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REVIEW PAPERS

The Role of Sterols in Morphogenetic Processes and Dimorphism in Fungi

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Abstract—The review considers the fundamental biological problem of fungal dimorphism as an adaptive reaction to adverse impacts. Primary attention is paid to sterols, phospholipids, storage lipids, and fatty acids. The structural and biological functions of sterols are considered, as well as their role in membrane stabilization under stress and their relation to morphogenetic processes in mycelial fungi, of which many are pathogenic. Data on the biosynthesis of the main fungal sterol, ergosterol, are presented, as well as data on the inhibitors of this process and on the mutants deficient in its particular stages. Ergosterol biosynthesis is also considered in terms of its relation to the composition of the fungal cell wall, which is the cell shape-determining structure, and to the intensity of chitin synthesis, a process in which azole derivatives play a role. Data obtained by the authors are presented that show the role of changes in the composition of sterols, phospholipids, storage lipids, and unsaturated fatty acids of resting cells in the induction of yeastlike growth in mucoraceous fungi.

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Key words: dimorphic fungi, ergosterol, phospholipids, dimorphism, morphogenesis.

INTRODUCTION

In response to unfavorable conditions, many mycelial fungi realize their capacity for dimorphism: they switch from hyphal growth to growth in the form of spherical multipolar budding yeastlike cells.

The factors that affect the morphology of fungi are extremely diverse. Environmental signals (pH, temperature, concentrations of oxygen and glucose) are the primary stimuli that switch on genetic programs involving the expression of specific genes [1–4] and the synthesis of regulatory proteins and enzymes [5–7], which form the biochemical basis of dimorphic growth. They change the enzymatic balance in the cell, cause metabolic rearrangement, and promote adaptation of the corresponding morphological variant to the environmental conditions [2, 8, 9]. Numerous studies have been conducted on the biochemical peculiarities of yeastlike cells and mycelium, the composition and structure of their cell walls, their rates of protein and RNA syntheses, the composition and content of cytochromes, cAMP and polyamine levels, etc. [10]. It has been shown that certain inhibitors of bioenergetic processes occurring in mitochondria (sodium cyanide, antimycin A, oligomycin, chlorinated anilines, etc.) can initiate changes in the morphology of fungal cells under aerobic conditions [11, 12]. Some studies have been concerned with the investigation of lipids and their relation to fungal morphogenesis, since lipids are important structural and regulatory component of the cell [13–15].

Fungal morphogenesis is known to be accompanied by changes in the composition of phospholipids, free and esterified sterols, triacylglycerols, and fatty acids [16, 17]; however, data on the relation of the lipid content to dimorphism are contradictory [10]. Certain regularities are common to many fungi; thus, the content of lipids, including sterols and unsaturated fatty acids, is higher in mycelium than in yeastlike cells [15, 18, 19]. In the mycopathogen *Sporothrix schenkii* the yeastlike and mycelial forms differ in the content of fatty acids and composition of cerebrosides [20]. For the transition of fungi of the genus *Mucor* from yeastlike growth to mycelial growth, the synthesis of lipids, phospholipids first of all, is required [13]. It has been shown that blocking phospholipid synthesis by the polyenic antibiotic cerulenin, which inhibits fatty acid synthetase, makes *M. racemosus* incapable of the transition from yeastlike growth to hyphal growth; however, this effect could be abolished by the addition of fatty acids (Twin 80) to the growth medium.

A tight correlation exists between the metabolic processes and the processes of growth and survival. The mycelial and yeastlike forms of dimorphic fungi that are pathogenic to plants, animals, and humans exhibit different virulence [21–23]. Therefore, all-round investigation of the biosynthesis and metabolism of lipids, including sterols, and of the regulation of these processes, are of considerable importance for modern

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medicine, veterinary, and agriculture. Our review is devoted to the role of sterols and other lipids of fungi in morphogenetic processes and in the phenomenon of dimorphism.

FUNGAL STEROLS: SYNTHESIS, FUNCTIONS, AND ROLE IN MORPHOGENESIS

Functions of sterols. Sterols are derivatives of cyclopentane perhydrophenanthrene; they are formed only under aerobic conditions via the widespread isoprenoid pathway. Sterols are extremely diverse in their structure. They have been found in most fungi. The main functions of sterols are the nonspecific structural one (the so-called *bulk function*) and the specific regulatory function (*sparking function*) [24]. In addition, sterols are precursors of steroid hormones, which are involved the regulation of the sexual reproduction of mycelial fungi [25, 26].

The content of sterols in fungi depends on the cultivation conditions (aeration, medium composition, temperature) and growth stage [27]. The sterol most frequently occurring in yeast and mycelial fungi is ergosterol (24-methyl-cholesta-5,7,22-trien-3β-ol). It has been shown for *Saccharomyces cerevisiae* and *Mucor circinelloides* that, under anaerobic conditions, the presence of ergosterol and monounsaturated fatty acids in the medium is required for growth [14, 28]. Heme competence has been shown to be a prerequisite for ergosterol biosynthesis [24, 29]: hemes or intermediates of their biosynthesis are necessary for the biosynthesis of sterol under aerobic conditions and for sterol uptake under anaerobic conditions, when sterol biosynthesis is impossible [30].

Ergosterol biosynthesis. The genes encoding enzymes of ergosterol biosynthesis are subject to positive regulation at the transcription level. Upon decrease in the ergosterol level, two transcriptional factors, Upc2p and Ecm22p, bind to promoters of most *erg*-genes, thus activating them and providing for biosynthesis of ergosterol or its uptake under conditions of hypoxia or in the presence of ergosterol biosynthesis inhibitors [31].

At the initial stage of the biosynthesis, mevalonic acid, the precursor of all isoprenoids, is the first intermediate. It is formed with the involvement of 3-hydroxy-3-methylglutaryl-CoA reductase, which is the key enzyme of sterol biosynthesis. Of the two genes coding for this protein, one is positively regulated by heme [32]. A subsequent series of molecular condensations of isopentyl pyrophosphate leads to the formation of the hydrocarbon squalene. Squalene undergoes epoxidation with the formation of 2,3-oxidosqualene, which, as a result of cyclization, is converted to the triterpene lanosterol (4,4,14-trimethyl-cholesta-8,24-diene-3β-ol).

Ergosterol is the end product of a complicated system of transformation of lanosterol, the key precursor of a large number of sterols. The biosynthesis pathway includes (1) a series of reactions of oxidative demethylation, affecting the two methyl groups at C-4 and the methyl group at C-14; (2) methylation at C-24; (3) dehydrogenation; (4) isomerization of the double bond Δ8 into Δ7; (5) introduction of the double bonds Δ5 and Δ22; and (6) reduction of the double bond Δ24(28) [33]. The introduction of the methyl group at C-24 in the side chain may also occur before the demethylation at C-4 and C-14, dehydration, and isomerization; this biosynthetic pattern results in the formation of the triterpene eburicol [34].

In many mycelial fungi and yeasts, including *Mucor hiemalis*, *Candida albicans*, *Cryptococcus neoformans* and the melanized fungi *Hortaea werneckii*, *Alternaria alternata*, *Cladosporium cladosporioides*, and *Aureobasidium pullulans*, the compound undergoing oxidative demethylation is eburicol rather than lanosterol [35–38]; thus, the transmethylation reaction in these organisms occurs prior to the demethylation reactions.

Moreover, in fungi exhibiting ecophysiological peculiarities (halophilic and halotolerant ones), several variants of ergosterol biosynthesis exist, which provide for the necessary level of cell membrane fluidity under the extreme conditions of the habitat [38, 39].

In the process of ergosterol synthesis, the methyl groups are donated by *S*-adenosylmethionine, which influences morphogenesis [40–42]. *C*-methylation of sterols is energy-dependent (14 ATP molecules per one methyl group [43]); this fact testifies to the importance of this process for the formation of 24-alkyl sterols in fungi. The addition of 24-epiaminolanosterol, an inhibitor of methylation at C-24 in the side chain, results in aberrations in the mycelial membranes, growth inhibition, and induction of asexual sporulation in *Gibberella fujikuroi* [44].

The intermediates produced by sterol methyltransferases are further transformed to end products that control physiological processes in fungi. In actively growing cells, the ratio of 4,4-dimethyl- and 4-monomethylsterols to 4,4-desmethylsterols equals 1 : 9 [45]. A shift of this ratio affects growth and development [44, 46, 47]. Thus, this ratio is equal to 1 : 9 in *M. hiemalis* mycelium and to 1 : 4 in the arthrospores formed on this mycelium [35]. In young *M. hiemalis* sporangiospores, which are able to germinate with the formation of mycelium, the ratio equals 1 : 38, whereas in old spores, whose germination results in yeastlike growth, it is equal to 2 : 3 [48].

It is known that lanosterol does not support *S. cerevisiae* growth under anaerobic conditions [46]. Successive elimination of three methyl groups (14α-methyl, 4α-methyl, and 4β-methyl), which is termed oxidative demethylation, renders to the sterol molecule the form necessary to promote the activity of many enzymes.

Ergosterol transportation. Sterol synthesis is associated with membranes. The details of the process of sterol transportation from the site of their synthesis

in the membrane to the site of sterol functioning remain unclear. Data have been obtained suggesting that this transportation may be mediated by both sterol-transporting proteins [49–50] and vesicular transport involving microtubules and microfilaments [51]. It has been shown that intracellular transportation of free and esterified sterols is mediated by different carriers, and that the esters of sterols that are ergosterol biosynthesis intermediates are not transported to membranes of the endoplasmic reticulum but stay in lipid granules [52].

It has been established that, in yeasts, nonvesicular transportation of sterol is performed by protein Osh4p/Kes1p, a member of a large family of lipid-binding proteins (ORPs, encoded by the *ohs* genes). The activity of this protein is regulated by phosphatidylinositides; in phosphatidylinositide mutants, sterol transportation is decelerated [50]. In addition, oxysterol-binding proteins are involved in the maintenance of the composition of sterols and sphingolipids in *S. cerevisiae* membranes and exert an indirect effect on the cell morphology [53, 54]. In *ohs* mutants, impairment of ergosterol transportation due to impairment of these genes led to changes in the lipid composition of membranes and in intracellular distribution of sterols and other lipids, to fragmentation of vacuoles, accumulation of lipid drops in the cytoplasm, and impairment of endocytosis, as well as to defects in budding, determined by abnormal deposition of chitin in the cell wall [54].

Inhibition of sterol biosynthesis. Inhibitors of sterol synthesis, as well as mutants impaired in its particular stages, are convenient instruments to study the relation of sterol structure and function [55, 56].

A wide range of biologically active compounds are known to interact with lipids and affect their biosynthesis. Among them are polyenic macrolides (produced by some *Streptomyces* species), which interact with membrane sterols (amphotericin B, candicidin, nystatin, pimaricin), and azole-containing compounds—imidazole and triazole derivatives and others. The antifungal activity of azole derivatives is determined by their interaction with sterol 14α-demethylase, which catalyzes oxidative demethylation at C-14 of lanosterol molecule in the process of ergosterol biosynthesis. Another group of antifungal agents—allylamines (naphthifine, terbinafine [57, 58]) and thiocarbamates [59]—impair biosynthesis of fungal sterols by inhibiting squalene oxidase. One of the first antifungal antibiotics discovered, griseofulvin, inhibits microtubule motility, which impairs transportation of newly formed cell-wall components and leads to the deformation of hyphae [60].

The toxicity of polyenic antibiotics to fungi is determined by their ability to bind to sterols incorporated in cell membranes. The physical state of the membrane thereby changes; its functions are impaired, the permeability of the membrane to protons increases, and loss of K^+, Ca^{2+}, and PO_4^{3-} ions occurs [61–63]. Polyenes not only inhibit growth of fungi but also change their lipid composition. In the presence of amphotericin B, the content of total lipids and sterols in yeastlike cells of the dimorphic fungus *S. schenckii* decreases [64]. The polyenic antibiotic nystatin retards growth and development of the mycelial fungi *Fusarium solani* and *Mucor lusitanicus*, which is accompanied by an increase in the polar lipids/sterols ratio, a decrease in the fractions of sterols and storage lipids, and an increase in the unsaturation degree of fatty acids [65, 66]. In polar lipids of *M. lusitanicus*, an elevated level of phosphatidylserine (PS) and an extremely low level of phosphatidylethanolamine (PEA) were recorded, indicating a decrease in the operation rate of the serine pathway of phospholipid biosynthesis.

Selective interaction of azole derivatives and other nitrogen-containing heterocyclic compounds with cytochrome P450 underlies the inhibition of ergosterol synthesis. In *C. albicans*, the depletion of ergosterol, accompanied by 14α-methylsterol accumulation, results in changes in the membrane functions, in synthesis and activity of membrane-bound enzymes, including mitochondrial ones, and in noncoordinated increase in chitin synthase activity [67].

Cytochrome P450–containing hemoproteins are involved in oxidative demethylation of lanosterol and Δ22 desaturation of the side chain, and cytochrome b_5 is additionally involved in Δ9 desaturation of stearic and palmitic acids, which yields oleic and palmitoleic acids, respectively. When *C. albicans* was incubated in the presence of demethylase inhibitors, it was either incapable of fatty acid synthesis or a shift in their synthesis occurred, from the formation of the unsaturated oleic and linoleic acids to the formation of the saturated palmitic acid [68, 69]. The enhancement of the synthesis of saturated fatty acids indicates a decrease in the activity of Δ9 desaturase—a microsomal enzyme complex containing NADH, NADH–cytochrome b_5 reductase, cytochrome b_5, and a cyanide-sensitive factor whose normal functioning requires phospholipids. The change in the degree of unsaturation of lipids is believed to be related to the inhibition of ergosterol biosynthesis [69]. Azoles inhibit desaturation and elongation of fatty acids, and this results in a change in the membrane fluidity and chelation of iron, which is necessary for redox reactions occurring in the course of desaturation [70].

Certain inhibitors (miconazole, ketoconazole) may directly interact with membrane lipids, penetrating hydrophobic or hydrophilic layers by portions of their molecules and destabilizing the lipid bilayer structure [71]. A decrease in the ergosterol content and accumulation of 14α methylsterols may cause changes in the lipid environment and thus affect the activity of membrane-bound enzymes, such as Mg^{2+}-, Na^+-, and K^+-ATPases [67, 72] and cytochrome *c* peroxidase [73]. It cannot, however, be stated with confidence that azoles affect the properties of all membrane-bound oxidases, including the NADH-dependent cyanide-insensitive oxidase. Accumulation of toxic concentrations of

hydrogen peroxide, formed as a result of an increase in the NADH oxidase activity, and elimination of the activities of catalase and peroxidase result in degeneration of subcellular structures.

The antifungal activity of azoles is determined by a complicated process launched by the inhibition of the two cytochromes P450 involved in sterol biosynthesis, namely, the P450 that catalyzes 14α-demethylation (CYP51, encoded by the *erg1* gene), and the Δ22 desaturase of the side chain (CYP61, encoded by the *erg5* gene) [74–76]. The interaction of azoles with 14α-demethylase (CYP51) results in a decrease in the ergosterol content and accumulation of 14-methylsterols, such as 14α-methylergosta-8,24(28)-diene-3β–6α-diol (3,6-diol) [36, 77]; in *C. neoformans* and *Hystoplasma capsulatum*, accumulation of the 14-methylated 3-ketosteroids obtusifolione and/or 14-methylfecosterone occurs [36, 78], suggesting that azoles also influence 3-ketoreductase, the product of the *erg27* gene. Certain azoles (itraconazole) inhibit, in addition, the last demethylation reaction (at C-4) [36]. The products thereby formed (eburicol and obtusifolione) cannot support growth of *C. neoformans*, and 3-ketosteroids destroy membranes. It should be noted that not all of the 14-methylsterols cause membrane destruction; e.g., 14-methylfecosterol can in part fulfill some functions of ergosterol in resistant isolates of *S. cerevisiae* [79]. The resistance of fungi to inhibitors of ergosterol biosynthesis may be determined by mutations of the genes encoding 14α-demethylase (*cyp51A*) [80] or Δ5(6)-desaturase [81], modifications in the regulation of genes related to ergosterol biosynthesis [82–85], enhanced extrusion of the inhibitors from cells via membrane transport [86], or by the use of alternative sterols (cholesterol in particular) for substitution of ergosterol in the membranes [87]. It should be noted that polyenes and sterol biosynthesis inhibitors are inefficient against the pseudo fungi of the genus *Pythium*, whose membranes contain no sterols [88].

By using various yeast mutants, particularly, *S. cerevisiae* GL7, deficient in sterol synthesis, it was shown that, in the absence of ergosterol, cell division terminates in the G_1 phase of the cell cycle [89, 90]. A minimal ("hormonal") amount of ergosterol, required for the regulatory ("sparking") function, caused, in the G_1 phase, transformation of polyphosphoinositides and/or protein phosphorylation, which launches mitotic division [89, 91].

Investigations of the structural features of sterols necessary for their regulatory functions to be fulfilled, which employed anaerobic yeasts grown in the presence of a 2,3-oxidosqualene cyclase inhibitor, demonstrated the necessity for the yeasts of sterols methylated at C-24 (ergosterol is among these sterols) [92]. In mutant GL7, the activity of certain enzymes of phospholipid biosynthesis, such as PEA–phosphatidylcholine (PC) *N*-methyltransferase and acyl-CoA α-glycerol-3-phosphate transacylase, was higher in cells grown in the presence of ergosterol than in cells grown in the presence of cholesterol [93], which lacks a methyl group at C-24 and the Δ7 and Δ22 double bonds. By using a yeast mutant deficient in Δ5 desaturation, it was shown that the capacity to desaturate sterols at the C-5 position is necessary for growth. In addition, the presence of a double bond at C-22 and a methyl group at C-24 improves the fulfillment of the regulatory function by ergosterol present in minimal amounts [94].

Effect of sterols on the composition and structure of the fungal cell wall and morphology. Inhibitors of sterol biosynthesis, apart from affecting the enzymes involved in this process, exert influence on the activity of many other membrane-bound enzymes, membrane transport, respiration, fatty acid synthesis, and cell morphology. The changes in cell morphology involve thickening of the cell wall, formation of small vesicles between the cell wall and the cytoplasmic membrane, and deposition of the vesicular material in the thickened cell wall [33]. In addition, the formation of septa is not completed, or their organization is changed; membranes of the endoplasmic reticulum and mitochondria are destroyed, lipid granules are accumulated, and vacuolization increases [95].

Fungal morphology is largely determined by the composition and structure of the cell wall. Chitin is the major structural component of the fungal cell wall [96, 97], and chitin synthase is the key enzyme of its biosynthesis. It is associated with the plasma membrane and, in the zymogenic form, is contained in cytoplasmic vesicles, chitosomes [98]. In *Neurospora crassa*, impairment of the expression of the gene encoding chitin synthase I (*chs1*) results in the appearance of aberrant hyphae [99]. Since all chitin synthases (ChsI, ChsII, ChsIII) are integral membrane proteins and require phospholipids (PC, PS) [100–104] and ergosterol for normal functioning, the chitin synthase activity depends on the state of the membrane and is modulated by stress impacts on the membrane [105. 106]. In the presence of ergosterol biosynthesis inhibitors, the organization of the membrane lipid stroma is impaired: the temperature of the dipalmitoyl–PC phase transition of the multilayered vesicles shifts toward lower values without changes in the inner melting temperature [61, 71, 107]. It has been established that the synthesis of chitin is inhibited by high and stimulated low ergosterol content: polyene-resistant strains of *C. albicans* with an increased ergosterol content exhibited higher chitin synthase activity [108, 109]. In this context, it is easily understandable why some of the azoles, although targeting ergosterol synthesis, produce an indirect effect on fungal morphology. For example, some imidazoles were shown to inhibit the yeast ⟶ mycelium transition [73, 110]. Chitin synthesis is more important for the mycelial form, since the yeastlike form contains less chitin in the cell wall; e.g., in *C. albicans*, yeastlike and mycelial cell walls contain 1% and 5% chitin, respectively [111], and the chitin synthase activity in the mycelium is twice as high as in yeastlike cells [112]. The noncoordinated increase in the chitin syn-

thase activity causes more pronounced changes in the chitin distribution in mycelium than in yeastlike cells, and, consequently, the mycelial form is more sensitive to azoles. The hyphal growth of *C. albicans* was shown to be inhibited by miconazole and ketoconazole; the culture grew in an yeastlike form [113]. Apart from the change in the chitin synthase activity, this effect may be due to 14-methylsterol accumulation, which may result in the impairment of the membrane's structural integrity. Changes in the sterol structure may also cause deviations in the regulation, which lead to the transition from the mycelial to yeastlike form.

Treatment with a low concentration of ergosterol biosynthesis inhibitors led to an increase in the synthesis of chitin and to its uneven distribution in the cell wall in the mycelial fungi *Ustilago maydis, Penicillium italicum, Aspergillus fumigatus*, and *C. albicans* [67, 68, 114–117], as well as to the formation of chains and clusters of unseparated cells [116]. In addition, in *C. albicans*, azoles inhibited the branching of hyphae and the formation of growth tubes and caused the production of aberrant growth forms and yeastlike cells [69, 118, 119]; in *A. fumigatus* and *Uncinola necator*, azoles induced enhanced branching and swelling of the hyphal apex [68, 120]. In *Fusarium culmorum*, tebuconazole also inhibited mycelial growth and caused irregular swelling and enhanced branching of hyphae, which was accompanied by thickening of the cell wall, enhanced septation, vacuolization, accumulation of lipid granules, and degradation of cytoplasm [121].

Cell shape is determined not only by the cell wall—"the exoskeleton"—but also by the cytoskeleton, among the main components of which is actin. The interactions between the cytoskeleton, plasma membrane, and extracellular matrix largely determine the character of hyphal growth; these interactions are mediated by the cAMP-dependent transduction of signals, which involves the activation of protein kinase A [122, 123]. Actin and tubulin filaments run through the entire cell volume; they are in the state of permanent movement and direct the transportation of secretory vesicles in the process of morphogenesis [124, 125]. In fungi, actin is involved in morphogenesis and growth of the hyphal apex, and determination of the site of formation of a new cell wall [126, 127], as well as in the formation of septa and the contractible actomyosin ring during budding [128, 129]. In various temperature-sensitive actin mutants of *S. cerevisiae* incapable of the formation of the actomyosin ring and complete cytokinesis, the distribution of chitin may change. These mutants undergo multiple cycles of budding and grow in a quasi-mycelial form (cdc3, cdc10, cdc11, cdc12) or as cells with multiple buds (cdc4) [127]. These studies allow a conclusion to be made that the effect of azole derivatives on ergosterol synthesis is determined by the effect they produce on the fluidity of the critical domains in the membrane, which leads to impairment of actin assemblage and, consequently, to a change in the distribution of chitin, as well as to the accumulation of secretory vesicles.

Structural and regulatory functions of ergosterol. The role played by sterols in membranes is determined by their effect on the membrane's physical properties—the regularity of the arrangement of molecules and gel to liquid crystal phase transitions—as well as on membrane permeability. These changes in the composition and structure of the membranes result in the inhibition of membrane-bound enzymes and leakage of intracellular components.

Sterols are indispensable components of the fungal plasma membrane. Thus, ergosterol promotes membrane stabilization in an *erg1* mutant of *S. cerevisiae* and increases its tolerance to heat shock and shock caused by ethanol treatment [130]. It is known that membrane phospholipids are involved in the reversible temperature-phase gel-to-liquid crystal transitions. Sterols increase the regularity of the arrangement of membrane phospholipids at temperatures higher than the temperature of the phase transition, and decrease the regularity at lower temperatures [131]. Since the liquid-crystal state of the membrane is the state favorable for cell growth and functioning, the structure of fungal sterols significantly influences membrane functions and the activity of membrane-bound enzymes [132–134]. Using the mutant *S. cerevisiae* GL7 as a model, it was shown that the fulfillment of membrane functions by sterols requires the presence of a 3β-OH group at C-3, a flat sterol nucleus without methyl groups at C-4, and a freely rotating side chain with the 20R configuration [133]. The presence of a double bond at C-24 in the side chain is important for the activity of Δ24(25)-sterol methyltransferase [134].

Impairments of ergosterol biosynthesis are often accompanied by the development of calcium dependence; therefore, it is believed that ergosterol is necessary for the normal functioning of the protein Pmrlp of the calcium pump, located in the membranes of the Golgi complex [135], as well as for calcium-mediated signal transduction [136]. Mutations in the *erg24* gene or treatment of yeasts with sterol biosynthesis inhibitors cause cells to be dependent on calcium, suggesting a relationship between impairment of ergosterol biosynthesis and disturbance of calcium homeostasis. Calcium is necessary for protein transportation in secretory systems [137, 138]. It has been reported that in the *S. cerevisiae* mutants impaired in the *erg24, erg26, scs1*, and *scs2* genes, the Ca^{2+}-dependent phenotype (altered sphingolipid composition and altered sensitivity to sterol biosynthesis inhibitors, pH, and high temperature) is connected with defects in ergosterol biosynthesis and accumulation of aberrant sterols [135, 139, 140]. It has been suggested that alterations in ergosterol biosynthesis cause impairment of translocation of proteins across the membrane, similar to the impairment resulting from mutations of the *pmr1* gene, which lead to sensitivity to calcium due to the inability

to retain its excess in vacuoles [135]. The similarity of phenotypes of sphingolipid and ergosterol mutants is thought to be related to the functioning of OsbP proteins, involved in the regulation of secretory transport and maintenance of the composition of sterols and sphingolipids in the membranes of *S. cerevisiae* [53, 54], as well as to the fact that lipids of these classes are primarily located in membranes and maintain their functionality [140].

Ergosterol regulates membrane permeability and the activity of membrane-bound enzymes [141]; it is the major component of secretory vesicles and plays an important role in respiration and oxidative phosphorylation in mitochondria [63, 67, 68, 142, 143]. Blockage of ergosterol biosynthesis by azoles leads to changes in the activity of cytochrome *c* oxidase in the mitochondrial membranes of *C. albicans* and to inhibition of biosynthesis of mitochondrial membrane-bound enzymes in *S. cerevisiae* [144. 145]. Changes in the level of ergosterol or in sterol structure affect the operation of certain metabolic pathways. Thus, in the presence of miconazole, palmitic acid accumulates in *C. albicans* lipids instead of oleic acid, indicating the inhibition of Δ9-desaturase of fatty acids [67, 69].

ROLE OF STEROLS IN THE MORPHOGENESIS OF MUCORACEOUS FUNGI

The species *M. hiemalis* is commonly considered to be monomorphic, i.e., capable of only mycelial growth [3], whereas the species *lusitanicus* (syn. *M. circinelloides* var. *lusitanicus*; *M. racemosus*) is believed to be dimorphic. However, in the course of our studies of the effect of environmental stressors (presence of chloroanilines, depletion of nutrient sources, etc.) on mucoraceous fungi, it was established that some *M. hiemalis* representatives are also capable of yeastlike growth after germination of arthrospores [12, 48, 146, 147]. Yeastlike growth in these fungi was more difficult to induce than in *M. lusitanicus* representatives; most probably, this is due to the higher resistance of *M. hiemalis* to stressor impacts, which is explained by the genetically determined composition of lipids in its sporangiospores.

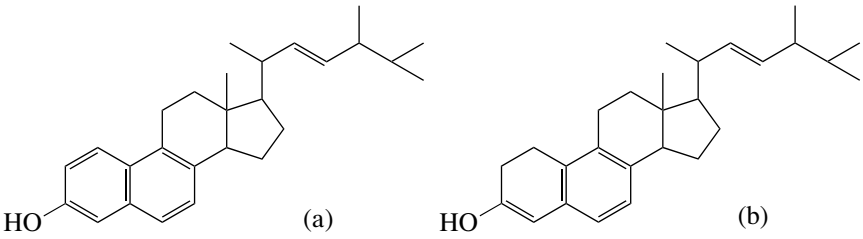
Prolonged cultivation of mucoraceous fungi on solid media leads to depletion of nutrient sources and a decrease in humidity, and culminates in the production of asexual resting forms—sporangiospores, whose function is species dispersion rather than prolonged survival. Sporangiospores are not characterized by deep dormancy, and this is reflected in their lipid composition. The realization of different growth strategies by mucoraceous fungi, *M. hiemalis* and *M. lusitanicus* 12M in particular, results in variations in the composition of sterols, fatty acids, and other lipids in their sporangiospores [48, 148, 149].

Lipids of *M. hiemalis* sporangiospores and morphogenesis of the fungus. The sporangiospores of *M. hiemalis* formed by cultures of different ages exhibited different survival rates and lipid and sterol compositions. They also differed in their ability to develop in a yeastlike form upon germination. During yeastlike growth, the content of sterols and storage lipids decreased, and the PEA/PC and phospholipids (PL)/ glycolipids (GL) ratios and unsaturation level of fatty acids changed [48]. The decrease in the PL/GL ratio (from 8.5 to 1.9) in sporangiospores formed in an old (20-day) culture (compared to the ratio characteristic of 3-, 7-, or 10-day cultures) must have led to changes in the structural organization and functionality of the membranes. Loss of viability by old sporangiospores was accompanied by an increase in the contents of linoleic and γ-linolenic acids. During the growth of a sporulating culture, the PEA/PC ratio in sporangiospores decreased from 0.9 to 0.4 (on the tenth day) at the expense of accumulation of the main phospholipid (PC) and then again increased to a value of 0.9 (on the 20th day) due to PC mobilization. Thus, the levels of phospholipids were the same in the sporangiospores of old and young cultures; however, sporangiospores of old cultures produced yeastlike rather than mycelial growth upon germination. In our opinion, the change in the sporangiospore sterol composition, namely, the decrease in the ergosterol content in sporangiospores of old cultures, is among the factors determining the growth pattern of the fungus.

Ergosterol was the main sterol of *M. hiemalis*; with aging of the sporogenous culture, its content decreased from 95% of total sterols on the 7th day to 51.4% on the 20th day [148]. Concomitantly, an increase occurred in the content of minor intermediates of sterol biosynthesis, both demethylated (fecosterol and episterol) and methylated ones (24-methylene-4αmethylcholest-8-en-3β-ol, eburicol, and 4,4-dimethylfecosterol). This fact, along with other ones (such as the decrease in the content of cardiolipin, the main phospholipid of mitochondrial membranes, from 15 to 9% of total polar lipids), suggests changes in the functional state of the membranes, which require 4,14-desmethylsterols for normal functioning [46, 150].

In young sporangiospores of *M. hiemalis*, which are able to produce mycelium upon germination, the ratio of methylated sterols to demethylated sterols was 1 : 38; in old spores, yielding yeastlike growth upon germination, this ratio equaled 2 : 3 [48]. The ratio of esterified and free sterols (ES/S) in old spores decreased from 2.0 to 1.35, indicating depletion of the pool of available ES. Sporangiospores with such changes in the lipid composition lost 70% of their germination ability; viable sporangiospores in which the lipid pool was depleted in the process of prolonged cultivation and the content of ergosterol was low produced both mycelial and yeastlike forms upon germination.

Thus, the distinctions between young and old *M. hielmalis* sporangiospores in the composition of sterols, fatty acids, and certain lipid classes correlated with their survival rates and ability to yield yeastlike



Structure of unusual sterols detected in the lipid composition of *M. hiemalis*: (a) 19-*nor*-24-methyl-cholesta-3,5(10),6,8(9),22-pentaene-3β-ol (1-dihydro-dehydroneoergosterol) and (b) 19-*nor*-24-methyl-cholesta-1,3,5(10),6,8(9),22-hexaene-3β-ol (dehydroneoergosterol) [35].

cells and produce arthrospores on grown mycelium; this correlation demonstrates the interrelation between lipogenesis and morphogenesis in fungi.

Lipids of *M. hiemalis* mycelium and arthrospores. The onset of conditions unfavorable for the growth of fungi serves as a signal for sporulation. However, mucoraceous fungi do not sporulate in submerged cultures. Under these conditions, when multiplication by sporangiospores is impossible, the ability to produce arthrospores acquires particular importance. Polymorphism of the resting forms is considered to be an adaptive mechanism that allows fungi (and other microorganisms) to survive under unfavorable conditions. Arthrospores are asexual propagative structures that represent a separate stage in the life cycle of many fungal species. The reasons for arthrospore formation and the fine mechanisms involved are still poorly studied. Arthrospore formation is usually related to submerged conditions favorable for fermentation.

However, upon inoculation of liquid nutrient medium with sporangiospores formed by an old *M. hiemalis* culture, production in the course of the resulting growth of arthrospores—round cells arranged in chains on hyphal tips—occurred even under aerobic conditions. Upon germination, these arthrospores produced yeastlike budding cells.

The arthrospore lipids differed from mycelium lipids in a higher content of sterol esters; the levels of free sterols did not differ significantly [35]. Esterified sterols are classified as storage lipids by many defensive [151, 152]; however, they may also play a regulatory role in the cell [153]. It has been shown that sterol esters of *S. cerevisiae* contain more lanosterol than free sterols (represented primarily by ergosterol) do [153, 154]. Ester formation binds and distracts from active metabolism a considerable portion of both free fatty acids and ergosterol precursors, which are membrane-active compounds. Bound fatty acids and ergosterol precursors are no longer subject to metabolic conversions [153].

The sterols of arthrospores contained less ergosterol than mycelial sterols (56% and 78%, respectively); among ergosterol precursors, methylated sterols—eburicol and 4,4-dimethylfecosterol—were the most abundant in arthrospores [35]. The ratio of methylated sterols to demethylated ones was 1 : 9 in mycelium and 1 : 4 in arthrospores formed on this mycelium.

In *M. hiemalis*, two unusual and extremely rare sterols were found: 1-dihydro-dehydroneoergosterol and dehydroneoergosterol, which are products of ergosterol transformation and contain in their A and B rings a complicated system of conjugated double bonds (see figure). It is known that some microorganisms capable of sterol transformation can catalyze splitting of the 19th carbon atom and introduction of the Δ1, Δ3, Δ5(10), Δ7(8), and Δ8(9) double bonds in the nucleus (*Pseudomonas*, *Nocardia restrictis*), as well as isomerization of the Δ7 bond into Δ6 bond (*Arthrobacter simplex*). For fungi, these reactions are not typical, however, some of them have been noted in some species of *Fusarium, Rhizopus, Curvularia, Penicillium*, and *Aspergillus* [155]. It is possible that ergosterol transformation products, whose content in *M. hiemalis* arthrospores is 82% higher than in mycelium, may be involved in the morphogenetic processes of this fungus.

No drastic distinctions have been found in the contents of individual fatty acids in the mycelial and arthrospore lipids; however, in arthrospores, the content of palmitic acid was higher, and the content of linoleic acid was lower than in the mycelium, which resulted in a decrease in the unsaturation degree of lipids [35]. The content of triacylglycerols (TAG) in arthrospores was lower, and the content of polar lipids, free fatty acids, and sterol esters was higher. In polar lipids of arthrospores, more glycolipids and phosphatidylserine were contained, and the contents of phosphatidic acid, cerebroside, and phosphatidylethanolamine were lower than in mycelial polar lipids. The distinctions in the lipid compositions were indicative of a decreased activity of metabolic processes in arthrospores.

Lipids of *M. lusitanicus* sporangiospores and morphogenesis of the fungus. In the fungus *M. lusitanicus* 12M, we also observed a correlation between the lipid composition of sporangiospores, their viability, and their tendency toward production of yeastlike growth [149, 156]. In *M. lusitanicus* 12M, yeastlike growth occurred even upon a small decrease in the TAG fraction in the lipids and an increase in the level of diacylglycerols (DAG). In the process of prolonged cultivation, the contents of TAG, ES, and major phospholip-

ids (PC, PEA, PS) decreased in sporangiospore lipids. The PEA/PC ratio increased from 0.39 to 0.94, and the PL/GL ratio decreased from 1.2 to 0.5; this indicated critical changes in the structure and functionality of membranes and correlated with a decrease in sporangiospore viability. An increase was observed in the contents of free fatty acids, DAG, and free sterols, half of which were represented by methyl derivatives. As mentioned above, methylsterols cannot successfully support membrane functions that require a high content of ergosterol (the main desmethylsterol of mucoraceous fungi). The depletion of the reserve pool of ergosterol (in ES) was evidenced by a decrease in the ES/free sterols ratio from 1.27 to 0.23. Sporangiospores with such a lipid composition completely lost their viability.

Based on the data presented, it may be concluded that estimation of the potential capacity of mucoraceous fungi for dimorphism requires consideration of the following parameters of the lipid composition of sporangiospores: (1) increased PEA/PC ratio; (2) increased methylsterols/desmethylsterols ratio; (3) decreased PL/GL ratio; and (4) decreased ES/S ratio. These parameters may be considered to be criteria of sporangiospore viability and of their capacity to produce yeastlike growth.

Thus, the capacity of mucoraceous fungi for yeastlike growth depends on, among other factors, the spore age, and correlates with changes in the composition and content of lipids: the decrease in the total lipid pool, the ergosterol and phospholipid fractions, and storage lipids, and the increase in the level of glycolipids and precursors of ergosterol or products of its degradation. The ergosterol level is one of the most important markers of this morphogenetic process, which provides for better adaptation and survival of dimorphic fungi under stresses.

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