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Mucor Dimorphism

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INTRODUCTION TO *MUCOR* SPP.

According to Ainsworth (3), the first illustrations clearly recognizable as representatives of the genus *Mucor* appeared in Robert Hooke's *Micrographia* in 1665 and in Marcello Malpighi's *Anatome Plantarum* in 1679. By 1838, the morphological variability of *Mucor* was being noted (11, 71), though for many years it was mistakenly believed that the filamentous form of the organism originated from *Saccharomyces* spp. by species transmutation (11). This erroneous notion was put to rest by Louis Pasteur and his contemporaries. In his classic *Études sur la Bière* published in 1876, Pasteur first implicated *Mucor* spp. as an important fungal contaminant of diseased beer (71). Pasteur noted the potent fermentative capacity of *Mucor* spp., described the dimorphic habit of *Mucor racemosus*, and related morphological conversions to the oxygen tension of the growth medium (71). Various studies from the late 19th through the mid-20th century (71) created a confusing picture with regard to the true environmental determinants of *Mucor* morphology, until the subject was resolved through the systematic investigations of Bartnicki-Garcia in the early 1960s (11).

The genus *Mucor* continues to be the focus of study by a considerable number of microbiologists and mycologists with a broad spectrum of interests in both the basic and applied areas of biological research. *Mucor* spp. are generally highly saccharolytic and proteolytic, resulting in their ubiquitous appearance as food spoilage agents. Meat, poultry, baked goods, dairy products, and citrus fruits often serve as a meal for the fungus (72, 107). Sometimes the metabolic action of *Mucor* spp. on a potential foodstuff is put to good use. These organisms are commonly used to produce wines fermented from rice in the Orient and ethyl alcohol in Europe (203). Strains have been domesticated to produce sufu and tempeh (fermented soybean food products), alpha-amylase, fusidic acid, and the proteolytic enzyme rennin, used in cheesemaking (6, 94, 97, 235). *Mucor* spp. are also associated with retting flax and hemp, ripening snuff, and decomposing leather (203).

Though *Mucor* spp. are usually not pathogens, several species are among the various zygomycetes identified as causing a type of opportunistic fungal infection called mucormycosis (8, 232). The level of severity of such an infection can vary from a relatively mild dermatophytic colonization of the skin, nailbed, or hair follicle (112, 125) to a deep systemic invasion of the pulmonary or central nervous systems via the nasopharynx (66, 125). Though the former condition is usually amenable to treatment with antiseptics or antibiotics (214), the latter is often refractory to even the most potent fungicides, such as amphotericin B, resulting in a lethal outcome (66). It is the hyphal form of *Mucor* spp. that is usually found associated with diseased tissue (66).

Members of the genus *Mucor* have been important experimental objects in studies of mating and biological regulatory mechanisms at the molecular level. The role of trisporic acid in zygospore formation was elucidated by using *M. mucedo* (84). *M. racemosus* has been employed to study the relationship between overall cellular growth rate, protein synthesis, and ribosome function (41d, 154, 189) as well as the coupling of RNA and protein synthesis during amino acid starvation (152). *M. rouxii* has served in the elucidation of the role of the chitosome in cell wall synthesis (15).

In most regards, *Mucor* spp. display properties typical of other zygomycetes. There is, however, one characteristic which makes several, but not all, *Mucor* spp. quite distinct from other zygomycetes, namely, the property of dimorphism. Though all *Mucor* spp. display a variety of differentiated hyphal morphologies, mainly associated with the production of arthrospores, sporangiospores, or zygospores, only those that can grow in the form of spherical multipolar budding yeasts are referred to as dimorphic. Examples of dimorphic *Mucor* spp. would include *M. racemosus*, *M. rouxii*, *M. genevensis*, *M. bacilliformis*, and *M. subtilissimus* (certain strains). Species of *Mucor* genetically constrained to a monomorphic existence include *M. mucedo*, *M. hiemalis*, *M. miehei*, *M. pusillus*, *M. rammanianus*, and *M. subtilissimus* (certain strains). Only two other genera of dimorphic

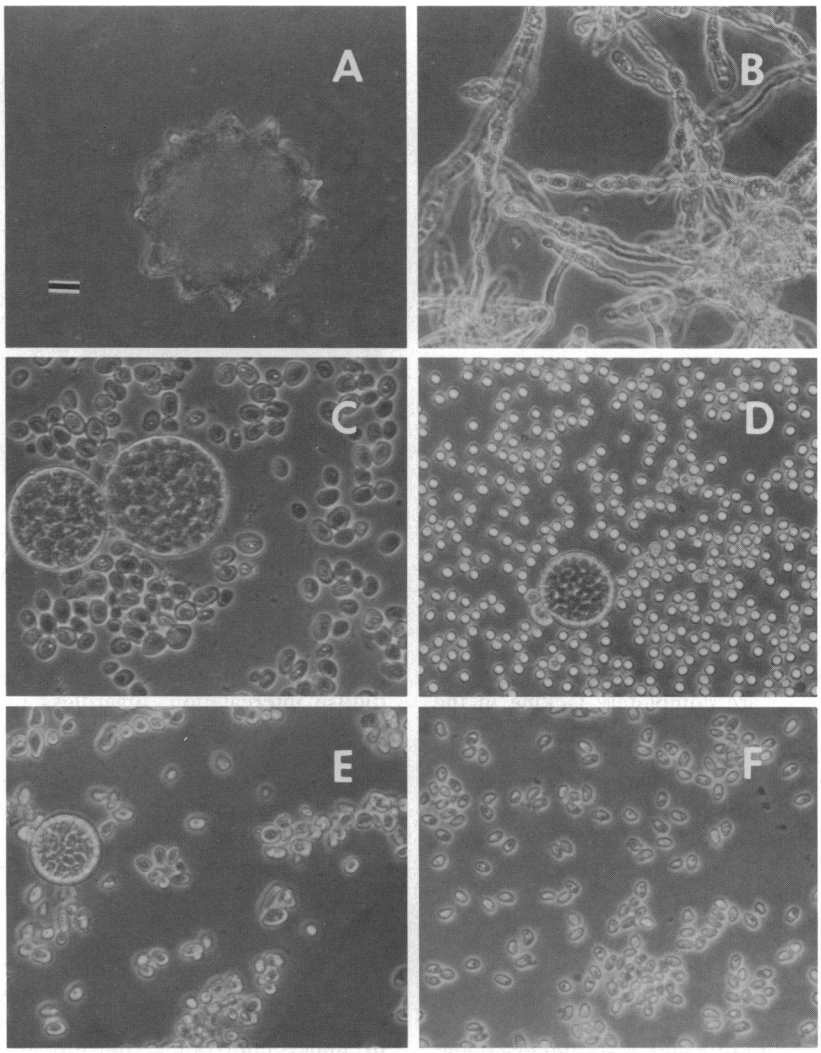

FIG. 1. Types of spores formed by *Mucor* spp. (A) Zygospore of *M. hiemalis*; (B) arthrospores of *M. racemosus*; (C) sporangiospores of *M. mucedo*; (D) sporangiospores of *M. genevensis*; (E) sporangiospores of *M. hiemalis*; (F) sporangiospores of *M. racemosus*. Large spherical structures in panels C through E are intact sporangia. All specimens are shown at the same magnification. Bar = 20 μm. Photomicrographs by Charles P. Chapman.

zygomycetes have been described. These are *Mycotypha* (90, 199) and *Cokoromyces* (108, 185), both within the order *Mucorales*.

MORPHOLOGICAL FORMS AND MORPHOGENETIC TRANSFORMATIONS OF *MUCOR* SPP.

Fungal morphogenesis in a cyclical process with no beginning or end. However, it is usually convenient to consider the spore as the point of reference. *Mucor* spp. are capable of constructing three different types of spores, each with its own raison d'être. The zygospore effects genetic recombination, the sporangiospore serves as a dispersal mechanism, and the arthrospore ensures survival under environmental duress.

The zygospore is a spiny, black, thick-walled structure (Fig. 1A) formed from the hyphal fusion of heterothallic or homothallic mating types. It remains dormant for many weeks to months before germinating to produce a single haploid germ sporangiophore with a terminal sporangium (80). The sporangiospores within this structure are predominantly uniparental in genetic makeup, prompting speculation about the selective forces that might account for such an inefficient mechanism of genetic exchange (106, 220). Since the zygospore arises only from hyphal thalli and gives rise only to a typical hyphal structure, it will not be dealt with any further here.

The sporangiospore is found in large numbers inside a sac (sporangium) at the terminus of an aerial hypha (sporangiophore). These structures are formed only on a solid substratum under an aerobic atmosphere. *Mucor* sporangiospores are characteristically ellipsoidal in shape, but their dimensions and mass can vary greatly depending on the species (Fig. 1C through F), *M. bacilliformis* displaying the smallest and *M. mucedo* the largest sporangiospores of the commonly studied taxons (208). The frequently studied *M. racemosus* constructs a sporangiospore midway between these extremes, being approximately 6 by 5 μm in size (212) and 9.6 pg in dry weight (208). The great majority (90 to 99%) of sporangiospores from *Mucor* spp. are uninucleate (208, 217);

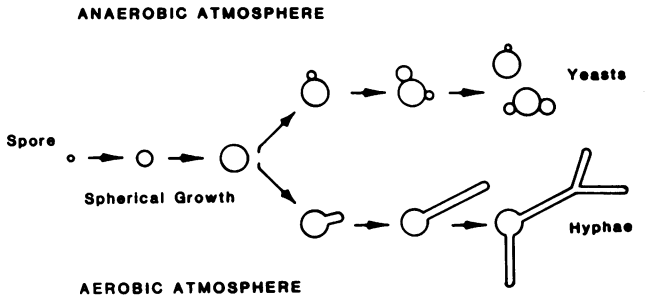

FIG. 2. Alternative morphogenetic fates of sporangiospores from dimorphic *Mucor* spp.

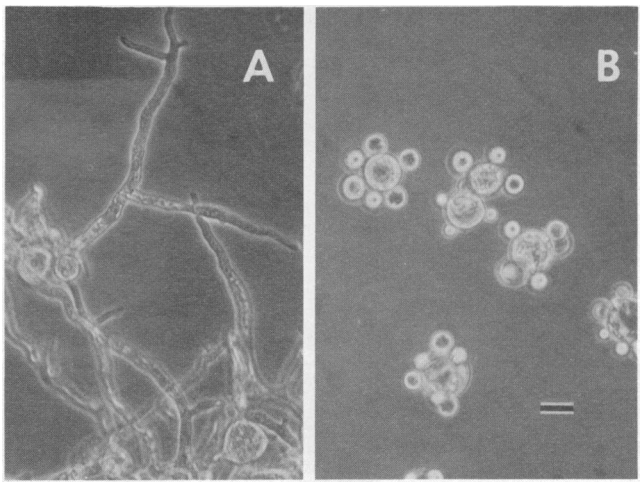

FIG. 3. Vegetative morphologies of dimorphic *Mucor* spp. Coenocytic hyphae (A) and multipolar budding yeasts (B) of *M. racemosus* are shown. All specimens are shown at the same magnification. Bar = 20 μm. Photomicrographs by Charles P. Chapman.

however, *M. mucedo* has recently been reported as a multinucleate exception (91). The outer wall of the sporangiospore is quite distinct from the vegetative cell wall both chemically and morphologically. It is relatively enriched in protein and lipid, and it contains large amounts of glucan and melanin, which are completely absent from yeast or hyphal forms of *Mucor* spp. (13). In contrast, the levels of chitin, chitosan, glucuronic acid, and phosphate are much reduced in sporangiospores relative to their levels in vegetative cells. Other components found in the walls of yeasts or hyphae (e.g., galactose and fucose) are completely lacking in the walls of sporangiospores (13).

Though it derives exclusively from the mycelial habit of *Mucor* spp., the sporangiospore is capable of developing into either the yeast or hyphal form upon germination (Fig. 2), the precise morphological direction taken being dependent only on the nutritional and gaseous environments (11, 23, 37, 212). This capability establishes *Mucor* spp. as unique among the commonly studied microbial models of development in that they are faced with a bifurcation in the morphogenetic sequence at which critical regulatory responses presumably direct the organism to construct one or the other of alternative morphologies. This property, together with the fact that macromolecular synthesis and accretion are obligatory accompaniments of cytological differentiation, makes *Mucor* sporangiospore germination perhaps a more valid model of metaphyte and metazoan morphogenesis than the more frequently studied sporulation systems in *Bacillus* spp. or *Saccharomyces cerevisiae*. Since large populations of cells can be made to undergo a fairly rapid and mostly synchronous morphogenesis in the *Mucor* system, it should prove more tractable than even the cellular slime mold *Dictyostelium discoideum* or the nematode *Caenorhabditis elegans*, frequently studied eukaryotic systems in which cytodifferentiation occurs but separation and collection of divergent cell lineages are cumbersome.

Whether the sporangiospore ultimately develops into a budding yeast cell or a hyphal germ tube, it first undergoes a common period of spherical growth (Fig. 2). This is not mere swelling due to rehydration but true logarithmic growth due to macromolecular synthesis (11, 23). The quantity and quality of macromolecules made at this time may be critical, because they presumably could determine or direct vegetative morphology. During spherical growth, new vegetative cell wall is synthesized underneath the existing spore wall. The composition of the vegetative wall is different from that of the spore wall and is specific to the incipient cell morphology. The spore wall stretches and cracks above the expanding germ sphere, its fragments adhering to the new surface but having no molecular continuity with it (23). The pattern of new-wall deposition during spherical growth is invariably diffuse intercalation, regardless of whether buds or germ tubes ultimately emerge from the germ sphere. Upon such emergence, new-wall deposition is uniformly dispersed in yeasts and apically localized in hyphae (18, 21). Both the bud wall and the germ tube wall represent a direct continuation of the germ sphere wall (117). The vegetative wall deposited is much thicker in spores developing as yeasts and differs in microfibrillar structure from spores converting to hyphae (11, 13, 23).

The arthrospore is the least studied, most poorly understood type of cell made by *Mucor* spp. (Fig. 1B). It is conjectured to serve essentially as a survival mechanism because it is formed after the cessation of logarithmic growth or under unfavorable nutritional or other environmental conditions (9, 24, 25). Arthrospores are formed in submerged cultures through septation of normally coenocytic hyphae and the deposition of a new three-layered wall beneath and distinct from the original hyphal wall (9). This septate chain ultimately fragments, releasing spherical cells which superficially resemble and are frequently mistaken for *Mucor* yeast cells (64). They critically differ from yeasts, however, in lacking buds and failing to accrete biomass. Virtually nothing is known about the physiology or metabolism of these spores. No systematic studies have been conducted to determine their resistance properties or the duration of their viability. Though they derive only from hyphae, arthrospores from dimorphic species of *Mucor* have the ability to germinate into either yeasts or hyphae depending on their environment. Such morphogenetic conversions have not yet been a focus of investigation.

Mucor hyphae (Fig. 3A) can develop from any of the spore types mentioned above and from *Mucor* yeasts. A typical hypha initially emerges from the spherical cell in the form of a short projection called a germ tube. Factors which may determine the site of emergence are not yet understood. The wall and plasma membrane of yeasts and sporangiospore-derived germ spheres remain ultrastructurally undifferentiated until germ tube formation commences (23). A multinucleate nonseptate tubular structure known as a coenocytic hypha develops from the germ tube by a process of apical

extension. The growing tip is the only site of new-cell-wall synthesis (18, 21) and is highly susceptible to osmotically induced rupture (20). Vesicles resembling the spitzenkörper of various ascomycetes and basidiomycetes reside just under the plasma membrane at the growing hyphal tip and have been postulated to play a role in new-wall deposition (22). Branching occurs at frequent but unpredictable intervals along the length of the hypha. Branching involves a transient departure from strict apical growth and, as such, may be considered a very simple case of cell morphogenesis. Elucidation of the mechanism controlling branching would therefore be of considerable interest. The length and width of the branches and the frequency of branching vary considerably for *Mucor* hyphae. Variable hyphal morphology displayed by *M. racemosus* has been related to the carbon source (212) and may be associated with the composition or geometry of the cell wall. The cell walls of *Mucor* hyphae are notably denser, less fibrous, and less differentiated into layers than those of *Mucor* yeasts (11).

Mucor yeasts (Fig. 3B) can develop from sporangiospores, arthrospores, and hyphae of dimorphic species (11, 212). They are not known to originate directly from zygospores. *Mucor* yeasts are rather large (approximately 20 μm in diameter), spherical, multinucleate cells that grow and propagate by the production of multiple buds. Buds appear to be initiated at random locations over the surface of the mother cell and sometimes number a dozen or more (11, 212). Bud detachment from the mother cell seems to be a random event following septum formation: a broad distribution of size is displayed for both free and attached buds. The cell walls in *Mucor* yeasts are much thicker, more diffuse, and more fibrous than the cell walls of *Mucor* hyphae. In addition, the yeast wall is differentiated into two discrete layers, whereas the hyphal wall is structurally uniform (11, 26). Deposition of new walls in *Mucor* yeasts is by diffuse intercalation: radiolabeled precursors appear to be inserted uniformly over the cell surface (18). The initial phase of yeast development from sporangiospores is indistinguishable from that described above for hyphal development from sporangiospores. The ellipsoidal spore increases manyfold in mass and girth, forming a spherical germ cell. The germ cell produces one or more buds rather than germ tubes under the appropriate environmental stimuli, which will be discussed in the next section. As in the case of germ tube formation, bud formation is not preceded by any visible cytodifferentiation. Factors that may specify the site of bud emergence in *Mucor* spores and yeasts are still a mystery. No formal description has been made of the process of yeast development from arthrospores. The formation of yeasts from hyphae involves spherical bud production on the sides and tips of the filaments, which themselves remain morphologically unchanged (212). Hyphal fragments persist as a significant portion of the cell population for a considerable time after the initiation of hypha-to-yeast morphogenesis. The resulting mixed-cell populations have dissuaded researchers from studying morphological conversions in this direction.

ENVIRONMENTAL FACTORS INFLUENCING *MUCOR* MORPHOGENESIS

Dimorphic species of *Mucor* are not all the same in their morphological response to the environment; however, there are two generalities that usually apply in this association: (i) a fermentable hexose is always required for growth in the yeast form (11, 212); and (ii) anaerobiosis generally favors growth in the yeast form, whereas an aerobic atmosphere usually induces growth in the hyphal phase (11, 212). A given set of environmental conditions will usually evoke the same morphological response from sporangiospores, arthrospores, and vegetative cells and on solid as well as liquid growth media (212).

Various workers have investigated a large number of *Mucor* species and strains with respect to their morphological responses to the environment (12, 24, 25, 64, 65, 78, 88, 145, 184). These investigators have found enough variation to preclude unqualified extrapolation of experimental results from one system to another (212). Though *M. rouxii* will not grow anaerobically in the yeast form without the presence of a fermentable hexose (24, 25), *M. racemosus* will not grow anaerobically at all unless a hexose is provided (212). High levels of hexose (>100 mM glucose) cause *M. genevensis* to grow in the yeast form even under an aerobic atmosphere (184).

In *M. rouxii* and *M. subtilissimus*, the morphological form of growth under anaerobiosis seems to stem from a complex balance between hexose concentration and the partial pressure of CO_2 in the culture fluid. At a low hexose concentration (0.1% glucose) under a nitrogen atmosphere, a high partial CO_2 pressure (pCO_2) (>0.3 atm) can induce growth in the yeast form, whereas at a high enough hexose concentration (8% glucose), growth occurs in the yeast form even under a 100% nitrogen atmosphere (12). In contrast, *M. racemosus* grows exclusively in the yeast form under a 100% nitrogen atmosphere at glucose concentrations ranging from 0.1 to 20% (145). Thus, CO_2 is not the critical effector of dimorphism in *M. racemosus* that it is in *M. rouxii*.

Any amount of oxygen introduced into cultures of *M. racemosus* growing under nitrogen, down to the detectable limit of 1.2 μM, causes the organism to grow exclusively in the hyphal form (175). Mooney and Sypherd (145) mistakenly attributed the prevalence of hyphal forms at low nitrogen-sparging rates to an endogenously synthesized volatile morphopoietic pheromone, but Phillips and Borgia (175) correctly identified the critical factor as trace amounts of contaminating oxygen diffusing through the Teflon tubing used to deliver catalytically reduced nitrogen. In contrast, continuous cultures of *M. genevensis* containing a steady-state concentration of <25 μM glucose and sparged with nitrogen-oxygen mixtures resulting in dissolved oxygen concentrations of 0.1 to 12.5 μM display a linear relationship between pO_2 and the percentage of hyphal forms in the culture (184). The effects of additional glucose easily override those of atmospheric composition in the *M. genevensis* system. In the *M. rouxii* system, which is comparatively unresponsive to glucose except at very high concentrations under pure nitrogen, the introduction of oxygen into a CO_2 atmosphere causes the production of hyphae at an incidence inversely proportional to the pCO_2 (24).

In addition to the morphopoietic agents oxygen, CO_2, and hexoses, which occur naturally in the external environment, a variety of synthetic compounds have been reported to alter *Mucor* cell morphology. Some of these have proven useful in studying the regulation of *Mucor* morphogenesis.

Substances that inhibit mitochondrial energy-generating functions such as electron transfer (e.g., potassium cyanide and antimycin A) and oxidative phosphorylation (e.g, oligomycin) lock *Mucor* spp., or their relatives the *Mycotypha* spp., into the yeast form under aerobic conditions (73, 199). Similarly, chloramphenicol, which inhibits protein synthesis on 70S ribosomes and thus prevents the production of cytochrome oxidase, cytochrome *b*, and other key components of the mitochondrion, constrains growth of *Mucor* spp.

to the yeast morphology in air (48, 238). Chloramphenicol exerts this effect most completely in *M. rouxii* and least effectively in *M. racemosus*.

Phenethyl alcohol (PEA) has been used to induce the aerobic growth of yeast forms in *M. rouxii* (219) and *M. genevensis* (85). It reportedly does so by stimulating alcoholic fermentation and inhibiting oxidative phosphorylation (219). However, the multiplicity of biological effects ascribed to PEA (177, 202) dictates caution in interpretation of these data.

EDTA and related chelating agents reportedly elicit the hyphal morphology of *M. rouxii* and *M. subtilissimus* under a CO_2 atmosphere (25). This effect is reversed by the addition of various transition-group metal ions but not by alkaline-earth metal ions. Another study disputes that the action of EDTA is due to its properties as a chelator, since a mineral-depleted medium could not mimic the EDTA effect (238). The EDTA effect has not been reported for *M. racemosus* or *M. genevensis*.

Cerulenin, an inhibitor of fatty acid synthetases, blocks conversion of *M. racemosus* yeasts to hyphae under air without stopping growth (105). This effect is overcome by the addition of Tween 80 (a mixture of fatty acids) to the medium. An analysis of lipid fractions suggested that the drug acts by preventing an increase in the turnover of phospholipids which may be essential to morphogenesis (105).

Cycloleucine is an inhibitor of *S*-adenosylmethionine (SAM) synthetase. When added to cultures of *M. racemosus*, it prevents the conversion of yeasts to hyphae under air (78). This observation, together with pool analyses and enzyme assays, suggested that elevated levels of SAM are required for the yeast-to-hypha morphogenesis (78).

Cyclic AMP is an important small regulatory molecule endogenously made within all cells (182). When added to cultures of *M. racemosus* (118), *M. rouxii* (167), or *M. genevensis* (70), it constrains growth to the yeast form regardless of atmospheric composition. This nucleotide is known to act primarily as an effector of protein kinases in eukaryotic cells (183), a fact which has given impetus to a line of research in *Mucor* spp. (147–149) that will be discussed in a later section of this review.

All of the morphopoietic agents with the capacity to induce aerobic growth in the yeast form can do so only if a hexose is present in the medium. If the carbon source is changed to a pentose, a disaccharide, or anything else, growth will occur only in the hyphal form. In many of the studies cited above, an elevated level of fermentative metabolism was noted in cells chemically induced to grow as yeasts under aerobic conditions, suggesting a linkage between alcoholic fermentation and yeast morphology. This is still an unresolved issue and will be discussed in the appropriate section below.

COMPOSITION AND CONSTRUCTION OF THE CELL WALL IN *MUCOR* YEASTS AND HYPHAE

S. Bartnicki-Garcia, J. Ruiz-Herrera, V. D. Villa, and their co-workers have made extensive contributions to our understanding of the cell wall and its role in *Mucor* dimorphism (13–22, 26, 27, 31, 35, 50, 53, 56–63, 68, 93, 117, 141, 142, 178, 191–193). Bartnicki-Garcia concisely stated the philosophy that has governed his approach to the study of *Mucor* morphogenesis: "In simplified terms, morphological development of fungi may be reduced to a question of cell wall morphogenesis" (13). In the last analysis, one must agree that whatever differential gene activations, regulatory mechanisms, or metabolic shifts play roles essential to morphogenesis, the ultimate focus of action must be the cell wall. Saying this, however, does not minimize the significance of studying the many other physiological and biochemical changes correlated with the total differentiated state of the cell. It may be a very different beast indeed that resides within the contours of a yeast cell rather than under a hyphal veneer. Furthermore, one cannot predict by what elaborate strategy a structure as complex as a fungal cell wall is assembled. Having identified the essential building blocks of *Mucor* cell walls (13), the aforementioned researchers have investigated the devices by which these building blocks are brought together.

TABLE 1. Chemical differentiation of the cell wall in the life cycle of *M. rouxii*[a]

Wall component	% Dry wt of cell wall			
	Yeasts	Hyphae	Sporangiophores	Spores
Chitin	8.4	9.4	18.0	2.1[b]
Chitosan	27.9	32.7	20.6	9.5[b]
Mannose	8.9	1.6	0.9	4.8
Fucose	3.2	3.8	2.1	0.0
Galactose	1.1	1.6	0.8	0.0
Glucuronic acid	12.2	11.8	25.0	1.9
Glucose	0.0	0.0	0.1	42.6
Protein	10.3	6.3	9.2	16.1
Lipid	5.7	7.8	4.8	9.8
Phosphate	22.1	23.3	0.8	2.6
Melanin	0.0	0.0	0.0	10.3

[a] Reproduced with permission from the *Annual Review of Microbiology*, Annual Reviews, Inc. (13).

[b] Not confirmed by X ray. Value of spore chitin represents N-acetylated glucosamine; chitosan is nonacetylated glucosamine.

Table 1 displays the comparative composition of cell walls from *Mucor* yeasts, hyphae, sporangiophores, and sporangiospores. The main structural components of both yeast and hyphal walls are chitin and chitosan fibrils embedded within a matrix of the polyuronides mucoran and mucoric acid, glycoproteins, and glycopeptides (26, 27, 57, 60). The contribution of the fibrillar components to the total mass of the cell wall is not much different in yeasts and hyphae. Neither is the amount of glucuronic acid, a major component of mucoran and mucoric acid, much different in yeasts and hyphae. The main compositional differences appear to be within the fractions containing mannose and protein, which are major constituents of the mucoran portion of the matrix. There is much less of this material in hyphae than in yeasts. In contrast, the contribution of fucose- and galactose-containing polymers is greater in hyphae than in yeasts (60). The large amount of phosphate present in about equal amounts in the walls of both vegetative forms is thought to neutralize the polycationic chitin and chitosan (212). How the observed alterations in cell wall chemistry may relate to the fine structure and overall geometry of the cell wall is not yet known. It is entirely possible that the chemical composition of the wall has nothing to do with the ultimate shape this structure assumes. The pattern or direction of polymer deposition may be paramount in this determination.

Electron microscopic examination of *Mucor* cells reveals that the walls of hyphae are relatively thin, dense, nonfibrous, and undifferentiated (Fig. 4A). In contrast, the walls of yeasts are relatively thick, diffuse, and fibrous and are

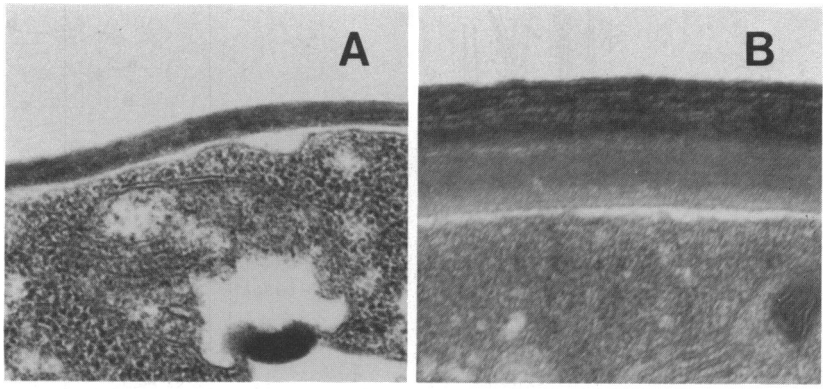

FIG. 4. Cell wall structure in dimorphic *Mucor* spp. Hyphae (A) and yeasts (B) of *M. racemosus* are shown. Electron micrographs of thin sections were stained with OsO_4. Magnification, ×30,000. Courtesy of Rodney K. Nelson.

differentiated into two distinct layers (Fig. 4B). Whole-cell autoradiography of *N*-acetyl-D-[^{3}H]glucosamine-labeled yeasts and hyphae revealed that wall deposition in the former is by means of diffuse intercalation and in the latter by means of strict apical extension (18). Germ tube emergence from a yeast or germinating sporangiospore is primarily the result of a vectorial change in wall deposition (21). Apical vesicles similar in structure to the spitzenkörper described for ascomycetes and basidiomycetes (86) have been reported in *Mucor* hyphae and postulated to play a major role in new-wall deposition (22). No such structures have been described in *Mucor* yeasts.

Hyphal growth of *Mucor* spp. has been characterized as a dynamic balance between wall synthesis and degradation at the elongating tip (20). The advancing hyphal apex is particularly fragile, being ruptured by exposure to a variety of physical and chemical agents including distilled water, acids, neutral salts, chelators, organic solvents, detergents, and sharp temperature fluctuations (20, 59). The remainder of the hyphal filament is refractory to such challenge. Treatment of *Mucor* hyphae with polyoxin D, an inhibitor of chitin synthetase, also results in eventual destruction of this specialized growing region, with no concomitant effect on the lateral walls of the hyphal cylinder (19). Integrity of the *Mucor* yeast wall is not affected by any of the mentioned physical or chemical agents or by polyoxin D. *Mucor* hyphae cannot be converted to yeasts by treatment with sublethal concentrations of polyoxin D, which suggested to Bartnicki-Garcia and Lippman (19) that the overall rate of chitin synthesis is not the critical factor in dimorphic development. Domek and Borgia (55), however, have examined in vivo rates of chitin-plus-chitosan biosynthesis in *Mucor* by measuring *N*-acetyl-D-[^{3}H]glucosamine incorporation into the polymers, and they report a threefold-higher rate of synthesis in hyphae than in yeasts. They claim that this phenomenon is independent of the cellular growth rate (which is somewhat higher in hyphae than in yeasts) and that exogenous cyclic AMP lowers the rate of synthesis in aerobically grown yeasts. Thus, they argue, the rate of chitin-plus-chitosan synthesis is linked to morphogenesis.

Synthesis and deposition of the chitin-chitosan fraction, referred to by many as the "skeleton" of the *Mucor* cell wall (24, 25, 44, 212), has received the greatest attention by researchers. Bartnicki-Garcia and his associates have made the following major contributions to our understanding of several aspects of the process: (i) elucidation of the biosynthetic reactions involved in chitin and chitosan synthesis; (ii) characterization of the properties and control of the enzyme chitin synthetase; and (iii) characterization of a specialized organelle, termed the "chitosome," which serves as the site of chitin synthesis and perhaps as the vehicle of chitin and chitosan deposition.

The immediate precursor of chitin, uridine diphosphate (UDP) *N*-acetylglucosamine (GlcNAc), is synthesized in the cytosol of *Mucor* spp. through the sequential actions of three soluble enzymes: (i) an ATP-dependent GlcNAc kinase, (ii) phosphoacetylglucosamine mutase, and (iii) UDP-GlcNAc pyrophosphorylase (142). The UDP-GlcNAc molecules produced by this pathway are added as GlcNAc residues to nascent chains of chitin by the particulate enzyme chitin synthetase (19, 141, 192, 195).

Early studies of chitin synthetase activity were performed with hyphal wall-associated fractions or microsomal fractions which contained approximately 85 and 10% of total measurable activity, respectively (141, 142). Free GlcNAc was found to be a strong positive effector for the enzyme. Chitin synthetase from *Mucor* yeasts proved to be more stable than the enzyme from hyphae and was used to effect the first reported in vitro synthesis of chitin microfibrils indistinguishable from those made in the living fungus (191). Treatment of yeast cell wall-associated fractions with UDP-GlcNAc and GlcNAc was found to release the enzyme from its particulate matrix in the form of granules 35 to 100 nm in diameter. These granules have a distinct microvesicular appearance and undergo a characteristic transformation during in vitro microfibril synthesis. They have been named chitosomes by their discoverers (31). Chitosomes were later demonstrated in *Mucor* hyphae and in many other species of fungi (16, 17).

Chitosomes have been extensively characterized in terms of ultrastructure, physicochemical properties, and biosynthetic abilities (15, 16). Chitosomes contain about 85% of the total chitin synthetase activity of the cell. They are quite stable once purified. These structures are about two-thirds protein and one-third lipid, and they display a membranous vesicular morphology when viewed with the electron microscope (93). They yield several characteristic protein bands upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the most prominent one having a molecular mass of 55,000 Da (68). Isopycnic centrifugation of chitosomes yields a single sharp band well separated from other cell components and having a buoyant density of 1.145 g/cm^3 in sucrose. Treatment with digitonin disperses chitosomes into uniform 16S-subunit particles (193). This disso-

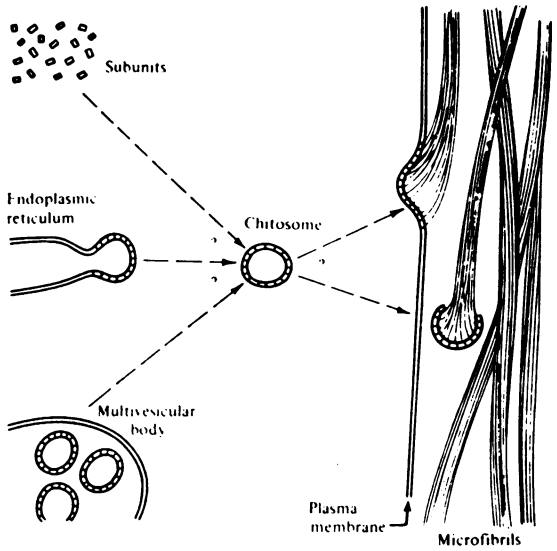

FIG. 5. Schematic representation of plausible views on the origin and fate of chitosomes. From reference 15, with permission.

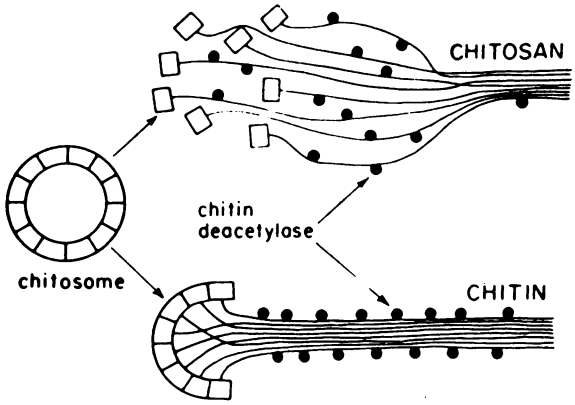

FIG. 6. Model for the mechanism and regulation of chitosan synthesis in *M. rouxii*. From reference 50, with permission.

ciation apparently acts on the ergosterol component of the chitosome, is totally reversible, and does not affect chitin synthetase activity in the subunit particles. Bartnicki-Garcia (15) proposes that the chitosome is essentially a membranous shell composed of multiple chitin synthetase complexes (i.e., 16S subunits) identical in apparent molecular mass (500,000 Da) and composition.

In vitro incubation of chitosomes with substrate (UDP-GlcNAc) and activators (GlcNAc and protease) yields microfibrils visible by electron microscopy (31, 191). It is thought that each 16S subunit within the chitosome synthesizes an individual chitin chain, and these chains collectively are assembled into one microfibril. Vectorial synthesis within the chitosome results in the formation of a microfibrillar coil which is released in a linear configuration as the chitosome disintegrates. Within the living cell, this release would be on the external surface of the plasma membrane. Whether actual chitin synthesis takes place with the chitosome residing in the cytoplasm, plasma membrane, or periplasmic space is not known, though the second possibility enjoys the most favor today (16).

The various possible origins and fates of chitosomes are shown in Fig. 5. Figure 6 presents a model to suggest how chitosan may be formed from chitin by deacetylation. The extent of disaggregation of the chitosome subunits may determine how accessible the individual chitin chains are to the action of chitin deacetylase (50). A recent study has detected chitin deacetylase in a membrane fraction of *M. rouxii* that must be in direct association with chitosomes to generate chitosan in vitro but does not copurify with these organelles (35). Bartnicki-Garcia (15) has suggested that *Mucor* vegetative morphology is determined by the pattern of wall polymer deposition and that this pattern is governed by the distribution of wall-synthesizing vesicles. Chitin-chitosan microfibrils with their origins in chitosomes may be the most important example of such a mechanism.

Another layer of complexity in this system that may be superimposed on the putative role of intracellular chitosome distribution in dimorphism—or may entirely supersede it—is the profound difference observed in both the activity and stability of chitin synthetase from the two morphological forms. Yeast cell preparations possess the enzyme in a highly stable but relatively inactive zymogen form (192). In contrast, hyphal extracts display very active but relatively unstable enzyme (192). An analogy with the mechanism thought to regulate the activity of chitin synthetase in *Saccharomyces* spp. (33) has been proposed (192). Chitin synthetase activities are determined by a balance between the amount of an apparent proteolytic activator and a heat-stable, dialyzable inhibitor present in the soluble fraction of the cytoplasm. In hyphae, protease levels are high and inhibitor levels are low; in yeasts, inhibitor concentrations are high and protease activities are low (192). This situation might dictate that chitin synthetase in the region of the hyphal apex would be very active but only for a brief period. As the hyphal apex traverses a linear path, each completed increment of cell wall is transformed from growing tip to nongrowing lateral wall. In yeast cells, however, every bit of wall surface is continuously on the leading edge of expansion. Enzyme activity is appropriately maintained at a constant level. Such a strategy might account for the slower rates of chitin synthesis but thicker deposition of wall observed in yeasts relative to hyphae. Though the hypothetical activating protease has not yet been identified, an acid protease that rapidly inactivates chitin synthetase has recently been isolated from *M. rouxii* (53).

The wall lytic activity indirectly demonstrated in *M. rouxii* by Bartnicki-Garcia and Lippman (19, 20) may have been characterized in *M. mucedo* by Humphreys and Gooday (99, 100). These researchers have identified chitinase activity in the supernatant and microsomal fractions of surface-grown hyphae. In most properties these activities are indistinguishable. However, the microsomal enzyme behaves as a membrane-bound zymogen: it can be activated by proteases and inhibited by phenylmethanesulfonyl fluoride or by phospholipases (99). The supernatant chitinase and the microsomal enzyme solubilized by treatment with nondenaturing detergents cannot be activated with proteases. Further experiments showed that the phospholipid environment is important in regulating membrane-bound chitinase activity but that the supernatant chitinase activity remains unaffected by the presence of phospholipids (100). These activities have yet to be described in any dimorphic species of *Mucor*.

Whereas Bartnicki-Garcia and Ruiz-Herrera have looked to the chitin-chitosan microfibrillar skeleton of the *Mucor*

cell wall for the key to explaining dimorphism, Dow, Villa, and colleagues have focused on the polyuronide matrix components of the wall in search of correlates with morphogenesis (57, 60, 61). It is an entirely reasonable proposition that the properties of the mortar may be as important as those of the brick in defining the potential architecture of an edifice. Both *Mucor* yeasts and hyphae contain readily extractable acidic matrix heteropolymers (mucorans) which have D-glucuronic acid as the main sugar component and lesser amounts of L-fucose, D-galactose, and D-mannose (57, 60). Nonextractable poly-D-glucuronic acid (mucoric acid) is also an important component of the matrix in both *Mucor* yeasts and hyphae (57, 60).

UDP-glucuronic acid is the most important precursor of the cell wall polyuronides mucoran and mucoric acid. UDP-glucose dehydrogenase, the enzyme responsible for synthesis of UDP-glucuronic acid, has been purified from *Mucor* yeasts and hyphae (56). Enzymes from the two sources differed in molecular weights and kinetic properties. It was not established whether these observations were due to alternative multimeric forms of the same enzyme subunits or the synthesis of separate gene products.

Pulse-chase experiments employing D-[^{14}C]glucose as label have shown that radioactive oligoglucuronides quickly appear in the supernatant fluid of *Mucor* cultures. The kinetics of label incorporation into soluble oligoglucuronides and cell wall polymers suggested that ongoing wall lysis is necessary for active hyphal extension (61). Radiolabeled polyuronides and glycoproteins with compositions similar to those of these polymers as they occur in the cell wall appear in membrane fractions of *M. rouxii* but are rapidly lost from these fractions following a chase. The radioactive polyuronides reappear as somewhat larger polymers localized in the cell wall after the chase. Molecular weights of the glycoproteins, in contrast, are not altered when these molecules leave the membrane and are deposited in the wall (56). The relevant membrane fractions have not been characterized with respect to fine structure or molecular composition. They have been studied with respect to catalytic activity. Glucuronosyl transferase, which catalyzes the transfer of UDP-glucuronic acid to high-molecular-weight acceptors, is highly active in the membrane fractions from both yeasts and hyphae of *M. rouxii*. Enzymes prepared from the two cell types differ in a number of properties. Neither requires the involvement of a lipid intermediate in transfer of glucuronosyl residues, and neither is stimulated by the addition of exogenous mucoran or mucoric acid (58). An endoglucuronidase capable of degrading mucoric acid has recently been purified from the hyphal form of *M. rouxii* (62).

It has been suggested that the eukaryotic cytoskeletal elements, mainly actin and tubulin, which have been well characterized in animal systems (123, 162), may play a role in *Mucor* dimorphism (206). These elements have been shown to play an essential part in the pattern of wall deposition in plant cells (87, 135). Microtubules are required for cytoplasmic streaming (115), which can be observed in *Mucor* hyphae with a phase-contrast microscope. However, these structures have not been observed in electron micrographs of *Mucor* spp. (9, 215, 216), and fluorescent-antibody techniques (123) have not yet been tried with *Mucor* spp. Treatment of *Mucor* cells with inhibitors of microtubule and microfilament assembly, such as colchicine and cytochalasin B, has yielded inconclusive results (150a).

Endogenously generated ion currents have been reported to precede and predict the emergence of hyphal branches in *Neurospora crassa* and *Achlya ambisexualis*. An inward flow of protons and calcium ions was thought to localize growth via an unknown mechanism (116, 198a). The originator of this model has recently disavowed it (92a). It had been speculated earlier that an electrophoretic force may serve to direct apical vesicles to the growing tips of *Mucor* hyphae (14), but no research has been done with this organism to examine the possibility. Recent studies of the dimorphic zygomycete *Mycotypha africana* have shown that external direct-current electrical fields cause hyperbranching towards the anode in young germlings. In contrast, electrical current strongly inhibits budding and glucose fermentation in the yeast form of the fungus (234).

A contemporary model put forward by Bartnicki-Garcia and tested by computer simulation (17a) proposes that vesicles carrying essential enzymes and wall precursors to an expanding fungal surface are released randomly in all directions from a unique vesicle supply center (VSC). A stationary VSC would result in uniform wall accretion in all directions and a spherical cell. Linear displacement of the VSC would generate the typical cylindrical shape of fungal hyphae, with a tapered dome at the apex. This model requires no ion currents, microfilaments, or microtubules to direct vesicles to specific sites on the cell surface. The actin-myosin or tubulin molecular motors so well characterized in the cells of other eukaryotic organisms could potentially recruit vesicles from numerous Golgi throughout the cytoplasm into the single VSC. A cytoskeletal link between the VSC and the advancing hyphal tip has been suggested as a possible motive force for linear displacement of the center (17a).

CORRELATES OF MORPHOGENESIS

Perspective

In the endeavor to understand cell morphogenesis, all manner of properties and parameters are considered and quantified, lest something important be passed over. Those characteristics that do not change over the course of morphogenesis are usually considered by developmental biologists to be extraneous to the process and inherently uninteresting.

A potential problem with this assumption may be posed in systems possessing alternative genes specifying the same or a similar protein. Differential gene activation may not be readily apparent if levels of the equivalent products fluctuate in a reciprocal relationship. With the ascendancy of modern molecular genetics and the availability of hybridization probes such as cloned genomic DNA and cDNA, several proteins are known to be encoded in multigene families (113, 128). It is now possible to analyze mRNA populations by hybridization with cloned DNA and to demonstrate differential gene activation where none was suspected before.

Another mistake would be to assume that all changes that accompany morphogenesis are an integral part of the developmental process. Many changes may merely represent a physiological adaptation to whatever environmental perturbation stimulated morphogenesis. Inducing morphogenesis with an alternative stimulus may produce an entirely different set of physiological responses. In such a case, those changes irrelevant to morphogenesis per se would be obvious. Though a hypothetical causal relationship can be effectively disproven by one negative result, rigorous substantiation of such a linkage is rarely attainable in biological systems. One usually attempts to marshal as much evidence as possible that is consistent with the hypothetical linkage

between some biological trait and morphogenesis. The kinds of evidence usually include (i) concomitant changes in morphology and the parameter at issue under all conditions and circumstances that result in morphogenesis; (ii) stasis of the parameter under conditions that fail to induce or that inhibit morphogenesis, whether such inhibition be caused by chemical interference or by mutation; (iii) blockage or derangement of morphogenesis under all conditions and circumstances, environmental or mutagenic, that destroy or significantly alter the normal regulatory response or function of the parameter in question; and (iv) reversibility of items ii and iii.

Fulfillment of all four of these criteria has been documented for only a small number of biological systems. For these, researchers can proceed as though a causal relationship between morphogenesis and the relevant parameter were true (contingent upon the continued absence of falsifying data). For most developmental systems (*Mucor* spp. included), the evidence that would definitively link observed changes in cellular properties with morphogenesis is far from complete. The evidence for most systems is especially deficient in areas ii and iii described above. The true status of most changes observed to occur during morphogenesis may be described as a correlation, that is, a consistent parallel or accompaniment but only tentatively a sine qua non. Because the experimental evidence is so incomplete, especially at the genetic level, I shall frame all of my discussions of the molecular and physiological changes that are known to accompany *Mucor* dimorphism in the context of the preceding discussion and term these changes correlates of morphogenesis. Fortunately, the *Mucor* system is highly malleable, responding to many natural and artificial morphopoietic agents. This makes it possible to ascertain how consistently a character correlates with morphogenesis. In spite of these ample opportunities, the question of obligatory linkage to morphogenesis has still not been settled convincingly for many parameters, as will be made clear in individual discussions below.

Carbon and Energy Metabolism

Most of the essential background bearing on the role of carbon and energy metabolism in *Mucor* dimorphism has already been presented above in the discussion of environmental factors influencing *Mucor* morphogenesis. Further information concerning regulation of some relevant catabolic pathways may be gleaned from the discussion of enzymes in a subsequent section. Most of the early observations were generally consistent with the notion that oxygen and aerobic respiration are essential for hyphal development, whereas anaerobiosis and alcoholic fermentation of a hexose are necessary for growth in the yeast form. However, the more these relationships have been tested, the more dubious they have become.

The issues were first clouded by the realization that all dimorphic species of *Mucor* do not respond to critical environmental factors in quite the same way. For example, in *M. rouxii*, anaerobiosis and the mere presence of a hexose are alone not enough to stimulate growth in the yeast form. Either a high pCO_2 (>0.3 atm) or a high hexose concentration (>8%) are required to produce yeasts. Otherwise the organism will grow anaerobically as hyphae (24, 25). In *M. genevensis*, high hexose concentrations can induce yeast morphology even under an air atmosphere (184). *M. racemosus*, in contrast, does not require CO_2 or a glucose concentration greater than 0.1% in order to produce anaerobic yeasts. However, it cannot grow at all anaerobically in the absence of a hexose (212).

Several different experimental approaches have been employed in the attempt to collect evidence supportive of a link between *Mucor* morphology and the mode of carbon and energy metabolism. Inhibitors of electron transport or oxidative phosphorylation in mitochondria (73) and inhibitors of protein synthesis within mitochondria (238) constrain growth of dimorphic *Mucor* spp. to the yeast phase. Similarly, mutants deficient in aerobic respiration are reported to be locked into the yeast morphology (208), and mutants selected as monomorphic are reportedly all respiratory deficient (196). All these findings might suggest an obligatory link between aerobic respiration and *Mucor* morphology. However, the reported mutants were apparently pleiotropic and were characterized under only a limited set of environmental conditions. Furthermore, the existence of conditional yeast mutants (28, 212) with undiminished respiratory competence offers one argument against morphogenetic control at this site. There are several others.

PEA, which was reported to stimulate alcoholic fermentation and inhibit oxidative phosphorylation in *M. rouxii*, constrains growth to the yeast form. Oxygen consumption and cytochrome content of the mitochondria are reportedly not affected by PEA (219). However, unless the carbon and energy source is a hexose, the organism grows as hyphae even in the presence of PEA. Evidently, oxidative phosphorylation, which should be just as depressed in the presence of the alternative carbon sources employed (xylose, maltose, or sucrose), cannot be a critical determinant of morphology.

EDTA induces the hyphal morphology of *M. rouxii* under anaerobic conditions (238). These organisms are fully fermentative, suggesting that this capability cannot be the critical determinant of morphology. Unless extraordinary precautions were taken to exclude all traces of oxygen from the growth vessels, as cautioned by Phillips and Borgia (175), these experiments cannot be used to discount the role of this gas as a critical effector of morphogenesis. The authors of the cited study claimed that respiratory capacity, stimulated by EDTA-induced mitochondriogenesis, is the critical determinant of *Mucor* morphology. However, Paznokas and Sypherd (169) have shown that both yeasts and hyphae can develop when respiratory capacity is low and that cyclic-AMP-induced aerobic yeasts have respiratory capacities equal to those of aerobic hyphae. Clearly, respiratory capacity is not the critical morphological determinant.

Mitochondrial development and oxidative metabolism were shown to be essentially irrelevant to dimorphism in *M. genevensis* (184). High glucose concentrations induce the yeast morphology of this organism in air but have minimal effect on the number, appearance, and functioning of the mitochondria. Furthermore, chloramphenicol prevents mitochondrial respiratory development in glucose-limited cultures but does not induce the yeast morphology, indicating that this metabolic ability is not essential for hyphal development (48). Anaerobically grown yeasts of *M. genevensis* reportedly possess mitochondria with well-defined cristae, although they were said to lack cytochromes (47). More recently, the same complement of cytochromes was shown to exist in both aerobic hyphae and anaerobic yeasts of *M. rouxii* (38). Fully developed mitochondria exist in yeasts of *M. racemosus* grown under a nitrogen atmosphere (108a). Furthermore, the respiratory capacity of these cells is nearly as high as it is in aerobically grown hyphae (150b). Analysis by two-dimensional PAGE (2-D PAGE) reveals that mito-

chondria isolated from anaerobic yeasts of *M. racemosus* do not lack any proteins extractable from mitochondria of aerobic hyphae (108a). Because the phospholipid composition of mitochondria differed in yeasts and hyphae of *M. genevensis* (85) and because cerulenin inhibits yeast-to-hypha morphogenesis and attendant changes in cell phospholipid composition (105), it has been suggested that there may be some nonrespiratory function of the mitochondrion required for hyphal development (38, 44, 105).

Inderlied and Sypherd (103) radioisotopically determined the participation of catabolic pathways in *M. racemosus* as a function of morphology and growth conditions. They found that anaerobic yeasts catabolize 86% of glucose through the Embden-Meyerhof-Parnas pathway and 14% through the pentose phosphate cycle. Aerobic hyphae catabolize essentially all glucose through terminal respiration. Aerobic hyphae that have developed from yeasts, however, have a basically fermentative metabolism, as do anaerobic hyphae grown under a nitrogen atmosphere. Cyclic-AMP-induced aerobic yeasts maintain a high fermentative metabolism. Thus, fermentation consistently accompanies yeast morphology but cannot be said to determine it. Garcia and Villa (79) suggested that ethanol produced during glycolytic fermentation may determine *Mucor* morphology. If this were true, fermentative hyphae, which produce copious ethanol, would not exist, and yet they do.

Phillips and Borgia (175) recently reexamined the effect of nitrogen flow rate on the morphology of *M. racemosus*. Contrary to Mooney and Sypherd (145), they found no evidence of a volatile factor inducing hyphal growth at low flow rates of gas. Instead, they found that oxygen permeates the Tygon or Teflon tubing generally used in these experiments, and the cultures then become microaerobic. These microaerobic cells are completely fermentative, eliminating this variable as the determinant of cell morphology. These authors hypothesize that the only variable in the system, trace amounts of oxygen, is the critical morphogenetic signal and, because of its limited availability, must operate at a level other than oxidative energy metabolism.

Borgia et al. (28) have isolated a respiratory-competent developmental mutant of *M. racemosus* that behaves like the wild type in terms of its morphology and energy-yielding catabolic pathways except in the presence of glucose. Under this condition, the organism is highly fermentative and grows as a yeast form whether or not oxygen is present. Respiration is not inhibited under these circumstances, implying that it is not a critical determinant of morphology. However, oxygen is not recognized as a morphogenetic signal in this mutant. The investigators propose that oxygen is the primary regulatory signal that independently controls expression of genes specifying catabolic and morphogenetic functions.

The failure of *Mucor* yeasts to catabolize disaccharides may be related to repression of the relevant enzymes by glucose and/or cyclic AMP or to the failure of the enzymes to be inserted into their functional site in the periplasmic space. Thus, this property of *Mucor* yeasts may be neither a cause nor an effect of morphogenesis but merely a fortuitous accompaniment. These topics are treated in greater detail in the section on enzymes.

Nitrogen Metabolism

The nutritional requirements of *Mucor* yeasts are, in general, more specific than those of *Mucor* hyphae. Growth in the yeast phase demands the obligate presence not only of a hexose as the carbon and energy source but also of an aminated organic compound as the nitrogen source (24, 25, 64, 65, 173, 174). Whereas an ammonium salt alone is sufficient to support the growth of hyphae, a minimal medium must include glutamate to support the growth of *M. racemosus* in the yeast form (173, 174). These observations suggest that nitrogen assimilation may proceed via different paths in the two forms of *Mucor* spp. One might predict a significant deficiency in the activity of biosynthetic (NADP-linked) glutamate dehydrogenase (GDH) in the yeast form of the organism.

Both the biosynthetic and catabolic (NAD-linked) forms of the enzyme were found in *M. racemosus* (174). However, it is the NAD-dependent enzyme which displays depressed activity in yeasts. The levels of this enzyme are 10-fold lower in yeasts than in hyphae. Exogenous cyclic AMP, which induces the yeast morphology under an aerobic atmosphere, represses the activity of NAD-linked GDH to levels found in anaerobic yeasts, suggesting a possible correlation of enzyme activity and cell morphology. The NADP-dependent GDH activity remains approximately the same throughout yeast-to-hypha conversions. Glucose strongly represses expression of the NAD-linked enzyme in hyphae but not down to the levels found in yeasts. The activities of both forms of GDH are generally higher in cells growing on complex organic nitrogen sources than in cells growing on inorganic nitrogen salts. The last two observations led to the conclusion that both forms of GDH function in a catabolic direction in *Mucor* spp. (174). It was later found that the organism assimilates ammonia by a two-step pathway involving glutamine synthetase and glutamate synthase (212).

The complex nitrogen sources arginine and urea are notable exceptions to the general correlations noted above in that they strongly repress NAD-linked GDH levels in hyphal cells to the low levels commonly observed in yeasts (102). These data must exclude NAD-linked GDH activity as a general correlate of dimorphism. Nonetheless, Inderlied et al. (102) propose a model in which urea acts as a central signal required for hyphal development. In this scheme, the function of NAD-dependent GDH is to provide ammonia for the synthesis of urea. High concentrations of exogenous urea or arginine, which can be converted to urea in a one-step reaction involving arginase, thus obviate the role of this enzyme and repress its expression. The model, largely speculative, proposes that cytosolic glutamate enters the mitochondria, where NAD-linked GDH is localized. There the ammonia derived from deamination of the glutamate serves as a substrate for carbamoylphosphate synthetase, the first enzyme in the urea cycle. Urea is ultimately produced through the action of arginase, the last enzyme in this pathway.

Inderlied et al. (102) asserted, in support of the stated hypothesis, that mutant PB104 of *M. racemosus* is respiratory deficient, possesses defective mitochondria, cannot convert to the hyphal morphology, and displays low NAD-linked GDH activity even under an aerobic atmosphere. However, Borgia et al. (28) have more recently characterized this mutant and indicated that it is, in fact, a respiratory-competent, conditional developmental mutant possessing functional mitochondria. It grows in the hyphal form on minimal medium with glutamate as the sole carbon and energy source. Only at high glucose concentrations is the hyphal form repressed under air (28). Inderlied et al. (102) reported that the addition of urea to cultures of mutant PB104 overcomes the morphological block, but only if yeast extract is excluded from the medium. They also related that

urea can overcome the morphopoietic effect of exogenous cyclic AMP in the wild-type strain of *M. racemosus*, but the conversion of yeasts to hyphae is much slower than normal.

Respiratory-deficient monomorphic mutants have been isolated from *M. bacilliformis* by Ruiz-Herrera et al. (196). Two of these mutants display a reduced level of NAD-dependent GDH activity, and both possess high levels of intracellular cyclic AMP. These results suggested to the investigators that NAD-dependent GDH activities are not morphology related but rather are linked to cyclic-AMP concentrations. Furthermore, these researchers observed a response in the wild type and five mutant strains of *M. bacilliformis* different from that observed in *M. racemosus* with respect to NAD-dependent GDH activity as a function of nitrogen source complexity. Enzyme activity in the presence of glutamate was lower than in the presence of ammonium salts as sole nitrogen source.

Paznokas et al. (171) isolated morphology mutants of *M. racemosus* that raise serious doubts about the hypothetical role of NAD-dependent GDH in dimorphism. These mutants are refractory to the usual morphopoietic influence of exogenous cyclic AMP, maintaining the hyphal morphology under air in the presence of cyclic AMP. The pattern of NAD-linked GDH activity must be considered uncoupled from morphogenesis in this case, because the morphology mutants continue to display a normal repression of enzyme activity by cyclic AMP (171). Consistent with the professed hypothesis is the observation that arginase activity increases 50-fold during hyphal development irrespective of the nutritional environment (102).

In summation, it must be said that experimental support for the current model linking nitrogen metabolism and *Mucor* dimorphism is mostly indirect and not totally satisfying. It is not clear that NAD-linked GDH exists to provide for the synthesis of urea during morphogenesis, nor is it clear just how urea might function in its hypothetical role as a morphogenetic signal. The fact that there is no useful radioisotope of nitrogen certainly limits our ability to trace the ultimate fate of the amino group donated by glutamate. However, the metabolic fate of urea or its potential role as a regulatory ligand could easily be pursued. The potential relationship between *Mucor* morphogenesis and nitrogen metabolism is intriguing and worthy of investigation, but it remains an unresolved issue.

Endogenous Small Molecules

The "second-messenger hypothesis" put forward by Sutherland and his co-workers (183) describes how an environmental stimulus may be transduced into a physiological or morphogenetic response via a small-molecule intermediary. The original paradigm proposed cyclic AMP as this intermediary, or "second messenger," but accumulated fact and theory have expanded the roster of potential intracellular signals to include, among others, cyclic GMP (83), guanosine tetraphosphate (75), calcium (179), ammonia (198), *N,N*-dimethyl-guanosine (7), and an array of highly phosphorylated nucleotides (181).

As indicated earlier, the most powerful naturally occurring environmental factors to influence *Mucor* morphology are CO_2, oxygen, and hexose sugars. The role of cyclic AMP in the reversal of catabolite repression by glucose and anaerobiosis in *Escherichia coli* (1) suggested to Larsen and Sypherd (118) that this nucleotide might act as an intracellular signal in *Mucor* dimorphism. These researchers noted a consistent correlation between cell morphology and internal cyclic-AMP levels in *M. racemosus*. Yeast cells grown under a 100% CO_2 atmosphere were found to contain high levels of cyclic AMP in contrast to aerobic hyphae, which possess low levels of the nucleotide. A threefold drop in internal cyclic-AMP concentration was observed to precede the conversion of yeasts into hyphae stimulated by a change of atmosphere from CO_2 to air. Introduction of cyclic AMP into the culture medium was noted to prevent the normal air-induced transformation of yeasts into hyphae. Aerobic hyphae are converted into aerobic yeasts upon the addition of cyclic AMP to the culture medium. In both instances, a hexose must be present in the medium in order for cyclic AMP to work its morphopoietic effect. Similar observations made later by Paveto et al. (167) linked intracellular cyclic-AMP concentration and the vegetative morphology of *M. rouxii*.

In related studies, cyclic-AMP pools were measured during aerobic sporangiospore germination of *M. racemosus* (169), *M. mucedo* (153), *M. genevensis* (153), and *M. rouxii* (52, 221). All investigators found cyclic-AMP pools to increase rapidly and substantially during the period of spherical growth of the spores. Maximum levels are attained within 2 to 4 min after *M. rouxii* spores are introduced to a medium containing glucose (221). During the emergence of hyphal germ tubes, the intracellular concentration of the nucleotide falls rapidly; it remains low throughout hyphal growth. The addition of cyclic AMP to cultures of germinating *M. racemosus* sporangiospores prevents the emergence of germ tubes but allows spherical growth to proceed indefinitely, producing giant cells but no buds (151, 231). In fact, the nucleotide actually stimulates the rate of spherical growth observed in the earliest stages of germination (231). The same conditions in cultures of germinating sporangiospores of dimorphic *M. genevensis* and monomorphic *M. mucedo* cause the ultimate formation in both systems of round, swollen, septated structures rather than normal hyphae (153). A similar effect of exogenous cyclic AMP was noted on germinating spores of the monomorphic species *M. hiemalis* (110). The consistent feature among all of these observations is that a high cyclic-AMP concentration appears to be antithetical to hyphal development. It is possible that high cyclic-AMP levels play a similar role in both budding yeasts and expanding germ spheres by inhibiting polar growth.

Early studies by Paznokas and Sypherd (169) seemed to disqualify the internal cyclic-AMP concentration as a universal correlate of *Mucor* dimorphism when these researchers reported that intracellular levels of the nucleotide are always low in cultures grown under a 100% nitrogen atmosphere, regardless of the cellular morphology. This would imply that high intracellular cyclic-AMP levels are not an absolute requisite for construction of the yeast form. However, Orlowski and Ross (155) reexamined this issue and corroborated the initial assertion of Larsen and Sypherd (118) that *Mucor* morphology and intracellular cyclic-AMP levels are inextricably linked. The cyclic-AMP levels reported in the study by Paznokas and Sypherd (169) seem anomalously high when compared with results obtained by other investigators (118, 155, 167, 196). An explanation for this disparity may possibly be found in the assay methods used: the first group (169) conducted radioimmunoassays, whereas all of the other groups performed binding-protein assays of cyclic AMP.

Orlowski and Ross (155) determined that *Mucor* yeasts grown under a 100% CO_2 or a 100% nitrogen atmosphere have identical intracellular cyclic-AMP concentrations.

These concentrations drop precipitously when either type of culture is shifted to an air atmosphere. The levels of cyclic AMP stay the same if the CO_2 is changed to nitrogen or vice versa, implying that the decline in nucleotide concentration observed during morphogenesis is not due simply to a reduction in CO_2 tension. Other experiments in this study that used nutritionally rich and poor media showed that the typical change in cyclic-AMP concentration that accompanies morphogenesis is independent of the cellular growth rate and independent of the nutritional milieu of the organism. By growing the methionine-requiring morphology mutant *coy-1* (213) in the absence of methionine, it is possible to shift cells from an anaerobic to an aerobic atmosphere while maintaining yeast morphology. Under such conditions, internal cyclic-AMP levels remain at constant high values, indicating that the decrease noted during development is not caused by the lifting of anaerobiosis. That intracellular cyclic-AMP levels are quite confidently considered the strongest correlate of *Mucor* dimorphism (102) is underscored by the fact that the morphopoietic effect of cyclic AMP has been used to test numerous other parameters as correlates of dimorphism (30, 157, 174).

Cyclic GMP is the only other cyclic nucleotide known to occur in living cells (92), and it has been proposed to act in a direction opposing that of the regulatory effects of cyclic AMP (83). Cyclic GMP occurs in all cell forms of *M. racemosus* but does not appear to be related to dimorphism (156). Levels of the nucleotide do not change during yeast-to-hypha transformations, and exogenous cyclic GMP has no effect on cell morphology. Moreover, the presence of exogenous cyclic GMP does not antagonize the morphopoietic effect of cyclic AMP. Rather large fluctuations in the intracellular concentration of cyclic GMP were observed during sporangiospore germination in *M. racemosus*, suggesting that the nucleotide may have a regulatory role in that process (156).

Adenylate cyclase (AC) and cyclic-AMP phosphodiesterase (PDE) are the key enzymes of cyclic-AMP metabolism, the former catalyzing the synthesis of cyclic AMP from ATP and the latter causing the hydrolysis of cyclic AMP to AMP. These enzymes have been characterized in the *M. rouxii* system by Passeron and her colleagues and are more thoroughly discussed in another section of this review. PDE activity correlates well with measured intracellular cyclic-AMP levels, being four- to sixfold higher in hyphae than in yeasts. AC, in contrast, remains at the same level of activity in both yeasts and hyphae (40, 167). Regulation of intracellular cyclic-AMP concentration during sporangiospore germination appears to be exerted at the level of AC activity in *M. mucedo* and *M. genevensis* (153) and by alterations in the activities of both enzymes in *M. rouxii* (39, 221).

The primary role of cyclic AMP in eukaryotic cells is as an effector of cyclic-AMP-dependent protein kinases (183). Using a radioisotopically labeled photoaffinity analog of cyclic AMP, Orlowski and Forte have identified cyclic-AMP-binding proteins in several species of *Mucor* (70, 151). These 51,000- and 65,000-Da proteins most likely represent regulatory subunits of cyclic-AMP-dependent protein kinases. Protein kinase activities have been well characterized in the *M. rouxii* system by Passeron and her associates (149) and are described in another section of this review.

Polyamines are polyvalent organic cations ubiquitous in all living cells. Their precise physiological role has been debated for some time, but they seem to influence the rates and fidelity of transcription and translation (2, 4). Because the documented interactions between polyamines and the biosynthetic machinery imply regulatory potential, the behavior of intracellular polyamine levels during development and its influence on morphology have been frequently studied (43, 143, 144, 233).

The polyamine contents of the yeast and hyphal forms of several dimorphic fungi, including *M. bacilliformis* and *Mycotypha microspora*, were examined, but no relationship between polyamine composition and fungal morphology emerged (139). Spermine has not been detected in any members of the *Mucorales* examined (34, 77, 139, 150). Spermidine levels are approximately the same in yeast and hyphal forms of *M. bacilliformis*, whereas putrescine levels are fourfold higher in the yeast form than in the hyphal form of this organism (139). Identical results were observed in *M. racemosus* with respect to spermine and spermidine; however, putrescine was found at levels five times higher in hyphae than in yeasts (77). Both spermidine and putrescine levels transiently double during yeast-to-hypha morphogenesis of *M. rouxii* (34). Ornithine decarboxylase (ODC), which catalyzes the formation of putrescine from ornithine, displays a very large increase in specific activity during the air-induced conversion of yeasts into hyphae in *M. racemosus* (101), *M. bacilliformis* (34), and *M. rouxii* (34). Treatment of *Mucor* cells with diamino butanone, a competitive inhibitor of ODC, blocks both the increase in ODC activity and the yeast-to-hypha transition (140, 194).

The changes in both putrescine levels and ODC activity have been consistent correlates of morphogenesis (34, 77, 105, 196), yet the roles of these substances can only be speculated upon at this time. Perhaps elevated putrescine levels facilitate the increase in the rate of translation observed during *Mucor* yeast-to-hypha morphogenesis (158). Polyamines are known inhibitors of AC (5) and PDE (49) and have exhibited inhibitory effects on these activities in cell extracts of *M. racemosus* (154a). Cyclic AMP has been known to repress ODC synthesis in eukaryotic cells (104) and was observed to do so in *M. racemosus* (105). Perhaps cyclic AMP and the polyamines, notably putrescine, exist as regulatory antagonists influencing the direction of *Mucor* morphogenesis. It has recently been reported that diamino butanone not only inhibits the increase in putrescine levels normally accompanying aerobic germination of *M. rouxii* sporangiospores (194) but also blocks germ tube emergence and the normally concomitant demethylation of nuclear DNA (36). The investigators have speculated that polyamine levels determine the extent of DNA methylation, which in turn determines whether growth will be polarized or spherical (36, 194).

SAM functions primarily as a methyl-group donor in all organisms. It also serves as a propylamine donor in spermidine biosynthesis. SAM was reported to be essential in *Saccharomyces* ascospore germination and outgrowth in some capacity other than polyamine synthesis (32). An increase in SAM levels was observed to accompany the yeast-to-hypha conversion in *M. racemosus* (77). Present evidence suggests that this change correlates with development per se rather than metabolic adaptation to the environment (78). The increase in SAM levels does not result in increased spermidine production; instead, qualitative as well as quantitative changes in the pattern of protein methylation occur (77). Further work has focused on one protein whose pattern of methylation changes profoundly during morphogenesis and may play a critical role in the process (95).

The influence of L-amino acids on dimorphism and sporangiospore germination was examined in *M. rouxii* (126) and *M. racemosus* (225). Certain amino acids were more

effective than others in stimulating germination when offered as the sole carbon source (225). Whether the spores had been raised on minimal or complex medium was a critical factor in the germination response and probably affected the composition of endogenous reserves that are tapped during germination (225). Some amino acids favored growth in the yeast morphology, whereas others favored growth in the hyphal form when offered as the sole nitrogen source (126). The pH influenced both morphological responses (126, 225). There is presently no single theory that satisfactorily explains the diverse effects of amino acids on *Mucor* morphogenesis.

Lipid Synthesis

The discussion in this review of the *Mucor* cell wall (see above) considered work bearing on the potential role of the cell membrane (56) and a special membrane-bound organelle, the chitosome (15), in the synthesis and deposition of the cell wall. Though the membrane and the chitosome are clearly involved in polyuronide (56) and chitin (15) synthesis, respectively, there are few facts to explain how these membranous structures effect the different patterns of wall deposition observed in *Mucor* yeasts and hyphae. It is possible that the lipid compositions of the relevant membranes are important in this regard. Studies of the lipid composition of chitosomes from *M. rouxii* yeasts have established that the membrane of this organelle is highly differentiated from the plasma membrane (93, 136). Other work suggests that the phospholipid environment may play a role in regulating membrane-bound chitinase activity in *M. mucedo* (99, 100).

Compositions of lipid fractions were compared in yeasts and hyphae of *M. genevensis* (85) and *M. rouxii* (197). Total lipid fractions from hyphae of *M. genevensis* were found to have much higher levels of sterols and fatty acids than the same fractions from yeasts. Furthermore, fatty acids in hyphae were predominantly unsaturated, whereas in yeasts they were mostly saturated. The fact that cells induced to grow aerobically as yeasts in the presence of PEA displayed the typical hyphal pattern of lipid composition prompted the investigators to conclude that *Mucor* dimorphism is not directly related to lipid composition (85). Lipids from crude cell wall and cytoplasmic fractions of *M. rouxii* were analyzed (197). The cytoplasmic fraction contained a relatively larger amount and a qualitatively different assortment of lipids than did the wall fraction. Consistent with the previous study, sterols and fatty acids were present at much higher levels in both cytoplasmic and wall fractions from aerobic hyphae than from anaerobic yeasts. Also consistent with the first study, the lipids in cytoplasmic membrane fractions from PEA-induced aerobic yeasts were very similar to those in the same fractions from aerobic hyphae. However, wall fractions from PEA-induced aerobic yeasts had lipid compositions more similar to those from anaerobic yeasts than from aerobic hyphae (197). The high levels of sterols in the hyphal form of *M. rouxii* appear to be largely dispensable and inconsequential to cell morphology, since triazole inhibitors such as propiconazole and etaconazole can reduce sterol synthesis to levels typical of the yeast form without affecting hyphal growth or morphology (230). Fatty acid synthetase has been purified from *M. rouxii*, but only from the yeast phase (186).

The polyene antibiotic cerulenin, which is known to block lipid synthesis by inhibiting fatty acid synthetases, was administered to cultures of *M. racemosus* at sublethal concentrations (105). Under these conditions, growth continued at an unaltered rate, but the organism was not able to effect the yeast-to-hypha transformation. Furthermore, other correlates of cell morphogenesis, such as the characteristic increases in the rates of protein and RNA synthesis (157–159) and ODC activity (101), were blocked by this morphopoietic agent. Supplementation of the culture medium with Tween 80, a complex mixture of fatty acids, completely countered the effects of cerulenin on morphogenesis and its correlates. Cerulenin was found to inhibit the increased rate of phospholipid synthesis that normally accompanies the *Mucor* yeast-to-hypha morphogenesis, but the drug even more significantly depressed the rates of turnover of phosphatidyl choline and phosphatidyl ethanolamine relative to the rates in untreated controls. In contrast, the effect of cerulenin on turnover of diphosphatidyl glycerol (cardiolipin) was minimal. The investigators concluded that an increase in the rate of lipid synthesis and an acceleration of phospholipid turnover are essential for the *Mucor* yeast-to-hypha transition as well as for its biochemical correlates (105). These claims need strengthening with an examination of lipid synthesis under additional conditions. The findings do justify a closer inspection of individual subcellular membrane systems (e.g., plasma membrane, nuclear membrane, chitosomes, mitochondria, peroxisomes, endoplasmic reticulum, etc.) in order to pinpoint changes that may occur during morphogenesis.

Enzymes

A large number of enzymes have been investigated as potential correlates of *Mucor* dimorphism. They have been examined both in terms of the physiological roles they may play in morphogenesis and with respect to the mechanisms by which their expression may be controlled. Many of these enzymes (e.g., AC, PDE, ODC, and SAM synthetase) are involved in the metabolism of substances that are well-established correlates of dimorphism. Other enzymes examined (e.g., GDH, pyruvate kinase, and RNA polymerase [RNAP]) have a more tentative relationship to dimorphism.

The enzymes of cyclic-AMP metabolism have been extensively studied by Passeron and her associates. The activities of both AC and PDE have been measured in vitro (167) and in situ (40, 138) during dimorphic transformations of *M. rouxii*. PDE activity was observed to rise as cyclic-AMP levels declined during hyphal development. AC activities remained constant during this process. The physicochemical and kinetic properties of AC (41) and PDE (76, 146, 221a) have been characterized. Phosphorylation of PDE by a cyclic-AMP-dependent protein kinase reversibly activates the enzyme. It is reversibly deactivated by a phosphoprotein phosphatase. Proteolysis with trypsin irreversibly activates the enzyme and decreases its sedimentation coefficient (76, 146). Two phosphoprotein phosphatases (200) and two heat-stable inhibitors of these enzymes (201) have been purified from *M. rouxii* and characterized. Three protein kinase activities, one of which is activated by cyclic AMP, were resolved in extracts of *M. rouxii* (149). The cyclic-AMP-dependent protein kinase was studied in detail (147, 148, 165). The structural model which has emerged from these studies describes a tetramer composed of two regulatory subunits of 75,000 Da and two catalytic subunits of 41,000 Da (166). Thus, the cyclic-AMP-binding proteins are somewhat different in size from the 50,000- and 60,000-Da proteins described for *Saccharomyces* spp. (211) and the 51,000- and 65,000-Da proteins described for *M. racemosus* (70). No

formal hypothesis linking alterations in protein kinase activity with *Mucor* morphogenesis has yet been proposed.

The role of ODC in putrescine synthesis (101) and the consistent correlation of ODC activity with *Mucor* morphogenesis (34, 77, 105, 196) have been discussed elsewhere in this review. The regulation of ODC expression in *Mucor* spp. has been studied (34, 101). Synthesis of the enzyme may be controlled at the translational level (101). The regulation of NAD- and NADP-dependent GDH activities during *Mucor* morphogenesis was also discussed earlier (102, 174) in another context. A preponderance of evidence discounts these enzyme activities as obligatory correlates of dimorphism. The activity of SAM synthetase was found to parallel the intracellular concentration of SAM, a universal methyl donor and a strong correlate of *Mucor* dimorphism, as noted elsewhere (77, 78). Cycloleucine, an inhibitor of SAM synthetase, was found to lower intracellular SAM levels and to prevent morphogenesis (78).

An array of enzymes involved in intermediary metabolism has been studied with respect to *Mucor* dimorphism. Two major forms of pyruvate kinase are found in extracts of *M. rouxii* (164); one of these (type II) occurs only in hyphae (74, 163, 218). The same isozymes of pyruvate kinase are also demonstrable in extracts of *M. racemosus* (170). They most likely represent separate gene products (98); however, the differential expression observed in yeasts and hyphae is not related to morphogenesis but simply to glucose repression under anaerobiosis (170). Phosphofructokinase occurs at levels 30 to 40 times higher in extracts of anaerobic yeasts than in extracts of aerobic hyphae of *M. rouxii*. The enzyme is inhibited in vitro by ATP and citrate, but the inhibition is reversed by cyclic AMP. Glucose stimulates activity only slightly in aerobic hyphae (168). These data indicate a possible link between enzyme activity and morphology; however, the critical experiments to demonstrate a definitive correlation between the two (e.g., assay of enzyme activity in aerobic yeasts) have not been done. Pyruvate decarboxylase activity was shown to be controlled essentially by the level of glucose in the medium rather than by the morphological state of *M. rouxii* (10). An unpublished study by Stearns and Paznokas (168a) revealed two isozymes of alcohol dehydrogenase in *M. racemosus*, each specific to one morphological form. Mutants selected for a defective hyphal form of the enzyme are unable to grow anaerobically, suggesting that both isozymes are products of the same gene. The change in isozyme profile is dependent on protein synthesis, suggesting that at least one additional gene product, perhaps linked to development, is required for the expression of isozyme differentiation.

Several enzymes that function in disaccharide catabolism have been examined in relation to *Mucor* dimorphism. *Mucor* spp. are normally unable to utilize disaccharides anaerobically. Thus, the yeast morphology is usually correlated with an inability to grow on substrates such as maltose, cellobiose, and trehalose. α-Glucosidase, which cleaves maltose, has been found in cytoplasmic and wall-associated fractions of *M. rouxii*. Enzymes from the two fractions have the same physicochemical and kinetic properties; however, it has not been established whether they represent the same gene product (67). The cytoplasmic enzyme is induced by maltose under aerobic or anaerobic conditions regardless of cell morphology. However, the wall-associated enzyme, which may function in the periplasmic space, is elicited by maltose only in aerobic hyphae or in EDTA-induced anaerobic hyphae (204). It never appears in anaerobic or aerobic yeasts because of repression by cyclic AMP (30). The inability of *Mucor* yeasts to grow on maltose may be related to the lack of an uptake mechanism for this compound in the organism (180). If the enzyme is not inserted into the periplasmic space of *Mucor* yeasts, it will not have access to the substrate.

β-Glucosidase, which cleaves cellobiose, exists in soluble and wall-associated fractions of *M. racemosus*. The enzymes from these two fractions have identical physicochemical and kinetic properties, but it is not known if they are specified by the same gene (29). Enzyme activity is expressed during growth on all carbon sources except hexoses. Hexoses and cyclic AMP severely repress synthesis of the enzyme. In addition, cyclic AMP quickly, but reversibly, inactivates the enzyme, presumably via the mechanism of a protein kinase (30). Thus, this enzyme cannot be expressed in *Mucor* yeasts, which require the presence of a hexose and invariably possess high intracellular concentrations of cyclic AMP. Since it can be totally repressed in hyphae, the enzyme cannot be an obligatory correlate of morphogenesis.

Trehalose, which cleaves trehalose, has been detected in cell extracts of aerobically and anaerobically germinating sporangiospores of *M. rouxii* (229). The appearance of enzyme activity in either system is dependent on exogenous glucose or cyclic AMP. Evidence suggests that activation of preexisting enzyme by a cyclic-AMP-dependent protein kinase is responsible for the appearance of enzyme activity (52). It is of interest that trehalose cannot support the growth of anaerobic *Mucor* yeasts, yet the enzyme responsible for catabolism of this disaccharide of glucose can be detected during the development of such cells. Further work is needed to determine the intracellular location of trehalose and the permeability of yeast cells to trehalose.

Several distinct aminopeptidases and carboxypeptidases have recently been characterized in *M. racemosus* (54). Some of the carboxypeptidases are morphology specific. Peptidase activity in general increases during yeast-to-hypha conversions and germ tube emergence from sporangiospores (54).

There is precedent among prokaryotic systems for transcriptional regulation exerted at the level of RNAP during spore and phage development (82, 137, 223). Young and Whiteley (236, 237) purified and characterized RNAP activities from yeasts and hyphae of *M. rouxii*. Three classes of RNAP, typical of all eukaryotic cells, were identified in each cell type. Each class of enzyme whether from yeasts or hyphae, is identical in all properties. RNAP-I, which is responsible for synthesis of rRNA, and RNAP-II, which directs mRNA synthesis, increase substantially in specific activity during yeast-to-hypha morphogenesis. RNAP-III, which catalyzes tRNA and 5S RNA transcription, remains at a constant level of activity during development. The specific increases in RNAP activities described by these investigators correlate with the increased rates of rRNA and mRNA synthesis reported to occur during yeast-to-hypha morphogenesis in *M. racemosus* by Orlowski and Sypherd (159).

Macromolecular Synthesis

Growth (and by implication macromolecular synthesis) is an obligatory accompaniment of any morphological change in *Mucor*. It may also be true that, under most circumstances, morphological differentiation is the ultimate programmed outcome of growth. Hyphal growth on a solid medium inevitably results in sporangiospore production. Batch cultivation in liquid medium inevitably results in septation of hyphae and arthrospore formation. Continuous

cultivation of *Mucor* hyphae is not possible but if attempted, results in septation of hyphae and arthrospore production in the growing mycelial mass (190a). Individual yeasts eventually accumulate so many bud scars that they can no longer reproduce. These aged mother cells are frequently very large and internally granulated in appearance. There is a much higher percentage of such cells at stationary phase in batch culture than in continuous culture (190a).

In *Mucor* spp., the stoppage of growth by various physical agents (37), chemical inhibitors (37, 88, 89, 161), substrate analogs (224), or starvation of an auxotroph (152) prevents or arrests morphogenesis. Prevention of morphogenesis can be brought about by various morphopoietic agents without causing a cessation of growth; however, the growth rate is usually altered considerably under such conditions (73, 105, 118). The growth rate may change substantially or very slightly during *Mucor* development, depending on the culture conditions (189, 190). However, a change in the growth rate does not in itself dictate a morphological alteration (189, 190).

Haidle and Storck (88) first observed that aerobically induced yeast-to-hypha morphogenesis is prevented in *M. rouxii* by cycloheximide, as is growth. They demonstrated that cycloheximide acts primarily by inhibiting protein synthesis, though it has a delayed effect on RNA synthesis as well (89). These investigators radioisotopically measured the kinetics of RNA and protein accumulation in the converting cells under steady-state conditions and observed that they were commensurate with the increase in culture turbidity (88).

Orlowski and Sypherd (157) performed pulse-labeling experiments to measure the instantaneous rate of protein synthesis at intervals throughout CO_2-to-air or CO_2-to-nitrogen atmospheric shifts inducing yeast-to-hypha conversions of *M. racemosus*. This parameter was invariably observed to increase temporarily and then decline, maximal synthetic activity coinciding with the emergence of germ tubes. The same temporal relationship between the instantaneous rate of protein synthesis and germ tube emergence was observed during germination of *M. racemosus* sporangiospores in air (161). The molecular mechanism responsible for adjusting the overall rate of protein synthesis was found to be a dynamic balance between (i) the cellular ribosome concentration, (ii) the percentage of ribosomes recruited into the translation process, and (iii) the rate of polypeptide chain elongation (158, 161). Since different gene transcripts reportedly display differential stability in eukaryotic microorganisms (42, 114), an adjustable rate of nascent peptide growth may impinge on not only the quantity but also the quality of protein that is made. Moreover, since the steady-state level of any protein represents the difference between the rates of its synthesis and degradation and since rates of proteolytic degradation vary considerably for different gene products (176), the presently described system offers considerable potential for effecting differential gene expression.

Several other parameters generally thought to be involved in the regulation of protein synthesis were observed to change in a coordinate fashion with the measured rate of protein synthesis during *Mucor* morphogenesis stimulated by CO_2-to-air shifts. A basic protein (designated S6) from the small ribosomal subunit displays various degrees of phosphorylation, which is a strong correlate of the level of protein synthetic activity in most eukaryotic organisms (122, 124). The degree of S6 phosphorylation shows a direct correlation with cell morphology, rate of protein synthesis, and intracellular ATP concentration during CO_2-to-air-induced yeast-to-hypha conversions and sporangiospore germination in *M. racemosus* (119, 120).

Intracellular polyamine levels are thought to affect the rate and fidelity of protein synthesis (2, 4). The intracellular concentration of putrescine and the specific activity of ODC, the enzyme which synthesizes this compound, fluctuate on the same time course as the parameters just considered (77, 101). Both putrescine and ODC have been discussed at length in other sections of this review.

In a study by Garcia et al. (77), increases in intracellular SAM concentration and the degree of generalized protein methylation were observed to correlate with putrescine levels and morphogenesis. When methylated proteins were subjected to 2-D PAGE, approximately 20 gene products were resolved. The degree of methylation of most of these was much greater in hyphae than in yeasts. One protein alone accounted for most of the difference (77). This highly methylated protein was identified as protein synthesis elongation factor 1α (EF-1α) by Hiatt et al. (95). Levels of the protein were reported to be much higher in aerobic hyphae than in yeasts grown under CO_2 or nitrogen. In fact, EF-1α represents the most abundant protein in hyphae. The degree of methylation of the protein was also noted to strongly correlate with cell morphology and, by implication, with protein synthesis activity. EF-1α is virtually nonmethylated in dormant spores. The level of methylation increases markedly during germination and hyphal growth, achieving a maximum of eight or nine mono-, di-, or trimethylated lysine residues per protein molecule in hyphae. Moreover, the specific activity of EF-1α, measured as stimulation of poly(U)-directed polyphenylalanine synthesis in crude cell extracts, increases severalfold during this period of development. The increase in factor activity occurs in the absence of any increases in the levels of EF-1α-specific mRNA or EF-1α protein and is apparently regulated solely by the degree of methylation (69).

A general model that explains regulation of the rate of protein synthesis during *Mucor* morphogenesis and encompasses many of the known correlates of development discussed in this review might be conceived. A potential role could be envisaged for cyclic AMP and a cyclic-AMP-modulated protein kinase in controlling the phosphorylation state of ribosomal protein S6, which in turn, at the appropriate polyamine concentration and in the presence of variably methylated EF-1α, could adjust the rate of translation. The extent of EF-1α methylation could be influenced by the intracellular SAM concentration, which in turn could be determined by SAM synthetase activity. Additional elements might play a role in the overall process. For example, the composition of the initiation factor population may influence the recruitment of ribosomal subunits into polysomes.

Some mechanism of this general nature may very well operate in *Mucor* spp. to control the rate of protein synthesis. However, there are a few anomalous experimental results which suggest that some of the correlates discussed above may be related primarily to alterations in the growth rate rather than to morphogenesis per se. *M. racemosus* cells shifted from a nitrogen atmosphere to air convert from yeasts to hyphae; however, the instantaneous rate of protein synthesis, the percentage of ribosome recruitment into polysomes, and the rate of peptide bond formation show little increase over the relatively high levels normally found in yeasts grown under nitrogen (155, 189, 190). The degree of S6 phosphorylation was described as nutrition and growth rate dependent under circumstances in which no morpho-

genesis takes place (120). It is possible that the mere absence of CO_2 derepresses certain functions necessary for yeast-to-hypha morphogenesis, some perhaps related to the rate of protein synthesis, but they alone are not sufficient to allow morphogenesis to go on in the absence of oxygen.

In addition to an increased rate of protein synthesis accompanying the yeast-to-hypha conversion induced by a CO_2-to-air atmospheric shift, other studies have noted similar increases in the synthesis of DNA (102), RNA (159), chitin plus chitosan (55), and lipids (105). Cano and Ruiz-Herrera (37) have noted the requirement for DNA synthesis and nuclear division prior to the initiation of germ tube formation in aerobic germination of *M. rouxii* and *M. bacilliformis*. Inhibition of DNA synthesis at high temperature or in the presence of hydroxyurea causes unregulated spherical growth and the production of giant germ spheres (37). In addition to these general classes of macromolecules, many specific enzymes (see the appropriate section of this review) have been reported to undergo during morphogenesis an increase in specific activity caused by a demonstrated or assumed increase in the rate of synthesis. Unfortunately, in no case was the morphological transition induced by a nitrogen-to-air atmospheric shift and the results compared with data from a CO_2-to-air shift in order to take account of possible growth rate effects. The site of de novo protein synthesis was studied during yeast-to-hypha morphogenesis and aerobic sporangiospore germination by means of whole-cell autoradiography. Though the net accretion of biomass occurs only at the growing tip, the synthesis of new protein takes place throughout the entire coenocytic structure (160).

Qualitative changes in protein synthesis during yeast-to-hypha transformations of *M. racemosus* were examined by means of radioisotopic labeling, 2-D PAGE, and autoradiography (96). Approximately 500 gene products were resolved in samples from CO_2-induced yeasts, nitrogen-induced hyphae, and air-induced hyphae. Although many quantitative differences were observed, only a few polypeptides showed a qualitative correlation with morphological form. On the basis of similarity of protein patterns observed in the two hyphal cultures, the investigators argued that the majority of the quantitative and qualitative differences noted were related to morphogenesis per se and were not a specific response to oxygen (96). When Phillips and Borgia (175) reexamined the system for inducing hyphal development under a nitrogen atmosphere, they discovered such cultures to be contaminated with trace amounts of oxygen. This microaerobic environment is sufficient to evoke hyphal forms possessing a fermentative metabolism. Hyphae cannot, in fact, develop under strict anaerobic conditions. In 2-D PAGE analyses, as described above, these researchers found many differences between the polypeptide population synthesized in aerobic hyphae and the populations synthesized in microaerobic hyphae and strictly anaerobic yeasts (grown under a pure N_2 atmosphere). These differences correlate with the pathways of energy metabolism. Microaerobic hyphae and anaerobic yeasts display only a few differences in polypeptide composition. Air-induced hyphae are identical to microaerobic hyphae, and nitrogen-induced yeasts are identical to CO_2-induced yeasts with respect to this limited group of proteins, which may represent bona fide correlates of morphogenesis (175).

Sporangiospores of dimorphic *Mucor* spp. have the capacity to form either yeasts or hyphae depending on the environment. The spores of *M. racemosus* were shown to contain a large pool of stable polyadenylated mRNA which is synthesized during spore formation but remains unused until dormancy is broken by introduction of the spores into nutrient medium (131). Before germination is initiated, most ribosomes are in the form of free inactive 40S and 60S subunits. Within 10 min of exposure to nutrient medium, 85% of the ribosomes are recruited into actively translating polysomes. De novo synthesis of RNA does not occur during the first 20 min of germination, and newly made mRNA is not available for translation until at least 30 min have passed; however, a large number of proteins are synthesized from the preformed mRNA templates at this time (131). Inhibitor studies have indicated that sporangiospores of *M. rouxii* can partially complete spherical growth using only the stored mRNA. However, new RNA must be synthesized during spherical growth in order to allow the subsequent formation of buds or germ tubes (37).

An analysis of the protein products synthesized in vivo during sporangiospore formation and germination was performed by radioisotopic labeling, 2-D PAGE, and autoradiography (132). It was found that the population of proteins accumulated during spore formation differ significantly from those proteins synthesized during spore germination. Several proteins are synthesized during the former but not the latter morphogenetic transformation. Conversely, other proteins are synthesized during the first 30 min of germination but not during spore formation, even though the mRNA specifying these proteins must have been synthesized and stored in the dormant spore. A posttranscriptional regulatory mechanism that directs selective translation thus appears to exist in the developing spore. Many proteins, although made throughout germination, display significant alterations in their individual rates of synthesis over this time. At least one gene product appears to undergo a posttranscriptional modification during the first hour of germination (132).

mRNA pools in dormant and germinating sporangiospores were analyzed by in vitro translation, 2-D PAGE, and autoradiography (133). Gene products encoded in mRNA were compared with gene products actually synthesized in vivo at a given stage of development. It was determined that (i) most of the differential gene expression displayed at the level of protein synthesis during germination results from concomitant changes in functional mRNA levels, (ii) some of the stored mRNA species may be activated and others inactivated by posttranscriptional processing mechanisms, and (iii) a small population of gene products may be regulated at the level of selective translation of preexisting messages (133).

Radioisotopically labeled in vivo translation products from *M. racemosus* sporangiospores germinating under an air or a nitrogen atmosphere were compared by means of 2-D PAGE and autoradiography (133a). The population of stable preformed mRNAs available for translation within the first 30 min of germination is identical in each case. Most of the protein products made within the first hour of germination are identical in the two systems. There is, however, a small number of proteins made that are unique to each system. Synthesis of these unique proteins persists throughout germination and is also characteristic of the eventual vegetative morphology achieved. Whether the observed differences are specifically linked to morphology or metabolism is not yet known. In either event, differential gene expression is controlled during the first stages of germination by selective translation of a specific subset of the total set of preformed mRNAs stored in the dormant spore. The sporangiospore may maximize its developmental options by storing mRNAs essential to the formation of both types of vegetative mor-

phology and ultimately expressing only those appropriate to the morphogenetic sequence dictated by the environment.

Stable preformed mRNA is stored in sporangiospores of *M. racemosus* in the form of messenger ribonucleoprotein particles (mRNPs). The mRNPs contain polyadenylated RNA with a heterodisperse size distribution (10S to 23S) and approximately a dozen specific proteins. The particles are about 30S in size and display buoyant densities of 1.595 g/cm^3 in CsCl and 1.295 g/cm^3 in metrizamide. The mRNPs do not represent translation initiation complexes of mRNA and 40S ribosomal subunits. The protein compositions of mRNPs and 40S ribosomal subunits are quite different. Within 5 min after the initiation of germination, the preformed polyadenylated mRNA, which is identified by hybridization to radioactive poly(U), leaves the 30S mRNPs and appears in the newly assembled polysome fraction of the cell (41c). It is not yet known what happens to the protein portion of the mRNPs after translation of the mRNA has begun, nor is it known whether distinct populations of mRNPs are mobilized for translation in sporangiospores germinated under an aerobic versus an anaerobic atmosphere.

MOLECULAR GENETIC STUDIES OF *MUCOR* SPP.

The science of genetics has served as an indispensable tool for probing cellular structure and function at the molecular level. The ability to alter and recombine genetic information has allowed molecular biologists to describe the central mechanism of information transfer from genotype to phenotype and to deduce many of the devices regulating such transfer. The tools of genetic analysis have gradually evolved and improved from classical Mendelian analysis to modern recombinant DNA technology. Developmental biologists presently employ genetic techniques in attempting to fathom the network of interrelated and interdependent molecular processes that control morphogenesis. The remainder of this review will report the progress to date in the genetic analysis of *Mucor* spp. and dimorphism.

All *Mucor* spp. are presumably capable of mating and forming zygospores. Zygospores are formed by heterothallic species through the fusion of gametangia produced by opposite mating types. Zygospores are formed in homothallic species by the fusion of gametangia produced in different locations on the same thallus. Gametangia are formed in response to the presence of trisporic acid, which is synthesized from complementary prohormone precursors secreted by the opposite mating types. Karyogamy and meiosis occur within the developing zygospore (84).

Of the three *Mucor* spp. most commonly studied with respect to dimorphism, *M. rouxii* and *M. genevensis* are homothallic and thus incapable of genetic exchange through mating. *M. racemosus* is heterothallic, but zygospores from this species have not been subjected to genetic analysis. Most of the information available on heterothallic mating in *Mucor* spp. comes from studies of *M. mucedo* and *M. hiemalis* (106). The results of such matings are not encouraging for the would-be geneticist. Many zygospores formed of such unions never germinate, and those that do lie dormant for an average of 2 months or more. Furthermore, most germ sporangia contain only one genotype. Heterokaryotic germ sporangiospores are quite rare.

The production of mutant strains of *Mucor* spp. has offered no major problems. Peters and Sypherd (173) used several freeze-thaw treatments to enrich for mutants of *M. racemosus* created by the action of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). Selection is based on the 1,000-fold-greater resistance to freeze-killing of growth-limited sporangiospores over actively germinating sporangiospores. In a similar fashion, Roncero (187) used the polyene antibiotic *N*-glycosylpolifungin to select against sporangiospores of *M. racemosus* and *M. miehei* not carrying a mutation caused by UV irradiation. Ruiz-Herrera and co-workers (196) succeeded in isolating NTG-induced mutants of *M. bacilliformis* without using a selective enrichment step. Borgia and his colleagues (28) have enriched for NTG-induced morphology mutants of *M. racemosus* by filtering cells through Miracloth, which passes yeasts while trapping hyphae. The use of one or another of these selection procedures has made it possible to isolate mutants that are auxotrophic (173, 187), monomorphic (196), conditionally monomorphic (28, 213), respiratory deficient (207), or refractory to the normal effects of exogenous cyclic AMP on morphology (171).

Since formidable obstacles stand in the way of exploiting the natural mating system in *Mucor* spp. to perform complementation and dominance testing of mutant strains, Borgia and his co-workers have attempted to circumvent this problem by means of protoplast fusion (81, 121). This technique has been used to produce stable hybrid organisms within and across generic boundaries in bacteria, plants, and fungi (172). The feasibility of producing stable spheroplasts of *M. rouxii* by treatment of hyphae or germlings with a mixture of commercial chitinase and chitosanase purified from a *Streptomyces* sp. had been documented by several groups (111, 178, 209). Germlings of *M. racemosus* were converted to spheroplasts by exposure to commercial chitinase and chitosanase purified from *Myxobacter* AL-1 (81). Fusion of different auxotrophic strains was stimulated with polyethylene glycol and $CaCl_2$. Following regeneration of the cell wall, prototrophic heterokaryons were formed at a frequency of about 5% (121). Karyogamy does not occur in these fusion products. Complementation is between genetically dissimilar nuclei within the coenocytic hyphae. The parental types are completely segregated during the formation of haploid sporangiospores. The total absence of genetic reassortment severely limits the usefulness of this system. Determination of simple linkage relationships, let alone chromosomal maps, is not possible using classical genetic analyses here. Much better results have obtained in analyses of the *Mucor* genome by recombinant DNA techniques and other procedures of molecular genetics, which will be discussed below.

Van Heeswijck (226) demonstrated that the generation of protoplasts through the action of chitosanase is applicable to many *Mucor* spp. In further work, Van Heeswijck and Roncero (228) transformed protoplasts of leucine auxotrophs of *M. racemosus* with the *Saccharomyces cerevisiae-E. coli* shuttle vector YRp17 carrying inserted random fragments of *Mucor* genomic DNA. The *Mucor* transformants were prototrophic for leucine and were shown to harbor vector DNA. *E. coli* cells transformed with DNA from the *Mucor* transformants displayed ampicillin resistance encoded by the vector. They were determined to harbor plasmid DNA containing a 10.5-kb insert of *Mucor* genomic DNA capable of complementing the *leu* mutation in *M. racemosus*. Initial attempts to transform *E. coli* strains carrying the *leuB6* allele or to complement a *Saccharomyces* strain carrying a mutation in the *leu-2* gene (both encoding isopropylmalate dehydrogenase) were unsuccessful (228). More-recent work has revealed that the *Mucor* gene, contained within a 4.4-kb *Pst*I subfragment of the original insert, encodes isopropylmalate

isomerase (188). The plasmid was demonstrated to replicate without integration into the nuclear genome (227). An autonomous replication sequence (*ARS*) has been located in a region of the 4.4-kb *Pst*I fragment having a moderate degree of similarity with the reported core consensus sequence of the *S. cerevisiae ARS* (188). The reported frequency of transformation was high (600 leu^+ transformants per μg of DNA per 3.2×10^6 viable cells), offering the promise that many genetic lesions may be analyzed by similar complementation experiments.

A different approach had been taken earlier by Cihlar and Sypherd (46) in an attempt to identify *Mucor* genes encoding leucine biosynthetic enzymes. *M. racemosus* genomic DNA was inserted into *E. coli* plasmid pBR322. This recombinant plasmid complemented the *leuB6* mutation in *E. coli*, which specifies a defective form of isopropylmalate dehydrogenase. However, the complementation was due not to substitution of a functional *Mucor* enzyme for the defective *E. coli* gene product but rather to suppression by an unknown mechanism that restores a functional *E. coli* enzyme. None of several other strains of *E. coli* leucine auxotrophs were complemented by any part of the *Mucor* genome, perhaps because *E. coli* cannot assemble functional mRNA from the eukaryotic DNA template.

A number of well-characterized gene products have been so highly conserved through the course of evolution that the cloned gene from one organism may serve as an effective hybridization probe for the homologous gene in an unrelated organism. This allows a genomic library to be screened for the particular gene and the appropriate clones to be selected. In a similar fashion, various cloned genes have served as probes to detect multigene families (113, 128) and pseudogenes (127) within the same organism. Several studies have resulted in highly conserved genes being cloned or detected in *M. racemosus*. Known examples presently include the genes for actin (44), α- and β-tubulin (142a), and *ras* oncogene homologs (41a, 41b). Using a cloned *S. cerevisiae* actin gene as a probe, Cihlar has identified an unspecified number of actin genes from *M. racemosus* (44). Using chicken fibroblast cDNA probes, Paznokas and his colleagues have shown that there are at least two and possibly three loci in the *M. racemosus* genome encoding α-tubulin and three distinct genes specifying β-tubulins (168a). Using *S. cerevisiae YRAS* gene probes, Linz and his co-workers have cloned three distinct *ras* genes from *M. racemosus*. The nucleotide sequence of each has been determined. One of the genes (*MRAS2*) appears not to be transcribed in developing or germinating sporangiospores or in either vegetative form. The levels of transcription of the other two (*MRAS1* and *MRAS3*) fluctuate independently during spore formation, spore germination, and yeast-to-hypha morphogenesis (41b).

Cihlar and his associates have cloned an interesting dispersed repetitive DNA sequence, designated MuR, from the genome of *M. racemosus* (51). MuR initially was noticed as a recurring sequence in many unrelated clones from a *Mucor* genomic library in pBR322. The sequence is approximately 3.0 kb in length, is reiterated 25 to 35 times, is not clustered in any region of the *M. racemosus* genome, and has been characterized with respect to at least 15 restriction sites. Sequences homologous to MuR were detected by hybridization in *M. genevensis*, *M. mucedo*, and *M. hiemalis*, suggesting that the gene is highly conserved in the genus. However, MuR is not related to sigma, a highly reiterated DNA sequence in *S. cerevisiae* (51). It has been speculated that MuR represents a transposon (44, 51). Should this prove to be true, the element might serve in the construction of an integrative cloning vector. It was recently reported that germinating spores of *M. racemosus* contain a heterogeneously sized population of polyadenylated RNA molecules that collectively hybridize with the entire length of MuR. RNA complementary to MuR is completely absent from *Mucor* yeasts but is present at low levels in mature hyphae (50a).

The gene specifying *Mucor* rennin was cloned from *M. pusillus* by using synthetic tetradecamer and septadecamer oligonucleotide probes based on a partial amino acid sequence of the enzyme (222). The nucleotide sequence of the gene was determined (222), and the gene was inserted into a *S. cerevisiae* plasmid (pSS21) under the control of the *S. cerevisiae GAL7* promoter (235). Enzyme protein was secreted from *S. cerevisiae* as an inactive zymogen which was subsequently activated in the culture medium by autocatalytic proteolysis (2a, 97). Thus, it should prove possible to study the regulation of isolated *Mucor* genes in the more precisely defined and manipulatable genetic environment of *S. cerevisiae*.

Because hyphal development from germinating sporangiospores and from *Mucor* yeasts shifted to an aerobic atmosphere is invariably accompanied by a marked change in the rate of translation (158, 161), a number of laboratories have begun analyses of various components of the translation machinery using recombinant DNA technology. The genes specifying the 5S, 5.8S, 18S, and 25S rRNA species have been cloned from *Bam*HI and *Hin*dIII restriction fragments of a genomic library of *M. racemosus* in the plasmid pBR322 (45). More recently, the same genes have been inserted into plasmids pUC19 and pACYC184 from *Eco*RI and *Hin*dIII restriction fragments of an rDNA fraction separable from nuclear DNA by cesium chloride isopycnic centrifugation (109). Purified rRNA served as the hybridization probe in the former study, whereas the cloned rDNA repeat unit from *S. cerevisiae* served the same purpose in the latter case. Both studies indicated that the genes from *M. racemosus* occur in a series of direct repeats having the order 5S-18S-5.8S-25S. The repeat unit is 9.76 kb in length and has been mapped with respect to 12 restriction recognition sites (109). The nucleotide sequence of the complete 25S rRNA gene and flanking regions was determined (109). A potential secondary structure of the putative RNA transcript has been constructed by computer modeling (109). Similar procedures have been used to clone and map *Mucor* genes coding for numerous tRNA molecules (44).

By means of cDNA probes copied from *Mucor* mRNA, three genes for EF-1α have been cloned from the *M. racemosus* genome (129). A 110-bp fragment from a cDNA clone sharing sequences with an mRNA population specifying the in vitro synthesis of a protein that comigrates with pure EF-1α in 2-D PAGE was used to isolate an 850-kb *Eco*RI fragment of *Mucor* genomic DNA which showed significant homology to the EF-1α gene from *S. cerevisiae* and which was subsequently subcloned into plasmid pBR322. This fragment was used as a probe to screen an *M. racemosus* genomic library in bacteriophage lambda for complete EF-1α genes. DNA from phage clones displaying hybridization to the probe could be grouped into three distinct restriction patterns, suggesting the existence of three genes for EF-1α. Subcloned restriction fragments were hybridized with mRNA and subjected to S1 nuclease digestion to identify the ends of the genes. Flanking regions of the three genes were found to hybridize with unique *Hin*dIII fragments of the *Mucor* genome, suggesting separate loca-

tions for each gene. The nucleotide sequences of all three genes (designated *TEF-1*, *TEF-2*, and *TEF-3*) (210) and the primary structures and functional domains of their protein products (130, 210) have been determined. The three *Mucor* genes share substantial sequence similarity, including highly conserved introns, with one another as well as with the homologous gene from *S. cerevisiae* (210). Thus, the EF-1α genes appear to represent a highly conserved family of genes such as the genes for actin, tubulin, and the *ras* proteins mentioned above. Regulated expression of EF-1α activity was shown to be a correlate of *Mucor* morphogenesis and of the rate of protein synthesis (69). Each of the EF-1α genes possesses relatively short, unique sequences at the 3′ ends of the genes that are transcribed but not translated (210). Nonadecamer oligonucleotides specific to these unique regions were synthesized and used as hybridization probes to study expression of the three genes during morphogenesis. All genes were transcribed in all cell types examined; however, their individual levels of transcription differed considerably and fluctuated independently during morphogenesis (134). The potential role of differential activation within this multigene family in *Mucor* morphogenesis or in the modulation of translation rates is not yet understood.

Using a strategy similar to that employed in the isolation of the EF-1α genes, a ribosomal protein gene was cloned from a genomic library of *M. racemosus* DNA in bacteriophage lambda Charon 4A (205). The nucleotide sequence of the gene was determined. It was found to possess one intron, a 25-nucleotide untranscribed leader, and a 114-nucleotide untranscribed 3′ end. The amino acid sequence of the predicted protein product identified it as a putative homolog of ribosomal protein S19 of *Xenopus laevis*. The cloned gene hybridizes with three different genomic DNA fragments, suggesting the existence of three copies of the same or very similar genes. A comparison of nucleotide sequences upstream of the translation start site revealed considerable similarity to known promoter elements of genes encoding proteins of the translation apparatus in *Saccharomyces* spp. and other yeast genera (205).

The cloned genes specifying various components of the translation apparatus should allow researchers to examine the coordinate regulation of these genes during *Mucor* morphogenesis and during other adaptive responses of the organism in which protein synthesis is affected. Recombinant DNA technology is a powerful new tool that is being used to study *Mucor* morphogenesis with increasing frequency. Using this tool, researchers stand poised to investigate many of the correlates of dimorphism already described but not yet explained.

PERSPECTIVE

The genus *Mucor* has been an object of study for medical, industrial, biochemical, and developmental reasons for well over 100 years. It has proven useful in testing or establishing paradigms in all these areas. Its easily manipulated dimorphic character has made it especially practical for relating cell structure to composition, and morphological changes to differential gene expression. Seminal work by Bartnicki-Garcia and his collaborators discovered key differences in the composition and pattern of deposition of wall polymers related to *Mucor* cell morphology (13, 18, 26, 117, 142). Sypherd and his colleagues pioneered the application of molecular biology and molecular genetics to the study of *Mucor* morphogenesis (30, 45, 46, 95, 96, 101, 118, 119, 129, 134, 158). Both schools of mucorology, now having independent practitioners in over a score of laboratories around the world, have uncovered numerous facts about this organism that have proven to be of general relevance to other systems of fungal growth and development. The presence of chitosomes (15, 16, 31, 93) and the influence of internal cyclic-AMP levels on cell morphology (118, 155, 167), both discussed at length above, are two good examples of such findings. Several other areas mentioned in this review that are presently either under study or contemplated for study in *Mucor* spp. are of wide concern in all of developmental and molecular biology. Such lines of inquiry include the role of *ras* proteins in regulating growth and development (41a, 41b), the role of EF-1α proteins in modulating developmentally linked changes in the overall rate of translation (129, 130, 134, 210, 210a), a possible ultrastructural role for EF-1α (235a), the potential use of actin and tubulin proteins in cytoskeletons and molecular motors (17a, 92a, 142a), the utilization of polyamines as intracellular signals or effectors (36, 87a, 194), stored mRNAs and their differential expression by selective translation (133a), characterization of distinct populations of mRNPs in germinating spores (41c), and the potential role of ion currents in directing polarized growth (228a, 234). I anticipate that the major progress in *Mucor* research will be made on these fronts over the next decade. I also anticipate that *Mucor* spp., being one of the rare examples of a eukaryotic facultative anaerobe and displaying a pattern of mitochondrial development in response to the environment that is distinctly different from what is classically observed in the well-characterized *S. cerevisiae* (108a, 198b), will reveal an interesting alternative strategy for regulating the biosynthesis of these organelles.

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