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Influence of pH on the Rate of Ribosomal Ribonucleic Acid Synthesis During Sporulation in *Saccharomyces cerevisiae*

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The rate of synthesis of ribosomal RNA (rRNA) is much slower during sporulation than during vegetative growth of yeast. If sporulating cells are transferred from normal incubation conditions at pH 8.8 to the same medium adjusted to pH 7.0, the rate of rRNA synthesis increases to approach that observed in vegetative cells. The response to the pH change is quite rapid, occurring within 10 min. The pH-dependent, rate-limiting step appears to be in the processing of 35*S* ribosomal precursor RNA to the final 26*S* and 18*S* RNA species. A similar pH effect also was found for the rate of protein synthesis. However, no change in respiration was observed when the pH was lowered. These results indicate that the observed differences in rate of rRNA synthesis in vegetative and sporulating cells are a consequence of pH and are not intrinsic to sporulation. The results also support the correlation between rRNA processing and protein synthesis.

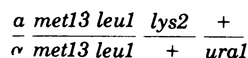
Sporulation of *Saccharomyces cerevisiae* occurs under conditions that are distinctly different from those that support vegetative growth. Unlike vegetative growth, sporulation is an obligately aerobic event that is triggered by the absence of nitrogen from the medium (3, 5, 9). During sporulation, the pH of the medium rises in the first hours from pH 7 to approximately pH 9. Vegetative cells grow very slowly above pH 7. Conversely, it has been shown that cells are completely inhibited from sporulating when maintained in sporulation medium buffered with phosphate at pH 6 (5, 9). (Recent experiments indicate that cells can in fact be sporulated in media buffered at pH 6 if buffers other than phosphate are employed [Haber and McCusker, manuscript in preparation].) The entire sporulation process is relatively slow; ascospore formation takes 24 h compared with a vegetative doubling time of 2 to 3 h. The rate of synthesis of macromolecules also appears to be considerably slower. In particular, the formation of 26*S* and 18*S* ribosomal ribonucleic acid (rRNA) from a common 35*S* precursor RNA occurs within 5 to 8 min during vegetative growth (13), but requires at least 20 to 30 min during sporulation (2).

During sporulation, uptake and incorporation of radioactive precursors into RNA is affected by a change in pH. At pH 9, the uptake and incorporation of radioactive adenine into nucleic acids is extremely inefficient. If, however, sporulating cells are transferred from pH 9 to sporulation medium at pH 6, uptake and incorporation are immediately enhanced (9). This method permits efficient labeling of RNA in sporulating cells for as long as 1 h without affecting ascospore formation when cells are returned to pH 9 medium (9).

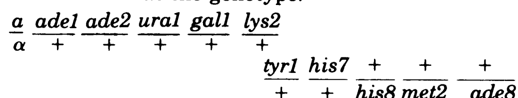
Changing the pH of sporulating cultures may affect other processes besides uptake. For example, Curiale and Mills (4) have noted differences in the RNA species labeled in the polysome fraction of low pH-labeled cells. We have found that lowering the pH of sporulating cultures affects not only the specific activity of newly synthesized rRNA, as Mills (9) first observed, but also increases the rate of appearance of stable rRNA species and the rate of protein synthesis. Respiration, as measured by the uptake of O_2, was not affected by lowering the pH of the media.

MATERIALS AND METHODS

Strains. Two diploid strains of *S. cerevisiae* were used in this study. Strain J1 (10) is methionine and leucine requiring and has the genotype:



Strain J659 has the genotype:



Parent haploid strains were from the Yeast Stock Center at Berkeley, Calif.

Cultivation. Strains were grown in liquid YEPD

medium (1% yeast extract, 2% peptone [Difco], and 2% dextrose) at 30 C.

Sporulation. Cells were grown to stationary phase (approximately 2.2 × 10^8 cells/ml) in YEPD medium. The cells were harvested, washed twice in distilled water, and suspended at a density of approximately 2 × 10^7 cells/ml in 1% potassium acetate, pH 7. Cells were aerated on a gyratory shaker at 30 C in Erlenmeyer flasks with a volume capacity 10 times larger than the volume of sporulation medium used. Sporulation was monitored by counting the percentage of asci.

Isolation of RNA. Cells, harvested by centrifugation, were broken in a French press by the method described by Bhargava and Halvorson (1), and RNA was extracted with cold, water-saturated phenol as described by Wejksnora and Haber (14).

Polyacrylamide gel electrophoresis. rRNA species were resolved by the electrophoresis of RNA on 3% polyacrylamide gels (9 mm diameter) subjected to 8 mA per gel for 12 h according to the method of Loening (8). For analysis, the gels were sliced into 1-mm sections on a Joyce Loebl gel slicer.

For gels that contained ^{32}P and ^{14}C, the gel slices were dried on filter paper strips, and the radioactivity was determined in a Beckman LS 250 liquid scintillation counter using PPO-POPOP toluene solution {4.0 g of 2,5-diphenyloxazole per liter and 0.5 g of 1,4-bis-[2]-(5-phenyloxazolyl)benzene per liter, dissolved in toluene}.

Gels containing tritium were analyzed by placing each slice in a disposable 3-dram (ca. 5-g) glass vial to which was added 1 ml of a solution containing 58.8% Protosol (New England Nuclear Corp.), 39% toluene, and 2.5% water (J. Warner, personal communication). The vials were covered and gently agitated on a platform mounted to a rotary water bath for 6 h. The soluble radioactivity was then determined in a liquid scintillation counter after the addition of 8.5 ml of PPO-POPOP toluene solution.

Measurement of uptake and incorporation of radioisotopes. To measure uptake of [^{3}H]adenine into whole cells, cells were filtered on nitrocellulose filters 25 mm in diameter, washed twice with 20 ml of cold water containing 20 μg of adenine per ml, and then washed twice with cold 95% ethanol containing 20 μg of adenine per ml. To measure the incorporation of isotope into RNA, samples of cells were mixed with equal volumes of 10% trichloroacetic acid and chilled on ice for 30 min (9). The solutions were filtered on nitrocellulose filters washed twice with 10 ml of cold 5% trichloroacetic acid containing 20 μg of adenine per ml and then washed with 10 ml of cold 95% ethanol. Filters were dried under a heat lamp and counted in 5 ml of PPO-POPOP solution.

To measure the incorporation of isotope into protein, samples of cells were mixed with equal volumes of 10% trichloroacetic acid and boiled for 20 min. The solutions were filtered on glass-fiber filters (GFC), washed twice with 10 ml of cold 5% trichloroacetic acid containing 20 μg of leucine per ml, and washed with 10 ml of cold ethanol. Filters were dried under a heat lamp and counted in 5 ml of PPO-POPOP solution.

Measurement of respiration. Oxygen consumption was measured using an oxygen probe mounted in a 3-ml closed chamber kept at a constant temperature of 30 C. Cells were grown to stationary phase and sporulated as described above. Samples were either taken directly from the flask and placed in the oxygen probe chamber or were centrifuged and resuspended in media adjusted to the desired pH. The sample in the chamber was agitated with a small magnetic stirring bar. Oxygen consumption was monitored until the oxygen level reached zero, as had been determined previously by the addition of sodium dithionite.

Adjustment of sporulation media pH. Sporulation medium of pH 7.0, into which cells were resuspended, was prepared by sporulating cells for about 6 h in 1% KAc and then removing the cells by two centrifugations and adjusting the pH to 7.0 by the addition of HCl. Equivalent sporulation medium at pH 8.8 was prepared as above but the pH was not adjusted, allowing it to remain at pH 8.8.

Radioisotopes. [8-^{14}C]adenine (6.53 mCi/mmol), [2-^{3}H]adenine (8.06 Ci/mmol, L-[4-5-^{3}H]leucine (5 Ci/mmol), and carrier-free [^{32}P]phosphoric acid were obtained from New England Nuclear Corp.

Materials. Acrylamide, bisacrylamide, and TEMED (*N,N,N',N'*-tetramethylethylenediamine) were purchased from Eastman Kodak; L-methionine, L-leucine, and 8-hydroxyquinoline were obtained from Sigma.

RESULTS

rRNA synthesis. A pH-dependent difference in the incorporation of radioactivity into newly synthesized RNA might occur either because of a change in the uptake of radioactive precursors or because of a change in the kinetics of RNA synthesis. To differentiate between enhanced equilibration of the label with a biosynthetic pool and an effect on the actual rate of RNA synthesis, we compared the rRNA species synthesized during 20 min at pH 7.0 with those synthesized at pH 8.8 and also followed the pH-dependent processing of labeled precursor rRNA. Cells of strain J1 were grown to stationary phase in YEPD medium containing 0.4 μCi of [^{14}C]adenine per ml, washed, and resuspended in sporulation medium. After 6.5 h, the cells were removed by centrifugation, divided, and resuspended in sporulation medium containing [^{3}H]adenine at pH 7.0 or 8.8. To minimize the difference in total adenine incorporation resulting from the effect of pH on uptake (9), the pH 8.8 medium contained 35 μCi of [^{3}H]adenine per ml, whereas the pH 7.0 medium contained 3 μCi of [^{3}H]adenine per ml. The cells were labeled for 20 min, after which RNA was extracted and examined electrophoretically.

There was a substantial difference in the patterns of ^{3}H-labeled rRNA under the two incubation conditions (Fig. 1). Most of the ^{3}H-labeled rRNA from cells incubated at pH 8.8 appeared as 35*S* rRNA precursor. Small amounts of 27*S*

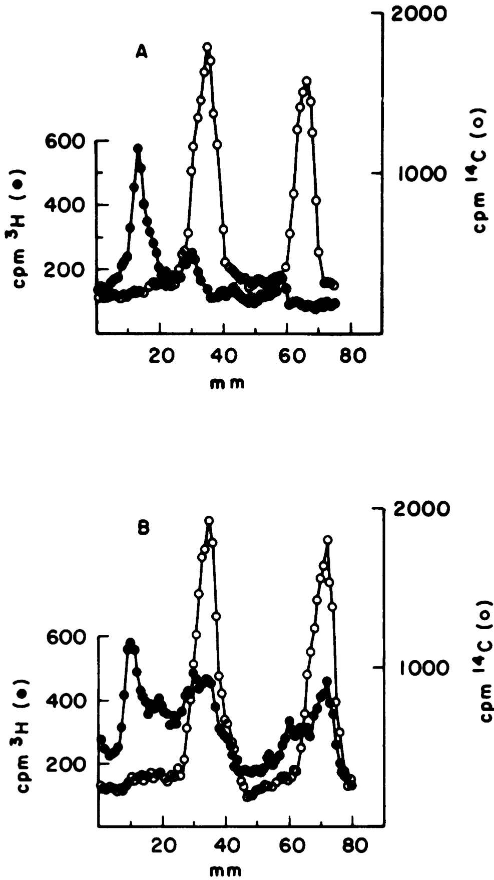


FIG. 1. *Synthesis of rRNA labeled for 20 min in sporulation media at pH 7.0 and pH 8.8. Cells of strain J1 were grown to stationary phase in the presence of 0.2 μCi of [¹⁴C]adenine per ml, washed, and resuspended in 1% potassium acetate, pH 7, containing 0.33 mM L-leucine and 0.13 mM L-methionine. After 6.5 h, the cells were removed by centrifugation and suspended in sporulation medium either at pH 8.8 (A) containing 35 μCi of [³H]adenine per ml, or at pH 7.0 (B) containing 3 μCi of [³H]adenine per ml. Cells were harvested 20 min later. The RNA was separated on 3% polyacrylamide gels. RNA synthesized during 20 min at pH 8.8 appears as labeled material only in the 35S, 27S, and 20S rRNA precursor regions. RNA labeled during 20 min at pH 7 appears as 35S, 27S and 20S precursor species as well as 18S and 26S rRNA.*

and 20S RNA, intermediate precursor forms of rRNA (13), were also present, but virtually no 26S or 18S rRNA was found (Fig. 1A). In contrast, approximately 40% of the RNA synthesized in 20 min at pH 7.0 was found in the 26S and 18S regions of the gel (Fig. 1B).

From these patterns, it was impossible to determine whether the rate of accumulation of 26S and 18S rRNA was limited by uptake or by an inherent difference in the rate of processing. To show that processing itself was influenced by pH, we sought to measure the effect of pH on the subsequent processing of 35S precursor rRNA that labeled before a change in pH. Cells were first labeled for 20 min at pH 8.8 to label 35S precursor rRNA (cf. Fig. 1A). When these cells were transferred to sporulation medium containing unlabeled adenine to prevent further incorporation of isotope into cells, the uptake of label into whole cells ceased abruptly at either pH (Fig. 2). The incorporation of labeled adenine into RNA leveled off within 10 min after resuspension (Fig. 3), and total accumulation of radioactivity was nearly identical for the two pH conditions. The amount of unlabeled adenine that was added in this experiment is sufficiently large to overcome the pH-dependent differences in the permeability of sporulating cells to adenine. Under these conditions, the expandable pools of cells at both pH 7.0 and 8.5 were flooded to the same extent (data not shown). In this experiment, then, any differences in processing of ribosomal precursor RNA cannot be attributed to differences in the total amount of unlabeled adenine taken up by sporulating cells at two different pH conditions.

The labeled RNA species present after 20 min of incubation in the presence of excess unla-

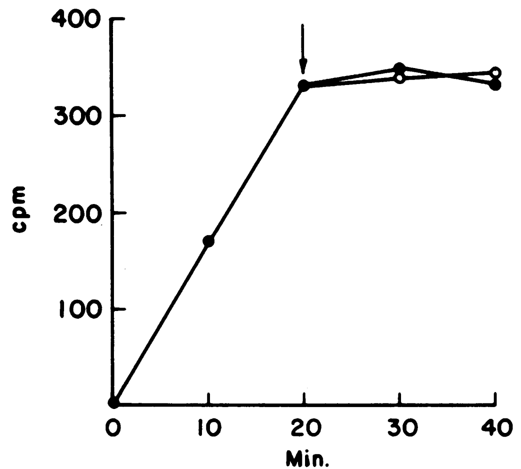


FIG. 2. *Uptake of label into sporulating cells. Sporulating cells of strain J1 were labeled at 6.5 h in pH 8.8 sporulation medium with 35 μCi of [³H]adenine per ml. Duplicate 0.1-ml aliquots were removed at 10-min intervals and washed in cold water as described in Materials and Methods. After 20 min (arrow) the cells were harvested, washed, and suspended in sporulation media at either pH 8.8 (●) or pH 7.0 (○) containing 200 μg of adenine per ml for another 20 min. The values shown are the average of two duplicate points that have been corrected for background radioactivity counts.*

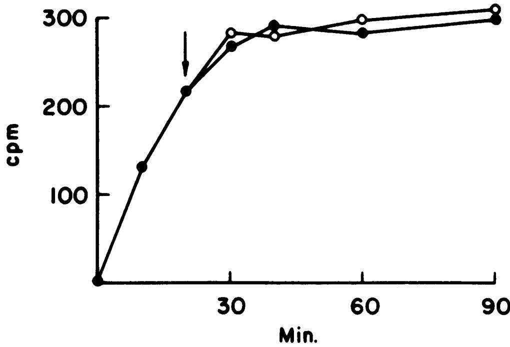


FIG. 3. *Incorporation of [^{3}H]adenine into RNA. Sporulating cells were labeled with [^{3}H]adenine as described in Fig. 2. Duplicate samples were removed and mixed with an equal volume of cold 10% trichloroacetic acid, and the incorporation of radioactivity into RNA was determined as described in Materials and Methods. The points shown are the average of duplicate assays, corrected for background radioactivity. Symbol: (○) cells transferred from pH 8.8 to pH 7.*

beled adenine are shown in Fig. 4. In cells incubated in the absence of exogenous label for 20 min at pH 8.8, processing of the 35S precursor RNA was not yet complete. Approximately 35% of the tritium was still in the 35S region of the gel (Fig. 4A). In cells incubated at pH 7.0, virtually all of the labeled material was found as mature 26S and 18S rRNA (Fig. 4B). Since the total amount of newly synthesized RNA relative to RNA labeled with ^{14}C during vegetative growth was virtually identical at the two different pH conditions, the 35S precursor rRNA still found in cells maintained at high pH did not result from continued incorporation of label. This labeled 35S RNA was slowly but completely processed to rRNA in the pH 8.8 cells that were incubated for 2 h.

This pH-dependent effect on the rate of mature rRNA production is not a consequence of labeling with adenine. If sporulating cells were incubated in the presence of ^{32}P for 20 min at different pH values, virtually all of the RNA in cells incubated at pH 8.8 was found as precursors, whereas at pH 7.0 most of the label appeared as 26S and 18S rRNA (Fig. 5). These results were similar to those obtained using adenine as label. Similar results also were obtained using strain J659 (data not shown).

Protein synthesis. Since continued protein synthesis has been shown to be essential to rRNA processing (14), we wished to see if the increased rate of rRNA processing observed at the lowered pH was correlated with a simultaneous increase in the rate of protein synthesis. Because the uptake of amino acids is enhanced when the cells are exposed to lowered pH, just as is the uptake of adenine (9), this difference in rate of uptake could obscure any difference in actual protein synthetic rate. However, it is possible to prelabel cells in high pH media,

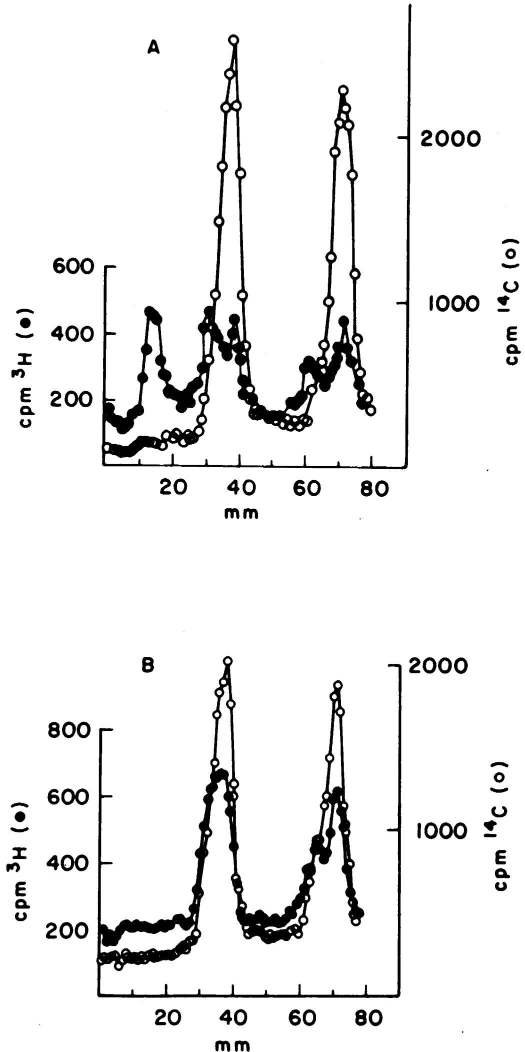


FIG. 4. *Processing of prelabeled RNA in 20 min at pH 8.8 and 7.0. Sporulating cells that had been labeled for 20 min in sporulation medium containing 35 μCi of [^{3}H]adenine per ml at pH 8.8 (see Fig. 1A) were harvested and resuspended in sporulation media at either pH 8.8 or 7.0 containing 200 μg of adenine/ml. After 20 min of incubation the cells were harvested, and the purified RNA was separated on 3% polyacrylamide gels. For cells remaining at pH 8.8 (Fig. 4A), a considerable amount of labeled RNA is found in the 35S, 27S, and 20S precursor regions of the gel. In contrast, the tritium-labeled RNA found in cells transferred to pH 7.0 is nearly all mature 26S and 18S rRNA species (Fig. 4B).*

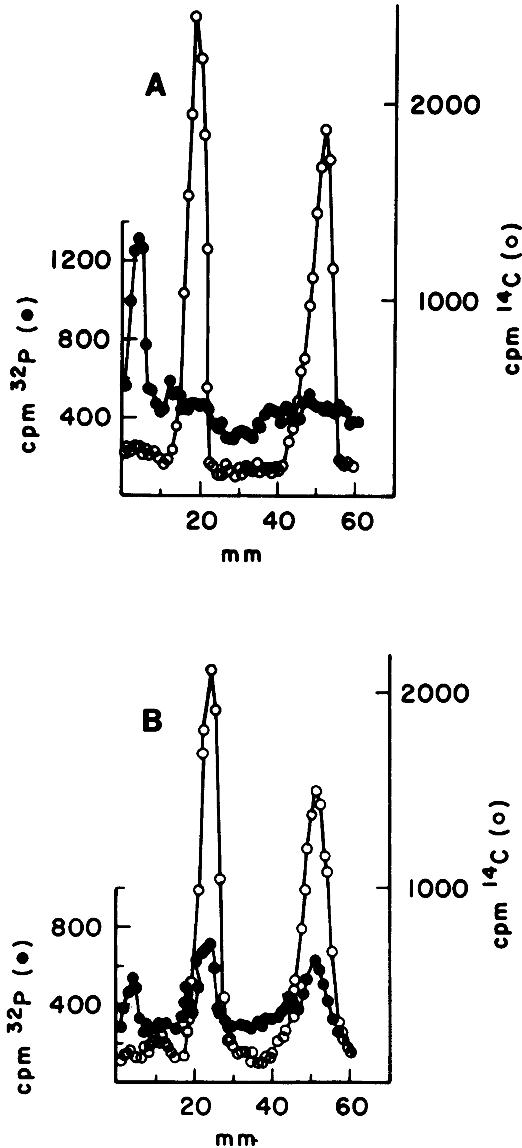


FIG. 5. *Synthesis of RNA labeled with* ^{32}P *at pH 7.0 and 8.8. Cells of strain J1 were grown to stationary phase in the presence of 0.2 μCi of [*^{14}C*]adenine per ml, washed and suspended in 1% potassium acetate, pH 7, containing 0.33 mM L-leucine and 0.13 mM L-methionine. After 6.5 h, the cells were harvested and resuspended in sporulating medium either at pH 8.8 (A) containing 15 μCi of* $H_3{}^{32}PO_4$ *per ml, or at pH 7.0 (B) containing 3 μCi of* $H_3{}^{32}PO_4$ *per ml. Cells were harvested 20 min later, and the purified RNA was separated on 3% polyacrylamide gels.*

loading their expandable pools with radioactive amino acids (6), and then to wash the cells and resuspend them in non-radioactive media that have been adjusted to pH 7.0 or 8.8. In this way, the specific activity of labeled amino acids available within the cells for protein synthesis at either pH will be identical. The radioactive amino acids would then be utilized for protein synthesis until the expandable pool was exhausted, at which point incorporation of label into protein would cease rather abruptly (6). Although this method is unable to detect changes specifically in the rate of ribosomal protein synthesis, we assumed that an acceleration or slowing of general synthesis would reflect similar changes in the synthesis of ribosomal proteins.

To examine the effect of lowered pH on the rate of protein synthesis, cells of strain J659 were placed in sporulation medium. After 6 h, the cells were labeled for 20 min with 4.0 μCi of L-[^{14}C]leucine per ml. Incorporation of label into protein was linear (Fig. 6B). After 20 min, the cells were washed quickly and resuspended in nonradioactive medium either at pH 8.8 or 7.0 for another 18 min. Samples were removed at the time of resuspension and at 1- or 2-min intervals thereafter. Although the total radioactivity incorporated at both pH values after 18 min was virtually identical, the initial rate of incorporation at pH 7.0 was 1.5 times faster than that observed in cells resuspended at pH 8.8 (Fig. 6A). Thus, exposure to lowered pH

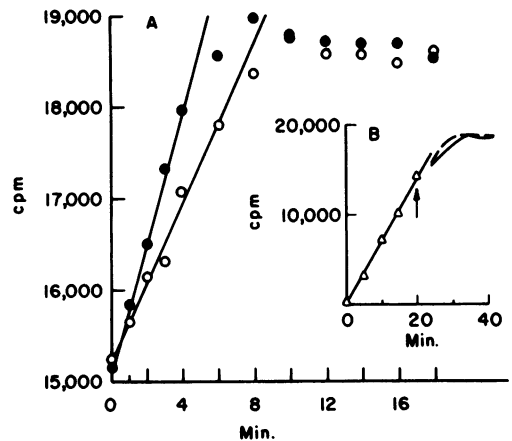


FIG. 6. *Incorporation of [*^{3}H*]leucine into protein. Sporulating cells at pH 8.8 were labeled with 4.0 μCi of [*^{3}H*]leucine per ml for 20 min at 6 h. Duplicate 0.5-ml samples were removed at 5-min intervals (insert B). After 20 min, the cells were centrifuged out of the medium, washed quickly with water and suspended either in medium at pH 7.0 (●) or pH 8.8 (○). Four 0.5-ml samples were removed at resuspension, and duplicate 0.5-ml samples were removed at 1- or 2-min intervals thereafter for 18 min. The points shown are averages of the duplicate or quadruple points that have been corrected for background radioactivity.*

medium after 6 h of sporulation results in an increase in the rate of protein synthesis.

Respiration. The possibility existed, however, that what we had observed was not a pH effect specific for protein synthesis and ribosomal processing, but rather a general increase in cellular metabolism resulting from lowered pH. As sporulating cells are unable to carry out fermentation, but rely on respiration exclusively, we measured the consumption of oxygen as a measure of metabolism. The oxygen consumption of sporulating cells was measured at 2, 6, and 7 h, at both pH 7 and 8.8 (Table 1).

Oxygen consumption after 2 h was very rapid, corresponding closely to results obtained by other workers (3, 7). However, for cells that had been sporulated 6 or 7 h and resuspended in sporulation medium at pH 7 and 8.8, the oxygen consumption was lower than for the cells measured at 2 h (again corresponding to previously published reports [3, 7]). There was no difference between samples adjusted to different pH values over a 20-min interval. This observation indicates that respiration is unaffected by a change in pH of the sporulation medium, and the observed phenomena of increased rates of rRNA processing and protein synthesis are not simply the results of higher respiration.

TABLE 1. *Oxygen consumption by sporulating cells at pH 7.0 and 8.8*[a]

Time in sporulation (h)	pH of medium	% O_2 consumed per min
2	7.9	33
6	8.8	18
6	7.0	18
7	8.8	15
7	7.0	15

[a] The effect of changing the pH of sporulation medium was measured at 6 and 7 h of sporulation. Cells sporulating at the normal, high pH were harvested and resuspended in either pH 7.0 or pH 8.8 medium, and the respiration rate was measured as described in Materials and Methods. The consumption of oxygen earlier in sporulation, after 2 h, is included for comparison.

DISCUSSION

Changing the pH of sporulation media has a rapid direct effect on the rates of synthesis of 26*S* and 18*S* rRNA and protein. The changes in rRNA processing and protein synthesis are clearly separable from pH-dependent effects on the uptake of radioactive precursors into the cell. Under conditions in which nearly identical amounts of label were incorporated into RNA at pH 7.0 or 8.8, very different ratios of mature rRNA to its precursors were found after labeling for a short interval. Further, when ribosomal precursor RNA is labeled at pH 8.8, its subsequent processing is greatly accelerated if the pH of the medium is lowered to pH 7.0. At the lower pH, the rate of 18*S* and 26*S* RNA appearance is quite similar to that observed in vegetative cells (13). Similarly, the rate of protein synthesis increases when cells are prelabeled with radioactive amino acids at pH 8.8 and exposed to pH 7.0 medium. Here again, when conditions are employed such that the rates of incorporation of labeled amino acids are not dependent on any difference in uptake, a clear change in the rate of biosynthesis is detected apart from any uptake effects. It does not seem likely that the increases in synthesis and processing rates we observed are part of an overall increase in cellular metabolism, as the rate of oxygen consumption is not increased by changing the pH of sporulation medium.

If rRNA processing depends on the availability of newly synthesized ribosomal proteins, as is suggested by the rapid block in rRNA processing in yeast when cycloheximide is added (13, 14), then an increase in ribosomal protein synthesis would result in an increased rate of processing of rRNA. Thus, in the experiment described above in which ribosomal precursor RNA was labeled before shifting the pH (cf. Fig. 4), approximately 35% of the labeled RNA remained as 35*S* precursors at pH 8.8, whereas virtually all was processed to 26*S* and 18*S* rRNA at pH 7.0. As shown in Fig. 6, the same shift in pH resulted in a 50% increase in protein synthesis. Assuming that this increase in general protein synthesis is also true for ribosomal proteins, the more complete processing of rRNA in a 20-min interval may be explained in terms of a regulation of processing by the increased availability of ribosomal proteins. However, we cannot rule out the possibility that the effects on protein synthesis are independent of those that control the assembly with protein or cleavage of precursor rRNA.

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LITERATURE CITED

1. **Bhargava, M. M., and H. O. Halvorson.** 1971. Isolation of nuclei from yeast. J. Cell Biol. 49:423–429.
2. **Chaffin, W. L., S. J. Sogin, and H. O. Halvorson.** 1974. Nature of ribonucleic acid synthesis during early sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. 120:872–879.
3. **Croes, A. F.** 1967. Induction of meiosis in yeast. I. The

timing of cytological and biochemical events. Planta 76:209-226.

4. **Curiale, M. S., and D. Mills.** 1975. Characterization of pulse-labeled ribonucleic acid in polysomes of sporulating yeast cells, p. 165-171. *In* P. Gerhardt, R. N. Costilow, and H. L. Sadoff (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
5. **Fowell, R. R.** 1969. Sporulation and hybridization of yeasts. *In* A. H. Rose and J. S. Harrison (ed.), The Yeasts, vol. I. Academic Press Inc., New York.
6. **Halvorson, H. O., and D. B. Cowie.** 1961. Metabolic pools of amino acids and protein synthesis in yeast. *In* Symposia CSAV. Membrane transport and metabolism. Publishing House of the Czechoslovak Academy of Sciences, Praha, Czechoslovakia.
7. **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.
8. **Loening, U. E.** 1967. Fractionation of high molecular weight ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J. 102:251-257.
9. **Mills, D.** 1972. Effect of pH on adenine and amino acid uptake during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. 112:519-526.
10. **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.
11. **Stahl, A. J. C., J. Bakes, J. H. Weil, and J. B. Ebel.** 1964. Etude du transfert du phosphore des polyphosphates inorganiques chez la levure II. Bull. Soc. Chim. Biol. 46:1017-1027.
12. **Turco, E., F. Altruda, A. Ponzetta, and G. Mangiarotti.** 1974. Ribosome biosynthesis in *Escherichia coli*. Concerning the limiting step. Biochemistry 13:4752-4757.
13. **Udem, S. A., and J. R. Warner.** 1972. Ribosomal RNA synthesis in *Saccharomyces cerevisiae*. J. Mol. Biol. 64:227-242.
14. **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. 120:1344-1356.