SYMPOSIUM ON BIOCHEMICAL BASES OF MORPHOGENESIS IN FUNGI[1]

III. Mold-Yeast Dimorphism of *Mucor*

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Introduction

In the development of many microscopic fungi, there are simple but well-defined examples of morphological differentiation which are amenable subjects for studying biochemistry of morphogenesis. In some of these cases, besides the convenience of rapid and facile cultivation in the laboratory, there is the important attribute of an exogenously controlled morphogensis. Since cells are in direct contact with the environment, a close and reproducible control of form development is possible through proper adjustment of specific chemical or physical factors of the external milieu. Furthermore, if a well-defined and specific chemical agent is found to affect morphogenesis, it immediately constitutes a reliable tool to probe the biochemical machinery of the cell, in a guided search for processes underlying form development.

The mold-yeast dimorphism exhibited by numerous species of fungi is one such example of elementary morphological differentiation. In response to a given environment, a fungus may grow in the characteristic manner of a mold, forming long, cylindrical, branched cells (mycelium). (The terms filamentous, mycelial, and moldlike are used equivalently.) In another environment, the same fungus may grow in the manner typical of a yeast, forming individual spherical or ellipsoidal cells which multiply by budding. Under intermediate environmental conditions, a gamut of transition forms is possible. Dimorphism may be experimentally controlled by changing a single factor in the environment. For instance, yeastlike development in the human pathogens *Blastomyces dermatitidis* and *Sporotrichum schenckii* may be induced by increasing temperature of incubation (21) or pCO_2 (11), respectively.

For our studies on biochemical bases of dimorphism, a strain of the saprophytic fungus *Mucor rouxii* was chosen. Detailed accounts of much of this work have appeared (4, 5, 6, 7). The present paper summarizes the results obtained and further extends the discussion thereof.

Historical Notes

Mold-yeast dimorphism of *Mucor* has historic interest for the microbiologist. The phenomenon was used last century to dispel ideas on species transmutation and to illustrate, in a most convincing manner, the ready capability of microorganisms to adapt to different environments by modifying their cellular constitution.

The morphological variability of *Mucor* was noticed over a century ago (8). At that relatively early stage in microbiology, the presence of drastically different morphologies in cultures of fungi led to erroneous interpretations. Thus, in 1857, Bail (2) described what appears to be the first clear example of mold-yeast dimorphism in fungi. He observed that saccharine cultures of *Mucor* (*M. racemosus?*) contained numerous

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spherical cells which multiplied by sprouting more spherical elements; he then concluded that the budding cells were ordinary brewers' yeasts, and that *Hormiscium cerevisiae* (former name for *Saccharomyces cerevisiae*) and *Mucor* were developmental stages of the same fungus. In 1861, Pasteur tacitly rejected such possibility of species transmutation, and some years later (27), on confirming Bail's observations, he provided a different interpretation. Accordingly, the morphology of the mold *Mucor* became yeastlike as a result of oxygen deprivation. On the surface of liquid cultures, where air was plentiful, the fungus developed ordinary mycelial turfs, whereas in the depth of the same cultures, where aeration was insufficient, the fungus assumed budding yeastlike shapes and conducted a strong alcoholic fermentation. Pasteur saw in *Mucor* a clear example of the capacity of an organism to adapt itself to a modified environment by modifying its own living machinery, an example which was more dramatic than that of *S. cerevisiae*, since in *Mucor* adaptation to "la vie sans l'air" involved conspicuous morphological as well as metabolic modifications.

Pasteur was neither the first nor the only one to interpret Bail's discovery correctly. Reess (28) and Fitz (12) had independently reached similar conclusions. About the same time, Brefeld (9) cultivated *M. racemosus* in the absence of oxygen under an atmosphere of hydrogen, and obtained filamentous, rather than yeastlike, cells. He concluded that formation of yeastlike cells was not caused by absence of oxygen but was due to the accumulation of metabolic CO_2 in the culture vessels. He considered the acidic properties of CO_2 to be responsible for the morphological effect. Klebs (20) was skeptical of Brefeld's views on the role of acidity in yeastlike development and showed that yeastlike cells were formed by *M. racemosus* in neutralized grape juice, provided air was completely excluded. Wehmer (34), examining cultures of *M. javanicus* and *M. racemosus*, found that absence of air was also the most important condition for the appearance of yeastlike growth. However, he pointed out that the yeastlike morphology of *Mucor* was not necessarily linked to its fermentative capacity, since both filamentous and yeastlike forms of the above-mentioned cultures produced similar amounts of ethanol. Furthermore, he showed that a strong alcoholic fermentation could occur in the presence, as well as in the absence, of air. Ritter (30) made a detailed study of mycelial fragmentation of *M. spinosus* and *M. racemosus*, concluding that three conditions were necessary for formation of spherical cells: (i) presence of sugar, (ii) absence of oxygen, and (iii) acid reaction of the medium. However, he showed that spherical cells could also be obtained in aerated, sugar-free peptone medium by the combined action of citric acid (0.5%) and NaCl (9.5%). It must be pointed out that Ritter's observations do not necessarily pertain to the formation of truly yeastlike cells, since he did not observe budding in these spherical cells; in fact, he considered budding to be a secondary process for which the presence of minute amounts of oxygen were necessary. By incubating cultures of *M. guilliermondii* under an atmosphere of CO_2, Nadson and Philippov (24) were able to obtain cultures composed entirely of yeastlike cells. They suggested that the effect of CO_2 was due to its acidic properties, and that organic acids such as tartaric acid could be substituted. It must be pointed out that the above-mentioned claims for a role of acidity in promoting yeastlike development of *Mucor* were made at a time when the concept of pH was either unknown or neglected.

In 1930, Lüers, Kühles, and Fink (22) undertook a comparative determination of some metabolic processes of filamentous and yeastlike forms of *M. guilliermondii*. Although some appreciable differences were detected in rates of respiration, fermentation, and several enzymatic activities, the authors did not elaborate on how the observed differences might be related to form development.

ENVIRONMENT AND FORM: ATMOSPHERIC CONTROL OF MORPHOGENESIS

From the foregoing historical notes, it is evident that the atmosphere of incubation plays a preponderant role in determining vegetative morphogenesis of *Mucor*. Yet, the diverse and apparently conflicting claims for the role of anaerobiosis, CO_2, acidity, and other agents create a confusing picture as to the nature of the inducer of yeastlike development.

The first objective in our studies of biochemistry of dimorphism of *Mucor* was the unequivocal determination of the inducer of yeastlike development, for which purpose the

selected organism *M. rouxii* was grown under conditions which permitted a strict control of the atmosphere of incubation. Shake liquid cultures were employed, and known and constant atmospheres were maintained by continuously flushing the gas under test during the entire incubation period (4). By this procedure, two atmospheric factors, CO_2 and O_2, were found to control dimorphism of *M. rouxii*. Carbon dioxide induces yeastlike development; oxygen inhibits it. Under an inert atmosphere (N_2), the fungus develops in the filamentous form; increasing tensions of CO_2 progressively shift morphogenesis from filamentous to yeastlike. At a pCO_2 of 0.3 atm (30% CO_2 + 70% N_2), or higher, cultures consisted exclusively of yeastlike cells. In the presence of air, however, the effect of CO_2 is annulled. Thus, at a pCO_2 of 0.5 atm in air (50% CO_2 + 50% air), cultures were mostly filamentous, and, in fact, only in the virtual absence of oxygen was filament formation completely prevented (4).

The effect of CO_2 was due to physically dissolved CO_2 and not to HCO_3^- (5).

Much of the confusion that exists in the literature concerning environmental control of yeastlike development stems from failure to distinguish arthrospores from true yeastlike cells. In the course of its development, the mycelium of *Mucor* reaches a point when fragmentation of hyphae appears inevitable. Under optimal growth conditions, *M. rouxii* attains this point about 12 hr after inoculation (4). Nonperforated septa are formed near the tips of filaments, and the resulting segments round off and eventually separate as individual spherical cells or chains thereof. Because of their mode of origin, these cells are termed "arthrospores" (a term preferred over "oidia"). After 2 days of incubation, a filamentous culture of *M. rouxii* may contain 10 to 20% (by weight) fragmented hyphae, but this fragmentary growth is not indicative of yeastlike development, since, by definition, yeastlike cells are those which originate and multiply by budding. Arthrospores and yeastlike cells of *M. rouxii* are further distinguished by the mechanisms which lead to their induction. Whereas yeastlike development is induced by CO_2 and inhibited by O_2, arthrospore formation is not dependent on these gases. Factors such as a high concentration of glucose (5) may stimulate increased arthrospore formation, but the initial inducer of arthrospore morphogenesis is as yet unknown. In conclusion, mere presence of spherical cells in a culture must not be taken as evidence of yeastlike development; a pattern of morphogenesis revealing *budding* of spherical cells must be observed. The relationship that exists among the different patterns of vegetative morphogenesis of *M. rouxii* is illustrated in Fig. 1. Notice that spore germination gives rise, initially, to spherical cells which may also be mistaken for yeastlike cells. This is especially true of heavily inoculated cultures in which a considerable number of spores do not germinate beyond the initial swelling into spherical elements (5).

Atmospheric control of dimorphism of *M. rouxii*, as described above, pertains to cultivation in complex yeast extract-peptone-glucose (YPG) medium at pH 4.5 with incubation at 28 C (4). Subsequently, studies were made to evaluate the influence on morphogenesis of some cultural variables, including temperature and time of incubation, surface vs. submerged cultivation, inoculum size, pH, quality and quantity of carbon sources, quality of nitrogen sources, and addition of various chemicals with reported action on morphogenesis of other fungi. All these variables were tested under three different atmospheres of incubation: air, N_2, and CO_2. Under this multiplicity of environments, the effects of the gaseous environment on morphogenesis were constant (except for some minor morphological modifications) and similar to that previously reported for YPG medium. Deviations were especially perceptible when the quality of nitrogen source was varied. Thus, use of individual nitrogen sources, such as threonine, serine, ammonium nitrate, etc , gave rise to a significant amount of filamentation in yeastlike cultures incubated under CO_2. Filament-free yeastlike development was observed only when a mixed amino acid diet was employed (3, 5).

A high concentration of glucose failed to stimulate yeastlike development of *M. rouxii*. However, it did enhance formation of arthospores (Fig. 2). This may explain the rather common notion that a high sugar concentration favors yeastlike growth of *Mucor* (1, 15, 30). Likewise, cultivation under acid pH or in the presence of carboxylic acids, reportedly stimulatory of

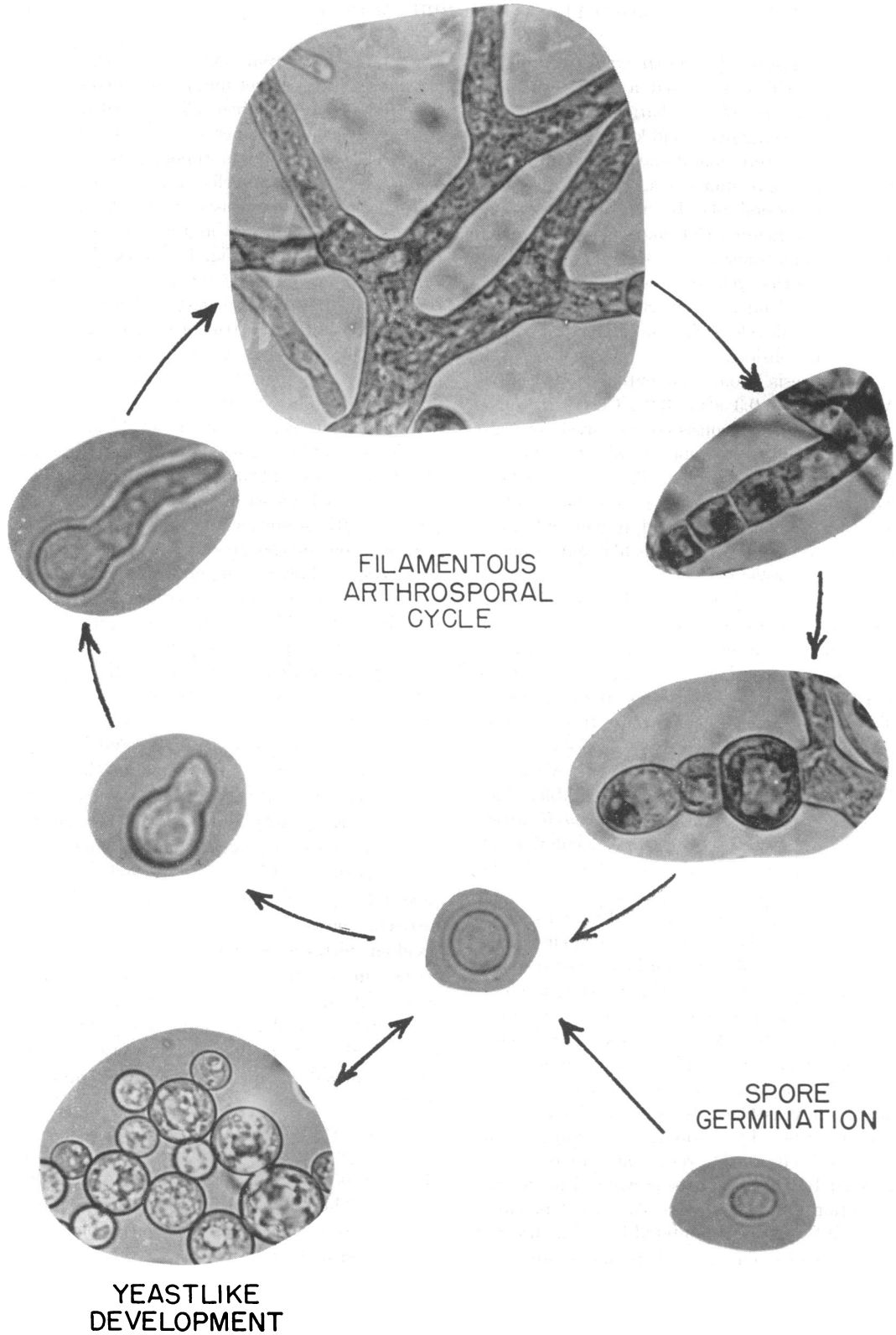

FIG. 1. *Patterns of vegetative morphogenesis in Mucor rouxii* (*3*).

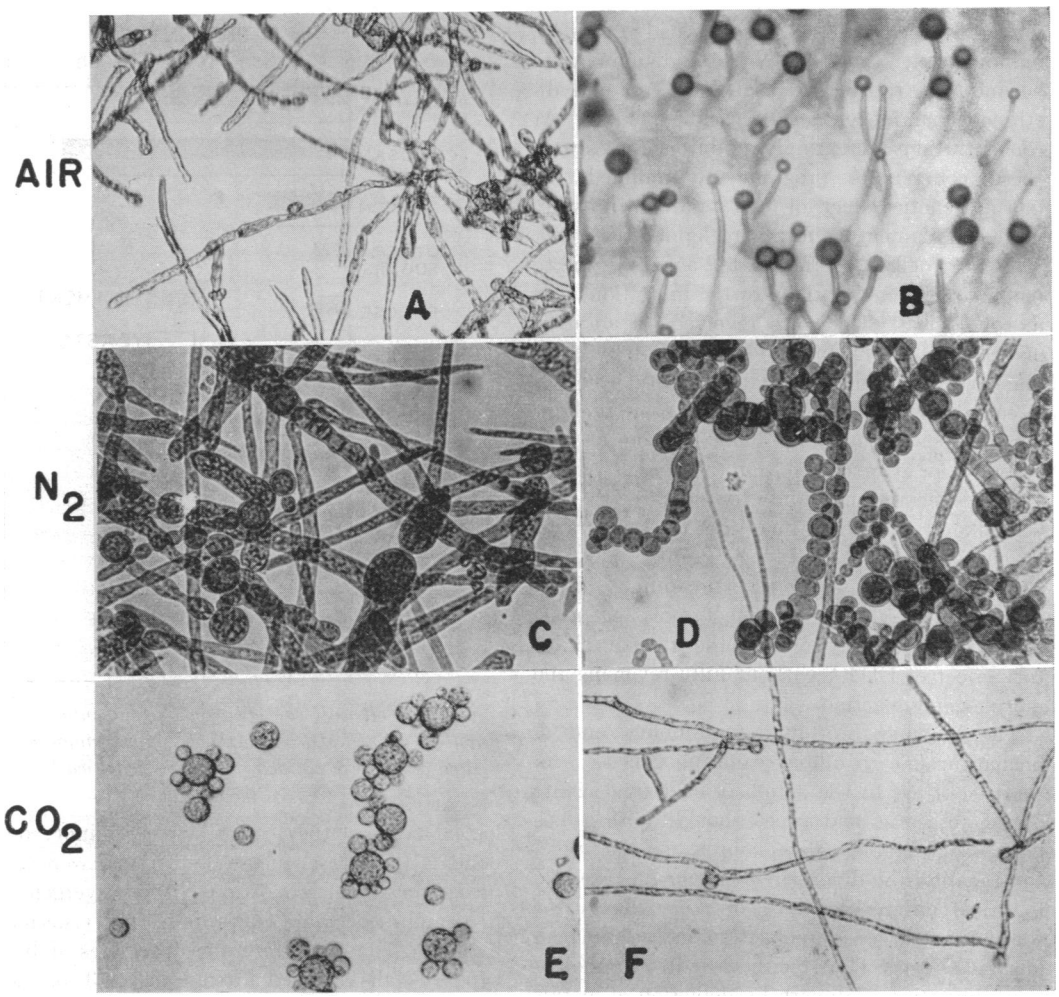

FIG. 2. *Environmental control of morphogenesis in Mucor rouxii. Cultures were made in yeast extract-peptone-glucose medium. The atmosphere of incubation is shown on the left. (A) Submerged filamentous development; (B) surface growth showing active sporogenesis; (C) filamentous mycelium obtained with low concentration of glucose (≦ 2%); (D) stimulation of arthrospore development by high concentration of glucose (10%); (E) yeastlike development; (F) inhibition of yeastlike morphogenesis by a chelating agent (DTPA). Magnifications: A, C, D, E, and F, 145 ×; B, 36 ×.*

yeastlike development of *Mucor* (9, 24, 30), was ineffective on *M. rouxii* (5).

A noteworthy by-product of these studies was the finding that anaerobic incubation under either N_2 or CO_2 greatly complicates the nutritional demands on the fungus. An exogenous supply of thiamine and nicotinic acid was essential for anaerobic growth of *M. rouxii*, though the fungus synthesized sufficient amounts of the two vitamins aerobically (3). Moreover, in the absence of O_2, carbon assimilation was restricted to hexoses, whereas aerobically a variety of organic compounds were utilized (5). The enhanced anaerobic nutritional demands, however, have no direct bearing on form development, since they were equally exhibited by filaments growing under N_2 and yeastlike cells growing under CO_2.

An examination of the action of metabolic inhibitors on *M. rouxii* (5) led to the discovery that morphogenesis was susceptible to control by chelating agents of the *N*-acetic acid type,

viz., ethylenediaminetetraacetic acid (EDTA), iminotriacetic acid (ITA), 1,2-diaminocyclohexane-*N*-*N'*-tetraacetic acid (CDTA), and diethylenetriaminepentaacetic acid (DTPA). At concentrations increasingly inhibitory for growth, these compounds progressively nullified the morphogenetic effect of CO_2, and cultures reverted to filamentous form development (Fig. 2). The effect of EDTA and related agents is due to metal chelation; both growth inhibition and morphological effect were reversed by addition of transition-group metal ions but not by alkaline-earth ions. The identity of the metal involved in each effect is still unknown, but some preliminary evidence suggests that depletion of zinc accounts for growth inhibition (5).

The concomitant inhibition of growth and yeastlike development may not be inexorably associated; other chelating agents, e.g., 8-hydroxyquinoline and *o*-phenanthroline, inhibited growth but did not affect morphogenesis. Moreover, zinc may reverse the growth-inhibitory effect of EDTA without fully reversing the morphogenetic action.

The inability of 8-hydroxyquinoline and *o*-phenanthroline to influence morphogenesis is in sharp contrast to the action of chelating agents of the *N*-acetic acid type, though both types have comparable metal-complexing activity. Presumably, the divergent behavior may be due to subtle differences in the relative affinity for specific metal ions involved in growth and in morphogenesis. Thus, with 8-hydroxyquinoline total inhibition of growth by depletion of metals required for growth may occur before the metal involved in morphogenesis is significantly complexed.

CHEMICAL AND ARCHITECTURAL BASES OF CELL MORPHOLOGY: CELL-WALL STRUCTURE

Mechanical rupture of filamentous or yeastlike cells of *M. rouxii* yielded cell walls, and fragments thereof, which retained the outline of the original cell. This indicates that a rigid cell wall determines morphology of both cellular types (6).

Cell walls of aerobic filamentous and yeastlike forms were prepared in a practically pure form; their comparative chemical composition is illustrated in Fig. 3. Three major cell-wall components, chitosan, chitin, and phosphate, were found in similar amounts in both morphological types. Other quantitatively minor components (galactose, fucose, and lipids) were also present in similar proportions. Major divergencies in composition were in protein, and especially mannose, content. Protein hydrolysates of both types of walls revealed a minimum of 13 common amino acids. No quantitative estimation of individual amino acids was made, but total protein content of yeast walls was considerably higher than that of filamentous walls. Mannose was present in the form of a mannan polysaccharide (mannan-protein complex?) in both types of cell walls but, whereas the walls of filamentous cells contained only small quantities, it was 5.6 times more abundant in walls of yeastlike cells (6).

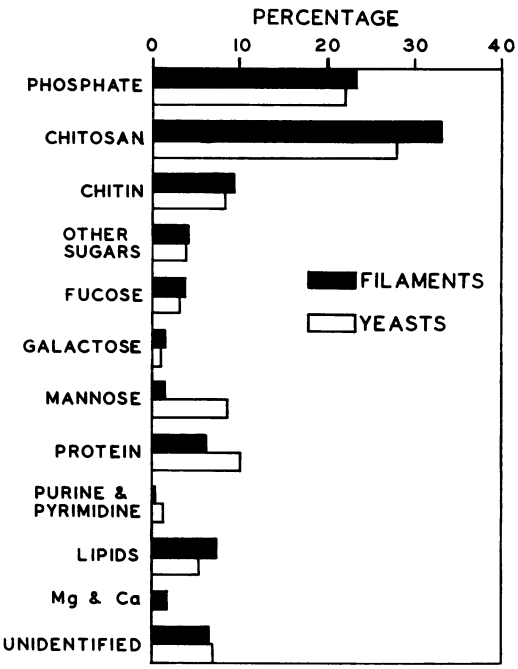

FIG. 3. *Cell-wall composition of filamentous and yeastlike cells of Mucor rouxii (6). Note: magnesium and calcium were not determined in yeast walls.*

In 1940, Garzuly-Janke (13) compared the polysaccharide composition of different species of yeasts and filamentous fungi and found that mannose was characteristically present in most species of yeasts but absent (or present in amounts too small to be detected by the relatively insensitive methods then employed) from all filamentous fungi studied. It is therefore of interest that within one organism, *M. rouxii*, the

same observation is valid; conversion from filamentous to yeastlike development occurs with a considerable augmentation of mannan content, thus strongly suggesting a morphogenetic role for this component.

Electron microscopy of ultrathin sections of whole cells of *M. rouxii* (6) revealed a great disparity in thickness and fine structure of walls of the yeastlike form as compared with filamentous walls (Fig. 4). Walls of the filamentous form are thin envelopes, about 0.05 to 0.1 μ thick, and apparently single layered. They contrast sharply with the walls of yeastlike cells, which are approximately five to ten times thicker and visibly double layered.

No individual microfibrils were discerned in sections of filamentous walls; seemingly these walls are compact structures of tightly packed elements. In contrast, individual microfibrils were readily seen, especially in the outer layer of yeast walls. The inner layer which accounts for most of the thickness exhibits a spongy texture. In over-all appearance, the yeast wall gives the impression of being a loosely packed envelope.

Calculations based on electron microscopic data revealed that the average volume occupied by the wall is 5 to 10% for filamentous cells and 30 to 40% for yeast cells. On a weight basis, these differences are much smaller. The wall of yeastlike cells comprises 18% of the total dry weight of the cell, and that of filamentous cells is 14%. This proximity of weight values readily indicates that, despite differences in volume and shape, the filamentous and yeastlike forms of *M. rouxii* accomplish a similar net synthesis of cell wall, therefore suggesting that differences in cell-wall morphology cannot be interpreted in terms of any substantial inhibition of over-all cell-wall synthesis. More likely, the observed difference in cell-wall structure originates from different manners of deposition of cell-wall polymers.

Biochemical Mechanisms Mediating Induction of Morphogenesis: Mode of Action of CO_2

What metabolic changes are induced by anaerobic incubation in the presence of a high pCO_2 which cause the observed alterations in cell-wall structure and the corresponding yeastlike appearance? In an effort to answer this question, the effect of CO_2 on various physiological processes was investigated. Early experiments revealed that carbon dioxide does not operate by inhibition of growth processes. In fact, the amount of growth (measured as dry weight) obtained in the presence of high tensions of CO_2 may be higher than that obtained anaerobically in the absence of CO_2 (4, 5). Similarly, studies on the anaerobic dissimilation of glucose indicated that no major changes resulted from

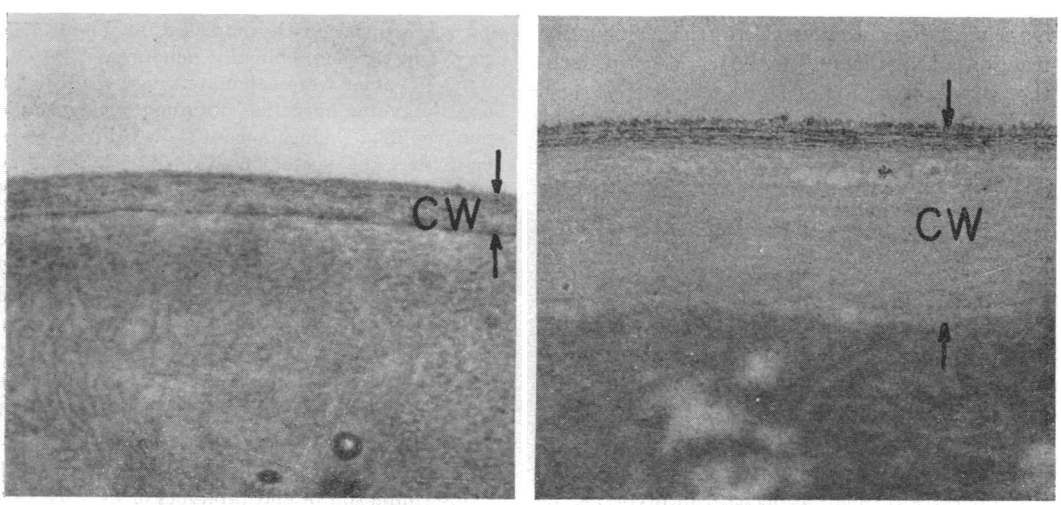

FIG. 4. *Electron micrographs of ultrathin sections of Mucor rouxii, stained with OsO_4, showing details of cell-wall structure in a filamentous hypha (left) and a yeastlike cell (right). Magnifications: 50,000 × and 53,000 ×, respectively.*

TABLE 1. *Effect of the atmosphere of incubation on metabolism of glucose by Mucor rouxii**

Atmosphere	Microscopic appearance	Cellular dry wt	Cellular nitrogen	Glucose metabolized		Economic coefficient†	Ethanol	
				Amount	Percentage		Amount	Percentage‡
		mg	%	*mg*			*mg*	
Air	Filamentous	547	9.6	1,800	35.2	30.4	40.1	2.9
N_2	Filamentous	94	10.0	1,620	31.7	5.8	509.5	41.0
CO_2	Yeastlike	64	9.7	1,120	21.8	5.7	447	47.0

* Cultures were incubated in 1-liter flasks at 28 C with continuous agitation for 24 hr. All determinations refer to 250 ml of culture medium. Culture medium contained the following ingredients per liter of distilled water: glucose, 20 g; KH_2PO_4, 3.0 g; $(NH_4)_2SO_4$, 2.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $ZnSO_4 \cdot 7H_2O$, 1.8 mg; $FeSO_4 \cdot 7H_2O$, 1.0 mg; $MnSO_4 \cdot H_2O$, 0.3 mg; $CuSO_4 \cdot 5H_2O$, 0.4 mg; thiamine, 2.0 mg; nicotinic acid, 2.0 mg. Initial pH was 4.5.

† Economic coefficient $= \frac{\text{cellular dry weight} \times 100}{\text{glucose metabolized}}$.

‡ Percentage of utilized glucose converted into ethanol.

incubation in an atmosphere of pure CO_2. Anaerobic filaments (incubated under N_2) and yeastlike cells (under CO_2) metabolized glucose with virtually the same efficiency (measured as dry weight of cellular substance synthesized per unit amount of glucose consumed) and converted nearly half of it into ethanol (Table 1). Likewise, manometric measurements of anaerobic fermentation showed that carbon dioxide evolution was independent of pCO_2. Similar Q_{CO_2} values were found throughout the pCO_2 range tested of 0 to 1 atm (Table 2), thereby disproving the possibility that a high tension of CO_2 affects morphogenesis through a mass inhibition, or reversal, of decarboxylation reactions. Experimental evidence also ruled out the possibility that CO_2 operates by mere acidification of the external milieu (5). Although internal changes in pH may occur as a result of incubation under a high pCO_2, the fact that the morphogenetic action of CO_2 can be reversed by chelating agents makes unlikely a mechanism based on pH.

In summary, it seems that carbon dioxide induces a drastic alternation in morphology of *M. rouxii* with little or no effect on major physiological processes. The metabolic changes underlying the difference in morphogenesis are probably more specific.

Studies on assimilation of CO_2 suggested that CO_2 operates through a mechanism involving its fixation into cellular metabolites (7). In support of this hypothesis was the observation that the quantitative effects of pCO_2 on morphology of *M. rouxii* and on carbon dioxide assimilation were strikingly similar; assimilation of CO_2 and extent of yeastlike development were nearly equal functions of pCO_2 (Fig. 5).

Determination of end products of CO_2 fixation at optimal pCO_2 (0.3 atm) revealed that two-thirds of the assimilated CO_2 was incorporated into protein components, chiefly as aspartic

TABLE 2. *Influence of pCO_2 on the over-all decarboxylation activity of Mucor rouxii*

Initial pCO_2*	Q_{CO_2}†	
	Filaments	Yeasts
0.00	124	122
0.05	114	126
0.10	102	125
0.25	132	128
0.50	128	138
1.00	122	149

* The initial pCO_2 of each Warburg flask was adjusted by introducing mixtures of CO_2 and N_2 with a total pressure of 1 atm. After 2 hr of incubation, the initial pCO_2 increased by about 0.04 atm owing to metabolically generated CO_2.

† Q_{CO_2} values were calculated on a dry weight basis by averaging values obtained during 2 consecutive hr. Endogenous CO_2 evolution was negligible. The system contained 2.85 mg (dry weight) of cells, 311 μmoles of glucose, and 44 μmoles of KH_2PO_4. Initial pH was 4.5; temperature was 30 C. Filamentous mycelium was harvested from a culture incubated under N_2 for 12 hr. Yeastlike cells were from a culture incubated under CO_2 for 12 hr.

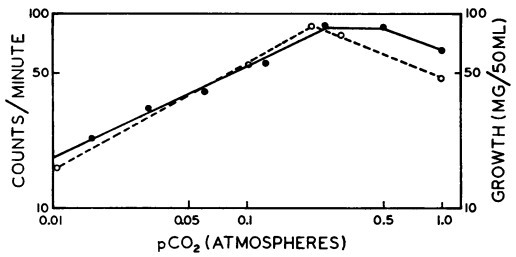

FIG. 5. *Comparative effect of pCO_2 on yeastlike growth (broken line) and $C^{14}O_2$ assimilation (solid line). Data from references 3 and 5.*

acid (75%) and, to a smaller extent, as glutamic acid and threonine (7).

Kinetic studies of Walker and collaborators (32, 33) on the effect of pCO_2 on carboxylation enzymes of higher plants indicated that the two principal systems of CO_2 fixation into C_4 dicarboxylic acids—that is, malic enzyme and phosphoenolpyruvate carboxylase—behaved quite differently. Malic enzyme is most active under relatively high tensions of CO_2, and its optimum lies at a pCO_2 of about 0.3 atm; in contrast, phosphoenolpyruvate carboxylase shows its maximal activity at a pCO_2 of about 0.005 atm and is progressively inhibited at higher pCO_2 values. Interestingly, the kinetics of malic enzyme bear sufficient resemblance to the kinetics of CO_2 assimilation (7) to justify the suggestion that assimilation of CO_2 by *M. rouxii* incubated at a high pCO_2 occurs via a malic enzyme system; this suggestion is consistent with the finding that the main product of CO_2 fixation is a C_4 dicarboxylic acid.

HYPOTHESES

Although our search for a biochemical interpretation of mold-yeast dimorphism of *Mucor* has merely begun, the information thus far collected, analyzed in the light of some preexisting knowledge and complemented with a few assumptions, may be used to construct a working hypothesis which establishes a surprising (and, hopefully, correct) relationship among the various experimental findings.

First, let us examine some cellular aspects of mold-yeast dimorphism. Upon shift from filamentous to yeastlike development, protoplasmic synthesis of *M. rouxii* does not necessarily change intensity; it merely changes direction. (Protoplasm is used in its broadest meaning encompassing all cellular components.) Thus, whereas in filamentous development protoplasm is preferentially deposited in the direction of elongation (polarized growth), in yeastlike development growth appears to lack orientation and the cell expands uniformly in all directions (nonpolarized growth). Time-lapse photographs comparing the two types of development vividly illustrate such mechanical differences in cell development (4). Periodically, during vegetative growth of *Mucor* by either elongation or uniform expansion, a point is reached whereupon new centers of morphogenesis are created more or less perpendicularly to the cell surface; incipient protuberances are formed which, continuing the original pattern of development, give rise to either lateral branches or spherical buds. Hence, hyphal branches and yeastlike buds, although morphologically different, may be considered physiologically equivalent. The cellular mechanisms determining appearance of new centers of morphogenesis may be similar, if not identical, for both morphological types, and probably involve the action on the cell wall of enzymatic processes equivalent to those described by Nickerson and Falcone (25, 26) for budding initiation in *S. cerevisiae* and *Candida albicans*.

With the idea that growth polarization is the crucial difference in mold-yeast dimorphism, and after taking into consideration previous hypotheses on plant cell growth (16) which suggest that growth of a cell results primarily from growth of its cell wall and furthermore that cell wall expansion is driven by internal osmotic pressure, one may formulate three fundamental problems of biochemistry of dimorphism: (i) the elucidation of the mechanism which polarizes cell-wall growth in filamentous development; (ii) how this polarized cell-wall growth, driven by internal osmotic pressure, fabricates a tubular envelope; and (iii) how anaerobic incubation under CO_2 abolishes polarization.

It has long been known that elongation of fungal hyphae takes place by tip growth (29). Consequently, the polarized development of molds is achieved by confining the regions of active growth to the hyphal tips. The fastest rate of cell-wall synthesis occurs precisely in the apex of a filament, and progressively diminishes toward the base of the tip where elongation ceases completely (10). The mechanism by which cell-wall formation is localized to the tip is not

clear, but two possibilities are open to consideration. First, the cell wall may contain, epigenetically, in its macromolecular constituents, all information necessary to accomplish its own synthesis (polymerization) and to determine, through the oriented deposition of individual polymers, its own shape; the cytoplasm may only act as a manufacturer of cell-wall precursors, and cytoplasmic streaming may insure an adequate supply at the tip. Maximal cell-wall synthesis occurs at the tip where macromolecules form part of a spherical or conical surface, and, somehow, in the topological transition from spherical to cylindrical orientation, net cell-wall synthesis fades out.

The second possibility is the existence of a cytoplasmic organelle, intimately associated with the tip and responsible for the synthesis of cell-wall macromolecules or precursors thereof. The suggestion has been made that some cytoplasmic organelles of unknown function, but intimately associated with the cell wall of bacteria, intervene in cell-wall synthesis (17). Also, organelles of unknown function have been observed directly underneath the cell wall of various fungi (23).

At any rate, it is not unreasonable to assume that development of hyphae involves an active morphogenetic mechanism which localizes or orients synthesis of cell-wall polymers. On the other hand, no such mechanism need be invoked to account for shape of yeastlike cells. Their remarkable sphericity suggests that shape is dictated merely by isotropic physical forces, such as osmotic pressure and surface tension, which of necessity determine a spherical cell. Consequently, the main postulate of our working hypothesis on the biochemistry of dimorphism is that yeastlike development results from the selective inhibition of, or interference with, the morphogenetic mechanism responsible for filamentous development.

The cell wall of the filamentous form of *M. rouxii*, for instance, may be considered as the end result of a complex coordination, in space and time, of numerous biochemical processes involved in the synthesis of cell-wall precursors, their transportation, and their orderly polymerization. Awareness of this amazing intricacy is sufficient evidence to postulate the existence of internal regulatory devices which maintain a harmonious operation despite variations in the external milieu. Control by selective permeability, enzyme repression (31), and feedback inhibition (14) is presently well established. However, the over-all regulatory capacity of a cell is not unlimited, and a large modification of the environment brings about metabolic changes which may ultimately affect one of the end products of biochemical operation, the cell wall. Thus, one may postulate that high concentrations of CO_2, a gas which readily penetrates the permeability barrier of a cell (23), may lead to excessive accumulation of fixation products which in turn upset the internal biochemical balance of filamentous mycelium. As proposed in the working hypothesis illustrated in Fig. 6, CO_2 assimilation at high pCO_2 results in a large accumulation of malic acid, which is metabolized (probably through oxaloacetic acid) and converted into aspartic acid, the main end product of CO_2 fixation. This increased aspartic acid content somehow stimulates the formation and accumulation of mannan-protein in the cell wall. The latter suggestion is consistent with the observation that the protein moiety of mannan-protein complexes isolated from yeast cell walls is

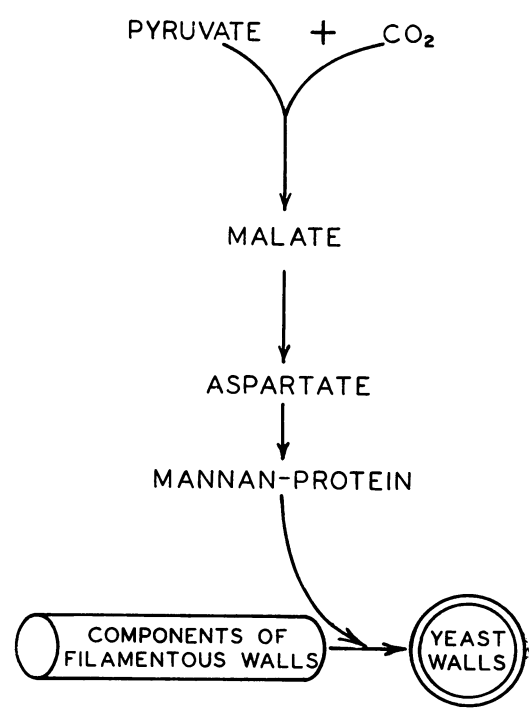

FIG. 6. *Working hypothesis on the mechanism of induction of yeastlike development in Mucor rouxii.*

conspicuously rich in aspartic acid (19). Since in *M. rouxii* the conversion from a filamentous to a yeastlike pattern of development represents mainly a marked increase in the cell-wall content of mannan and protein, one may further postulate that the increased presence of these macromolecules disrupts, or prevents, that orderly orientation of cell-wall polymers necessary for filamentous development. As a result, the growing cell acquires the spherical shape dictated by isotropic physical forces. In the above scheme, the inhibitory effect of O_2 on yeastlike development of *M. rouxii* is explained on the basis that, aerobically, operation of the tricarboxylic acid cycle may prevent any excessive accumulation of carboxylic acids resulting from CO_2 fixation.

To conclude, mention is made of the fact that although most species of *Mucor* thus far examined require CO_2 for induction of yeastlike development, a strain of *M. subtilissimus* has been found in which mere anaerobic incubation sufficed to induce a purely yeastlike growth (4). The existence of a CO_2-independent yeastlike development has been construed to support the view that the action of CO_2 on *M. rouxii* is not a direct one; instead it involves the onset of metabolic changes which eventually lead to the formation of an internal inducer of yeastlike development common to all *Mucor* species and whose formation in *M. subtilissimus* does not require externally supplied CO_2 (5).

Experimental support for this thesis of basically similar mechanisms of yeastlike development in all *Mucor* species stems from the observation that EDTA equally reverses yeastlike morphogenesis in both CO_2-dependent and -independent strains (5).

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