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Glucose Metabolism and Dimorphism in *Mucor*

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Mucor racemosus fermented glucose to ethanol, carbon dioxide, and glycerol. When this fungus was grown anaerobically in either the yeast or mycelial form, the catabolism of glucose was very similar. Yeast cells shifted to aerobic conditions maintained a high flux of glucose carbon through the glycolytic and pentose phosphate pathways. Mycelial cells grown aerobically catabolized glucose in a manner consistent with a respiratory metabolism. Although there was no consistent pattern of glucose metabolism in the mycelial form of _Mucor_, growth in the yeast form consistently was correlated with a high flux of glucose carbon through the catabolic pathways.

Mucor racemosus is a dimorphic phycomycete that can grow either as budding yeasts or as filamentous mycelia. Although the morphology of this fungus appears to be influenced by a variety of environmental factors (4, 14), the presence of a fermentable hexose is essential for the development of the yeast form (2). Larsen and Sypherd (8) showed, for example, that, although dibutyryl cyclic AMP (dbcAMP) induced yeast development in air, the presence of glucose was necessary to maintain the yeast form. Depending on the concentration of glucose in the growth medium, the morphology of *M. rouxii*, grown under anaerobic conditions (100% N_2), varied from the mycelial form to the yeast form (2). Mooney and Sypherd (10) reported, however, that effects of glucose concentration on morphology were not observed with *M. racemosus* if one carefully regulated the flow rate of nitrogen through the culture. When *M. genevensis* yeast cells were grown in continuous culture with excess glucose, mycelial cells were not formed even at high oxygen concentrations (13). Indeed, these aerobic yeast cells continued to produce ethanol and carbon dioxide at a rate indicative of a fermentative metabolism. These workers (13) concluded that respiratory capacity was not correlated with morphology. In addition, Paznokas and Sypherd (12) measured the respiratory capacity of aerobic mycelia and aerobic dbcAMP-treated yeast and concluded that morphology was independent of respiratory capacity. The present study was designed to determine the nature of the relationship between the patterns of glucose carbon metabolism and the morphology of *Mucor*. Changes in the distribution or flux of carbon into the glucose catabolic pathways could reveal changes in metabolic intermediates, enzymes, or end products important to the regulation of morphogenesis.

MATERIALS AND METHODS

Organism and culture conditions. *Mucor racemosus* (*M. lusitanicus*) ATCC 1216B was used in all experiments. Sporangiospores were prepared as described by Paznokas and Sypherd (12). Cells were grown in either a complex medium consisting of 0.3% (wt/vol) yeast extract (Difco), 1% (wt/vol) peptone (Difco), and 2% (wt/vol) D-glucose; or a minimal medium consisting of 0.05% (wt/vol) yeast nitrogen base (Difco), 2% (wt/vol) D-glucose, 10 mM L-glutamate, 10 mM L-alanine, 10 mM L-aspartate, and 10 mM NH_4Cl (J. Peters and P. S. Sypherd, J. Gen. Microbiol., in press). Media were adjusted to pH 4.5 with sulfuric acid. Cultures were inoculated with 3×10^5 spores per ml, incubated at 22°C, and shaken at 220 rpm.

Fermentation balance. The procedure described by Dawes et al. (5) was used to determine the glucose fermentation balance for yeast cells, except that carbonate was not added to the growth medium. After the fermentation stopped, cell material was removed by centrifugation. Volatile fermentation products, collected in cold traps, were added to the culture supernatant fluid and maintained at 5°C. Glucose was assayed with *o*-toluidine (Sigma bulletin 635, Sigma Chemical Co.). Ethanol was assayed enzymatically (Sigma bulletin 331-UV). Glycerol, acetaldehyde, pyruvate, and lactate were assayed enzymatically, and carbon dioxide was determined gravimetrically as described by Dawes et al. (5).

Cell extracts. Yeast or hyphal cells were collected on Teflon filters (type LC, Millipore Corp.; pore size, 10 μm) and washed with distilled water. The cells were suspended in 0.08 M Bicine-HCl buffer (pH 7.8) containing 0.04 M $MgCl_2$ and were disrupted in a French pressure cell. Broken-cell suspensions were centrifuged at 9,000 × *g* for 10 min, and the supernatant fractions (cell extracts) were used in the measurement of enzyme activities.

Enzyme assays. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.144) were assayed by the method of Wood (19). Protein was measured with the Folin phenol reagent (9).

Glucose carbon metabolism. The distribution of

glucose carbon between the glycolytic and pentose phosphate pathways was determined by measuring the rates of $^{14}CO_2$ evolution when cells were given differentially labeled D-[^{14}C]glucose. The procedure combined the method of Wang (18) and a technique described by Sissions (15). Yeast or hyphal cells were collected on Teflon filters (pore size, 10 μm) and washed with distilled water. The cells were suspended in distilled water to a cell density that gave 100 to 200 μg of protein per assay. Cells were collected, washed, and resuspended within 2 min. Measurements of $^{14}CO_2$ evolution were started by adding 50 μl of the cell suspension to 50 μl of the reaction mix in a tube (10 by 75 mm). The reaction mix consisted of either 0.1% (wt/vol) yeast nitrogen base (Difco) or complex medium (0.6% [wt/vol] yeast extract and 2% [wt/vol] peptone) with 5 μmol of D-glucose and 25 nCi of D-[1-^{14}C]glucose or 25 nCi of D-[3,4-^{14}C]glucose. The tube was attached to a scintillation vial (containing a glass-fiber filter and 200 μl of 1.3 M KOH) and a serum cap via a polypropylene T-connector (15). The reaction was terminated by adding 100 μl of 2 M $HClO_4$ with a syringe. A minimum of 2 h was allowed for the absorption of $^{14}CO_2$ onto the filters. A 12-ml volume of scintillation fluid (0.5% [wt/vol] 2,5-diphenyloxazole and 40% [vol/vol] methoxyethanol in toluene) was added to the scintillation vial and directly counted.

The perchloric acid-treated material was adjusted to pH 7.0 with NaOH and brought to 1.0 ml with water. Insoluble material was removed by centrifugation at 500 × *g* for 5 min. Portions of the supernatant were assayed for D-glucose with an ultramicro glucostat kit (Worthington Biochemicals Corp., Freehold, N.J.); one-half the prescribed volume of reagents was used. The total protein of the cell suspension at zero time was determined by the method of Herbert et al. (6). Alternatively, changes in cell protein during the assay period were monitored by measuring changes in the $HClO_4$-precipitated protein with the Folin phenol reagent (9). Because the initial rates of $^{14}CO_2$ release could be determined within 20 min, the maximum specific growth rate of *Mucor* (0.28 h^{-1} for cells grown aerobically in the yeast-peptone-glucose medium) did not significantly affect the observed rates of glucose metabolism. Assays run to completion released more than 90% of the added labeled substrate. Assays containing 100 to 200 μg of protein released $^{14}CO_2$ at the rate of 50 to 500 cpm/min, depending on the position of the label and the type of cell used. The distribution of glucose carbon into the glycolytic and pentose phosphate pathways was calculated from the initial rate of $^{14}CO_2$ release from D-[1-^{14}C]glucose and D-[3,4-^{14}C]glucose and the initial rate of D-glucose assimilation, with the equations described by Wang (18).

Chemicals. Radioactively labeled glucose—D-[3,4-^{14}C]glucose (10.32 mCi/mmol) and D-[1-^{14}C]glucose (55.56 mCi/mmol)—was obtained from the New England Nuclear Corp. Oxidized nicotinamide adenine dinucleotide phosphate, oxidized nicotinamide adenine dinucleotide, glucose-6-phosphate, 6-phosphogluconate, and dbcAMP were obtained from Sigma Chemical Co. All other chemicals were commercially available.

RESULTS

Fermentation balance. Most species of yeasts ferment glucose to ethanol and carbon dioxide via the glycolytic (Embden-Meyerhof-Parnas, EMP) and pentose phosphate (PP) pathways (1), but the distribution of glucose carbon through each of these pathways varies with the species. Some yeasts ferment glucose to end products other than ethanol and carbon dioxide. As a first approach to our study of glucose metabolism in *Mucor,* we determined the glucose fermentation balance for yeast cells grown in a complex medium (yeast-peptone-glucose). Mooney and Sypherd (10) showed that *Mucor* grows as a yeast in the complex medium when the culture is sparged with nitrogen at flow rates greater than 4 volumes per min per volume of culture medium. We inoculated spores into the medium; the culture flask was then placed in a fermentation train (see Materials and Methods) and sparged with oxygen- and carbon dioxide-free nitrogen at a rate that gave yeast growth. The fermentation was continued until the evolution of carbon dioxide from the culture stopped. The fermentation balance is shown in Table 1.

Glucose was fermented to ethanol, carbon dioxide, and glycerol. We did not detect pyruvate, lactate, acetate, or acetaldehyde. Although the ratio of oxidized and reduced fermentation products was quite reasonable, the percent carbon recovery was low. In a separate experiment (not shown), we were able to detect significant amounts of glycogen in yeast cells that were harvested in late stationary growth. The accumulation of glycogen during this stage of growth is common in yeasts and may account for the low carbon recovery in *Mucor* (1).

The fermentation balance indicated that *Mucor*, grown anaerobically as a yeast, has a typical alcoholic fermentative metabolism. Two important glycolytic enzymes have been described in *Mucor*: phosphofructokinase (16) and pyruvate kinase (11). We detected both glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) activities in both yeast and mycelial cell extracts (Table 2). Both enzymes are oxidized nicotinamide adenine dinucleotide phosphate-specific in

TABLE 1. *Fermentation balance for yeast-phase cells grown in complex medium under nitrogen*

Product	D-Glucose (mmol/100 mmol)
Ethanol	131.2
CO_2	156.4
Glycerol	28.3
Carbon recovered (%)	84.0
O/R[a]	1.08

[a] O/R, Ratio of oxidized to reduced fermentation products relative to glucose.

both types of cells. We concluded that yeast cells ferment glucose primarily to ethanol and carbon dioxide via the EMP and PP pathways.

Yeast glucose carbon metabolism. Wang (18) showed that the pattern of glucose carbon metabolism through the EMP and PP pathways can be determined by measuring the rate of release of $^{14}CO_2$ from D-[3,4-^{14}C]glucose and D-[1-^{14}C]glucose. Using an adaptation of this method (see Materials and Methods), we determined the pattern of glucose carbon metabolism for yeast cells grown in either a complex or minimal medium under nitrogen (Table 3). In both cases, more than 90% of the assimilated carbon was catabolized. For cells grown in the complex medium, the distribution of carbon into the EMP and PP pathways, 86 and 14%, respectively, was similar to that reported for *Saccharomyces* and other fungi (1).

Cells grown in a minimal medium assimilated glucose at a higher rate, although the rates of glycolysis were similar. Peters and Sypherd (in press) found that yeast growth in a minimal medium requires an organic nitrogen source and is stimulated by the addition of amino acids. This observation is consistent with our finding that only small amounts of glucose carbon were diverted to anabolic functions.

Mycelial glucose carbon metabolism. Mycelial cells, grown aerobically in either the complex or minimal media, had a pattern of glucose metabolism significantly different from that of anaerobic yeast cells (Table 4). In a complex medium, the assimilation rate was nearly 10-fold less than that of yeast cells, and this is primarily a reflection of the difference in glycolysis. Mycelial cells grown in a minimal medium also differed from yeast cells in their pattern of glucose carbon metabolism, although the differences were somewhat less pronounced than those for cells grown in complex medium. We concluded that aerobic mycelia metabolize glucose in a pattern consistent with a respiratory metabolism and that the differences in yeast and mycelial glucose metabolism (under these conditions) reflected the differences between a respiratory and a fermentative metabolism rather than differences in cell morphology.

Glucose carbon metabolism and morphology. Two methods were used to determine whether these patterns of glucose metabolism could be correlated with morphology. First, mycelial cells were grown under anaerobic conditions. Vegetative spores were inoculated into either yeast-peptone-glucose or minimal medium that had previously been flushed with oxygen- and carbon dioxide-free nitrogen. Anaerobiosis was monitored by including 0.0001% (wt/vol) resazurin in the medium (7). The medium, usually 80 ml, was contained in a 500-ml Klett flask with a KOH trap in the side arm. After inoculation, the culture was flushed with nitrogen for 30 min and quickly sealed with a rubber stopper. In this closed anaerobic environment, spores rapidly swelled and germinated as mycelia. The first germ tubes appeared 5 h after inoculation. The resazurin dye remained reduced for incubation periods up to 48 h. The respiratory capacity of these anaerobic mycelia

TABLE 2. *Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) activities in crude cell extracts of yeast and mycelia*

Morphology	G6PD (μmol/min per mg of protein)		6PGD (μmol/min per mg of protein)	
	NADP^{+a}	NAD^{+a}	NADP$^+$	NAD$^+$
Yeast	55.4	<0.1	66.5	<0.1
Mycelia	105.4	<0.1	98.6	<0.1

[a] NADP$^+$, Oxidized nicotinamide adenine dinucleotide phosphate; NAD$^+$, oxidized nicotinamide adenine dinucleotide.

TABLE 3. *Glucose metabolism by yeast-phase cells grown in 100% nitrogen*

Metabolic fate of glucose	Minimal medium[a]		Complex medium[a]	
	U/g[b]	%[c]	U/g[b]	%[c]
Assimilated	254.2	100	198.4	100
Catabolized	231.8	91	199.9	101
EMP pathway	178.8	70	172.0	86
PP pathway	53.0	21	27.9	14
Anabolized	22.4	9		

[a] Minimal medium (0.1% [wt/vol] yeast nitrogen base) or complex medium (0.6% [wt/vol] yeast extract and 2% [wt/vol] peptone) with 5 μmol of D-glucose and 25 nCi of D-[1-^{14}C]glucose or 25 nCi of D-[3,4-^{14}C]glucose in a total volume of 50 μl (referred to as the "assay mix" in the text).

[b] U/g, Micromoles per minute per gram of total cell protein.

[c] Fraction of total glucose assimilated, as measured by glucose disappearance, expressed as a percentage.

TABLE 4. *Glucose metabolism by mycelial cells grown in air*[a]

Metabolic fate of glucose	Minimal medium		Complex medium	
	U/g	%	U/g	%
Assimilated	87.3	100	21.6	100
Catabolized	48.2	55	18.4	85
EMP pathway	31.4	36	13.1	61
PP pathway	16.8	19	5.3	24
Anabolized	39.1	45	3.2	15

[a] All notations as described in footnotes to Table 3.

ranged from 10 to 21 ng-atoms of O_2 per min/mg of protein. This range is similar to that reported for anaerobic yeast by Paznokas and Sypherd (12).

Table 5 shows the pattern of glucose carbon metabolism by mycelia grown under nitrogen in either the complex or minimal medium. In both cases, the patterns indicate a fermentative metabolism similar to that of yeast cells. The rate of catabolism by anaerobic mycelia grown in complex medium was slower than that for yeast, but the percentage of distribution into the EMP and PP pathways was quite similar. The pattern of glucose carbon metabolism for anaerobic mycelia grown in a minimal medium was virtually identical to that for comparably grown yeast. In both cases, there was no glucose carbon diverted to anabolic functions.

The second method for determining the relationship between glucose metabolism and morphology was to examine the pattern of metabolism in yeast cells grown aerobically in the presence of dbcAMP. A yeast culture grown in complex medium under nitrogen was divided in early exponential growth into three fractions. One fraction was maintained under nitrogen, a second fraction was treated with 4 mM dbcAMP and incubated aerobically, and the third fraction was incubated aerobically. Five hours after the shift, the aerobic untreated cells had converted to mycelia (80%). The aerobic cells treated with dbcAMP remained as yeasts (100%), as did the anaerobic control culture. The cells from all three fractions were harvested to determine the patterns of glucose carbon metabolism (Table 6). At this time after the shift, the rate of glucose metabolism by both mycelial and yeast cells was 60% of the control culture rate. This oxygen inhibition of glycolysis, or Pasteur effect, has been observed in many fungi (1). However, the rates of glucose catabolism and the distribution of carbon into the two catabolic pathways, as compared with the pattern of glucose metabolism by aerobic mycelia (Table 4), indicated that cells maintained an essentially fermentative energy metabolism when shifted from anaerobic to aerobic conditions.

TABLE 5. *Glucose metabolism by mycelial cells grown in Nitrogen*[a]

Metabolic fate of glucose	Minimal medium		Complex medium	
	U/g	%	U/g	%
Assimilated	216.0	100	118.0	100
Catabolized	231.9	107	119.9	100
EMP pathway	168.5	73	105.9	88
PP pathway	63.4	27	14.0	12
Anabolized				

[a] All notations as described in footnotes to Table 3.

DISCUSSION

We have shown that mycelial cells grown in an anaerobic environment have a typical yeast fermentative metabolism. When yeast cells are shifted to an aerobic environment, the mycelia that develop have a fermentative metabolism, in terms of the flux and distribution of glucose carbon through the EMP and PP pathways. Furthermore, aerobic yeast cells grown in the presence of dbcAMP also maintain a fermentative metabolism. We conclude from these observations that in *Mucor* the morphological conversion of yeast to mycelia can occur without a radical change in catabolic carbon metabolism. This observation is consistent with the earlier observations of Paznokas and Sypherd (12) and Rogers et al. (13). The study reported here also shows that the yeast form is consistently correlated with a high flux of glucose carbon through the EMP and PP pathways, whereas the mycelial form does not show a consistent pattern in catabolic carbon metabolism. Since there is little difference in the flux or distribution of glucose carbon in comparably grown yeast and mycelial cells, catabolic intermediates and enzymes are probably less important than end products in the regulation of morphogenesis.

Many studies of *Mucor* dimorphism indicate that carbon dioxide is a potent morphogenetic signal that promotes yeast development (2, 3, 14, 15). Rogers et al. (13) noted, for example, that the yeast form of *M. genevensis* was always correlated with a high carbon dioxide concentration in the medium. The culture system we used

TABLE 6. *Glucose metabolism by cells 5 h after shift from nitrogen to air: effect of dbcAMP*[a]

Metabolic fate of glucose	+dbcAMP		−dbcAMP		Control (N_2)	
	U/g	%	U/g	%	U/g	%
Assimilated	93.3	100	103.6	100	152.1	100
Catabolized	92.7	99	98.3	95	165.5	109
EMP pathway	74.0	79	79.6	77	138.4	84
PP pathway	18.7	20	18.7	18	27.1	16
Anabolized	0.6	1	5.3	5		

[a] All notations as described in footnotes to Table 3.

to grow anaerobic mycelia may have facilitated the growth of mycelia by decreasing the carbon dioxide partial pressure (trapped KOH). Accumulation of a volatile factor associated with mycelial growth (10) may have been facilitated by sealing the culture. Currently, we are investigating the carbon dioxide regulation of certain decarboxylases that may be involved in the development of mycelia. The yeast requirement for a fermentable hexose may reflect the regulatory role of carbon dioxide; i.e., the yeast form develops when there is a high flux of carbon through the glycolytic and pentose phosphate pathways and a consequent high production of ethanol and carbon dioxide. The reason that certain potential substrates for yeast cells (e.g., maltose, cellobiose, and pentoses) cannot be fermented by *Mucor* may be that the rate of transport or cleavage of these compounds is not sufficient under anaerobic conditions to maintain the necessary high flux of carbon. We are also currently investigating the role of substrate transport in the regulation of morphology.

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LITERATURE CITED

1. **Barnett, J. A.** 1976. The utilization of sugars by yeasts. Adv. Carbohydr. Chem. Biochem. **32**:126-228.
2. **Bartnicki-Garcia, S.** 1968. Control of dimorphism in *Mucor* by hexoses: inhibition of hyphal morphogenesis. J. Bacteriol. **96**:1586-1594.
3. **Bartnicki-Garcia, S., and W. J. Nickerson.** 1962. Induction of yeastlike development in *Mucor* by carbon dioxide. J. Bacteriol. **84**:829-840.
4. **Bartnicki-Garcia, S., and W. J. Nickerson.** 1962. Nutrition, growth, and morphogenesis of *Mucor rouxii*. J. Bacteriol. **84**:841-858.
5. **Dawes, E. A., D. J. McGill, and M. Midgley.** 1971. Analysis of fermentation products. Methods Microbiol. **6A**:53-215.
6. **Herbert, D., P. J. Phipps, and R. E. Strange.** 1971. Chemical analysis of microbial cells. Methods Microbiol. **5B**:1266-1270.
7. **Hungate, R. E.** 1969. A roll tube method for cultivation of strict anaerobes. Methods Microbiol. **3B**:117-132.
8. **Larsen, A. D., and P. S. Sypherd.** 1974. Cyclic adenosine 3',5'-monophosphate and morphogenesis in *Mucor racemosus*. J. Bacteriol. **117**:432-438.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
10. **Mooney, D. T., and P. S. Sypherd.** 1976. Volatile factor involved in the dimorphism of *Mucor racemosus*. J. Bacteriol. **126**:1266-1270.
11. **Paveto, C., and S. Passeron.** 1977. Some kinetic properties of *Mucor rouxii* phosphofructokinase. Effect of cyclic adenosine 3',5'-monophosphate. Arch. Biochem. Biophys. **178**:1-7.
12. **Paznokas, J. L., and P. S. Sypherd.** 1975. Respiratory capacity, cyclic adenosine 3',5'-monophosphate, and morphogenesis of *Mucor racemosus*. J. Bacteriol. **124**:134-139.
13. **Rogers, P. J., G. D. Clark-Walker, and P. R. Stewart.** 1974. Effects of oxygen and glucose on energy metabolism and dimorphism of *Mucor genevensis* grown in continuous culture: reversibility of yeast-mycelium conversion. J. Bacteriol. **119**:282-293.
14. **Schulz, B. E., G. Kraepelin, and W. Hinkelmann.** 1974. Factors affecting dimorphism in *Mycotypha* (Mucorales): a correlation with the fermentation/respiration equilibrium. J. Gen. Microbiol. **82**:1-13.
15. **Sissions, C. H.** 1976. Improved technique for accurate and convenient assay of biological reactions liberating $^{14}CO_2$. Anal. Biochem. **70**:454-462.
16. **Terenzi, H. F., E. Roselino, and S. Passeron.** 1971. Two types of pyruvate kinase in *Mucor rouxii*. Changes in the enzymatic pattern related to aerobic development. Eur. J. Biochem. **18**:343-350.
17. **Terenzi, H. F., and R. Storck.** 1969. Stimulation of fermentation and yeast-like morphogenesis in *Mucor rouxii* by phenethyl alcohol. J. Bacteriol. **97**:1248-1261.
18. **Wang, C. H.** 1972. Radiorespirometric methods. Methods Microbiol. **6B**:185-230.
19. **Wood, W. A.** 1971. Assay of enzymes representative of metabolic pathways. Methods Microbiol. **6A**:411-424.