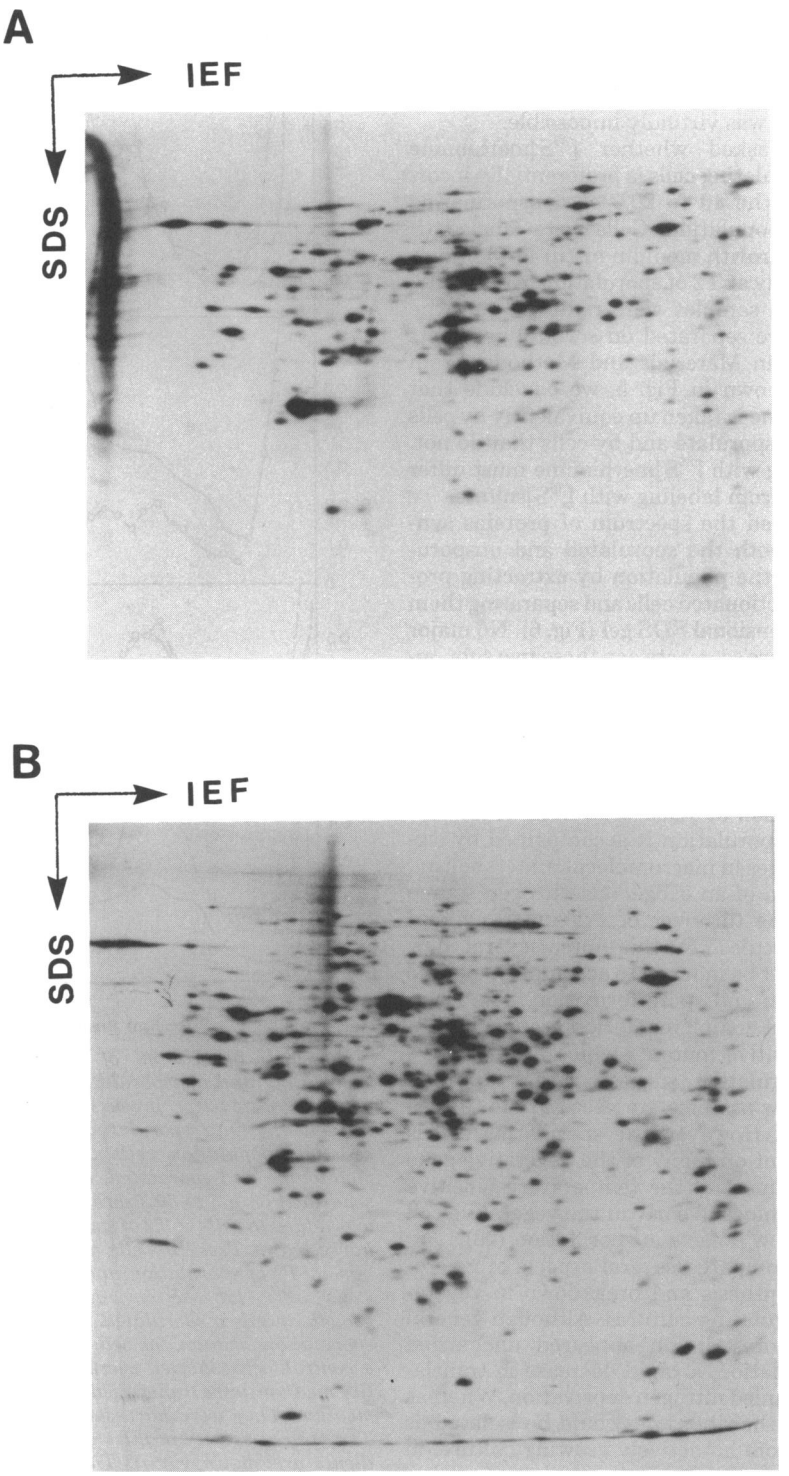

FIG. 4. *Labeling of sporulation proteins. Cells were labeled 4 h into sporulation with [^{35}S]methionine for 5 min (A) or 2 h (B). The extracted protein was fractionated on two-dimensional O'Farrell gels. The gels were fluorographed.*

during sporulation revealed many differences (data not shown). The proteins labeled were significantly different from those seen with the other procedures, and direct spot comparison for most proteins was virtually impossible.

We then asked whether [^{35}S]methionine added to sporulating cells is preferentially incorporated into the 10 to 20% of nonsporulating cells in the population. Cells were labeled (i) overnight in growth medium or (ii) for 5 min or (iii) indefinitely at T2 of sporulation. At T4, T12, T24 and T48, samples were removed, and the cell types were separated on sorbitol gradients as described in Materials and Methods. From the results shown in Fig. 5, we conclude that [^{35}S]methionine is taken up equivalently by cells that actually sporulate and by cells that do not. Thus, labeling with [^{35}S]methionine must differ in some way from labeling with [^{35}S]sulfate.

We examined the spectrum of proteins synthesized by both the sporulated and unsporulated cells in the population by extracting proteins from fractionated cells and separating them on a one-dimensional SDS gel (Fig. 6). No major differences were seen between these two subpopulations, suggesting that even cells that fail to complete sporulation carry out many of the same biosynthetic processes.

DISCUSSION

The transition of yeast cells from vegetative growth into sporulation is accompanied by significant changes in macromolecular metabolism. Cells deprived of an exogenous nitrogen source depend on the turnover of existing RNA and protein molecules. Furthermore, several new functions must be induced to accomplish meiotic recombination and spore formation. The isolation of several complementation groups of temperature-sensitive mutants which are defective only in sporulation suggested that approximately 50 new proteins are necessary for differentiation (4). However, the sporulating cell is also dependent on many of the vegetative gene products, as most of the temperature-sensitive *cdc* mutants blocked early in the vegetative cell cycle also show defects in sporulation (26).

We have compared several aspects of macromolecular synthesis and breakdown in vegetative and sporulating cultures. Although general aspects of transcription appeared unchanged during sporulation, a rapid decrease in translation accompanied nitrogen deprivation. Whereas 75 to 80% of the ribosomes could be isolated as polysomes from an actively growing culture, in sporulation most of the ribosomes were found as monosomes. We have shown that the loss of polysomes is not limited to strains genetically capable of completing spore formation, as aspo-

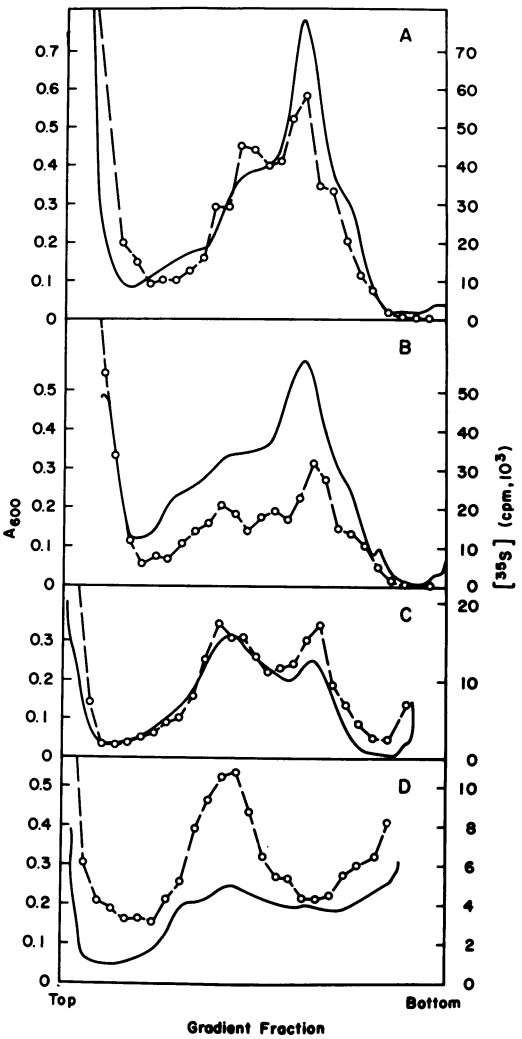

FIG. 5. *Incorporation of [^{35}S]methionine into sporulating and nonsporulating cells. To determine whether radioactive precursors were incorporated equivalently into sporulating and nonsporulating cells in a population, cells were labeled with [^{35}S]-methionine and sporulated, and the cell types were separated on a 5 to 50% sorbitol gradient. (A) Label was added to cells at T2 of sporulation; the cells were harvested at T24. (B) Cells were pulse-labeled for 5 min at T2 of sporulation and harvested at T24. (C) Cells were labeled overnight during vegetative growth and then sporulated. After 24 h in unlabeled sporulation medium, the cells were harvested. (D) As a control, cells, labeled overnight in defined vegetative medium, were transferred to unlabeled vegetative medium. They were harvested at stationary phase. The optical density profiles of the fractionated gradients are shown (——). The large peak in (A) is primarily four-spored asci; the peak in (D) is composed of nonsporulating, stationary-phase cells. The radioactivity in each fraction was determined, and the distribution of the counts is indicated* (○).

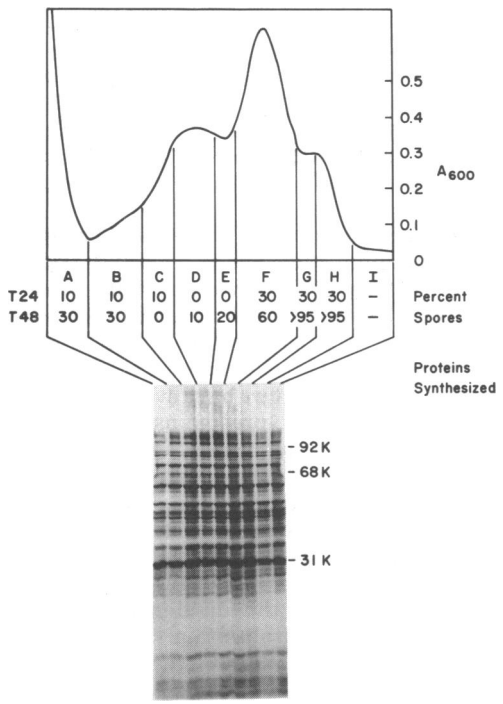

FIG. 6. *A comparison of proteins synthesized by sporulating and nonsporulating cells in the population. Cells were pulse-labeled for 5 min with [^{35}S]-methionine at T4 of sporulation. They were harvested at T24 and fractionated on a sorbitol gradient to separate asci from nonsporulated cells. The radiolabeled protein was extracted from the pooled fractions and separated by electrophoresis through an 11% polyacrylamide gel. The resulting autoradiograph is shown. The percentage of asci was determined microscopically for the corresponding fractions at T24 and for a similar gradient at T48.*

rogenous *MATα/MATα* cells placed in sporulation medium also became enriched in monosomes. Furthermore, yeast cells in late stationary phase of growth showed the same shift in the polysome profile, though they failed to sporulate. The ribosomes could be reincorporated into polysomes by adding nutrients to the media.

If a shift in ribosomes is a general response to starvation, there are several levels at which it might be mediated. First, there could be a limited amount of functional mRNA due to decreased synthesis, increased degradation, or inactivation. Second, some aspect of translation itself, such as initiation, could become rate limiting due to the loss or inactivation of a necessary factor. Several of the experiments described in this paper address this question.

First, the proportion of poly(A)-containing RNA was unchanged during sporulation, so a limitation in the amount of structural mRNA is not likely. However, the ability to translate this RNA in a foreign in vitro system did change during sporulation. RNA extracted from cells at T4 was 30% less efficient at stimulating in vitro translation than vegetative RNA. The apparent decrease in functional mRNA could be an artifact of extraction due to the increased nucleolytic activities during sporulation. However, if degraded mRNA was used to stimulate the cell-free translation, there was an increase in low-molecular-weight products (data not shown). This was not observed in the translation of sporulation mRNA, so massive degradation is unlikely. We have further independent evidence that specific mRNA's are less abundant during sporulation. By fractionating RNA, transferring it to diazobenzoxymethyl paper, and hybridizing to specific DNA probes, we have demonstrated a relative decrease in the mRNA homologous to *MATa*, *MATα*, and *URA3* during sporulation (manuscript in preparation). This finding, though consistent with a decrease in the proportion of functional mRNA during sporulation, is limited in scope, and the amount of mRNA for other genes may be unaltered. The decrease in translatable mRNA could account for only a portion of the loss of polysomes. The decrease in functional mRNA was gradual and continued at least through T8, whereas the loss of polysomes was rapid and complete within 120 min of the initiation of starvation. There was no difference in the average half-life of poly(A) RNA from vegetative and sporulating cells. The 20-min turnover time agrees with previous estimates for cells growing under various conditions (3, 14, 27). However, it must be remembered that we have examined the metabolism of the average mRNA and that specific half-lives may vary greatly (17). Though the sporulating culture fails to divide, we have assumed a doubling time of 720 min to reflect the slow increase in the cells (5). We selected 12 h, as it is the earliest time at which ascus formation is observed. However, a generation time of infinity results in a calculated half-life of 20 min. Therefore, with a short half-life and a relatively long doubling time, the measurement is valid even for a culture that fails to divide. We can conclude that there is no change in the general turnover of mRNA during starvation. Since there is less poly(A) on sporulating mRNA (Saunders, thesis) and the half-life is unchanged, the mRNA longevity is not dependent solely on poly(A) length. Furthermore, the mRNA half-life is unaffected by the increased RNase activity in sporulating cells.

The loss of polysomes in general may be caused either by decreased mRNA availability or by the loss of a factor necessary for initiation of protein synthesis, or both. However, the shift

in ribosomes differs in these two cases and may be used diagnostically (25). For example, a limitation in the mRNA supply induces the gradual loss of polyribosomes, as is observed in strain ts136, carrying the *rna1* mutation defective in RNA processing or transport (25). On the other hand, defective initiation, as seen in the mutant strain ts187, causes a rapid shift in ribosomes, analogous to the one observed during sporulation (25).

The drug cycloheximide, which reduces the rate of elongation, has been shown to have no effect on the proportion of polyribosomes when mRNA was limiting (10). However, in cells with lowered levels of initiation, the proportion of polysomes can be increased by cycloheximide. The drug apparently stabilizes those polysomes which are present (10) and also recruits monosomic ribosomes back into polysomes (6). Whereas no change in the distribution of polysomes was observed when cycloheximide was added to a vegetative culture, there was a significant effect in polysomes from sporulating cells. Both our results and those of Mills (22) show that the addition of cycloheximide to the culture before harvesting allows recovery of about 60% of the ribosomes in polysomes, as opposed to about 25% polysomes without drug addition. We suggest that the nitrogen starvation conditions of sporulation result in a decreased rate of initiation of translation which can be reversed by lowering the elongation rate with cycloheximide. Therefore, the observed sporulation ribosome profile is consistent with decreased initiation, but not with a severe mRNA limitation.

Recent measurements of the percentage of active ribosomes during sporulation suggested that many of the monosomic ribosomes may still be engaging in active translation (7). Although these studies were complicated by the presence of cycloheximide, they offer the intriguing possibility that a class of mRNA molecules specific for some process in sporulation may be selectively translated at a different rate on monosomes. We have translated mRNA extracted from monosomes and polysomes in vitro. A one-dimensional gel analysis of the most abundant protein products reveals no obvious mRNA sequestering or protection during sporulation (data not shown).

In slime molds, the percentage of polysomes decreases drastically upon initiation of differentiation and then increases slightly (1). Alton and Lodish (1) have proposed that the initial decline in polysomes after induction of *Dictyostelium* differentiation is caused, at least in part, by the masking of a fraction of the mRNA population. They have identified five mRNA species whose presence and functionality have been demonstrated in an in vitro translating system, but whose protein products are not synthesized in vivo. In slime molds, 25% of the synthesized proteins detected on two-dimensional gels change during differentiation. In yeast, we observe changes in fewer than 5% of the abundant proteins.

Changes in specific proteins. The two-dimensional protein gel analysis resolves approximately 400 spots. However, previous measurements of the yeast mRNA complexity suggested the presence of 4,000 different poly(A)-containing sequences (11). Therefore, the changes in protein synthesis that we have evaluated encompass only the most predominant 10% of the synthesized proteins. Specific alterations in the high-complexity/low-abundance mRNA class would not have been detected. The shift in ribosomes is therefore not reflected in the loss of a specific major class of proteins, although many changes in minor species might occur. We are currently evaluating the functional mRNA populations by in vitro translation.

Though the gel system used here fails to resolve basic proteins, other studies from this laboratory (23a) have demonstrated a fivefold decrease in the synthesis of ribosomal proteins relative to total protein synthesis during sporulation. However, loss of this specific class of smaller mRNA molecules would be reflected in the loss of predominantly small polysomes (19) and cannot itself account for the drastic decrease in polysomes observed during sporulation.

To insure that we were examining synthesis rather than processing or degradation of proteins, we labeled cells with [^{35}S]methionine for 5 min. Other studies, designed to examine protein accumulation by long-term labeling (24, 28), discovered similar classes of protein changes; once again all of the differences were also found in *MAT*α/*MAT*α cells. We have also compared short-term pulse-labeling, in which the spots represent primarily initial products of translation, with long-term labeling, where one also sees processing and differences in degradation. It is possible to see spots unique to each labeling protocol. Since we were interested in studying the translation process in a culture with depressed polysomes, we concluded that the most accurate analysis would be one with 5-min pulse labeling. We were therefore studying the predominant translation products as they were synthesized.

Previously, Wright and Dawes (32) reported that the 10 to 30% of the cells in the sporulating population which fail to form asci incorporate most of the [^{35}S]sulfate. They argued that labeling during sporulation fails to reveal changes in

that portion of the population undergoing differentiation. This was not the case when our strains were labeled with [^{35}S]methionine in sporulation medium buffered to pH 5.5.

[^{35}S]methionine is incorporated equivalently into sporulating and nonsporulating cells, and proteins synthesized by both types of cell were identical. Thus, there is no advantage to labeling during pregrowth. Possibly labeling with methionine is categorically different from labeling with sulfate during sporulation. In fact, we have found another example of such a difference. Sporulating cells incorporate adenine, but fail to take up uracil, whereas vegetative cultures can be labeled efficiently with either precursor (Kraig and Haber, unpublished data).

Neither our investigation with pulse-labeling nor other studies using long-term labeling (24, 28) during sporulation have detected sporulation (*MAT*a/*MAT*α)-specific proteins. Wright and Dawes (32) detected several *MAT*a/*MAT*α sporulation-specific spots. We suspect that this may have been due to differences in labeling, their use of galactose for pregrowth, or strain differences. Other studies (24, 28), using labeling during sporulation for various time periods, have failed to detect such sporulation (*MAT*a/*MAT*α)-specific proteins.

We are currently analyzing changes in protein synthesis in strains defective for sporulation. These studies, coupled with in vitro translation of sporulation mRNA, should produce further insight into the role of certain proteins in differentiation as well as the level of their regulation.

ACKNOWLEDGMENTS

We gratefully acknowledge Anita Hopper for the strains as well as helpful suggestions. We also thank James E. Hopper for providing the wheat germ, and we are grateful for his advice in the assembly of our cell-free translation system. We also thank Nancy Pearson and Michael Rosbash for numerous discussions and Chan Fulton, Lucy Rowe, David Rogers, John McCusker, Susan Raposa, Anne Comeau, and Barbara Weiffenbach for their comments on this paper.

E.K. was supported by Public Health Service training grant GM-7122 in Genetic and Biochemical Mechanisms of Regulation from the National Institutes of Health. This work was supported by Public Health Service grant GM-20056 and Biomedical Research Support Grant 6611 from the National Institutes of Health.

LITERATURE CITED

1. **Alton, T. H., and H. F. Lodish.** 1977. Translational control of protein synthesis during the early stages of differentiation of the cellular slime mold *Dictyostelium discoideum*. Cell **12**:301–310.
2. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
3. **Cannon, M., J. E. Davies, and A. Jimenez.** 1973. Inhibition by lomofungin of nucleic acid and protein synthesis in *Saccharomyces cerevisiae*. FEBS Lett. **32**: 277–280.
4. **Esposito, M. S., and R. E. Esposito.** 1974. Genes controlling meiosis and spore formation in yeast. Genetics **78**:215–225.
5. **Esposito, M. S., R. E. Esposito, M. Arnaud, and H. O. Halvorson.** 1969. Acetate utilization and macromolecular synthesis during sporulation of yeast. J. Bacteriol. **100**:180–186.
6. **Fan, H., and S. Penman.** 1970. Regulation of protein synthesis in mammalian cells. II. Inhibition of protein synthesis at the level of initiation during mitosis. J. Mol. Biol. **50**:655–670.
7. **Frank, K. R., and D. Mills.** 1978. Ribosome activity and degradation in meiotic cells of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **160**:59–65.
8. **Greenberg, J. R.** 1972. High stability of messenger RNA in growing cultured cells. Nature (London) **240**:102–104.
9. **Haber, J. E., P. J. Wejksnora, D. D. Wygal, and E. Y. Lai.** 1977. Controls of sporulation in *Saccharomyces cerevisiae*, p. 129–154. *In* D. H. O'Day and P. A. Horgen (ed.), Eukaryotic microbes as model developmental systems: microbiology series, vol. 2. Marcel Dekker, Inc., New York.
10. **Hartwell, L. H., H. T. Hutchison, T. M. Holland, and C. S. McLaughlin.** 1970. The effect of cycloheximide upon ribosome stability in two yeast mutants defective respectively in the initiation of polypeptide chains and in messenger RNA synthesis. Mol. Gen. Genet. **106**: 347–361.
11. **Hereford, L. M., and M. Rosbash.** 1977. Number and distribution of polyadenylated RNA sequences in yeast. Cell **20**:453–462.
12. **Hopper, A. K., P. T. Magee, S. K. Welch, M. Friedman, and B. D. Hall.** 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:619–628.
13. **Hopper, J. E., K. A. Bostian, L. B. Rowe, and D. J. Tipper.** 1977. Translation of the L-species dsRNA genome of the killer-associated virus-like particles of *Saccharomyces cerevisiae*. J. Biol. Chem. **252**:9010–9017.
14. **Hynes, N. E., and S. L. Phillips.** 1976. Turnover of polyadenylate-containing ribonucleic acid in *Saccharomyces cerevisiae*. J. Bacteriol. **125**:595–600.
15. **Jacobson, A., and H. F. Lodish.** 1975. Genetic control of development of the cellular slime mold *Dictyostelium discoideum*. Annu. Rev. Genet. **9**:145–185.
16. **Klar, A. J. S., and H. O. Halvorson.** 1975. Proteinase activities of *Saccharomyces cerevisiae* during sporulation. J. Bacteriol. **124**:863–869.
17. **Lawther, R. P., and T. G. Cooper.** 1975. Kinetics of induced and repressed enzyme synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **121**:1064–1073.
18. **Magee, P. T., and A. K. Hopper.** 1974. Protein synthesis in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:952–960.
19. **Mager, W. H., and R. J. Planta.** 1976. Yeast ribosomal proteins are synthesized on small polysomes. Eur. J. Biochem. **62**:193–197.
20. **McCusker, J. H., and J. E. Haber.** 1977. Efficient sporulation of yeast in media buffered near pH 6. J. Bacteriol. **132**:180–185.
21. **Mills, D.** 1972. Effect of pH on adenine and amino acid uptake during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **112**:519–526.
22. **Mills, D.** 1974. Isolation of polyribosomes from yeast during sporulation and vegetative growth. Appl. Microbiol. **27**:944–948.
23. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.

23a. **Pearson, N. J., and J. E. Haber.** 1980. Changes in regulation of ribosomal protein synthesis during vegetative growth and sporulation of *Saccharomyces cerevisiae*. J. Bacteriol. **143**:1411–1419.

24. **Petersen, J. G. L., M. C. Kielland-Brandt, and T. Nillson-Tillgren.** 1979. Protein patterns of yeast during sporulation. Carlsberg Res. Commun. **44**:149–162.
25. **Peterson, N. S., and C. S. McLaughlin.** 1974. Polysome metabolism in protein synthesis mutants of yeast. Mol. Gen. Genet. **129**:201–215.
26. **Shilo, V., G. Simchen, and B. Shilo.** 1978. Initiation of meiosis in cell cycle initiation mutants of *Saccharomyces cerevisiae*. Exp. Cell. Res. **112**:241–248.
27. **Tonnesen, T., and J. D. Friesen.** 1973. Inhibitors of ribonucleic acid synthesis in *Saccharomyces cerevisiae*: decay rate of messenger ribonucleic acid. J. Bacteriol. **115**:889–896.
28. **Trew, B. J., J. D. Friesen, and P. B. Moens.** 1979. Two-dimensional protein patterns during growth and sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **138**: 60–69.
29. **Tsuboi, M.** 1976. Correlation among turnover of nucleic acids, ribonuclease activity and sporulation ability of *Saccharomyces cerevisiae*. Arch. Microbiol. **111**:13–19.
30. **Walsh, C., and C. Fulton.** 1973. Transcription during cell differentiation in *Naegleria gruberi*. Preferential synthesis of messenger RNA. Biochim. Biophys. Acta **312**:52–71.
31. **Wejksnora, P. J., and J. E. Haber.** 1978. Ribonucleoprotein particle appearing during sporulation in yeast. J. Bacteriol. **134**:246–260.
32. **Wright, J. F., and I. W. Dawes.** 1979. Sporulation-specific protein changes in yeast. FEBS Lett. **104**:183–186.
33. **Zitomer, R. S., D. L. Montgomery, D. L. Nichols, and B. D. Hall.** 1979. Transcriptional regulation of the yeast cytochrome C gene. Proc. Natl. Acad. Sci. U.S.A. **76**: 3627–3631.
34. **Zubenko, G. S., A. P. Mitchell, and E. W. Jones.** 1979. Septum formation, cell division and sporulation in mutants of yeast deficient in proteinase B. Proc. Natl. Acad. Sci. U.S.A. **76**:2395–2399.