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Control of Dimorphism in *Mucor rouxii*

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ABSTRACT

HAIDLE, C. W. (The University of Texas, Austin), AND R. STORCK. Control of dimorphism in *Mucor rouxii*. J. Bacteriol. **92**:1236–1244. 1966.—Yeastlike cells of *Mucor rouxii* NRRL 1894 were converted to filaments in a medium containing glucose, mineral salts, casein hydrolysate, nicotinic acid, and thiamine when the gas phase was changed from CO_2-N_2 or N_2 alone to air. Germ tubes began to appear 3 to 4 hr after exposure to air. Ribonucleic acid (RNA) precursors were incorporated into RNA in a discontinuous fashion during this conversion, but the incorporation was continuous during the anaerobic growth of yeastlike cells and during the aerobic germination of sporangiospores. The incorporation of labeled amino acids during the conversion was exponential. Labeling of ribosomal RNA occurred as shortly as 5 min after replacement of CO_2-N_2 with air. However, P^{32}-labeled RNA isolated 20 min after exposure to air had a guanine plus cytosine (GC) content of 41% (mole %) as compared with the 47% found for labeled and unlabeled RNA isolated at other stages of the life cycle of this organism or later during the conversion. In addition, the overall base composition of this 20-min pulse-labeled RNA resembled that of deoxyribonucleic acid (GC = 39%), suggesting that a significant proportion of this RNA is of the messenger type. Furthermore, the synthesis of cytochrome oxidase was induced upon exposure of yeastlike cells to air. Cyanide, acriflavine, and cycloheximide, which inhibited the action or synthesis of cytochrome oxidase, also inhibited the yeast to filament transition.

Dimorphism, which has been known for more than a century, refers to that property exhibited by many fungal species to exist either as spherical yeastlike cells, which reproduce by budding, or as filaments. Dimorphism therefore can be considered a good example of primitive morphogenesis. Bartnicki-Garcia and Nickerson (2, 3, 4, 5, 6) found that this morphological difference can be characterized in *Mucor* by a quantitative variation in the chemical composition of the cell wall. The protein and mannan content, for example, were found to be, respectively, two and five times higher in yeast than in hyphal walls. These authors also discovered that some *Mucor* species, such as *Mucor rouxii*, exist in the yeast form only when air is replaced by a mixture of CO_2 and N_2. Bartnicki-Garcia (1) suggested that CO_2 operates in dimorphism through a mechanism involving its fixation into aspartic acid and the subsequent induction of mannan synthesis.

The apparent simplicity of this phenomenon and its environmental control prompted us to study the possibility that nucleic acids are involved in the conversion of yeastlike to filamentous cells. In the present publication, it is shown that this conversion is accompanied by the synthesis of ribonucleic acid (RNA), protein, and cytochrome oxidase.

MATERIALS AND METHODS

Production of sporangiospores. M. rouxii NRRL 1894 was kindly supplied by C. W. Hesseltine, Northern Utilization Research and Development Division, Peoria, Ill. Tomato-juice agar (50% commercial tomato juice plus 2.5% agar in distilled water) was used for the maintenance of the stock culture and for the production of sporangiospores. The organism was grown at 28 C for 7 days in Roux bottles. The spores were harvested aseptically by scraping the surface of the culture with a glass rod in the presence of distilled water. The resulting spore suspension was passed through four thicknesses of cheesecloth to remove hyphae and debris. The spores were washed by centrifugation with three volumes of sterile distilled water. The pellet was suspended in sterile distilled water and contained 10^9 spores per ml. This suspension was stored at 4 C for subsequent use as inoculum. Numbers of spores were determined by use of a standard hemacytometer. These counts, as well as dry weights of yeastlike cells, were correlated with Klett-Summerson colorimeter readings by use of the no. 56 filter.

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Production of yeastlike cells and conversion to filaments. The defined medium of Bartnicki-Garcia and Nickerson (4) was used after supplementation with 0.2% vitamin-free casein hydrolysate. Sporangiospores (10^6 per ml of medium) were converted to yeastlike cells after incubation for 18 hr at 25 C in Erlenmeyer flasks placed on a rotary shaker. The flasks were equipped with rubber stoppers containing an inlet and outlet permitting constant flushing over the liquid surface with either 0.1 liter per min of CO_2 plus 0.3 liter per min of N_2 or 0.3 liter per min of N_2 alone. After 18 hr, the gas was replaced by flushing a stream of air over the liquid surface for 1 min. The cells were incubated aerobically with stirring at 25 C, which resulted in the onset of filamentous growth after 3 to 4 hr.

Chemicals. $P^{32}O_4$, carrier-free, was purchased from Oak Ridge National Laboratory, tritiated uridine (6.58 c/mmole) from New England Nuclear Corp., Boston, Mass., C^{14}-labeled L-isoleucine (6.16 mc/mmole) from Nuclear-Chicago Corp., Des Plaines, Ill. Pancreatic ribonuclease, five times crystallized, was purchased from Calbiochem. A solution containing 20 mg/ml of ribonuclease was prepared in 0.15 M NaCl (*p*H 5.0) and heated at 80 C for 10 min. Cytochrome *c*, type III from horse heart, was purchased from Sigma Chemical Co., St. Louis, Mo. Cycloheximide was a gift from The Upjohn Co., Kalamazoo, Mich.

Preparation of labeled samples. Labeled nucleic acid and protein precipitates were prepared by rapidly mixing an equal volume of cells with cold 20% (w/v) trichloroacetic acid. After 30 min of storage in ice, the precipitates were collected and washed six times with 5-ml volumes of cold 10% trichloroacetic acid on Schleicher and Schuell membrane filters (27 mm in diameter, type B-6). The filters were air-dried and placed in scintillation vials, together with 15 ml of toluene containing 0.4% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP).

The specific activity of RNA was determined according to the procedure outlined by Nomura, Hall, and Spiegelman (16). Samples, containing 2 ml of labeled cells, were rapidly mixed with 2.0 ml of cold 20% TCA and held for 20 min in ice, followed by the addition of 6.0 ml of cold 10% TCA. The precipitates were sedimented and washed twice by centrifugation with 10.0-ml volumes of cold 10% TCA. The pellets were washed with 2.0 ml of 0.2 M sodium acetate suspended in 2.0 ml of 0.2 M sodium acetate plus 0.2 ml of a solution of ribonuclease (500 μg/ml) and incubated at 37 C for 30 min. After incubation, the samples were chilled, 0.1 ml of 5 M $HClO_4$ (PCA) was added, and the mixtures were stored in ice for 15 min. The precipitates were pelleted by centrifugation and discarded. The optical densities (OD) of the supernatant fractions were measured at 260 mμ, and the concentration of RNA was estimated by assuming

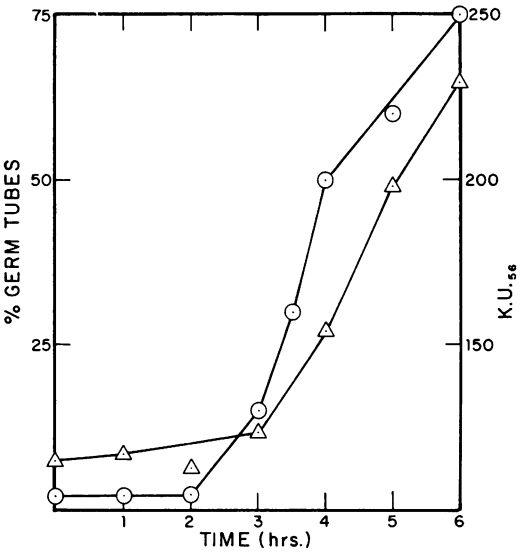

FIG. 1. *Time course of appearance of germ tubes. The yeastlike cells were grown under unpurified N_2 and exposed to air at 0 min. Symbols:* ○ = *per cent of germ tubes;* △ = *turbidity, KU_{56}.*

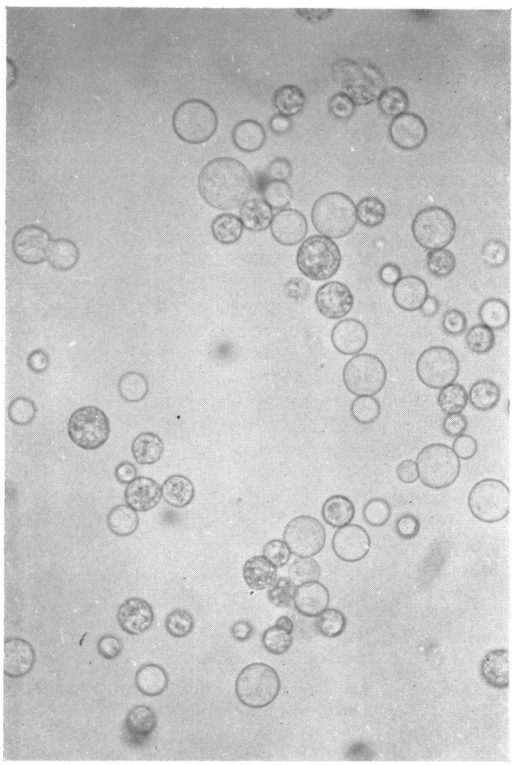

FIG. 2

FIG. 2–5. *Photomicrographs of converting yeastlike cells taken at 60, 180, 240, and 300 min, respectively, after exposure to air, magnification,* × *250. The yeastlike cells were grown under unpurified N_2.*

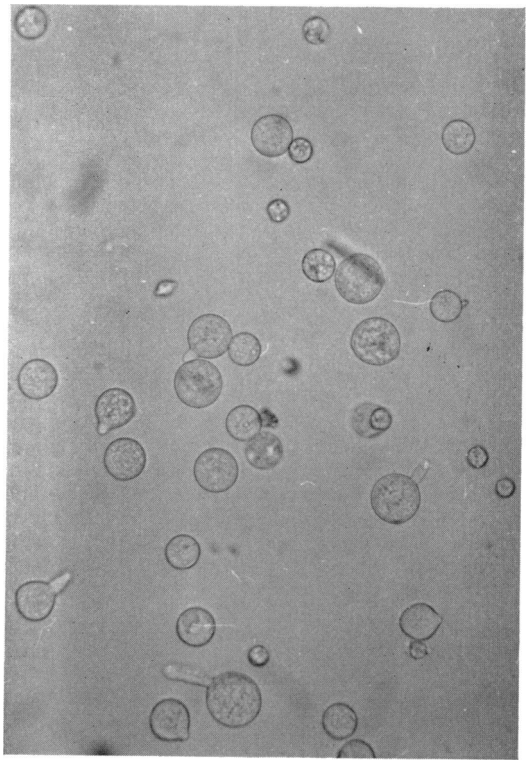

FIG. 3

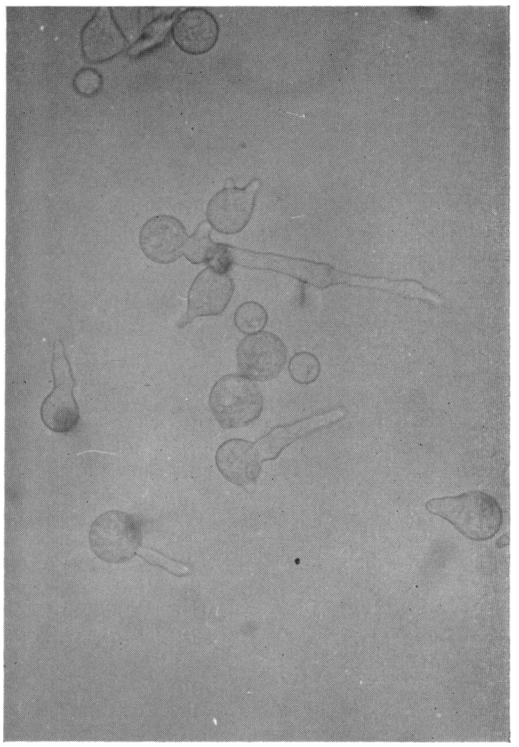

FIG. 4

that an OD_{260} of 1.0 was equal to 50 μg/ml. Samples of 1.0 ml were mixed with 15 ml of Bray's solution (7) in scintillation vials. All radioactivity measurements were determined by use of a Packard Tri-Carb liquid scintillation spectrometer.

Cell extracts and density gradient centrifugation. Labeled cells (50-ml volumes containing 1.0 to 1.8 mg/ml, dry weight, of cells) were collected and washed by filtration on a membrane filter (0.45-μ pore size, 47 mm in diameter; Millipore Filter Corp., Bedford, Mass.) with 200 ml of cold 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.4, containing 0.01 M $MgCl_2$ and 20 μg/ml polyvinylsulfate (TMP buffer). The cells were scraped from the filter and placed in a cold mortar together with 0.3 g of 50-mesh acid-washed sand; they were then ground for 5 min. The ruptured cells were transferred to a 15-ml glass centrifuge tube, 0.5 ml of TMP buffer was added, and the suspension was centrifuged in the cold at 15,000 $\times$ *g* for 10 min. The supernatant fluid was removed and 0.2 to 0.4 ml, containing 400 to 500 μg of RNA, was layered on a linear 5 to 20% sucrose density gradient prepared in TMP buffer and centrifuged for 60 min at 35,000 rev/min in the SW 39 rotor of a Spinco Model L ultracentrifuge. Fractions of 0.2 ml were collected after puncturing the botton of the centiifuge tube, 1.0 ml of TMO buffer was added to each fraction, and the OD_{260} was determined. For radioactivity measurements, 1.0-ml samples from each fraction were mixed with 250 μg of salmon sperm deoxyribonucleic acid (DNA), used as carrier, and further treated according to the membrane-filter technique previously described.

Determination of sedimentation coefficients. Sedimentation of ribosomes was studied at 4 C in a Beckman Model E analytical ultracentrifuge equipped with schlieren optics. Sedimentation coefficients were calculated according to Schachman (18), and correspond to infinite dilution and 20 C in water ($S^0_{20,w}$).

Cytochrome oxidase assay. Cultures to be assayed for cytochrome oxidase activity (40- to 50-ml volumes, dry weight = 1.0 to 1.8 mg/ml) were harvested by filtration through a Millipore filter (0.45 μ pore size, 47 mm in diameter) and washed on the filter with 2 volumes of cold 0.1 M phosphate buffer (*p*H 7.0). The cells were scraped from the filter and placed in a cold mortar together with 0.3 g of 50-mesh acid-washed sand and were ground for 2 min. The ruptured cells were transferred to a 15-ml glass centrifuge tube, 2.0 ml of the phosphate buffer containing 200 μg/ml cycloheximide was added, and the suspension was centrifuged in the cold at 2,000 $\times$ *g* for 5 min. The supernatant fluid was removed for enzyme assay according to the procedure described by Smith (21). Protein concentration was estimated by use of the *biuret* method described by Layne (15).

Determination of base ratios of RNA. After incor-

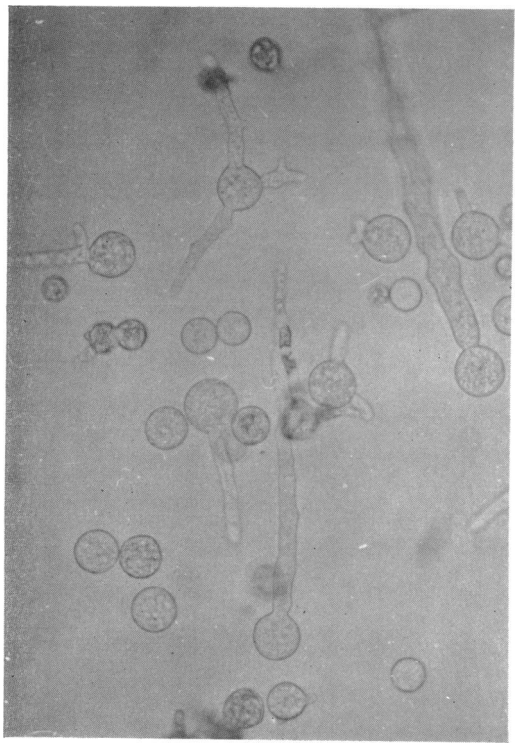

FIG. 5

poration of $P^{32}O_4$, 200 ml of cells (dry weight = 1.0 to 1.8 mg/ml) were made 0.5 M with respect to PCA and maintained in ice for 30 min. The precipitate was washed by centrifugation with 700 ml of cold 0.5 M PCA followed by 250 ml of cold 0.2 M sodium acetate. The final pellet was suspended in 10 ml of 0.2 M sodium acetate; 0.5 ml of a solution of ribonuclease (20 mg/ml) was added and the suspension was incubated with stirring at 37 C for 60 min. The insoluble residue was removed by centrifugation; the supernatant fluid was neutralized with 9 M KOH and was made 0.3 M with respect to KOH. This solution was incubated at 37 C for 18 hr, after which cold PCA was added to a final concentration of 0.5 M. After 15 min in ice, the precipitate (ribonuclease plus $KClO_4$) was removed by centrifugation; the *p*H of the supernatant fluid was brought to 10 and the second precipitate ($KClO_4$) was sedimented by centrifugation. The nucleotides, contained in the final supernatant fraction, were separated by column chromatography on Dowex 1-X8-formate resin 200–400 mesh (8). The column was 5 cm in length and 1.2 cm in diameter. The elution was performed as described elsewhere (14). The OD of the effluent was monitored at 260 and 280 mμ with a Gilford Multiple Sample Absorbance recorder; the flow rate and the amount of each nucleotide recovered were determined as described by Storck (23). Fractions of 4.5 ml were collected, and 1.0 ml was removed from each fraction for radioactivity measurement. The nucleotide composition, based on radioactivity, was obtained after determining the relative proportion of counts per minute associated with each nucleotide. Corrections were made for decay of P^{32} and for the quenching characteristics of each eluent. The base ratios found using the above method were the same as those reported by Storck (23) with use of direct KOH hydrolysis of cell extracts. It is worthwhile to emphasize the fact that the above procedure not only minimizes the action of endogenous nucleases but also gives a threefold increase in the yield of RNA.

RESULTS

Description of the converting system. Yeastlike cells could be produced from spores by incubation in the presence of CO_2 and N_2 as originally described by Bartnicki-Garcia and Nickerson (3), or by incubation in the presence of N_2 alone provided that traces of O_2 were removed by passage through bright copper filings heated to 450 C. When unpurified N_2 was used, 1 to 2% of the cells exhibited short germ tubes.

Figure 1 shows the time of appearance of germ tubes when yeastlike cells, which had been grown

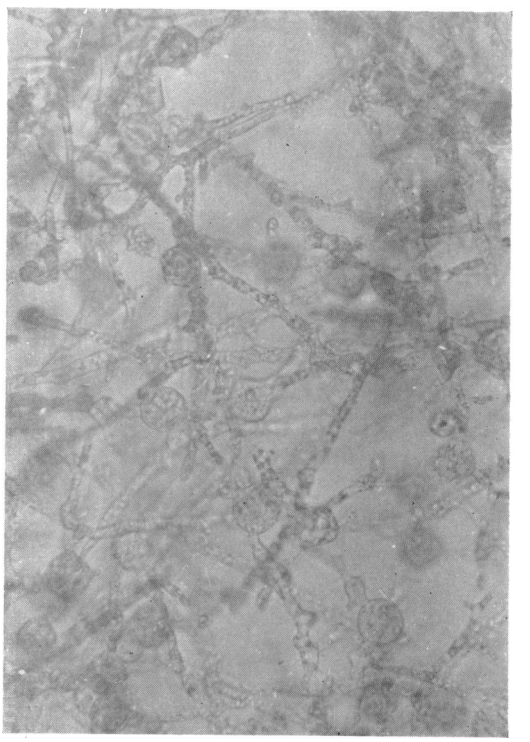

FIG. 6. *Hyphae grown aerobically for 16 hr, magnification, × 250.*

in an atmosphere of N_2, were exposed to air. Germ tubes began to appear approximately 3 hr after exposure to air, and the number of cells exhibiting germ tubes increased to 50% after 4 hr. This increase was paralleled by an increase in the turbidity of the culture. When yeastlike cells were produced in an atmosphere of CO_2 and N_2 followed by exposure to air, the appearance of germ tubes was delayed by 1 hr.

Photomicrographs taken at intervals during the conversion are presented in Fig. 2 to 7. After 1 hr of exposure to air (Fig. 2), the budding yeastlike cells were indistinguishable from those growing under anaerobiosis. Figures 3, 4, and 5, representing cells at 3, 4, and 5 hr, respectively, after exposure to air, show an increase in the number of cells with germ tubes, as well as an increase in the length of the tubes. Figures 6 and 7, respectively, show a typical filamentous culture which was grown aerobically for 16 hr and sporangiospores of *M. rouxii*.

Incorporation of labeled precursors. Figure 8 shows the incorporation of H^3-uridine into yeastlike cells during the conversion to filaments. The rate of incorporation was discontinuous whether one measured the radioactivity incorporated into total trichloroacetic acid-precipitable material or that incorporated specifically into RNA. These interruptions of H^3-uridine incorporation were observed in all 20 experiments performed, but the time of their appearance, as well as their duration, varied somewhat. Although saturating concentrations of uridine were used in the experiments represented by Fig. 8, the discontinuities also were apparent at lower and higher uridine concentrations. These variations in the rate of H^3-uridine incorporation during the conversion also were observed after yeastlike cells were transferred to fresh medium, and when the yeastlike cells were grown under N_2 alone prior to exposure to air. This phenomenon appears to be peculiar to yeastlike cells undergoing morphogenesis, since, as shown in Fig. 8, no such discontinuities exist when H^3-uridine was incorporated into yeastlike cells growing in anaerobiosis. Also, during the aerobic germination of sporangiospores, the incorporation of H^3-uridine was continuous. Furthermore, as illustrated in Fig. 9, the specific activity of P^{32}-labeled RNA also increased discontinuously during the conversion. In contrast, the incorporation of C^{14}-labeled L-isoleucine was continuous and exponential (Fig. 10).

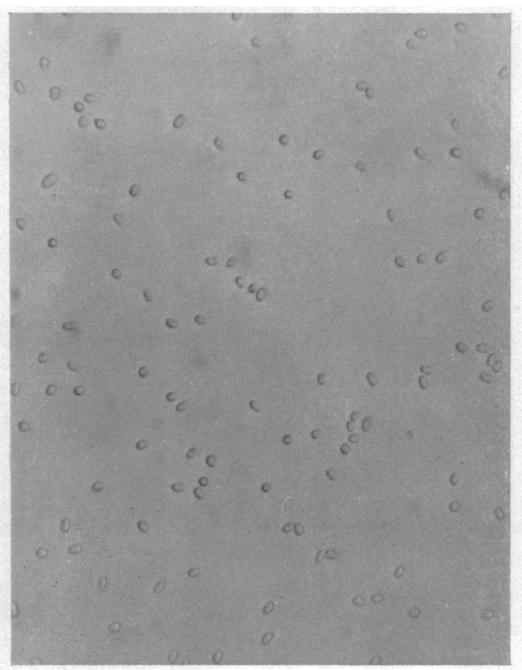

FIG. 7. *Sporangiospores, magnification, × 250.*

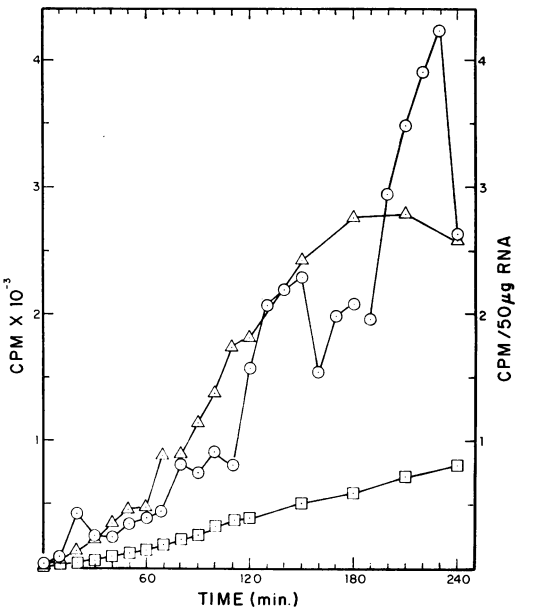

FIG. 8. *Incorporation of H^3-uridine into converting and nonconverting yeastlike cells. All cultures were grown under CO_2-N_2 and exposed to tracer at 0 min. Symbols: ⊙ = exposed to air at 0 min, 0.4 μc of H^3-uridine per ml of culture. Radioactivity incorporated into cold trichloroacetic acid-precipitable material was measured; △ = exposed to air at 0 min, 1.0 μc of H^3-uridine per ml of culture. Radioactivity incorporated into RNA (specific activity) was measured; ⊡ = not exposed to air, i.e., continued yeastlike growth, 1.0 μc of H^3-uridine per ml of culture. Specific activity of RNA was measured.*

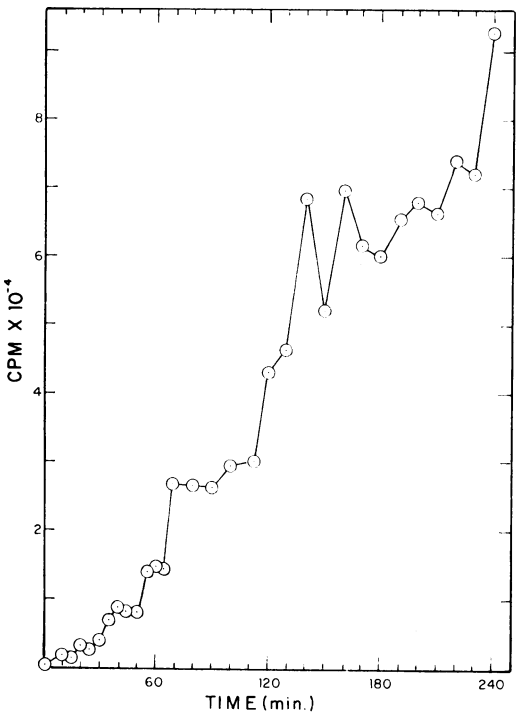

FIG. 9. *Incorporation of P^{32} into the RNA fraction of converting yeastlike cells grown under CO_2-N_2. Air and tracer (10 μc/ml of culture) were added at 0 min. Specific activity of RNA was measured.*

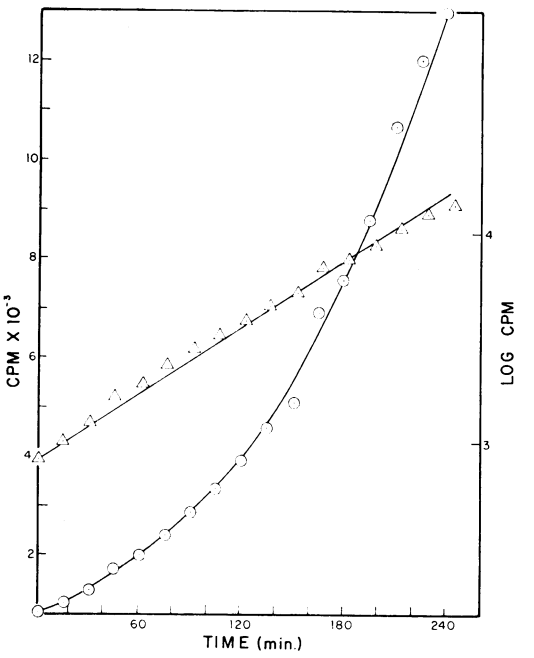

FIG. 10. *Incorporation of C^{14}-labeled L-isoleucine into cold trichloroacetic acid-precipitable fraction of converting yeastlike cells grown under CO_2-N_2. Air and tracer (0.2 μc/ml of culture) were added at 0 min. Symbols: ⊙ = arithmetic plot; △ = semilogarithmic plot.*

To determine the rapidity with which H^3-uridine becomes distributed among the various RNA classes, a series of sucrose density gradient analyses were performed during the early stages of the conversion. Figure 11 shows the results of three such analyses; the ribosome region, fractions 7 through 17, representing particles which have sedimentation coefficients between 120*S* and 84*S*, became labeled as shortly as 5 min after exposure to air and tracer and continued to receive label through 20 min. The label associated with the ribosome region could not be removed by treating the extract with 1.0 μg/ml of ribonuclease for 10 min at 37 C prior to density gradient centrifugation.

Base composition of RNA. As shown in Table 1, it is apparent that the RNA synthesized during the first 20 min after exposure to air was different from that synthesized between 20 and 40 min. The base composition of the latter was similar to that for total RNA, the bulk of which was ribosomal. In contrast, the RNA synthesized during the initial 20 min had a composition mimicking that of DNA and was almost identical to the RNA synthesized in the presence of the antibiotic cycloheximide (12). This antibiotic, which prevents the conversion of yeast to filaments in *M. rouxii* (12), appears to specifically inhibit the synthesis of ribosomal RNA, as well as protein (9, 10, 11, 12, 20).

Respiratory induction during the conversion. Figure 12 shows the time course of the synthesis of cytochrome oxidase after exposure to air of a yeastlike culture grown under purified N_2. Germ tubes began to appear at 3 hr after a 10-fold increase in the amount of the enzyme. Aerobically grown hyphae contained approximately 50 times more enzyme than yeastlike cells.

In addition, cyanide (0.10 M), acriflavine (100 μg/ml), and cycloheximide (200 μg/ml) inhibited the yeast to filament conversion and also inhibited the action or synthesis of cytochrome oxidase. Phenethyl alcohol (0.2%), however, inhibited the conversion, but had little or no effect on the synthesis or action of cytochrome oxidase (Haidle, Tetenai, and Storck, *in preparation*).

DISCUSSION

Pasteur demonstrated that the yeast phase of *Mucor* could be characterized by a strong alcohol fermentation (17). This observation was confirmed in 1963 by Bartnicki-Garcia (1), who

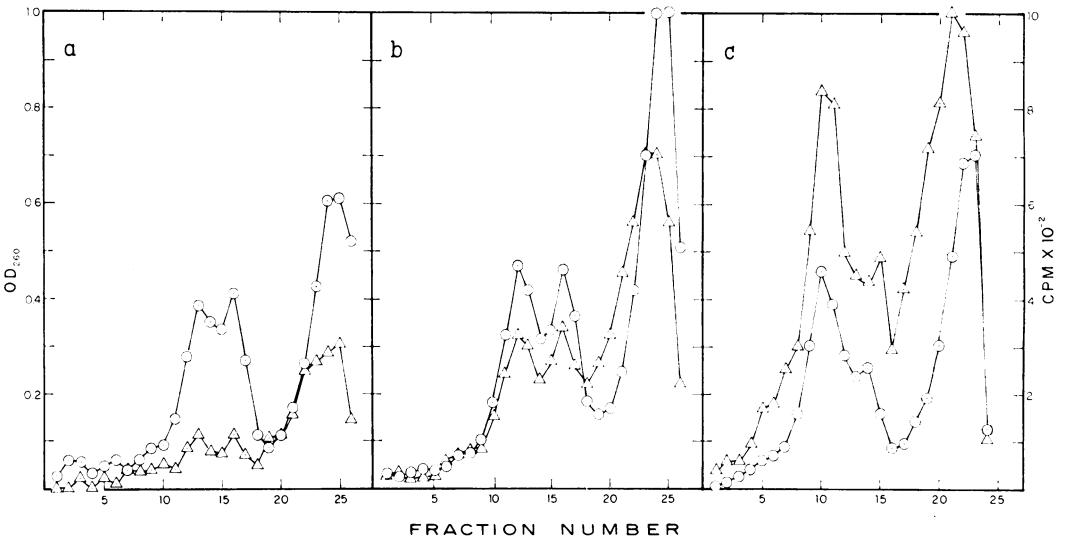

FIG. 11. *Sucrose density gradient centrifugation profiles of extracts of H^3-uridine-labeled converting yeast-like cells grown under CO_2-N_2. The cultures were exposed to air and tracer (0.4 μc/ml of culture) at 0 min; 50-ml samples were removed for density gradient analyses at the indicated times: a = 5 min, b = 10 min, c = 20 min. Symbols: ⊙ = OD at 260 mμ; △ = counts per minute.*

TABLE 1. *Base composition of total and pulse-labeled RNA*

Determination	Nucleotides (mole %)					
	Method	C	A	U(T)	G	GC
Total RNA	OD	20.8	27.2	25.2	26.8	47.6
Total RNA	P^{32}[a]	22.0	28.5	25.3	24.2	46.2
0–20 min pulse	P^{32}[b]	20.1	31.2	27.7	21.1	41.2
20–40 min pulse	P^{32}	19.5	29.2	24.8	26.5	46.0
0–40 min pulse + cycloheximide (250 μg/ml)	P^{32}	21.7	29.7	27.4	21.2	42.9
DNA	—[c]	19.5	30.5	30.5	19.5	39.0

[a] Determined on hyphal cells grown for 16 hr in the presence of tracer (1.0 μc of P^{32} per ml of culture) from a spore inoculum.

[b] In pulse labeling experiments, 35 μc of P^{32} per ml of culture was used.

[c] Determined by CsCl buoyant density centrifugation and melting point analyses (24). The mole % of the individual nucleotides was calculated by assuming an equivalence of A to T and G to C.

showed that *M. rouxii*, in the absence of air, converted 47% of the glucose utilized to ethyl alcohol, whereas in the presence of air, this amount was only 2.9%.

Our work is compatible with the idea that there is a shift from an anaerobic to an aerobic metabolism accompanying the conversion of yeasts to filaments in *M. rouxii*, since the appearance of germ tubes coincides with a 10-fold increase in the specific activity of cytochrome oxidase. This enzyme is present at trace levels in the yeast phase but increases 50-fold in the filamentous phase.

The induction of the synthesis of a terminal respiratory enzyme after exposure to air is similar to the so-called respiratory adaptation observed by several workers in *Saccharomyces cerevisiae* (19, 22). In *M. rouxii*, as in *S. cerevisiae*, the base composition of P^{32}-labeled RNA synthesized immediately after exposure to air is different from that of total and ribosomal RNA. This finding, with use of *S. cerevisiae*, led Fukuhara (11) to suggest that this newly synthesized population of RNA molecules might contain a certain proportion of messenger RNA required for the synthesis of these respiratory enzymes. That this may be the case for *M. rouxii* is suggested by the fact that the base composition of P^{32} RNA synthesized during the first 20 min after exposure to air resembles that of DNA. The GC content of DNA is 39% and that of P^{32} pulse-labeled RNA is 41%, whereas that of total RNA is 46%. This close resemblance is also seen in the respective propor-

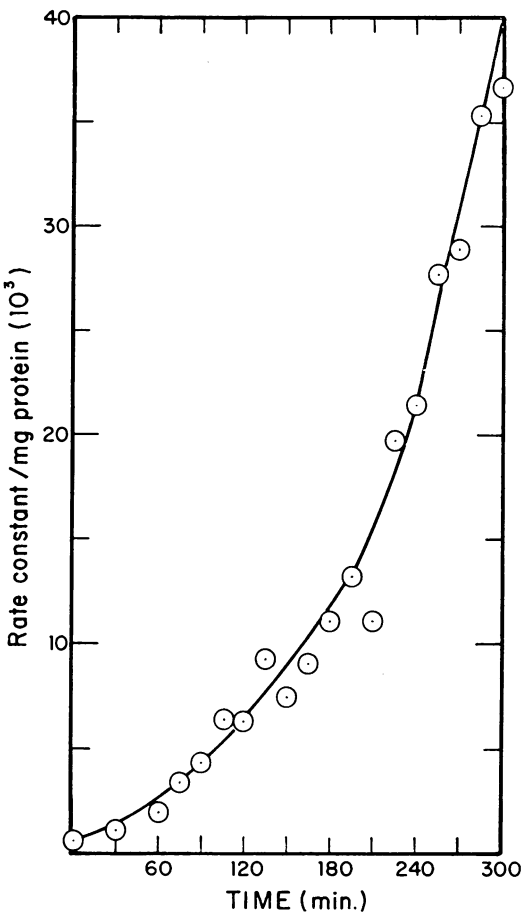

FIG. 12. *Induction of cytochrome oxidase synthesis. Yeastlike cells grown under purified N_2 were exposed to air at 0 min.*

tions of the individual bases. In addition, the GC content of ribosomal RNA is 48% and that of transfer RNA is 54% (H. Terenzi, *personal communication*). This suggests that the ribosomal RNA synthesis observed during the period immediately after exposure to air is not as great as that of the RNA with a DNA-like base composition. As suggested by Hayashi and Spiegelman (13), such a population of RNA molecules is likely to contain a high proportion of molecules of the messenger type. As we have shown previously (12), there is little or no inhibition of H^3-uridine incorporation during the first 30 min of the conversion in the presence of cycloheximide, which is thought to preferentially inhibit the synthesis of ribosomal RNA (9). And, as in the case of *S. carlsbergensis*, the P^{32}-labeled RNA which accumulates in the presence of the antibiotic has a base composition resembling that of DNA (9). It appears therefore that the synthesis of respiratory enzymes in both *Saccharomyces* and *Mucor* is accompanied by the synthesis of messenger RNA.

After the exposure to air, the budding yeastlike cells of *M. rouxii* are progressively converted to filaments concomitant with the formation of cytochrome oxidase. Is the synthesis of cytochrome oxidase and related enzymes a necessary and sufficient condition for this morphological change? That it may be necessary is suggested by the fact that cyanide, acriflavine, and cycloheximide, which inhibit the activity or synthesis of cytochrome oxidase, also prevent the conversion. That it is not sufficient is shown by the effect of phenethyl alcohol, which inhibits filamentous but not yeastlike growth, and has no effect on the activity or synthesis of this enzyme. Therefore, it is necessary to conclude that other enzyme systems might be required and that their activity or synthesis is inhibited by phenethyl alcohol. We are currently investigating the mode of action of this inhibitor to verify our hypothesis.

Bartnicki-Garcia and Nickerson (3) found that a mixture of CO_2 and N_2 was necessary for the production of the yeast phase in several strains of *M rouxii*. However, with N_2 alone, mostly filaments were produced. In our work, when N_2 alone was used, provided traces of O_2 were eliminated, *M. rouxii* NRRL 1894 was exclusively yeast-like. It is important to note that Bartnicki-Garcia and Nickerson used a medium containing yeast extract and peptone, whereas we employed a synthetic medium. If we used the yeast extract-peptone medium or a tomato-juice medium, some filaments were produced under N_2 alone. These observations indicate that nutritional factors are involved in the control of dimorphism. From their studies, Bartnicki-Garcia and Nickerson (3) concluded that CO_2 plays a specific role in the maintenance of yeast growth. Our data rule out this hypothesis, at least for *M. rouxii* NRRL 1894. It is entirely possible that rich media supply intermediates required for the conversion which normally would be produced only in aerobiosis.

The discontinuous synthesis of RNA was detected only during the yeast to filament transition, and was observed when the radioactivity of whole-cell trichloroacetic acid precipitates, as well as the corresponding specific activities, were measured. When total radioactivity was measured, the interruptions of incorporation were manifested by an apparent decrease in radioactivity, suggesting an actual destruction of labeled RNA. However, when the specific activity of RNA was measured, the cessation of incorporation was characterized by a plateau. This difference can be explained if one assumes that: (i) RNA synthesis is not continuous during the conversion, i.e., there occurs a cessation of total RNA synthesis, (ii) there is a

degradation of RNA either continuously or following the cessation of synthesis, and (iii) the degradation is not specific for newly synthesized (labeled) RNA. Thus when the radioactivity of trichloroacetic acid precipitates is measured, the concomitant cessation of synthesis and degradation of RNA would appear as a decrease in the amount of radioactivity, whereas when specific activity (counts per minute per milligram) of RNA is measured, this cessation and degradation appears as a plateau. Although the discontinuous incorporation of RNA precursors was observed only during the morphogenesis, it may not necessarily be an expression of the conversion phenomenon, but result from a condition of unbalanced growth or perhaps may reflect a state of synchrony resulting from the drastic change in environmental conditions. We are currently attempting to study DNA replication, which is rendered difficult by the fact that neither thymine nor thymidine can be incorporated.

ACKNOWLEDGMENTS

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