DIMORPHISM IN THE FUNGI

by

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INTRODUCTION

In most higher fungi, the thallus generally assumes one of two basic morphological forms: a unicellular yeast or yeast-like form or a multicellular (or multinucleate) mycelial form. The yeast forms are ellipsoidal or spherical cells which carry on cell division and reproduction by a process known as budding. Here, a protuberance (which later enlarges to form a daughter cell) is formed on the parent cell; the cytoplasm between the parent and daughter cells is constricted and a cross wall is laid down. Then the two cells generally separate. Mycelial forms are long filaments or hyphae which can be septate (multicellular and usually uninucleate) or nonseptate (unicellular but multinucleate). Cell division occurs in septate hyphae by extension of the cell wall at the hyphal tip followed by cross wall formation between the two cells. There is no constriction of the cytoplasm between the cells in this form. In nonseptate hyphae, the cell elongates by growth at the hyphal tip, but no cross wall is formed so the hypha is one multinucleate cell.

Some fungi exhibit the ability to exist in either of these two forms depending upon the environment in which they are growing. In these fungi, both the yeast and mycelial morphologies are a part of the life cycle. There is, however, no sharp line of distinction in nature between the yeast and mycelial forms, and many intermediate forms have been observed. These intermediates result from cell division in which there is a constriction of the cytoplasm between the parent and daughter cell, but it is not a complete constriction, and the two cells are not separated. The resultant form is a long chain of yeast-like cells which remain attached. Such a form is termed a pseudomycelium. Varying degrees of constriction between parent and daughter

cells can occur which lead to gradations in form from the yeast-like to true mycelial morphology.

The ability of a fungus to exhibit this two-fold nature in its phenotype is generally termed dimorphism. Since, as will be noted below, most of the fungi that exhibit dimorphism (especially those studied in the earlier researches) are animal and human pathogens, the term dimorphism has been defined with specific reference to these pathogens. This can be seen in Ainsworth's (2) definition as he refers to dimorphism as "the condition in which there is a yeast-like parasitic phase and a mycelial saprophytic phase."

This limited definition does not, however, include other fungi which do not behave this way as pathogens or those which are completely non-pathogenic but can exhibit both yeast and mycelial phases in their life cycles. In this report, as in the reviews of Scherr and Weaver (52) and Romano (45), the term dimorphism will be used in the broad sense and defined as "an environmentally controlled reversible interconversion of yeast and mycelial forms, and denoted as Y $\leftrightarrow$ M" (45).

The dimorphic fungi are not included in any one taxonomic grouping. In fact, examples of dimorphism can be found in all three classes of the fungi and in the form class Fungi Imperfecti. The animal pathogens which are dimorphic include both sporogenous and asporogenous yeasts (2, 3, 52). Saccharomyces cerevisiae (50, 52) and S. ellipsoideus (57, 58, 59, 60), two common sporogenous yeasts, have both been shown to develop filamentous forms under certain conditions. Species of Taphrina in the Ascomycetes and members of the Ustilaginales in the Basidiomycetes are plant pathogens which show dimorphic tendencies. They differ from the animal pathogens, however, in that the mycelial phase is parasitic, and the yeast-like phase is the one which

is saprophytic (2). In the Phycomycetes, certain species of Mucor can grow in the yeast phase under certain conditions while these organisms are, under most circumstances, mycelial (4, 5, 6, 7, 8). Besides the asporogenous yeasts mentioned above, certain other imperfect fungi such as Fusarium oxysporum have been observed to grow as yeasts under certain environmental conditions (56).

The purpose of this report is to examine the physiological and biochemical bases responsible for the dimorphic phenomenon in the fungi as reported in the literature. For this reason, this report does not present a comprehensive list of all the incidents of dimorphism so far reported for the fungi, but tries to bring together those papers which might present some insight on the cause and mechanism of this phenomenon.

REVIEW OF LITERATURE

Action of Temperature.

For some time, temperature has been recognized as an important factor in controlling the M → Y conversion. Early workers noticed that brewer's yeast elongated somewhat when the temperature of incubation was below optimum, but grew as the normal yeast morphology under conditions near or above the optimum temperature (52).

The pathogen, Blastomyces dermatitidis is one dimorphic fungus for which temperature seems to be the most important factor controlling the morphological form. This fungus grows as the yeast phase in the animal host tissue or on blood agar at 37°C incubation temperature. The mycelial form predominates on a variety of media when incubated at room temperature. Using a glucose peptone medium, Levine and Ordal (21) found that the mycelial form grew at temperatures ranging

from room temperature to 33°C with the optimum at 31°C. The yeast form grew at incubation temperatures ranging from 35°C to 37°C with the optimum at 35°C. These results were obtained whether the inoculum consisted of a suspension of yeast cells or spores. It was also noticed that amino acid nitrogen sources enhanced the amount of growth over ammonium nitrogen sources, but this did not affect the phase of growth at any temperature. Salvin (48) obtained similar results with both B. dermatitidis and B. brasiliensis. He also showed that no growth factor was necessary for the maintenance of the yeast phase. Both of these authors concluded that the temperature of incubation is the most important factor in determining the morphology of the organism.

Nickerson and Edwards (51) called this "Thermal Dimorphism" and proposed that the transition from Y $\rightarrow$ M is due to selective inhibition of cell division without a decrease in growth. They studied oxygen uptake at 5° intervals over the temperature range of 5° to 40°C in order to see if there were differences between the yeast and mycelial growth. It was found that oxygen uptake increased with increasing temperature with a maximum around 40°C. Correspondingly, the yeast form consumed 5 to 6 times as much oxygen as the mycelial form.

Taylor (53) studied the level of nucleic acids present in both phases of Blastomyces. He found that although the DNA content of both morphological forms remained essentially at a low and constant level as would be expected, the RNA level fluctuated. The yeast phase exhibited a large increase in RNA from the sixth to the tenth day after inoculation; after the tenth day, it decreased a corresponding amount and remained constant for the remainder of the incubation time. The RNA level in the mycelial phase showed little fluctuation and was much

lower than that of the yeast phase. The higher content of RNA in the yeast cells was attributed to a faster rate of growth and division than was occurring in the mycelial cells. On the basis of preliminary work, Taylor proposed a causal role for RNA in dimorphism of Blastomyces.

Action of Temperature and Nutrition.

In some cases of dimorphism, incubation of the fungus at higher temperatures is not sufficient to maintain and/or convert it to the yeast phase. The compounds present in the substrate and the composition of the atmosphere in which the organism is grown are also important factors in determining the phase of growth. Species of Histoplasma, an animal pathogen, are good examples of the effect that both temperature and nutrition can have in determining the morphology of the organism.

Bullen (10) found that, in Histoplasma farciminosum, the yeast phase was produced if the atmosphere contained 15 to 30% carbon dioxide. Several media were used, and it was found that only mycelial development was obtained if the cultures were incubated in the air. However, if these cultures were placed in an atmosphere of high carbon dioxide content (15-30%), a gradual change to the yeast-like form was accomplished. After two or three subcultures in an atmosphere with this composition, the growth was almost entirely yeast-like. Cultures were also placed under atmospheres containing a high percentage of nitrogen to see whether the requirement was for increased carbon dioxide partial pressure or for decreased oxygen content. Mycelial cells were the only forms produced, indicating that increased carbon dioxide concentration was the critical factor in conversion to the yeast phase in this organism.

In another species, Histoplasma capsulatum, the M → Y conversion takes place under different conditions. The yeast-like phase occurs in animal tissues, and if removed in this phase, can be maintained on blood agar at 37°C for a short time. The mycelial phase can be grown on a variety of media at lower temperatures (13).

In earlier work, conversion to the yeast phase was rather difficult to achieve and was only accomplished by reinfecting the animal host, (46) or by growing it on a complex medium, such as glucose cystine blood agar at 37°C (12) or on potato flour-egg medium. (20) In later studies, however, several compounds were found which were necessary for the conversion, and the yeast phase could be maintained on a synthetic medium containing these compounds even at temperatures lower than 37°C (40, 41, 43, 46, 47, 51).

Salvin (46) found that a semisolid medium containing peptone, tryptone, glucose, and inorganic salts maintained yeast growth while a liquid medium with the same nutrients did not. The best growth was obtained when the semisolid medium contained 0.1 to 0.5% agar. Several tests were constructed to determine if the agar contained some kind of growth substance, or if the higher viscosity of the medium produced the yeast-like growth. The agar was continuously washed to remove any growth factors which might be present, and silica gel was used to eliminate the effect of any organic compound which might be present. In both of these cultures, growth was similar in amount and morphology to that obtained on unwashed agar. It was speculated that the semisolid medium produced a zone where the carbon dioxide and oxygen partial pressures were more favorable for yeast growth. To test this hypothesis, cells were grown in atmospheres that ranged from those which were

completely anaerobic to those containing various concentrations of carbon dioxide (10 to 80%) to an atmosphere of 100% oxygen. The growth in all of these cultures was similar to that in the air, and it was therefor concluded that oxygen and carbon dioxide partial pressures did not play a deciding role in the dimorphism of H. capsulatum. Scherr (51) later proposed that the action of the semisolid medium was to lower the oxidation-reduction potential of the medium sufficiently to make it favorable for yeast development.

Salvin (47) also investigated the nitrogen requirements of both phases and found differences between them. Cells were inoculated on tubes of solid media which contained only one of the twenty common amino acids as a sole nitrogen source. The yeast phase grew only on the media which contained cysteine or cystine as the nitrogen source. The other amino acids supported only mycelial growth or no growth at all, and a mixture of sixteen nonsulfur-containing amino acids produced growth which was entirely mycelial. Other sulfur-containing molecules were then tested for their ability to maintain the yeast phase, and it was found that inorganic or oxidized sulfur compounds were ineffective. Reduced organic molecules (especially the small ones such as cysteine and cystine) were the best in inducing the yeast phase although glutathione, methionine, and sodium thioglycollate had some effect. A study of the vitamin requirements showed that the yeast phase required biotin while the mycelial phase had no requirements. Using this information, Salvin could then convert H. capsulatum from the mycelial to the yeast phase on a solid medium at a constant temperature by changing the medium from one with alanine as the nitrogen source to one with biotin plus cysteine as the nitrogen source.

If a large inoculum of yeast cells in the logarithmic phase of growth was used, yeast-like growth could be maintained on a liquid medium that contained a mixture of amino acids (casamino acids or peptone), glucose, inorganic salt, and biotin (49). The ratio of the surface area to the depth of the medium was found to be critically important and needed to be relatively high for the yeast morphology to be predominant.

Scherr (51) confirmed the importance of cysteine in the maintenance of the yeast phase of H. capsulatum on a synthetic medium with an ammonium salt as the nitrogen source. He found that the amount of mycelial growth was directly proportional to the decrease in the incubation temperature and inversely proportional to the increase in the concentration of cysteine. He also showed that the concentration of sulfhydryl groups was more critical than the temperature of incubation, since the yeast phase could be maintained at temperatures lower than 37°C if a higher concentration of cysteine was used. The higher temperature seemed to be required for optimum activity of certain enzymes necessary for M $\longrightarrow$ Y conversion. It was proposed by Scherr that the metabolic pathways which control the maintenance of the yeast phase are different from those which participate in the M $\longrightarrow$ Y conversion. Since sulfhydryl groups do not remain free in the medium very long, it was thought that they act to maintain the viability of the organism until growth begins. Then after the concentration of sulfhydryl groups (or oxidation-reduction potential) in the medium has determined the phase of growth in which the organism will exist, the metabolic activity of the particular phase of growth which results will in turn determine the oxidation-reduction potential of the med-

ium. This would imply that the yeast phase is more active metabolically which has been proven to be correct in several instances.

Pine (40) also worked with the involvement of cysteine in the dimorphism of H. capsulatum. He confirmed the previous results by showing that a synthetic medium containing cysteine supports growth in the yeast phase while one without cysteine does not. He noticed that the pH of the medium must be lower than 6.5 or the yeast phase would not grow. This was thought to be due to the oxidation of cysteine at higher pH's. Pine's results did not agree entirely with those of Salvin in that he found that glutathione was not effective in stimulating yeast growth and thought that perhaps the sulfhydryl group was not specifically required but that cysteine participated in some other manner. However, the following data were presented (41) which implicated the sulfhydryl group as being responsible for the maintenance of the yeast form: 1.) better growth occurred at a low pH, because cysteine was not oxidized; 2.) growth in the yeast phase was stimulated by the addition of whole red blood cells presumably because these cells supplied a constant source of sulfhydryl groups; 3.) growth was not affected by a higher pH (5.5 to 7.5) if red blood cells were present because they continued to supply sulfhydryl groups, and; 4.) glutathione, beef extract, and coenzyme A stimulated yeast growth if cysteine were present, because they maintained the sulfhydryl groups.

Pine's study (42) of the vitamin requirements showed that most of the strains tested required only thiamine for yeast phase development at 37°C. One of the strains tested required only biotin under the same conditions. It was also noticed that yeast forms grown at 37°C were more sensitive to vitamin requirements than those grown at 25°C.

The mycelial phase showed no vitamin requirements.

Both Pine (40) and Scherr (51) showed that the culture must contain at least 10^6 cells/ml before yeast growth would begin. Pine, (40) however, noticed that the size of the inoculum could be lowered if bovine albumin or starch were added to the medium. These macromolecules are known to form complexes with fatty acids and it was thought that they either carried a fatty acid essential for yeast growth into the medium or protected the cells from a toxic fatty acid already present in the medium. A similar action was proposed as the requirement for a large inoculum. The dead cells present could provide protection by combining with a toxic fatty acid in the medium or could provide an essential one upon autolysis.

Since yeast growth was poor on solid media, Pine (41) extracted the agar with a lipid solvent and added albumin and starch to it. The resultant growth was good, indicating the presence of a toxic fatty acid in the agar.

In a further study by Pine and Peacock, (43) it was shown that the addition of alpha ketoglutarate and citrate to the medium in place of glucose stimulated the conversion to and growth of the yeast phase. Mycelial growth was inhibited by citrate, but it was not affected by alpha ketoglutarate. The pH of the citrate-containing medium did not increase with time, indicating that it was not utilized but stimulated growth in some other way, possibly through its chelating ability. Other chelating agents such as glycylglycine and Versene were added to media to find out if chelating agents stimulate growth. Both these compounds were found to stimulate conversion to the yeast phase but were not as effective as citrate. In order to find out more about the action of chelating compounds on growth, various metal ions were added to try to

reverse the effect of citrate. Calcium ions reversed the inhibition of mycelial growth most effectively, and magnesium ions had some effect. These results implied a differential metal requirement between the yeast and mycelial forms. At 30°C magnesium and calcium ions stimulated mycelial growth: at 37°C only calcium did so in the presence of citrate. Yeast growth was also inhibited by calcium at this temperature. Zinc ions were found to stimulate only yeast growth. For conversion to the yeast phase of H. capsulatum, Pine formulated a medium which contained citrate, alpha ketoglutarate, and zinc sulfate.

In another study of this organism, Mahvi (22) compared the activities of various enzymes present in both the yeast and mycelial phases. He found that both phases had similar activities of hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic acid dehydrogenase, fructokinase, aldolase, transaldolase and transketolase.

From these results, he concluded that both phases have the capacity to carry on the Embden Meyerhof and hexose monophosphate shunt pathways. The only difference found between the two phases was that the yeast form had a higher activity in the TCA cycle enzymes.

The physiological basis for the dimorphic phenomenon in H. capsulatum as seen from the experiments reported is not clearly elucidated. There are several factors which seem to enhance formation of the yeast phase. Among these is the addition of cysteine to the medium for the proposed purpose of maintaining and providing reduced sulfhydryl groups. This corresponds closely with the proposed action of cysteine on Candida albicans which will be discussed below. However, it seems that the reduced sulfur group is not the only factor involved in the cysteine requirement, since glutathione and other sulfhydryl-

containing compounds have been shown to be less effective than cysteine. The action of chelating agents has also been shown to cause yeast formation. It has been noted that cysteine possesses some very strong chelating capacities (30), and it could be considered that this molecule is acting in a similar manner to that of citrate in removing cer tain ions which stimulate mycelial formation. This factor is considered in more detail below.

Action of Nutrition.

In some fungi, the morphological form assumed is entirely dependent on the nutrition. The temperature of incubation has no effect on the dimorphic response. Two examples of this type of dimorphism are discussed: dimorphism in Mucor in which the composition of the atmosphere determines the form produced and dimorphism in Candida in which the composition of the substrate is responsible for the form of the organism.

Dimorphism in Mucor. The morphology which certain species of Mucor exhibit is largely dependent upon the composition of the atmosphere in which the fungus is grown. Mucor rouxii is the species most extensively studied by Bartnicki-Garcia and Nickerson (5, 6, 7, 8). Under the normal "air" atmosphere this fungus grows as aseptate hyphae which are typical of the Mucorales (5). After about twelve hours of incubation, some of the hyphae break into single cells to form a small number of spherical cells in the culture. These cells are called arthrospores and are distinguished from the yeast cells which are characterized in the manner of their formation and proliferation by forming buds. If this fungus is incubated under an anaerobic atmosphere of nitrogen, filamentous hyphae are formed, but the amount of growth is

not as great as that under aerobic conditions. When the fungus is incubated in an anaerobic environment which consists of essentially pure carbon dioxide, only yeast cells which divide by multi-polar budding are formed. Under both aerobic and anaerobic conditions, an increase in the partial pressure of carbon dioxide results in a decrease in mycelial growth. A partial pressure of carbon dioxide as little as 0.3 atmosphere under anaerobic conditions is enough to cause the growth to be entirely yeast-like.

In order to show that the M $\longrightarrow$ Y conversion results from the phenotypic expression of the same genotype, plates which were inoculated with Mucor rouxii were grown aerobically and anaerobically under high concentrations of carbon dioxide (5). The yeast phase developed on the plates in the anaerobic plus carbon dioxide atmosphere, whereas mycelial growth was produced under the normal atmosphere. The plates which showed yeast growth were then exposed to the atmosphere for eight hours and examined microscopically. All of the 5,000 colonies examined at that time showed extensive mycelial formation.

The germination and growth of the spore also varies depending upon the type of atmosphere (5). In air the spore germinates by means of a germ tube in about three hours. Under high concentrations of carbon dioxide, germination takes longer (more than twelve hours), and a bud is formed instead of a tube.

Other members of the Mucorales were also examined (5) for this type of dimorphism. Four strains of Mucor rouxii developed as described above. Mucor subtilissimus developed yeast cells anaerobically under high concentrations of carbon dioxide and also in a nitrogen atmosphere. Species of Rhizopus grew in the mycelial form under all conditions and the other fungi tested did not grow at all under anaerobic conditions.

Several aspects of the physiology of Mucor rouxii were examined (6) to try to find a basis for the dimorphic response. No change in morphology was noticed when cultures were incubated in the air at 20°, 28°, and 37°C. Growth was mycelial at all temperatures. The pH of the medium in which the organism was grown had only a small effect on the morphology. In the air, growth was mycelial at most pH's. Below pH 3.5, however, the amount of growth decreased and the hyphae were shorter. In a carbon dioxide atmosphere the yeast form was predominant at low pH's; but near pH 7, short filaments were formed. The greatest change in morphology was observed when the fungus was grown in an anaerobic nitrogen atmosphere. Below pH 4.5 the cells were filamentous. The size of the inoculum also affected the morphology of the fungus grown under a nitrogen atmosphere. Cultures which were initiated with a small inoculum were filamentous, while those which began with a large inoculum were yeast-like. The latter was thought to be the accumulation of metabolic carbon dioxide from the larger number of cells.

The optimum concentration of the carbon source depended upon the conditions under which the fungus was grown (6). Maximum growth was obtained on 5% glucose in an air atmosphere, on 10% glucose in a nitrogen atmosphere, and on 2% glucose in a carbon dioxide atmosphere. A wide variety of carbon sources were utilized by the fungus if it were grown aerobically, but hexoses were the best carbon sources if it were grown under anaerobic conditions. There were also differences in the optimum nitrogen source depending upon the condition of growth. Aerobically, amino acids were found to be the most readily utilized, while ammonium salts were the best nitrogen sources for anaerobic growth.

Cysteine and carboxylic acids which had been reported to stimu-

late yeast formation in other fungi had no effect in stimulating the yeast phase of Mucor rouxii (6). However, the addition of disodium ethylenediaminetetraacetic acid (EDTA) to cultures of yeast-like cells growing in a carbon dioxide atmosphere, gave rise almost entirely to mycelial growth. Yeast-like growth was almost completely suppressed by the addition of 10^{-4} M. EDTA under these conditions. Since EDTA is a chelating agent, metal ions were added to the culture to try to restore yeast-like development. The transition metals (ferrous, manganous, cupric, zinc, aluminum, and cobaltous ions) were found to be most effective in restoring the yeast morphology. The amount of growth was also decreased with the addition of EDTA; zinc ions restored growth to its full capacity, but had no effect on the conversion back to yeast-like growth. This indicated another enzyme system involved in maintaining the yeast morphology.

Since carbon dioxide fixation and yeast development seemed to be linked, Bartnicki-Garcia and Nickerson (8) studied the uptake and assimilation of carbon dioxide by Mucor rouxii. The cells were grown in an atmosphere with radioactive carbon dioxide and were later analyzed for the presence of fixation products. Malic enzyme seemed to be the most likely point for carbon dioxide fixation. A 1 N. KOH extract of the purified cell wall fraction was found to contain some radioactivity. The macromolecule components of the fraction were hydrolyzed and chromotographed. The radioactivity was found mainly in aspartic acid, with lesser amounts in the glutamic acid and threonine fractions. The macromolecules extracted and hydrolyzed in this manner were shown to be mannan-protein complexes; the proteins associated in these complexes commonly contained high proportions of aspartic and glutamic acid.

Since the radioactivity of the fixed carbon dioxide was found in the protein of the mannan-protein complex of the cell wall, it was suggested that high concentration of carbon dioxide causes fixation which shifts the metabolism of the fungus in such a way as to enhance the M $\rightarrow$ Y conversion.

Cell wall fragments of both the yeast and mycelial phases were isolated, purified, and subjected to analysis (7). Several important differences were found between the two phases. The yeast cell wall contained 10.3% protein and 8.9% mannan; the cell wall of the mycelial phase contained only 6.3% protein and 1.6% mannan. It was also noted that the cell wall of the yeast occupies 30 to 40% of the volume of the cell and the cell wall of the mycelial form occupies only 5-10%.

Examination of electron micrographs of the cell walls of both forms has also shown differences. The wall of the mycelial phase seemed to be a compact envelope of one layer from 0.05 to 0.1 micron in thickness. It appeared to be composed of solidly packed microfibrils which had their main axis perpendicular to the surface of the cell. The yeast walls showed a much thicker envelope, 0.5 micron to 1.0 micron in thickness. Two distinct layers could be observed: the outer layer was thin with a fibrillar structure, while the inner layer was thicker and possessed a much less distinct internal structure.

The polarization of growth was considered by Bartnicki-Garcia (4) to be the critical difference between the yeast and mycelial phases. Bud formation and lateral branch formation were considered to be physiologically equivalent although they were different morphologically. Three fundamental problems were seen in further elucidation of the biochemistry of dimorphism in Mucor: 1.) discovering the mechanism causing polarized cell wall growth in mycelial forms; 2.) finding out

how a tubular envelope is made; and, 3.) discovering how anaerobic incubation under carbon dioxide abolishes polarization.

Using some existing evidence on these problems, Bartnicki-Garcia (4) has postulated possible answers to explain the dimorphic phenomenon in Mucor. The elongation of a hypha takes place at its tip and new cell wall material can be made in one of two ways: 1.) the cell wall makes itself in a primer reaction; or, 2.) a cytoplasmic organelle is responsible for the synthesis of the cell wall. The shape of the yeast cell is not, however, controlled by internal factors, but rather by outside physical forces (osmotic pressure and surface tension).

From this it seems likely that yeast-like development results from the inhibition of the mechanism responsible for mycelial development. When the cells are grown anaerobically under an atmosphere of carbon dioxide, a change can be postulated which interferes with mycelial development. The accumulation of carbon dioxide and the formation of fixation products upsets the metabolic balance of mycelial formation. It has been shown that carbon dioxide fixation results in a large accumulation of malic acid which is then converted to the main fixation product, aspartic acid. It can then be postulated that the accumulation of aspartic acid in some way stimulates the formation of the mannan-protein complex. The increased presence of this polymer disrupts the orientation of the cell wall molecules necessary for mycelial growth and yeast-like growth ensues. Although this model lacks good experimental evidence in many aspects, the assumptions are based on what is now known, and it gives a system to work with in later studies on this type of dimorphism.

Dimorphism in Candida. The conversion M ⟶ Y has been extensively studied in Candida albicans. The results of many of the experiments on this organism have led to the isolation of an enzyme presumably responsible for cell division and a proposal for the mechanism of budding in yeasts.

The dimorphic phenomenon in Candida albicans is clearly controlled by the nutrition of the organism. Two early theories (19) on the cause of dimorphism suggested that the availability of a carbon source in the medium was related to the form of growth. One proposal correlated the molecular weight of utilizable compounds to the morphology of the fungus, so that the higher molecular weight compounds caused a more complex form of growth (i.e. mycelial growth). Another stated that substances favoring rapid growth caused the yeast phase to predominate, while less favorable conditions for growth gave rise to mycelial forms.

Nickerson and Mankowski (36) studied the nutrition of C. albicans and its relation to the morphological form produced. This fungus grew as a yeast on a synthetic medium composed of glucose, ammonium sulfate, inorganic salts, and biotin. It was found that altering the carbon:nitrogen ratio had no effect on the morphology of the cells, nor did the substitution of sucrose as the sole carbon source or glycine as the nitrogen source. When phosphate was excluded from the medium, filaments were formed at the edge of the culture after about six days. It was also discovered that if slowly utilized carbon sources such as starch or glycogen were used as the carbon source, mycelial growth resulted. The polysaccharides first had to be exhaustively washed to remove any readily utilizable carbon sources for this to occur and the addition of glucose to the medium caused the culture to revert to the yeast form. The addition of cysteine in concentra-

tions of 10^{-2} to 10^{-3} M. to a polysaccharide medium also resulted in production of the yeast-like phase. McClary (25) also studied the nutrition of C. albicans and found that not only polysaccharides but other carbon sources such as galactose, which were not readily metabolized, produced good growth of the mycelial phase. He proposed that mycelial formation was favored by readily assimilable but not readily fermentable carbohydrates.

The action of cysteine in producing the yeast phase was not due to its action in lowering the oxidation-reduction potential of the medium (38). Other compounds such as ascorbic acid which had low oxidation-reduction potentials, but no sulfhydryl groups were without effect in altering the form of growth. This result implied that sulfhydryl groups of cysteine were the responsible site.

Nickerson (27) had earlier proposed that cell division in microorganisms was caused by no more than one unit enzyme system, and he had furthermore postulated that cell division was the result of the reduction of sulfur bonds in some structural protein in the cell wall. The action of cysteine on restoring cell division supported this proposal and Nickerson and Mankowski (36) concluded that the polysaccharides in the medium were not utilized rapidly enough to maintain the level of reduced sulfur groups in the cell to carry on cell division. However, the metabolism of glucose, like the addition of cysteine to the medium provided the cells with the necessary sulfhydryl groups to carry on cell division.

Two other substances were found to uncouple cell division without affecting the growth of the organism (38). Penicillin, which is known to act on sulfhydryl groups, and cobaltous ions enhanced mycelial growth. Addition of cysteine reversed the effect caused by both

of these substances.

A permanently filamentous mutant of C. albicans which seemed to be genetically blocked in the cell division mechanism, was compared to the parent strain (30). No differences were found in their ability to ferment sugars, in their vitamin requirements, in their endogenous respiration, or in their carbohydrate composition. The mutant strain synthesized cell material at a slightly slower rate than the parent strain. To see if a syntropism might exist between the two strains, Nickerson and Chung (30) streaked them out on an agar plate within six to ten millimeters of each other. Yeast cells were produced by the parent strain, which was in close proximity to the mutant, and filaments were formed only at the points farthest away from the mutant. This indicated that a diffusable product was being released by the mutant strain which caused yeast-like development in the parent.

If the two strains were placed even closer together (one to three millimeters), the cells of the mutant strain which was closest to the parent were more yeast-like than those farther away. Using an agar which contained a bismuthyl hydroxy-polysulfite complex, cysteine could be detected. Cysteine and this complex formed a blackened area around the point where cysteine was present whereas glutathione, ascorbic acid, sodium sulfide, and other reducing substances did not show this reaction. If the parent strain of C. albicans was grown on a medium containing this complex, a blackened area was produced around the places where the organism was growing, indicating that cysteine, (or a substance containing cysteine) was released into the medium after an extra-cellular enzymatic reaction. The material, presumably cysteine, then secreted by the parent strain partially overcame the genetic block in the mutant.

Nickerson and Chung (30) found that the mutant strain developed almost entirely as yeast cells if it were grown on a medium containing 10^{-2} M. cysteine. Without the cysteine addition, the growth was entirely mycelial on the same medium. Glutathione was found to be as effective as cysteine, but cystine and inorganic reducing substances were not. It was postulated by them that sulfhydryl substances acted by maintaining the yeast state against division-inhibiting conditions. The possiblility that cysteine also acted as a metal complexing agent which relieved an inhibition in the filamentous mutant was also considered.

Nickerson (28) found a striking difference between the mutant and parent strains: the ability of the mutant to accumulate and reduce tetrazolium dyes. Although the parent can accumulate these redox dyes, it reduces them only in the outer edges of an older colony in the filamentous cells which are produced. Cells which retain the yeast shape in the same culture show no tetrazolium reduction.

It has been shown that tetrazolium salts are reduced enzymatically by a flavoprotein system which also reduces coenzyme I (9). This was presumed to be the case in C. albicans. The location of the reduced dye was found to be located in granules inside the cells of the filamentous cells (28). These granules exhibited many of the properties of mitochondria.

The addition of the metal chelating agent, Na_2EDTA, to a culture of the parent strain resulted in the reduction of tetrazolium dyes by this strain. The action of the chelating agent was thought to be the combination and removal of a metal ion from an intercellular complex. By testing the ability of several metal-EDTA complexes to stimulate tetrazolium reduction, it was found that a ferrous complex was the

most likely to be uncoupled in the cell. Washed, resting cells of both strains reduced the tetrazolium dyes; the addition of glucose to these suspensions completely inhibited reduction by the parent, but did not effect the reducing capacity of the mutant strain.

Yeast cells have the ability to reduce tetrazolium dyes only after they have lost their ability to undergo cell division due to a genetic or a metabolic block. The tetrazolium reducing system of the cell was thought to be uncoupled from an oxidative chain, the operation of which is necessary for cell division. The point of uncoupling was brought about by the chelating action of EDTA in the removal of a metal, presumably iron, from a flavoprotein, or possibly by the formation of a double chelate.

A comparison of respiration of the normal strain and divisionless mutant showed that the mutant reduced oxygen more rapidly than did the parent strain (55). Respiration however, was found to be similar and it was not mediated by cytochrome oxidase. Because cytochrome oxidase was not resent, Ward and Nickerson proposed that the flavoprotein system was the terminal oxidase. In the parent strain then, a competition exists between the reduction of molecular oxygen and disulfide bonds. If the flavoprotein is altered by the removal of a metal ion, however, dye reduction can compete with oxygen reduction and cellular hydrogen can be channeled into this pathway.

In their study of the physiology of the two strains of C. albicans, Nickerson et. al. (37) found that selenite causes inhibition of mycelial formation and thereby enhances yeast-like growth of both the parent and mutant strains. The inhibition of mycelial development increases with increasing selenite concentration. A back mutation is not

responsible for this action of selenite on the mutant strain; for when this compound is removed from the medium, mycelial growth is produced. Two explanations for the action of selenite were proposed: 1.) selective inhibition of mycelial growth; or 2.) prevention of the uncoupling of cell division from growth. Selenium has several properties in common with sulfur, and there is some evidence that selenium-containing amino acids are incorporated into proteins. The -SeH group has been reported to be present in proteins, and an organic selenium compound has been found in isolated cell walls of the mutant strain which had been growing on a selenite-containing medium. It is therefore thought that the action of selenite in reverting the mycelial to the yeast-like forms is similar to the action of sulfhydryl groups in that they both prevent the uncoupling of cell division from growth. In the divisionless mutant this action is probably by the stability of the -SeH groups. If the mutant is grown for several transfers on a medium deficient in sulfur, it grows as a budding yeast (54). This was explained by the fact that probably less sulfhydryl groups were incorporated into the cell wall, and the lower enzyme content of the mutant was then able to reduce enough to make division by budding possible.

The next step in the elucidation of the mechanism responsible for dimorphism in C. albicans taken by Nickerson and his co-workers was the isolation, purification, and characterization of the cell wall (14, 19, 29, 55). For these studies, cell walls of Saccharomyces cerevisiae and both strains of C. albicans were used. The cells were mechanically ruptured in a Waring blender with glass beads; and the cell wall fragments were isolated by differential centrifugation and repeated washings with distilled water, 0.25 M. sucrose, and phosphate buffer.

After a series of fifty washings and centrifugations, a preparation was obtained which was free of cellular debris as determined by light and electron microscopy. These cell wall fragments were analyzed for total nitrogen, sugars, amino acids, and lipids.

A previous study by Northcot and Horne (39) showed the composition of the cell wall of yeast to consist of 29% glucan, 51% mannan, 13% protein, 8.5% lipid, and 3% ash. In their procedure, 16% of the material was not accounted for. Nickerson et. al. (35) found 7% protein, 85% polysaccharide and 3% chitin in the cell wall of C. albicans. In both C. albicans and S. cerevisiae a high sulfur:protein ratio (2.1%) was found, indicating that the protein was probably a pseudokeratin type. Amino acid chromatography of a hydrolysate of the protein showed the presence of 16 amino acids and a high percentage (31-39%) of acidic amino acids.

A procedure for the extraction of the macromolecular components was also formulated (29). First, lipids were extracted from the purified cell wall fragments (14, 19, 29, 35). A three stage extraction was necessary and two types of lipid components were found to exist in the cell wall: 1.) the readily extracted lipid which was thought to exist either as a lipopolysaccharide or as a lipopeptide; and 2.) the bound lipid which required an acid solvent for extraction. This component was thought to exist as a lipopolysaccharide in the cell wall. Although there is very little evidence for the role of the lipid component in the cell wall, it is thought to contribute to maintaining the shape of the cell. A dried yeast cell is not flattened if viewed through the electron microscope, but remains as a prolate spheroid in shape. If these cells are extracted for lipids, further examination

in the electron microscope shows a marked flattening, indicating that these compounds play some role in maintaining the shape of the cell.

After removal of the lipid fraction, the cell wall fragments were treated with 1 N. KOH (14). This dissolved part of the cell wall and left behind a residue. Analysis of the residual fraction showed it consisted of a carbohydrate composed only of glucose residues plus a protein. This glucan protein (GP) accounted for 40% of the weight of the cell wall. The glucan portion was considered to be a highly branched polymer with side chains varying in length from eight to fifteen glucose residues (24).

The portion of the cell wall which was soluble in alkalai was shown to contain two sugars, glucose and mannose; further analysis showed that this solution consisted of two polymers (14, 35). These were separated by treatment with a saturated ammonium sulfate solution. The complex, glucomannan-protein 1 (GMP-I is precipitated by this procedure and glucomannan-protein II (GMP-II) is left in solution. The polysaccharide portion of GMP-1 is composed of mannose and glucose in the ratio of 1:1; this complex makes up about 4% of the cell wall. The GMP-II polysaccharide has a 2:1 ratio of mannose to glucose and accounts for 30% of the cell wall material.

The polysaccharides and proteins appeared to be tightly bound together in these complexes, since the carbohydrate moiety could not be precipitated as the copper complex by treatment with cold Fehling's solution (14). Because the protein has been shown to contain a high percentage of acidic amino acids, it was thought that ester linkages were likely between the protein and polysaccharide. If the complexes were subjected to enzymatic degradation and the amino acids chromato-

graphed, very little aspartic and glutamic acid were found, indicating that they remained bound to the polysaccharide. The hydroxylamine reaction for ester linkages also showed that this type of bonding was likely (29).

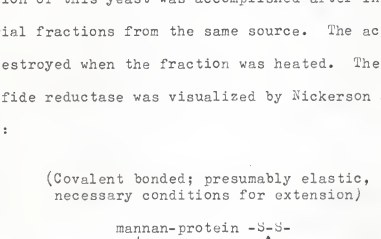
Examination of electron micrographs of yeast cell walls gave further data on the arrangement of these macromolecular complexes in the cell wall. Agar and Douglas (1) noticed that the cell walls consisted of two distinct layers. The outer layer was made of an electron dense material with a fibrillar appearance with a thickness of 0.05 microns. The inner layer possessed a less electron dense appearance and was about 0.2 microns in thickness. In this layer electron dense lamellae were noticed. Treatment with dilute alkalai caused the inner layer to dissolve. Comparison of electron micrographs of purified GMP-II with those of this layer also suggest that the inner layer is composed of GMP-II (35).

Using the above isolation procedure to obtain a substrate that consisted of purified cell wall fragments, Nickerson and Falcone (33,34) measured enzyme activities of fractions from both the parent and mutant strains of C. albicans for the reduction of disulfide bonds. Since the site of tetrazolium reduction in this fungus had been found to be a mitochondria-like particle, the mitochondrial particulate fraction was tested as an enzyme source. The amount of sulfhydryl formation was measured by a spectrophotometric determination of mercaptide formation with p-chloromercuribenzoate. Each enzyme fraction was incubated with the cell wall material for two hours at 37°C before the increase in the amount of reduced sulfur was measured.

The enzyme fraction from the parent strain incubated with the

cell wall fraction from the same source showed a relatively large increase in the free sulfhydryl groups formed. This enzyme system also showed considerable activity on cell wall fragments from the mutant strain. The enzyme system from the divisionless mutant exhibited no activity on the cell wall of the parent strain and only slight activity on its own cell wall. From these results, Nickerson and Falcone (32) proposed that the enzyme of the mitochondrial particulates (which reduced these disulfide bonds in the cell wall protein) was responsible for cell division in C. albicans, and called it protein disulfide (PDS) reductase. These workers (32) also tested Saccharomyces cerevisiae for activity of this enzyme. Reduction of disulfide bonds in the cell wall fraction of this yeast was accomplished after incubation with mitochondrial fractions from the same source. The activity of the enzyme was destroyed when the fraction was heated. The role of the protein disulfide reductase was visualized by Nickerson and Falcone (34) as follows:

(Covalent bonded; presumably elastic,
necessary conditions for extension)



(May undergo plastic deformation and fibrillar
ordering--necessary conditions for division)

From the action of the PDS reductase in vitro, Nickerson and co-workers (29, 34, 35) accumulated evidence to show how the enzyme could operate in vivo and to explain the phenomenon of cell division

or budding in yeasts.

The mitochondria are particles in the cell which, due to cytoplasmic streaming, are in constant motion throughout the cell. Since a fluid in motion exerts its maximum force on the region of greatest curvature of its container, it could be expected that the mitochondria, containing PDS reductase, would come in contact with the cell wall mainly at points in this region. It has also been shown that new buds arise in the region of maximum curvature, (i.e. near the ends of ovoid cells). Burns, (11) in studying the division process in yeasts of different ploidy, provided further evidence suggesting that the mitochondrial particulates are involved in cell division. He found that the rate limiting process in cell division is controlled by nongenetic units, and the distribution of these units during the division of the cell determines the relative division times of the two daughter cells. He suggests that the mitochondrial particulates containing the enzyme of Nickerson and Falcone fit these specifications, and could be this nongenetic unit.

As the mitochondrial particulates come in contact with the cell wall, the PDS reductase reduces the disulfide bonds of the protein and there is a cleavage in these covalent bonds (15, 29, 34, 35).

This breakage causes a weakness of the cell wall at this point and the osmotic pressure of the cell exerts a force on the cell wall which opens the fibrils enough to allow a small naked bud to be extruded. Using dark field, time lapse cinematography, Nickerson has observed the naked bud being produced on the mother cell in this manner. There is a thirty second time lapse between the frames of the film which shows the intact mother cell and the naked bud. A short time

after it is produced, cell wall material from the mother cell is synthesized around the bud.

The evidence for this mechanism has been disputed over this point. McClary and Bowers (26) claim that the bud cannot begin as an "explosive blow out," and Nickerson has only observed a change in focus which brings the bud into full view when the cell rotates. They also suggest that the cell wall cannot be observed using dark field microscopy, but only the cytoplasm of the cell can be observed. Electron micrographs through sections of budding yeast cells, showed cell wall material to be uniformly thick around the mother cell and bud at all stages of development. They have not been able to demonstrate a naked bud with electron micrographs. Romano(45) suggested that this bud might be destroyed in preparing the specimen for the electron microscope.

Robson and Stockley (44) on the other hand, provided cytological evidence supporting Nickerson's proposal that sulfhydryl groups are formed during budding. The position of free sulfhydryl groups of four fungi including C. albicans was studied by adding labelled phenyl-mercuric chloride to the cells. This compound combined with all the free sulfhydryl groups and its location could then be detected using an autoradiographic technique. Sulfhydryl groups were shown to exist in the cell walls of C. albicans in the yeast form, while the filamentous fungi showed only a weak reaction in their cell walls. It was also noticed that a higher proportion of sulfhydryl groups were associated with bud formation than on mature cell walls supporting the proposal that during bud formation, sulfhydryl groups are formed.

Nickerson et. al. (15, 35) have also studied the fibrillar configuration around the bud scars in electron micrographs of isolated

cell walls. These bud scars generally appear as a ridge on the outside of the cell wall with the fibrils arranged in a ring around the scar. The shape and orientation in these bud scars is very similar to that which would be predicted for a purely physical system in which a liquid force is exerted at right angles to a substance.

Although more experimental evidence is needed to assign the protein disulfide reductase to the major role in cell division, it is a very exciting possibility. Since the discovery of this enzyme in yeast, it has also been reported to exist in seeds of broad bean, wheat, barley, maize, peas, and oats, and in the shoots and roots of the seedlings of many of these plants (17). No role in cell division has been postulated here, however.

Action of Plant Growth Regulators

Early reports on the effect of auxin on yeast cells were rather controversial. Several instances were reported in which plant growth regulators were tested on various fungi and were found to act like toxic substances, rather than growth stimulators (16). Turfitt, (54) on the other hand, found that several auxins (indole acetic acid and napthalene acetic acid) stimulated growth and fermentation in yeast. He reported no elongating effect. Scherr (50) studied the effect of carcinogens and several plant growth regulators on Saccharomyces cerevisiae. Although the action of napthalene acetic acid and indole acetic acid was not a definite promotion of elongation, he presented the following evidence for this action on yeast cells: 1.) the auxin activity of filtrates from cultures containing a high percentage of elongated cells; 2.) tryptophan, a precursor of IAA, induces the formation

of a larger number of elongated cells than if tryptophan is not added; 3.) increasing the hydrogen ion concentration of cultures containing tryptophan enhances elongation, presumably by releasing the free acid form of IAA; and 4.) the effect of alpha NAA appeared to be that of increasing the number of elongated cells without affecting the rate of cell multiplication. Other auxins, such as IAA showed no effect, however, when added to cultures of this yeast. Since enlarged cells are also obtained when toxic compounds are added to cultures of yeast, Gruen (16) proposed that the elongation or increase in cell size reported for auxins in yeast could not be an auxin effect since the same type of response was obtained when these unrelated toxic compounds were added.

In 1963, however, Yanagishima (57) reported definite elongation of cells of a strain of Saccharomyces ellipsoideus after the addition of auxin to cultures. A cytoplasmic mutant deficient in aerobic respiration (strain P602) was found to show marked elongation without auxin in a maltose, amino acid, vitamin medium. When the plant growth regulators, IAA and NAA were added to this medium, the cells showed even greater elongation. The elongation caused by these regulators was also found to be reversed by an antiauxin (2, 4, 6, -T.). The action of auxin on this yeast strain was found to be similar in several ways to the action of auxin on higher plant cells: 1.) only elongation was stimulated, while the width remained uninfluenced, 2.) the effect was reversed by an antiauxin and 3.) the effective concentration ranges (10-20 mg/l) were similar in both instances. It was also noticed that the number of cells was not increased, only the elongation of the cells was stimulated.

The parent strain (KV2) was also examined for its response to auxins. No elongation was induced by the addition of either NAA or IAA. However, it was discovered that this strain would elongate in the presence of auxin if the cells were pretreated with gibberellic acid (GA) or if gibberellic acid and auxin were added to the culture at the same time. (58) Gibberellic acid seemed to make the cells susceptible to the action of auxin, and attempts were made to find some cellular substance which was produced because of the presence of gibberellic acid that would make the cells responsive to auxin treatment.

Since GA has been shown to be involved in RNA metabolism, RNA extracts were prepared and purified from cells of KV2 which had been subjected to GA pretreatment and from those which had no previous treatment (59). These extracts were then added to cultures of the same strain KV2, in cultures which contained auxin and those which did not. After twenty hours of incubation, the cell lengths of the different cultures were measured and compared. The only cells which showed significant elongation were those which received RNA extracts from GA pretreated cells and had auxin additions in the medium. RNase addition to RNA extracts completely destroyed this effect. The strain KV2 could then be made to elongate in the presence of auxin if it were treated with GA or if it were treated with RNA from GA pretreated cells.

If cells of KV2 were treated with purified RNA extracts from the mutant strain P602, elongation was again induced in the presence of auxin and the effect was completely destroyed by incubating the RNA with RNase before addition to the culture.

RNA (60) extracts from other plant cells were also tested to see if the effect was more widespread. Cells of the Jerusalem artichoke

tuber have been shown to be responsive (23) to auxin treatment in a similar manner to that of strain KV2. (i.e. they must be treated with GA before they show elongation with auxins.) Purified RNA extracts from cells of the Jerusalem artichoke which had none were added to cultures of strain KV2. The yeast cells showed significant elongation only in the cultures which had RNA from GA pretreated artichoke cells and auxin. The other cultures showed no elongation.

Another type higher plant cell which responds to auxin in a manner similar to the mutant strain of S. ellipsoideus is the cell of Avena coleoptile. These cells elongate in the presence of auxin without GA treatment. As could be expected, RNA extracts from these cells cause elongation of KV2 cells in cultures containing auxin.

From these results it can be seen that RNA has a causal role in the elongation of yeast cells by the action of auxin. Whether this action is connected with the mechanism of cell division or whether it is an entirely different mechanism, is not known. Yanagishima and Masuda (60) hope that the use of these yeast strains will help to provide more evidence to explain the action of auxin in elongating plant cells.

DISCUSSION

It is apparent that the M $\rightarrow$ Y conversion can be caused by different factors in the various species of the dimorphic fungi. One factor, however, that of differential metal requirements, appears to exist in several of the examples of dimorphism that have been most extensively studied. The effect of chelating agents on the organism does not seem to be similar, since Pine and Peacock (43) found that citrate and alpha ketoglutarate inhibited mycelial formation by their ability to make calcium and magnesium ions unavailable for Histoplasma

capsulatum; and Nickerson (28) uncoupled cell division from growth, thereby enhancing mycelial formation with the removal of a metal ion, presumably ferrous, from a flavoprotein with the action of EDTA in Candida albicans. In Mucor rouxii, which seems to be unrelated as to the mechanism of the dimorphic response, EDTA also acted to stimulate mycelial formation while inhibiting yeast-like growth (6). These examples seem to indicate that many of the enzyme systems responsible for the maintenance of the morphology in these fungi, have a metal prosthetic group which can be dissociated rather easily. It would be helpful if more of the fungi which exhibit dimorphic tendencies were studied on the basis of their metal requirements.

The action of cysteine also occupies a central role in the elucidation of the mechanism of dimorphism. Cysteine has been found to stimulate yeast formation in both H. capsulatum (40, 47, 51) and C. albicans (36, 37). This has been proposed to be due to the sulfhydryl group of this molecule, although there are contradicting reports on the action of other -SH-containing compounds and they are certainly not as effective as cysteine. It has been suggested that cysteine might act in its capacity to bind metal ions (30) and, therefore, be considered as the chelating agents discussed above. The metabolism of sulfhydryl groups in the cell, however, has been so strongly documented (29, 34, 35, 44) that it seems that the -SH group of cysteine must be the active part of the molecule. There have been few studies which indicate the action of metal ions on reversing the effect of cysteine.

RNA metabolism has been shown to be implicated in dimorphism in two instances. The fluctuation in the RNA content of the yeast phase of Blastomyces (53) and the induced responsiveness to auxin of cells

treated with RNA from responsive organisms (59, 60) seems to indicate that RNA might have some role in the morphological conversion. This is expected, for adherence to the central dogma would indicate that if enzyme systems were responsible for the conversion, RNA must control the manufacture of the enzymes. Much more work must be done on this relationship before any model could be proposed, however.

Finally the proposal of an enzyme protein disulfide reductase (29, 32, 33, 34) division in yeasts presents an exciting possibility. As stated earlier, more exact evidence on the action of this enzyme in the cell would provide a much greater understanding for the mechanism of dimorphism in C. albicans, at least, and probably in many of the fungi which exhibit dimorphic phenomenon.

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DIMORPHISM IN THE FUNGI

by

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ABSTRACT

Dimorphism in the fungi is the phenotypic ability of fungi to display either a yeast-like or mycelial morphology. The literature which has dealt with the possible physiological bases for this phenomenon has been reviewed in this report.

Four types of environmental influences on the interconversion of these morphological forms have been considered: 1.) the action of temperature, 2.) the action of temperature and nutrition, 3.) the action of nutrition, and 4.) the action of plant growth regulators.

In species of Blastomyces the dimorphic response is entirely dependent upon the incubation temperature. The mycelial form grows at lower temperatures, and the yeast phase predominates at temperatures above 35° C. It has been proposed that the enzymes responsible for maintaining each form have different optimum temperature requirements.

In Histoplasma capsulatum both the composition of the substrate and the temperature play a deciding role in the phenotypic expression of the morphology. Two different sets of conditions have been devised to accomplish the conversion to and maintenance of the yeast phase. One conversion medium containing biotin plus cysteine as the nitrogen source produces the yeast phase. The sulfhydryl-containing group of cysteine was postulated as a requirement for cell division. The second proposal implicated a differential [illegible] between the two forms, and the conversion medium contained citrate (a chelating agent), alpha ketoglutarate and zinc ions.

In Mucor rouxii and Candida albicans the change in morphology has been correlated to the action of various compounds in the atmosphere and substrate in which they are grown.

M. rouxii grows as the yeast form only if incubated anaerobically

under a high concentration of carbon dioxide. Studies on this organism have shown that the carbon dioxide is fixed and transformed into aspartic acid. Comparative analyses of the cell walls of yeast and mycelial forms showed that the yeast cell wall contained a higher percentage of mannan-protein complex and that the protein contained a high percentage of aspartic acid. It has been proposed that the formation of large amounts of aspartic acid caused this macromolecular component to be synthesized, and this in turn upset the orderly synthesis of the mycelial cell wall, thereby producing the yeast morphology.

In Candida albicans the morphological form was dependent upon the relative availability of carbon compounds. The more slowly metabolized compounds gave rise to the mycelial form, whereas the addition of cysteine reversed this effect. This fact led to the proposal that a level of cellular sulfhydryl groups must be maintained for cell division to occur and that slowly utilized compounds could not supply the necessary reducing power.

Characterization of the cell walls of the yeast phase of C. albicans showed that three polysaccharide-protein complexes made up its structural integrity. The activities of the mitochondrial particulate fractions of the normal strain and a permanently filamentous mutant in reducing disulfide bonds of the cell wall protein were compared. The enzyme activity (protein disulfide reductase) of the parent strain was found to be much greater than that of the mutant and was postulated as the enzyme system responsible for cell division in yeasts. Further evidence was also presented to show the possible action of this enzyme in vivo.

The action of plant growth regulators in inducing the elongation of filament formation in yeasts was also discussed. Recent experiments have shown that auxins and gibberellic acid are active in inducing such a response. These have implicated cellular RNA in elongation control.