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REVIEW

The synthesis, regulation, and functions of sterols in *Candida albicans*: Well-known but still lots to learn

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ABSTRACT
Sterols are the basal components of the membranes of the fungal pathogen *Candida albicans*, and these membranes determine the susceptibility of *C. albicans* cells to a variety of stresses, such as ionic, osmotic and oxidative pressures, and treatment with antifungal drugs. The common antifungal azoles in clinical use are targeted to the biosynthesis of ergosterol. In the past years, the synthesis, storage and metabolism of ergosterol in *Saccharomyces cerevisiae* has been characterized in some detail; however, these processes has not been as well investigated in the human opportunistic pathogen *C. albicans*. In this review, we summarize the genes involved in ergosterol synthesis and regulation in *C. albicans*. As well, genes in *S. cerevisiae* implicated in ergosterol storage and conversions with other lipids are noted, as these provide us clues and directions for the study of the homologous genes in *C. albicans*. In this report we have particularly focused on the essential roles of ergosterol in the dynamic process of cell biology and its fundamental status in the biological membrane system that includes lipid rafts, lipid droplets, vacuoles and mitochondria. We believe that a thorough understanding of this classic and essential pathway will give us new ideas about drug resistance and morphological switching in *C. albicans*.

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The relative evolutionary relationship between humans and their fungal pathogens has made the treatment of fungal infections with chemotherapeutic agents encounter great challenges. The majority of life-threatening fungal infections are caused by *C. albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, with *C. albicans* predominating with a death toll of over 400,000 each year (the mortality rate can approach 40% for systemic infections).[1] Worryingly, in the past years *C. albicans* infections have been extended from life-threatening systemic infections in immunocompromised patients to nosocomial fungus infections in healthy people.[2] Currently the treatment of candidiasis in the clinic is limited by the relatively small number of traditional types of antifungal drugs and by drug resistance, which has increased through antifungal drug abuse and the need for long-term treatment options in response to recurrent attacks.[3]

At present, the azoles, polyenes, and echinocandins have been widely used for the treatment of fungal infections.[4] Although there are limited reports of resistance to polyenes or echinocadins, azole resistance is still the most clinically relevant issue. Recently, fluconazole-(diflucan-)resistant *C. albicans*, which leads to about 46,000 hospitalized patients infected each year, has been classified as a serious pathogen by the Centers for Disease Control and Prevention.[5,6] There are 4 major mechanisms that generate resistance to azoles in *C. albicans*: transcriptional regulation or mutations in the ergosterol biosynthetic pathway, including the up-regulation or mutations of the *ERG11* gene whose product is the direct target for azoles;[7,8] reduction in intracellular drug concentrations by the overexpression of transmembrane efflux pump proteins, such as major facilitator superfamily (MFS) and ATP-binding cassette (ABC) transporter proteins;[9-11] cellular stress responses through changes in metabolic pathways, which depend on the regulation of transcription factors (Mdr1p, Tac1p, Crz1p) or protein kinase A;[12-14] and the formation of biofilms consisting of an extracellular exopolymeric matrix, yeast cells, hyphae and pseudohyphae.[15] Most of the drug-resistant isolates are related in some way to alterations in the content and composition of membrane sterols. To address the problem of drug resistance in *C. albicans*, we need a clear

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understanding of the synthesis of sterols and the transcriptional or post-transcriptional regulation of the process.

In yeast, the major sterol is ergosterol, which is mainly synthesized in the endoplasmic reticulum (ER) and in lipid droplets.[16] Sterols in yeast are indispensable factors that coordinate membrane heterogeneity, prevent water penetration, and maintain the integrity, rigidity and fluidity of the plasma membrane.[17] In a spin probe electron paramagnetic resonance (EPR) study, Sgherri et al. detected that azole treatment disrupted the formation of the ordered lipid bilayer and/or decreased the content of integral membrane proteins.[18] In addition, the membranes of organelles and the fusion of transport vesicles are dependent on the correct assemblies of sterols and other lipids. In this review, we present the synthesis of ergosterol in the opportunistic pathogenic fungus *C. albicans* and summarize key genetic mutations in ergosterol biosynthesis and transcriptional factors, focusing on their effects on drug sensitivity. As a basic component of all kinds of membranes, ergosterol depletion changes many cellular biological processes and destroys the normal membrane properties. The impact of ergosterol depletion on lipid rafts, vacuoles and mitochondria in *C. albicans* are also covered. Finally, we identify some issues that are worthy of study in *C. albicans* through reference to the formation and function of ergosterol in *S. cerevisiae*.

The biosynthesis and storage of sterols

The sterol biosynthetic pathway in eukaryotes is conserved. Both ergosterol as the final product in yeasts, and cholesterol as the final product in mammals, are synthesized from the same intermediate lanosterol, albeit using different enzymes and requiring different levels of oxygen consumption.[19] Previous work had not identified direct evidence for the uptake of sterols from the external environment in *C. albicans* until Zavrel et al. found that *C. albicans* takes up sterols only under aerobic conditions during the post-exponential-growth phase, a process independent of *UPC2*.[20,21] As a result, the pathway of steroid biosynthesis plays an important part in dealing with the external environmental stress. This pathway and the linked antifungal inhibitors are shown in Fig. 1, which identifies the major intermediates and the alternative biosynthesis routes bypassing *ERG11* or *ERG3*.[22,23] This pathway includes 2 rate-limiting enzymes. One is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is necessary in regulation of cholesterol synthesis in mammals, and the other one is the lanosterol 14α-demethylase, which is essential in the ergosterol synthesis in fungi. By using the technology of gene disruption and controlled gene expression, *ERG1*, *ERG7*, *ERG9*, *ERG25*, *ERG26*, and *ERG27* have been proved to be essential, while *ERG2*, *ERG3*, *ERG5*, *ERG6*, and *ERG24* are considered to be non-essential in ergosterol biosynthesis.[24-31] Interestingly, the direct target gene of azoles, *ERG11*, can only be disrupted in the *ERG3*-deficient strains or when strains are cultured in media with nystatin or amphotericin B, as the sterols with a C14-methyl group or diols especially for 14-methylergosta-8,24(28)-dien-3β,6α-diol accumulated by *ERG3* is lethal in *C. albicans*.[32-34] *NCP1* encodes a NADPH-cytochrome P450 reductase that, in conjunction with Erg11p, is required for sterol C14-demethylation. *NCP1* is an essential gene and highly expressed in cells treated with ketoconazole.[35,36] The ERG gene disruptions change the components and distribution of sterols. These changes can be measured by gas chromatography-mass spectrometer (GC-MS), which provides a solid evidence for the function of the ERG enzymes.[37] For instance, the clinical isolates displayed cross-resistant to azoles and amphotericin B have been detected to be the *ERG3* mutated strain. And the direct evidence is that the upstream products of Erg3p, episterol and fecosterol, have accumulated about 10%. In consistent with this, ergosta-7,22-dienol, which is the product of the alternative route bypassing the Erg3p accounts for about 50% of all the sterols. These sterol composition data has provided a powerful evidence that the cross-resistance of clinical isolates depends on the mutation in *ERG3*.[38]

Although *C. albicans* can survive the inhibition of ergosterol synthesis,[39] antifungals such as azoles, polyenes, and allylamines inhibit the growth of *C. albicans* severely. To be specific, azoles target to lanosterol 14-α demethylase (Cyp51p); polyenes disrupt the ergosterols distributed on the membrane of fungi; and allylamines are squalene synthase (Erg1p) inhibitors. Most of antifungal drugs in the clinic targeting the biosynthesis of ergosterol inhibit the activities of ERG enzymes and direct the biosynthesis of the ergosterol pathway to an alternative route (Fig. 1, B or C).[40] Their fungistatic properties are caused not only the reduction of ergosterol in the membranes but also by accumulation of aberrantly formed sterols with a C14-methyl group changing the properties of membranes.[17,41] The mutations or disruptions in ERG genes lead to increased or reduced sensitivity to the antifungal agents (Table 1). These sensitivity phenotypes provide clues for studying the uncharacterized genes or networks within the ergosterol biosynthetic pathway. Meanwhile, mutations in this pathway, causing alterations in enzymes activity or drug binding sites, are major factors which are related with azole resistance. Mutations in the *ERG11* and *ERG3* genes or the *UPC2* gene which encodes a transcription factor contribute to azole resistance.[22,42-46] Mutations in *ERG11* are not limited to the point mutations, as the clinical isolates with 2 extra copies of Chromosome 5L, as an isochromosome, conferred increased fluconazole resistance. Recently, a

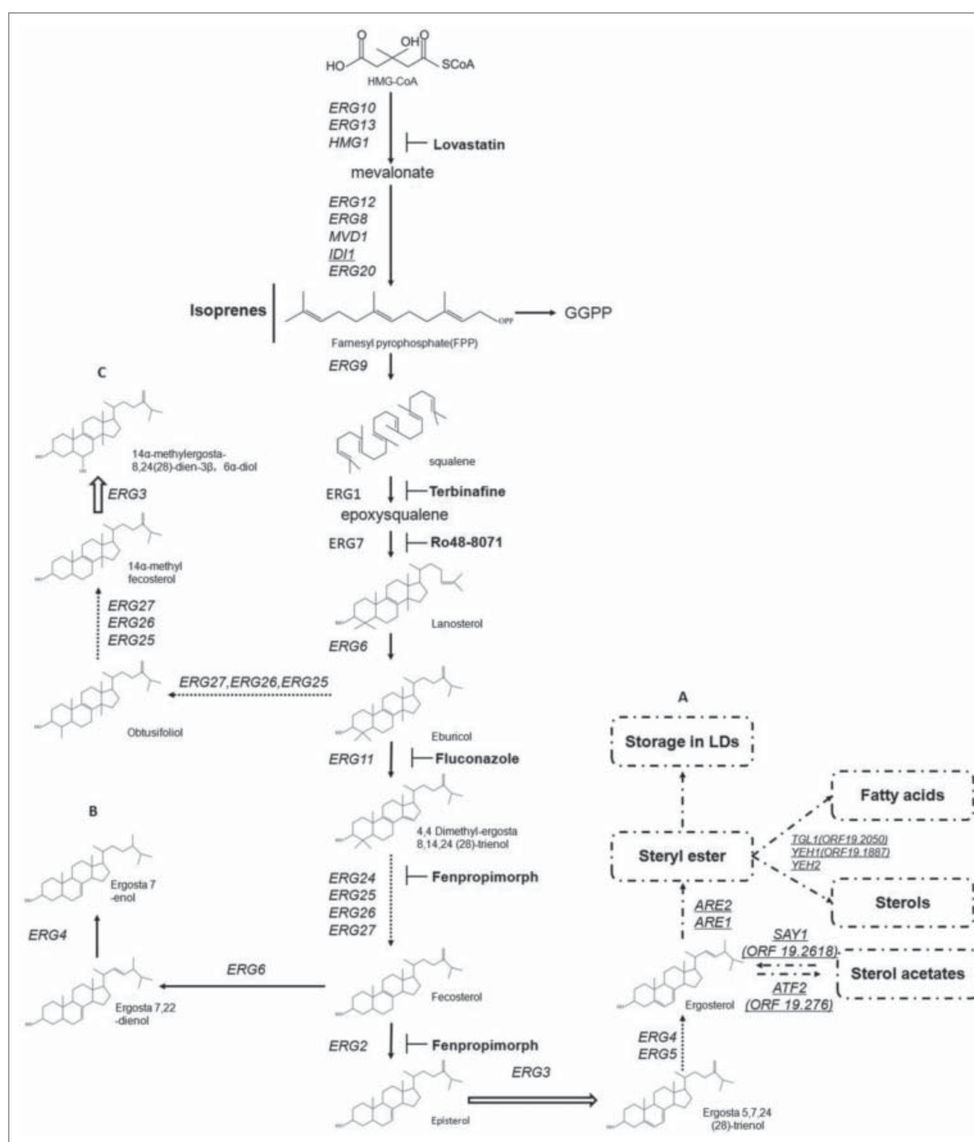

Figure 1. The conserved pathway of sterol synthesis, storage and transformation in *C. albicans* and *S. cerevisiae.* A. Schematic representation of the common ergosterol synthesis process including the enzymes and intermediate products in *C. albicans*. The storage and transformation between steryl esters and fatty acids or sterols is uncharacterized in *C. albicans* and labeled with dotted boxes, based on the homologous genes and their roles in *S. cerevisiae.* B. Accumulated intermediates when *ERG3* function is lost. C. Bypass pathway when *C. albicans* is treated with Erg11p inhibitors. The resulting aberrant sterol 14-methylergosta-8,24(28)-dien-3β,6α-diol is produced by *ERG3*. Solid arrows, single enzymatic process; dashed arrows, multiple enzymatic processes; underlined genes, *S. cerevisiae* genes and their homologous genes in *C. albicans* are shown in parentheses.

Table 1. Sensitivities of the mutant strains with the mutations or disruptions in ERG genes referred in this article.

Gene Description	Increased sensitivity	Reduced sensitivity
Met3p-ERG1	fluconazole, terbinafine, amphotericin B, cycloheximide, nystatin	
erg2Δ/erg2Δ	fluconazole, 4-nitroquinoline oxide, terbinafine, o-phenanthroline	
erg6Δ/erg6Δ	terbinafine, tridemorph, fenpropiomorph, fluphenazine, cycloheximide, cerulenin, brefeldin A	
erg24Δ/erg24Δ	terbinafine, cycloheximide, cerulenin, fluphenazine, brefeldin A	fluconazole (slightly)
erg3Δ/erg3Δ	amphotericin B, cycloheximide, fluphenazine, brefeldin A,	fluconazole
erg3Δ/erg3Δ-erg11Δ/erg11Δ	terbinafine, cycloheximide, fluphenazine, brefeldin A, hygromycin B	fluconazole, amphotericin B
ncp1Δ/ncp1	clotrimazole, fluconazole	
upc2Δ/upc2Δ	fluconazoles, terbinafine, fenpropimorph, lovastatin, nikkomycin Z, calcofluor white, SDS	
ndt80Δ/ndt80Δ	fluconazoles, caffeine, flucytosine, rapamycin, SDS	
efg1Δ/efg1Δ	fluconazoles, calcofluor white, congo red, amphotericin B, antimycin A, caspofungin, nystatin	

clinical isolate of *C. albicans* with mutations in *ERG11* and *ERG5* was reported to be cross-resistant to azoles and amphotericin B.[29]

The synthesis of ergosterol in *C. albicans* has been demonstrated clearly. However, the storage of ergosterol, the transformation between sterols and fatty acids and the secretion of sterols have not been extensively investigated. In *S. cerevisiae*, sterol and steryl ester metabolism and the involved enzymes have been researched thoroughly (Fig. 1). The genes used in the metabolism of steryl esters, such as *ARE2*, *ATF1*, *SAY1*, *TGL1*, and *YEH1*, have corresponding homologs in *C. albicans*. All these genes are uncharacterized in *C. albicans* except *ARE2*, which has been verified as a sterol acyltransferase that uses cholesterol and oleoyl-CoA as substrates and is induced by ketoconazole.[47] These genes control the storage and decomposition of sterols in lipid droplets, which in *S. cerevisiae* contain a neutral lipid core consisting of triacylglycerols (TG) and sterol esters (SE) surrounded by phospholipid.[48] Recently, it has been noted for the first time in *C. albicans* that cells treated with arylquinuclidine derivatives (squalene synthase inhibitors) showed a cytoplasmic accumulation of lipid droplets that could be labeled with Nile Red.[49] Lipid droplets not only play an important role in overcoming the potential toxic effect of abnormal sterols and free fatty acids (FA), but also affect cellular energy homeostasis and lipid metabolism.[50,51] There are still few reports about the connections between lipid droplets and drug sensitivity in *C. albicans*, but only one literature refers that the loss-of-function of Pdr19p located in the lipid droplets in *Candida glabrata* renders yeast cells more sensitive to fluconazole.[52] In our opinion, paying more attention to the features of lipid droplets and improving the understanding of the storage and decomposition mechanisms of the sterols in *C. albicans* may provide shortcuts to solving the problem of drug resistance and provide new strategies to identify antifungal targets.

Regulation of sterol biosynthesis in C. albicans

The regulatory mechanism for the sterol biosynthesis pathway is also conserved between animals and fungi. Sterol regulatory-element binding protein (SREBP), which consists of a sterol-sensing complex, is a key transcription activator of both cellular sterol import and de novo biosynthesis.[19,53] Since the first fungal ortholog of SREBP, Sre1p, was identified in *Schizosaccharomyces pombe*, several SREBP-like proteins have been identified in *S. cerevisiae* and the pathogenic fungal species *Cryptococcus neoformans* and *Aspergillus fumigates* but no clear orthologs have been identified in *C. albicans* (Fig. 2).[54] Maguire et al. illustrated that SREBP-like proteins in *S.*

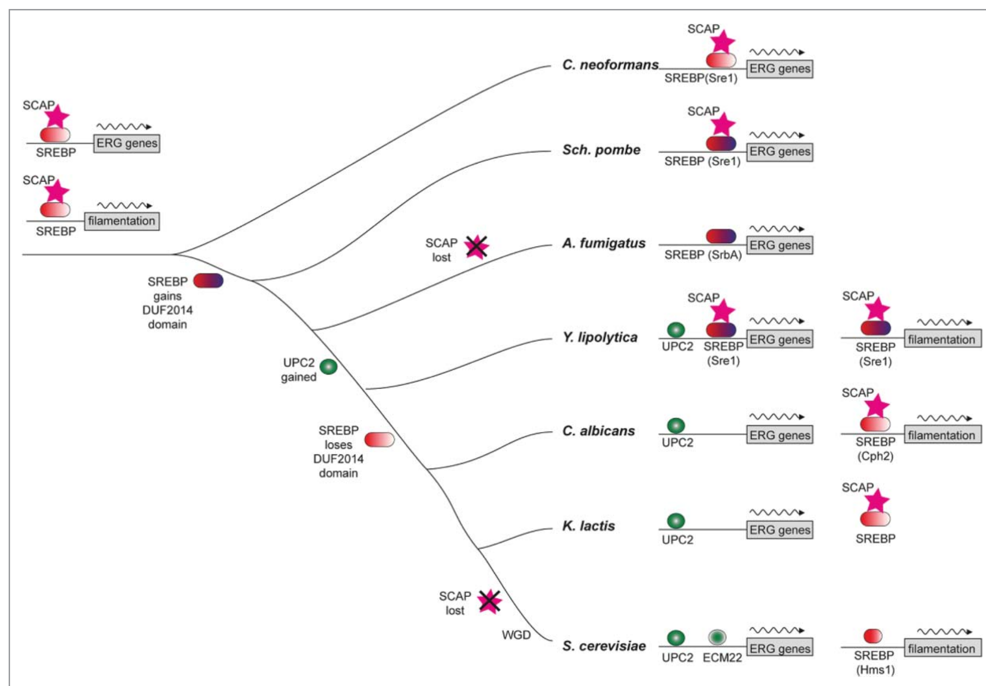

Figure 2. Model of sterol regulon evolution in Saccharomycotina illustrated by Maguire et al. in Plos Genetics (50). The ERG genes and filamentation genes are both regulated by SREBP and SCAP in the fungal progenitor. Upc2p gains binding sites in the promoters of ERG genes and the DUF2014 domain of SREBP, which may be important for interaction with Scap and retention of SREBP in the membrane, is lost but the protein still plays a role in regulation of filamentation in *C. albicans*.

cerevisiae (Hms1p) and *C. albicans* (Cph2p) have lost a DUF2014 domain (Fig. 2). As a consequence, the proteins lack the ability to regulate sterol synthesis, and instead they are implicated in regulation of filamentous growth. At the same time, *SRE1* appears to have an ancestral role in regulating hyphae growth, and has been replaced in its role in sterol gene regulation by the emergence of *UPC2* in the Saccharomycotina. Similar to the situation in *S. cerevisiae*, Upc2p is a global transcriptional activator of the ERG genes in *C. albicans* and has been shown to up-regulate gene expression as a compensatory mechanism in response to sterol stress.[55] Disruption of *UPC2* impaired cell growth in anaerobic environments and enhanced azole sensitivity. The high expression of *ERG* genes (*ERG2*, *ERG6*, *ERG7*, *ERG11*, *ERG24*, *ERG25*, and *ERG27*) and genes encoding efflux pumps (*CDR1*, *CDR2*, and *MDR1*) induced by fluconazole disappeared in the *upc2*Δ/*upc2*Δ mutant as measured by Northern blot and microarray analysis.[56] In contrast, gain-of-function mutations in *UPC2* often lead to high expression of *ERG11* and make a significant contribution to azole resistance.[46] These results suggest that *UPC2* is essential for azole resistance in *C. albicans*. In response to azoles, Upc2p has the ability of transcriptional self-regulation. Upc2p can always bind to a bipartite element within its own promoter, which is a domain including the sterol response element (SRE) and a partial homology to the SRE, named the short direct repeat (SDR).[57] Furthermore, the data suggest that an additional transcription factor controls sterol biosynthesis by regulating *UPC2* expression, but this gene has yet to be identified. In agreement with this, Gallo-Ebert et al. found that 3 inhibitors (VB00075177, VB00075853, and VB00074845) reduced the expression of both mRNA and protein levels of *UPC2* and demonstrated that these compounds' inhibiting effect of DNA-binding is irrelevant to the direct interaction with Upc2p.[58] Given these points, there likely is another transcription factor that positively regulates the expression of *UPC2* and the ERG genes.

As well as Upc2p, transcription factor Ndt80p can also bind to the promoters of ERG genes. By comparing transcriptional profiles of the *ndt80*Δ/*ndt80*Δ cells exposed to fluconazole or not, 7 of 10 ERG gene promoters have been identified as being transcriptionally dependent on Ndt80p, namely *ERG3*, *ERG4*, *ERG6*, *ERG7*, *ERG11*, *ERG24*, and *ERG25*, all of which are bound by Ndt80p directly.[59] Ndt80p is required for the transcriptional activation of the sterol synthesis pathway in response to fluconazole, but the upregulation of ERG genes is not completely abolished in the *ndt80*Δ/*ndt80*Δ strains. In agreement with the ERG gene down-regulation, the absence of Ndt80p enhances antifungal drug susceptibility. However, blocking *NDT80* plays little role in attempts to eliminate azole resistance in clinical isolates. This result is attributed to hyperactive forms of Mrr1p, Tac1p, and Upc2p, which up-regulate their target genes independent of Ndt80p and thereby mediate drug resistance in clinical strains.[60]

As well, there are other transcriptional factors that can regulate ERG gene expression and modify the sterol composition in *C. albicans*. The *efg1*Δ/*efg1*Δ mutant grown on solid media exhibited negative regulation of *ERG11* and a simultaneous positive regulation of *ERG3*, and became more sensitive to azoles.[61] All these transcription factors mentioned above regulate the sterol composition to promote cell growth and consequently control drug resistance and morphogenesis.

Ergosterol: The basis of normal biological membranes in C. albicans

Like cholesterol in mammals, ergosterol is an important component of membrane lipids and modulates the fluidity, permeability and integrity of the membrane in fungi. Through quantitative nano-electrospray ionization tandem mass spectrometry (nano-ESI-MS/MS) analysis of lipid extracts in *S. cerevisiae*, significant amounts of ergosterol have been identified in both the plasma membrane and the endomembrane system including peroxisomes, mitochondria, vacuoles and the ER.[62] We have summarized the distribution and the important role of ergosterol in *S.cerevisiae* or *C. albicans* (Fig. 3[63]).

Within the plasma membrane or the membranes of intracellular organelles, microdomains with higher amounts of sterols and saturated fatty acids than the rest of the membrane are called lipid rafts; these rafts are resistant to the action of non-anionic detergents.[64] The protein samples obtained from *C. albicans* lipid rafts were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and matrix assisted laser desorption ionization/time of flight (MALDI-TOF) mass spectroscopy. A total of 29 proteins were identified, including *PMA1* (the marker for lipid rafts in *S. cerevisiae*), *ECM33* (glycosylphosphatidylinositol (GPI)-anchored protein involved in cell wall biogenesis), *MNN7*, *PMT2*, *MNT1* (mannosyltransferases), *ERG11*, *SCS7* (proteins involved in lipid metabolism), *SSA1*, *HSP90* (heat shock proteins) and, *CDR1* (the ATP-binding cassette (ABC) multidrug transporter). The function of these proteins is consistent with the role that lipid rafts play in many biological processes, such as lipid metabolism, cell wall biogenesis, protein metabolism, electron transport, ATP synthesis and cellular stress.[65-67] In addition, ergosterol enriched in lipid rafts has been implicated in many cellular processes, such as sporulation,

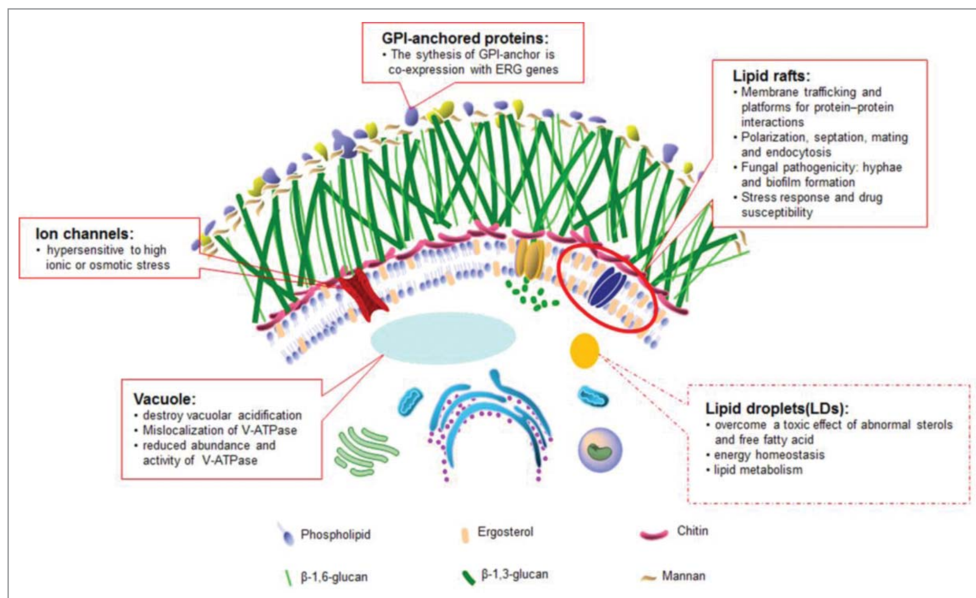

Figure 3. The effects of ergosterol are labeled on the *C. albicans* cell pattern diagram based on the picture from the Atlas of Fungal Infection.[63] The functions and cellular properties of ergosterol in *C. albicans* are described in solid boxes, while the functions verified in *S. cerevisiae* but yet uncharacterized in *C. albicans* are noted in dotted boxes. Candida albicans Mutations in the Ergosterol Biosynthetic Pathway and Resistance to Several Antifungal Agents.

pheromone signaling, polarization and membrane fusion during mating and endocytosis in *S. cerevisiae*.[68,69] Meanwhile, a highly polarized ergosterol-rich domain which splits the hyphae into several cavities is formed specifically during hyphal growth. The inhibition of either sterol biosynthesis with azoles or sphingolipid biosynthesis with myriocin leads to a loss of ergosterol polarization and disruptions in hyphae morphogenesis, suggesting that lipid rafts are necessary in filamentation.[63] Recently, O'Meara et al. illuminated the correlation between cell morphology and sterol synthesis using the *C. albicans* gene replacement and conditional expression (GRACE) collection. They identified that transcriptional disruption or repression of genes involved in the early phases of ergosterol synthesis, up to the production of episterol which was generated by *ERG2*, resulted in a hyphal growth deficiency, yet repression of genes functioning in the process of episterol to ergosterol modification did not.[70] This result from ERG gene deficient strains was confirmed using the ergosterol biosynthesis inhibitors terbinafine and fluconazole, as well as with amphotericin B which binds to ergosterol directly.[71] But the exact mechanisms of the antifungal-modulated morphogenesis phenotype have several explanations. One is that *C. albicans* cells treated with various azoles produce increased levels of the quorum sensing molecule farnesol, which suppresses the transition from yeast to hyphae.[72] The other is that blocking of sterol biosynthesis with azoles results in a loss of the ergosterol polarization needed to form the highly ergosterol-riched domains found in the cell tips.[61] Morphogenesis is a critical part of biofilm formation.[73] Biofilms generally possess distinct sterol patterns in diverse phases compared with planktonic cells. Sterol analyses show that ergosterol levels are significantly decreased in both intermediate (12-30h) and mature phases (31-72h), compared to those in early-phase (0-11h) biofilms, and this is connected to the decreased expression of ergosterol biosynthetic genes (*ERG25*, *ERG11*, and *ERG6*).[74-76]

Ergosterol exists in many membranes of organelles. But its role is not confined to that of a constituent part of membranes as it forms the basis of maintaining the localization and activities of some enzymes within the membrane. Vacuole as the largest organelle in yeast cells plays an important role in several cellular functions such as environment stresses response and the yeast-hyphae switch. To function normally, the vacuole need to maintain the intravacuolar homeostasis of pH and ion, which is regulated by the vacuolar proton-translocating ATPase.[77] Cells with a depletion of *ERG2* or *ERG24*, or treated with azoles or morpholines, reduce the degree of vacuolar acidification and exhibit an abnormal morphology of the vacuole. Meanwhile, *erg2*Δ/*erg2*Δ and *erg24*Δ/*erg24*Δ mutant strains have similar phenotypes to vacuolar deficient mutants in being hypersensitive to elevated temperature and to high ionic or osmotic stress.[78] Moreover, in the *ERG24*-defiecient strains, the V-ATPase that is required for the generation of a pH gradient in the vacuole and cytoplasm was mislocalized and reduced in abundance in the vacuolar membrane.[79] Surprisingly, ergosterol depletion diminishes the function of V-ATPase

in the vacuolar membrane but has no influence on Pma1, which encodes a plasma membrane H^+-ATPase. This research demonstrates that a specific lipid composition is required for normal membrane protein function. For other organelles, the relationship between the mitochondrial dysfunction and the decreased fluconazole susceptibility in *C. albicans* has been reported by several literatures. For instance, the inhibitors of mitochondrial complex I (CI) or complex V (CV) can always improve the outcome of fluconazole treatment in patients or the lab isolates. And the CI null mutants showed hypersusceptibility to fluconazole. In *C. albicans*, mitochondria are power houses which are needed for many cellular processes, such as drug susceptibility, cell wall integrity, phospholipid homeostasis, and virulence.[80] On one hand, mitochondrial dysfunction always cause downregulation of transporter genes (*CDR1* and *CDR2*) and the ergosterol synthesis gene family, which in turn decrease the cellular ergosterol levels. On the other hand, dysfunctional mitochondria deregulates iron metabolism caused by perturbed Fe-S cluster biogenesis.[81] Iron is a necessary cofactor for essential cellular processes as well as its toxicity (via hydroxyl radicals produced by the Fenton reaction) to proteins, lipids, and nucleic acids. *C. albicans* have a tight iron regulation system including 3 major transcriptional regulators, Sef1p, Sfu1p, and the Hap43p-associated CCAAT-binding complex. In addition, iron concentration is a significant signal to induce morphological transformation in *C. albicans*.[82] Iron deprivation could increase the membrane fluidity and permeability, and reduce drug sensitivity to azoles by decreasing *ERG1*, *ERG2*, *ERG11* and *ERG25* transcription and ergosterol levels.[83,84] There are few studies about the influence of ergosterol on other organelles in organisms other than in *S. cerevisiae*. In *S. cerevisiae*, ERG-deficient mutants exhibit defects in endocytosis, vacuolar fusion, mitochondrial morphogenesis and maintenance.[85,86] Some ERG-deficient strains even show perturbations in ion uptake and utilization that are similar to those in *C. albicans*. Ca^{2+} and Mg^{2+} can change the growth rate of the *erg24*Δ/*erg24*Δ mutant. Li^+ and Na^+ are absorbed more easily in the *erg6*Δ/*erg6*Δ mutant.[87,88] These functions of ergosterol demonstrated in *S. cerevisiae* are still obscure in *C. albicans*. We need to be cautious about how ergosterol affects the activity of enzymes on the membranes and the other membrane functions besides vacuolar acidification.

Perspectives

Since the first azoles targeting *ERG11* became available in 1960s, extensive research has been carried out to find new chemicals to inhibit the synthesis of ergosterol. Fluconazole does not have an antifungal effect as strong as amphotericin B, the latter which destroys the cell membrane through extracting ergosterol from lipid bilayers and is fungicidal.[89] Its antifungal mechanisms include ergosterol depletion, aberrant sterol accumulation and the hypothesis of unbalanced proportion between ergosterol and other sterols.[79] The fungistatic effects may result from the comprehensive factors, but there is no doubt that lipid mobilization plays an important role in *C. albicans* fighting against pressures from the outside environment. Due to the fact that *C. albicans* cannot take up sterols from external sources under anaerobic conditions, it is likely that studying the storage and metabolism of sterols in pathogenic fungi will be informative. Lipid droplets are a particularly interesting area for future investigation. By inhibiting the storage or release of sterols and synergizing with the sterol synthesis inhibition, we may find new strategies to kill fungi and solve the problems of azole resistance. Apart from the critical role of ergosterol, the regulation of the ERG genes exhibits cross talk with many other pathways, such as those controlling morphological transformation, biofilm formation, and GPI-anchor synthesis. Identifying the key signaling molecular process that adjusts the proportions of various kinds of sterols and what is the co-regulator that coordinates lipid metabolism and other biological processes will be critical. This information should bring us new ideas to further exploit the sterol synthesis pathway as an antifungal target.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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