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Thoughts on Quorum Sensing and Fungal Dimorphism

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Thoughts on Quorum Sensing and Fungal Dimorphism

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Abstract
Farnesol has been best studied for its role in regulating fungal dimorphism. However, farnesol is also a lipid and in this review we analyze data relevant to farnesol's function and synthesis from the perspective of farnesol and bacterial endotoxins acting as membrane active compounds. This analysis implicates the possible roles of: (1) endotoxins in the regulation of farnesol production by *C. albicans*; (2) farnesol in the interactions between *C. albicans* and the host during disseminated infections; and (3) ubiquinones in the mechanisms for unusually high resistance to farnesol by some *C. albicans* cell types. Finally we discuss the implications that the use of farnesol as both a signaling molecule and to antagonize competing microbials species has for the regulation of HMG-CoA reductase, the enzyme that is the usual rate limiting step in sterol/lipid synthesis.

1 Introduction

The role of farnesol in the dimorphism of *Candida albicans* was discovered by Hornby et al. (2001), reviewed by Nickerson et al. (2006), and updated by Langford et al. (2009) and Hogan and Muhlschlegel (2011). Up to now the theme of farnesol research has been farnesol as a signaling molecule and how it affects fungal polymorphism via signal transduction (Nickerson et al. 2006). However, in the process the role of farnesol as a lipid has been somewhat neglected. Thus, the present review will focus on how the lipid nature of farnesol contributes to its roles in virulence and pathogenicity, mating, and the interactions of *C. albicans* with both host macrophages and competing microbes.

For the fungi, we define quorum sensing as any cell density dependent phenomenon which is mediated by an extracellular molecule which is produced and excreted by the fungus in question. The name is, of course, borrowed from the classic review by Fuqua et al. (1994) which discussed bacterial homoserine lactones. We have extended the terminology slightly by introducing quorum sensing molecules or QSMs

(Nickerson et al. 2006). Critically, our use of the term QSM does not presuppose anything about its mode of action. In particular, it could include a situation where the fungal role is restricted to modifying an exogenously provided molecule, e.g. linoleic acid to 3- hydroxy tetradecaenoic acid (Nigam et al. 2010). The key point is that fungi also have mechanisms to sense their own population densities.

2 It Must Be Something in the Water

This story is both a cautionary tale and a possible area for future study on bacterial fungal interactions. The first published report on farnesol as a quorum sensing molecule (QSM) for *C. albicans* (Hornby et al. 2001) was delayed for roughly 2 years by issues of water quality. In 1996 the Nickerson lab moved from an old building, soon to be torn down (Lyman Hall), to a new state-of-the-art research center named for University of Nebraska graduate and Nobel Prize winner George W. Beadle. At the time we were using an activity directed purification scheme to identify the molecule in spent media which blocked germ tube formation in *C. albicans*. The assay worked perfectly in Lyman Hall but did not work in our new facilities. We eventually found that if we purchased bottled distilled water at the local supermarket and used that water to prepare our growth media then the assay worked perfectly again.

What was different about the distilled waters provided in the two buildings? And did the building distilled/deionized water in Beadle prevent the QSM (farnesol) from being formed or inactivate it after it had been formed? In this regard, we know that farnesol is markedly sensitive to air oxidation and, consequently, we always store our farnesol stock solutions under nitrogen. Shchepin et al. (2003) showed that the 10, 11 epoxide of farnesol has only 3% of farnesol's QSM activity, and at the time of our move the city of Lincoln had just switched from chlorine to ozone treatment as the penultimate step in their water treatment procedures. Could there be any residual ozone carried over? However, this possibility was eliminated by mixing the two spent media, one with QSM activity and one without, and observing that the resulting QSM activity was undiminished.

Thus, we were left with discovering what difference in the two waters regulated QSM/farnesol production by *C. albicans*. This question has not yet been fully resolved. A chemical analysis of the respective waters was indicated. There were two precedents. First, waters can differ dramatically in their mineral contents. Consider, for instance, Table 12.1 of Okafor's text on *Industrial Microbiology* (Okafor 2007) which

compares the mineral content of water in eight cities noted for their breweries. The concentrations of Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^- Cl^-, and HCO_3^- in the respective waters differed by 44-, 62-, 212, 62-, 120-, and 31-fold, respectively. Second, Cu^{2+} and Zn^{2+} cause morphological shifts in most dimorphic fungi, i.e. *Ceratocystis ulmi, Histoplasma capsulatum, Mucor rouxii, Sporothrix schenkii, Ustilago sphaerogena,* and *C. albicans*. The Zn^{2+} induced shifts are all in the same direction (mycelia to yeasts) but the concentrations of Zn^{2+} found to be effective varied. *C. ulmi* was typical in that it required 4–5 mM Zn^{2+} whereas *C. albicans* required only 10–20 mM Zn^{2+} (Yamaguchi 1975; Soll et al. 1981).

Accordingly, we obtained eight types of distilled water locally available in Lincoln and in Central Minnesota. Five of the eight permitted QSM accumulation, three did not. These eight water samples were analyzed by atomic absorption for 11 elements including Se and most of the transition metals. At that time ICP-MS was not available to us for the detailed analysis of the elemental composition of the waters. These analyses produced some surprises, such as two of the waters having 50-fold more Co^{2+} than the others, but there was no discernible correlation between elemental composition and QSM formation! We were stumped. At this point we decided to publish our data showing that farnesol was the QSM for *C. albicans* without having resolved the water issue. Thus, the Hornby et al. (2001) paper specifies that the GPP growth medium was always prepared with Kandiyohi distilled water (Kandiyohi Bottled Water Co., Willmar, Minn.). The Minnesota connection reflected the participation of visiting scientist Prof. Ellen Jensen from the College of St. Benedict's and St. John's University in Minnesota.

Some years later we learned that our standard water purification system (running distilled water through deionizing columns and activated charcoal columns followed by 0.22 mm filtration) might not remove bacterial endotoxins. We had not considered this possibility previously. Accordingly, we purchased an endotoxin detection kit and sampled the distilled/deionized water from three locations in the Beadle Center. They each contained 16–32 endotoxin units per ml. The Kandiyohi distilled water had no endotoxins and Crystal Glen distilled water had 1 endotoxin unit per ml. The other commercial distilled waters were no longer available. Samples of the Beadle distilled water taken prior to filtration contained 10–100 CFU/ml when plated on LB agar. As expected, these bacteria were Gram negative, presumably Pseudomonads. Our current scenario is that: rain water contains ca. 8 mg/L organics, Pseudomonads are notorious for the wide variety of organics they can metabolize (Stanier et al. 1966), and it is expected that large volumes of standing water with long residence times will experience some bacterial presence (McCoy 1980). Lyman Hall did not experience

these problems because the distilled water had been obtained from condensed steam and our current supplier Super-Saver has a very short residence time.

This cautionary tale also suggests a direction of research opportunity. There is increasing evidence, summarized by Langford et al. (2009), that bacteria and fungi wage a sophisticated, molecular level battle with one another. In particular, farnesol treatment kills or inhibits many bacteria, i.e. *Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus mutans*, while at least five bacteria, *A. baumannii, Burkholderia cenocepacia, P. aeruginosa, Salmonella enterica,* and *Xanthomonas campestris,* secrete molecules which inhibit filamentation by *C. albicans* (see Langford et al. 2009). The supposition that *Pseudomonas* endotoxins block farnesol production by *C. albicans* (see above) is consistent with these observations and casts them in a new light. The secreted, filament inhibiting bacterial molecules which have been identified include: dodecanol, 2-dodecenoic acid, 11-methyl-2-dodecenoic acid, and the C12-acyl homoserine lactone. Each of these molecules makes a better structural analog for the C10- and C12-fatty acyl side chains of a *Pseudomonas* endotoxin (Fig. 1) than it would for farnesol.

With regard to location, *C. albicans* is most likely to encounter bacterial endotoxins in the anaerobic mammalian guts. Thus, it is entirely consistent that under anaerobic growth conditions *C. albicans* does not produce detectable farnesol or respond to added farnesol (Dumitru et al. 2004). It would be of interest to compare the effects of bacterial endotoxins on *C. albicans* growing under both aerobic and anaerobic conditions.

3 A Potential Role for Farnesol in *C. albicans* Host Interactions

3.1 Plasticity of Morphology: A Virulence Factor

Biologically speaking, the interactions of *C. albicans* and the human host are much like a never-ending game of cat-and-mouse: the human host laying down defense mechanisms to keep the fungus in check, and the fungus breaking these walls down for pure survival. Understanding this interplay is important as scientists seek to tip the balance away from the pathogenic *C. albicans*. Throughout the body, *C. albicans* has adapted mechanisms to gather, interpret, and respond to signals provided by the host and the diverse terrain the host environment poses, much of which is still unknown. One of the key responses is simply changing its morphology. In vivo, different morphologies of *C. albicans*

have been associated with distinct degrees and locations of infection. From the benign colonization of the skin typically by white or opaque phase cells to benign infections of the oral cavity by white phase cells, chronic vaginal infections by white phase yeast cells, the hyphal and pseudohyphal growth found in the gastrointestinal tract and in many disseminated systemic infections, this morphological plasticity appears to act as a tolerance mechanism to counteract the changes in pH, nutrient availability, microflora composition, and oxygen levels the fungus will encounter on and within its human host (Lachke et al. 2003; Sobel 1997; Sudbery 2011).

C. albicans easily colonizes many locations around an immune-competent host without doing much harm to the individual but, in an immunocompromised host, the capability to switch between morphologies acts as a virulence factor and is central to its pathogenicity. However all forms of *C. albicans* do not convey this level of virulence. Some forms are more susceptible to macrophages and the other defenses of the human immune system than others. This difference in morphological susceptibility could be an important opportunity for the development of antifungal drugs that block systemic Candidiasis.

3.2 Farnesol and Virulence

The secretion of farnesol also plays a role in host–pathogen interactions in vivo. Hornby et al. (2001) discovered that in vitro farnesol acted to block the yeast to mycelia transition. Thus, in vitro farnesol acted as a QSM. But what would its role be in vivo? At that time we presented two possibilities. Firstly, if farnesol acted in vivo as it did in vitro, then farnesol and its analogs should prove to be effective antifungals because the yeast to mycelia transition is essential for virulence. It was on this premise that 50 analogs of farnesol were synthesized and tested for their in vitro QSM potency (Shchepin et al. 2005). However, we also suggested that farnesol production by *C. albicans* might instead function as a virulence factor (Hornby et al. 2001) and for the mouse tail vein injection model that proved to be the case (Navarathna et al. 2007). *C. albicans* mutants which produced 85% less farnesol were five times less pathogenic to mice than their parent cells. Also, when farnesol was administered orally to the mice prior to infection, their mortality increased as did the colonization of kidneys (Navarathna et al. 2007).

These observations pose the dilemma of finding a mechanism whereby a molecule which blocks the yeast to mycelia transition can also act as a virulence factor. In this regard, we note that there is as yet no evidence for farnesol blocking the yeast to mycelia transition in vivo

while there is evidence that farnesol behaves differently for surface infections, where it is protective (Hisajima et al. 2008), versus systemic infections where it is a virulence factor (Navarathna et al. 2007). A partial resolution of this dilemma comes from the realization that in vitro, in glass or plastic, excreted farnesol can accumulate whereas in vivo it would be soaked up by the mammalian cell membranes. Thus, different concentrations of farnesol should be present in vitro and in vivo.

3.3 A C. albicans Macrophage Chemoattractant: White Versus Opaque

Another partial resolution of this *in vitro* vs. *in vivo* dilemma concerns how farnesol affects the host innate immune system. The first level of defense the host has against candidal infection is through the innate immune system. Distinct morphologies elicit different responses by the host immune system. Both white and opaque cells are known to attract leukocytes to the site of infection, but only white cells produce and secrete a small molecular weight chemoattractant that draws the leukocyte directly towards the white cell (Geiger et al. 2004). Lohse and Johnson (2008) took this knowledge a step further by showing that not only were leukocytes more attracted to white cells than opaque cells, but because of the presence of a chemoattractant produced by the white cells, mouse macrophages engulfed white *C. albicans* cells much more efficiently than they did opaque cells (Lohse and Johnson 2008). Not only were the white cells engulfed at a higher rate but they were also less susceptible to killing by human macrophages and neutrophils than were opaque cells, possibly due to their increased capabilities of escape once phagocytosed or possibly due to another effect of the chemoattractant on the macrophages (Kolotila and Diamond 1990). The chemical identity of this chemoattractant is currently unknown, but the reason behind its continued secretion by the white form is intriguing. One likely candidate is farnesol (Langford et al. 2009). Macrophages are capable of detecting and responding to exogenous farnesol, specifically by stimulating secretion of proinflammatory and regulatory cytokines (IL-6, IL-1b, IL-10, and TNF-α; Ghosh et al. 2010). The production of these warning signals by macrophages is an important indicator of how the body ultimately hopes to clear the infection. Because of the cytotoxic effects farnesol has on macrophages (ROS and DNA fragmentation), farnesol suppresses the anti-Candida activity of macrophages (Abe et al. 2009), thus making it all the more difficult to eliminate the fungus early in infection.

This chain of events from attraction to engulfment to eventual killing of the macrophages is mediated at two points by different *C. albicans* morphologies. It is known that wild type, white cells of *C. albicans*

can escape from mouse macrophages by switching to the hyphal morphology 6–8 h post-engulfment, and effectively puncturing the macrophage from within (Ghosh et al. 2009). Those strains with delayed or dysfunctional hyphal formation (through disruption of the arginine biosynthetic pathway for instance) were unable to survive within and escape from the macrophage (Ghosh et al. 2009). It remains a perplexing mystery why certain morphologies such as the opaque cells are better able to elude host immune defenses, or retaliate such as the hyphae, while other morphologies such as the white yeast cells seek to be found through the production of a potent chemoattractant. This aspect of farnesol production by *C. albicans* is in part counterintuitive of the way we think a fungus should behave but it does have precedent in the form of some pathogenic intracellular bacteria.

3.4 Bacterial Analogs for Host Evasion

Direct targeting of tissue phagocytes to the site of infection by pathogenic microorganisms, in the hopes of being phagocytosed, is not a novel concept in the realm of microbial infections. A comprehensive review of bacterial evasion strategies can be found in Flannagan et al. (2009). For some "professional" intracellular bacteria, such as *Mycobacterium tuberculosis, Listeria monocytogenes,* and *Legionella pneumophila,* successful establishment of infection and dissemination throughout the host depends entirely on exploiting the natural responses of phagocytes. Following phagocytosis and entry into the phagosome, these bacteria have developed mechanisms to prevent further phagocyte killing and digestion, allowing for long term intraphagosomal survival within host cells, either through interfering with phagolysosome maturation, and the secretion of ROS and antibacterial proteases, or through counteracting the host cells' expression of MHC and loading of antigenic bacterial peptides, effectively eluding further host immune system detection.

The most researched of these pathogenic microbes that use macrophages to escape immune detection is *L. monocytogenes*. This model system could be used as a possible bacterial analog to understand what strategy *C. albicans* may utilize during infection. Phagocytosis of *L. monocytogenes* is mediated by a macrophage scavenger receptor that binds directly to the lipoteichoic acid on this Gram-positive bacterium (Dunne et al. 1994). Once within the phagosome of the macrophage, it uses an array of cholesterol-dependent cytolysins to prevent the further maturation of the phagosome by inhibiting its fusion with the lysosome. By sequestering inside the membrane vacuole, it is able to acquire the nutrients it requires to replicate directly from the host without host detection, prior to escape from the macrophage. Through this comparison,

much can be learned about possible intracellular signaling interactions between the phagocyte and the intracellular fungi during the phagocytosis process. We note that during the 6 h between their engulfment and escape, *C. albicans* can spread through the body as the macrophages migrate.

4 Evolutionary Adaptations to Farnesol as a Signaling and Antagonistic Molecule

4.1 C. albicans Resistance to the Antifungal Effects of Farnesol

Regardless of their capacity to produce farnesol, many fungi respond to farnesol in that they are inhibited or killed by it, although the exact molecular mechanism of farnesol induced cell death is still under investigation. As examples, farnesol induced apoptosis in Aspergillus nidulans (Semighini et al. 2006), inhibited Trichophyton rubrum in coculture with *C. albicans* (Jillson and Nickerson 1948), and antagonized many other fungi including *S. cerevisiae* (Machida et al. 1998). The exceptional resistance to farnesol shown by *C. albicans* is an interesting issue since it is the only known fungus which tolerates farnesol up to 300 mM while using it as a quorum sensing molecule. What makes *C. albicans* different from the rest of the fungi? We assume that *C. albicans* has a protective mechanism to safeguard itself from excessive farnesol, similar to the self defense mechanisms used by antibiotic-producing microorganisms to prevent them committing suicide by their own products. What's more, this resistance of *C. albicans* to farnesol is not just a constant suit of armor but a subtly variable protection. Anaerobically growing cells are resistant to farnesol right up to its solubility limit of 1–1.2 mM (Dumitru et al. 2004) while resistance is lost entirely in cells which have switched from the white phase to the opaque phase (Dumitru et al. 2007).

In terms of where to look for this variable resistance, the mitochondria are a likely target. As a result of aerobic respiration, all aerobic organisms produce Reactive Oxygen Species (ROS) which leads to oxidative destruction of cells. Consequently, all of these aerobes including yeasts developed efficient mechanisms to get rid of these unwelcome companions. In a study done to reveal the growth inhibitory effect of farnesol in *S. cerevisiae* (Machida et al. 1998), the level of farnesol induced ROS was found to increase in a dose dependent manner. Further, the inhibition of growth by farnesol could be prevented by the presence of antioxidants in the medium. Thus ROS generation leading to intracellular oxidative stress suggested involvement of the mitochondrial

electron transport chain as the target of farnesol. This view on the primary means of farnesol mediated death in *S. cerevisiae* by generation of reactive oxygen species was further confirmed by Fairn et al. through a study on the genomic effects of the chemical compounds, farnesol and geraniol (chemogenomic profiling; Fairn et al. 2007).

4.2 Ubiquinone and Its Variable Isoprenylation

There is a growing interest in mitochondrial respiratory mechanisms, especially the participating components in its electron transport chain. Ubiquinone, also known as coenzyme Q (abbreviated as UQ) is an isoprenoid quinone which 'ubiquitously' exists in all living organisms, functioning as membrane bound, mobile electron carrier in the electron transport chain (Shinkarev 2006). The structure of UQ (Fig. 2) has a redox active benzoquinone ring and an all-trans-polyprenyl side chain of variable length. During biosynthesis, a host specific enzyme, polyprenyl diphosphate synthase successively adds isoprenoid chains to the benzoquinone ring, which ultimately determines the type of UQ present in that organism. UQ with 4–10 isoprenes are common in microbiology. The longer isoprenoid tails would have a more highly folded structure and thus be more firmly embedded in the mitochondrial membrane. In contrast, UQs with shorter isoprenoid length would have some difficulty in grabbing electrons as they are more exposed on the surface (Olgun et al. 2003). Thus, we suggest that the shorter UQs have a critical effect on cells because they can be either dislodged or inactivated by farnesol.

For the organisms with which we are concerned, *S. cerevisiae* has UQ 6 and *C. albicans* has UQ 9 while almost all the rest of the Candida species produce UQ 7 (Suzuki and Nakase 2002). This variability in ubiquinone content depending on the length of isoprenoid side chain is thought to be taxonomically useful although there is as yet no specifically identified biological significance for these different chain lengths (Okada et al. 1998; Olgun et al. 2003). The farnesol sensitive *S. cerevisiae* has UQ 6 but research done with mutated strains by Okada et al. (1998) showed that the length of the UQ isoprenoid side chain did not act as a critical factor; no phenotypic variations were observed. However, the length of the isoprenoid tail may still have an unidentified function.

We hypothesize that *C. albicans*, as a part of evolving to being a farnesol excretor, shifted from using UQ 6 to using UQ 9 while all the other non-pathogenic Candidas stopped at UQ 7. If farnesol mediated cell death targets the ubiquinones, it is not surprising to observe the resistance of anaerobic cells of *C. albicans* to farnesol (Dumitru et al.

2004) since they lack mitochondrial respiration. Thus, a key question is whether there is a significant effect of UQ side chain length on farnesol sensitivity during aerobic respiration. This question could be answered by examining the farnesol sensitivity of *S. cerevisiae* which make UQ 9 instead of UQ 6 or *C. albicans* which made UQ 6/7 instead of UQ 9.

4.3 Possible Mechanisms for C. albicans White Cell Resistance to Farnesol

C. albicans can grow in both aerobic and anaerobic environments and it has two types of cells: white cells and opaque cells. These cell types respond to farnesol in different ways. Neither opaque cells nor anaerobic cells make farnesol (Dumitru et al. 2004, 2007). During anaerobic growth, *C. albicans* doesn't produce farnesol or respond to farnesol, even at concentration as high as 1.2 mM (Dumitru et al. 2004). Similarly, in the presence of farnesol, white cells are prevented from making germ tubes but they can tolerate farnesol at concentrations up to 300 mM, while opaque cells are lysed by farnesol at 50 mM (Dumitru et al. 2007). It is desirable to understand why the white cells of *C. albicans* are highly resistant to farnesol whereas opaque cells are very sensitive to it.

Different phases of white cell growth also differ in their tolerance to farnesol. Farnesol shows different activities towards *C. albicans* depending on the growth conditions and inoculum characteristics. In rich growth medium (YPD), *C. albicans* is very resistant to farnesol (Langford et al. 2010) while in defined media such as glucose-phosphate-proline (GPP) log-phase cells were significantly more sensitive to farnesol than were stationary phase cells. Consequently, when using an inoculum of stationary-phase cells, the growth curves are similar to those in YPD media (Langford et al. 2010) while using an inoculum of log phase cells resulted in significant delays due to farnesol induced cell lysis (Langford et al. 2010). Thus, the starting growth phase, media, and cell density of the inoculum are critical for the effect of farnesol on the cells. Finally, farnesol tolerance is an energy dependent process. Cells suspended in a farnesol buffer without an energy source lysed whereas those with an energy source did not (Langford et al. 2010). Together, the influence of cell type, growth conditions and inoculum characteristics suggest resistance to farnesol is an active and regulated process.

C. albicans may detoxify farnesol enzymatically. Using a spent medium assay for quorum sensing activity, i.e. the ability to block hypha formation, Hornby et al. (2001) found that the levels of farnesol present in the spent media roughly paralleled cell mass for 20 h after inoculation but then decreased rapidly after that. Farnesol contains three C□C

double bonds and exists in four isomers of which only (E, E) farnesol has QSM activity. Farnesol is a very unstable molecule and air oxidation results in a hydroxyl or epoxide of farnesol which causes a dramatic decrease in QSM activity (Shchepin et al. 2003). Thus, the decrease in QSM activity observed by Hornby et al. (2001) could have been either enzymatic or spontaneous. Additionally, the morphological response to farnesol in *C. albicans* appears to be very sensitive to other minor changes in the structure of farnesol. For instance, farnesol with three isoprene units has three methyl branches. If the 2-methyl branch is either removed or enlarged to a 2-ethyl branch then the QSM activity of the resulting farnesol analog is 20-fold lower (Shchepin et al. 2005). Thus, the decreased activity of farnesol with time observed by Hornby et al. (2001) could result from the modification of farnesol to a new compound with a lower QSM activity and/or lower toxicity for the cells.

4.4 Membrane Differences in White and Opaque Cells

C. albicans strain WO-1 is capable of switching at high frequencies between two phenotypes, white and opaque cells. Among the many differences between white and opaque cells, it has been shown that these cells have variations in their lipid and sterol contents (Ghannoum et al. 1990). White cells have 4-fold and 7.7-fold higher sterol contents than do opaque cells in mid-exponential and stationary phase, respectively (Ghannoum et al. 1990). Additionally, white cells contain more free sterols and less of the steryl glycoside and steryl ester sterol derivatives (Ghannoum et al. 1990). Finally, the sterols present in mid-exponential and stationary phase cultures of white cells were qualitatively different. The sterols present in midexponential phase cultures of white cells were primarily lanosterol (48 wt%) and 24-methylene dihydrolanosterol (26.2 wt%), while for mid-exponential phase opaque cells they were ergosterol (49.3 wt%), lanosterol (33.2 wt%) and squalene (17.5 wt%). During stationary phase, ergosterol was the major sterol in both white and opaque cells (Ghannoum et al. 1990). These observations suggest the twin hypotheses that a high sterol content in the membrane protects cells from farnesol and that the percentages of total sterols and of ergosterol in particular will differ for mid-exponential phase cells grown with and without farnesol. It also leads to a focus on the regulation of HMG CoA reductase (HMGR), the rate limiting step in sterol biosynthesis.

Farnesol is an isoprenoid produced by dephosphorylation of farnesyl pyrophosphate, an intermediate in the isoprenoid pathway (Hornby et al. 2003). The isoprenoid pathway is responsible for synthesis of sterols from acetyl-CoA (Fig. 3). Acetyl-CoA is converted to mevalonate by

reduction with NADPH by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMGR). Mevalonate leads to the synthesis of farnesyl pyrophosphate, a branch point in the sterol biosynthetic pathway. Farnesol pyrophosphate serves as a precursor for sterol biosynthesis and nonsterol isoprenoids. Sterols are an important structural component of cellular membranes. Nonsterol isoprenoids include geranylgeranyl pyrophosphate and farnesol. Farnesyl pyrophosphate and geranylgeranyl pyrophosphate are involved in the prenylation of proteins. Isoprenoids are also precursors for the prenyl side chains of ubiquinone.

HMGR is often the rate-limiting enzyme for the sterol biosynthetic pathway. Human HMGR is regulated by transcription, protein degradation and phosphorylation (Table 1). Phosphorylation decreases the enzyme efficiency. The rapid degradation of mammalian HMGR is triggered by cellular sterols and farnesol, farnesyl pyrophosphate and geranylgeranyl pyrophosphate (reviewed in Joo and Jetten 2010; Burg and Espenshade 2011). These signals function both in vivo and in vitro as triggers for rapid degradation. In fungi, regulation of HMGR has been studied in the yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. *S. cerevisiae* has two HMGR isozymes, Hmg1 and Hmg2. Both isozymes are subject to feedback regulation by nonsterol products of the isoprenoid pathway (Table 1). Hmg1 is the primary source of HMGR in aerobically grown cells and in these conditions it is mainly regulated at the level of translation (Dimster-Denk et al. 1994). Hmg2, like its mammalian counterpart, is primarily regulated by protein turnover. In vivo, geranylgeranyl pyrophosphate is the signal regulating Hmg2 degradation (Theesfeld et al. 2011). However, in vitro farnesol also causes Hmg2p to undergo a change to a less folded structure (Shearer and Hampton 2005). This in vitro conformational change is the same as that observed in vivo which triggers the degradation of Hmg2p (reviewed in Hampton and Garza 2009). Different mechanisms are used to regulate HMGR by different fungi. For example, the fission yeast *Schizosaccharomyces pombe* has a single HMGR enzyme which is regulated by phosphorylation (Table 1; Burg et al. 2008).

Like S. pombe, *C. albicans* has a single gene for the HMGR enzyme. Currently regulation of the *C. albicans* HMGR has not been investigated, however an understanding of this regulation is important because the nature of the regulation could have profound effects on sterol and/or farnesol synthesis. *C. albicans* is unique amongst the fungi examined because some cell types make very high levels of farnesol while others do not make farnesol at all. This suggests the *C. albicans* HMGR is most likely resistant to feedback regulation by farnesol. Consistent with that prediction, farnesol production is elevated 8- to 40-fold in the presence of sub-lethal levels of drugs that block sterol synthesis

in fungi (Hornby et al. 2003; Hornby and Nickerson 2004). Treatment with zaragozic acid B, fluconazole, clotrimazole, ketoconazole, or miconazole all caused a dose dependent increase in farnesol levels. These drugs inhibit steps downstream of farnesyl pyrophosphate in the synthesis of ergosterol, the fungal sterol and after the branch point for non-sterol isoprenoids (Fig. 3). At the time (Nickerson et al. 2006), these elevated levels of farnesol were attributed to increased pool sizes for farnesyl pyrophosphate. Thus these results imply that HMGR is not feedback regulated by non-sterol isoprenoids.

5 Summary

Farnesol and bacterial endotoxins are lipids and in this review we analyzed data relevant to farnesol's function and synthesis from the perspective of farnesol and the endotoxins acting as membrane active compounds. This analysis implicated the possible roles of: (1) endotoxins in the regulation of farnesol production by *C. albicans*; (2) farnesol in the interactions between *C. albicans* and the host during disseminated infections; and (3) ubiquinones in the mechanisms for unusually high resistance to farnesol by some *C. albicans* cell types. Finally we discuss the implications that the use of farnesol as both a signaling molecule and to antagonize competing microbial species has for the regulation of HMG-CoA reductase, the enzyme that is the usual rate limiting step in sterol/lipid synthesis. In the future, to fully understand farnesol's function as a membrane active compound, three research directions should be pursued. (1) The role of lipid signaling molecules in interspecies communication between *C. albicans* and the typical microbial neighbors that it encounters in its normal habitats. (2) The role of farnesol in the interactions between *C. albicans* and the human innate immune system, and (3) The role of isoprenoids in regulation of HMG-CoA reductase of *C. albicans*. We expect that this new understanding will uncover the basic biological principles that underlie interspecies signaling by these lipid molecules.

Table 1 Regulation of HMG-CoA reductase in different organisms

Organism	Enzyme	Type of regulation	Signal	Reference
Humans	HMGR	Transcription Phosphorylation Degradation	Sterols AMP/ATP ratio Farnesol, farnesyl pyrophosphate or geranylgeranyl pyrophosphate	Reviewed in Joo and Jetten (2010) and Burg and Espenshade (2011)
Saccharomyces cerevisiae	Hmg1	Translation	Mevalonate derived molecule produced before the synthesis of squalene	Dimster-Denk et al. (1994)
	Hmg2	Degradation	Geranylgeranyl pyrophosphate (in vivo and in vitro) Also farnesol (in vitro)	Theesfeld et al. (2011), Garza et al. (2009), and Shearer and Hampton (2005)
Schizosaccharomyces pombe	Hmg1	Inactivated by Phosphorylation	Nutrient stress Osmotic stress	Burg et al. (2008)

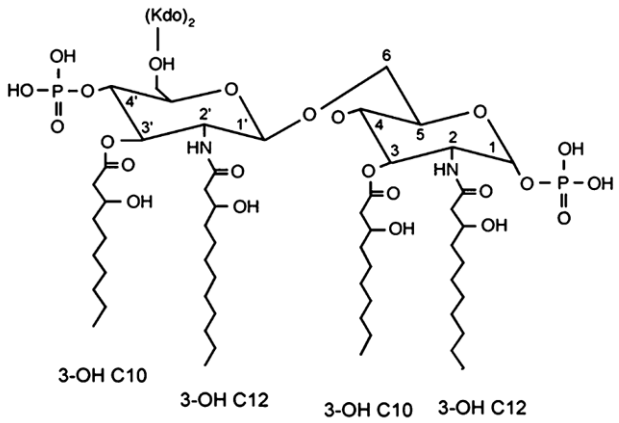

Fig. 1 Pseudomonas aeruginosa endotoxin structure (p245 of Wang and Quinn 2010)

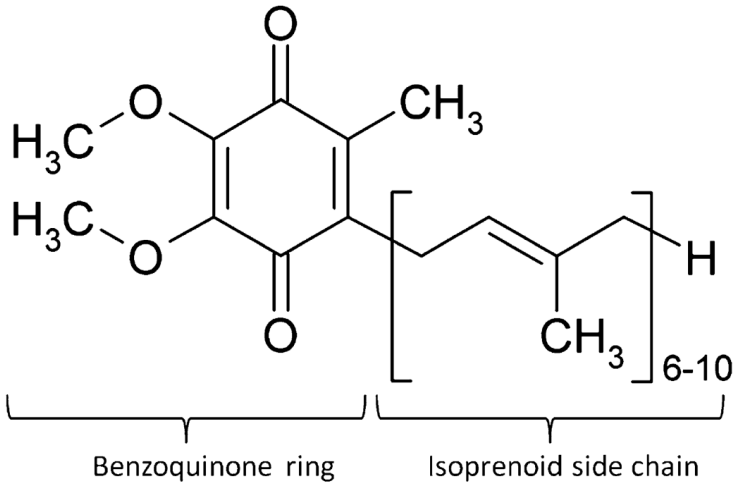

Fig. 2 The structure of ubiquinone. The number of isoprenoid side chains varies among different Ubiquinones.

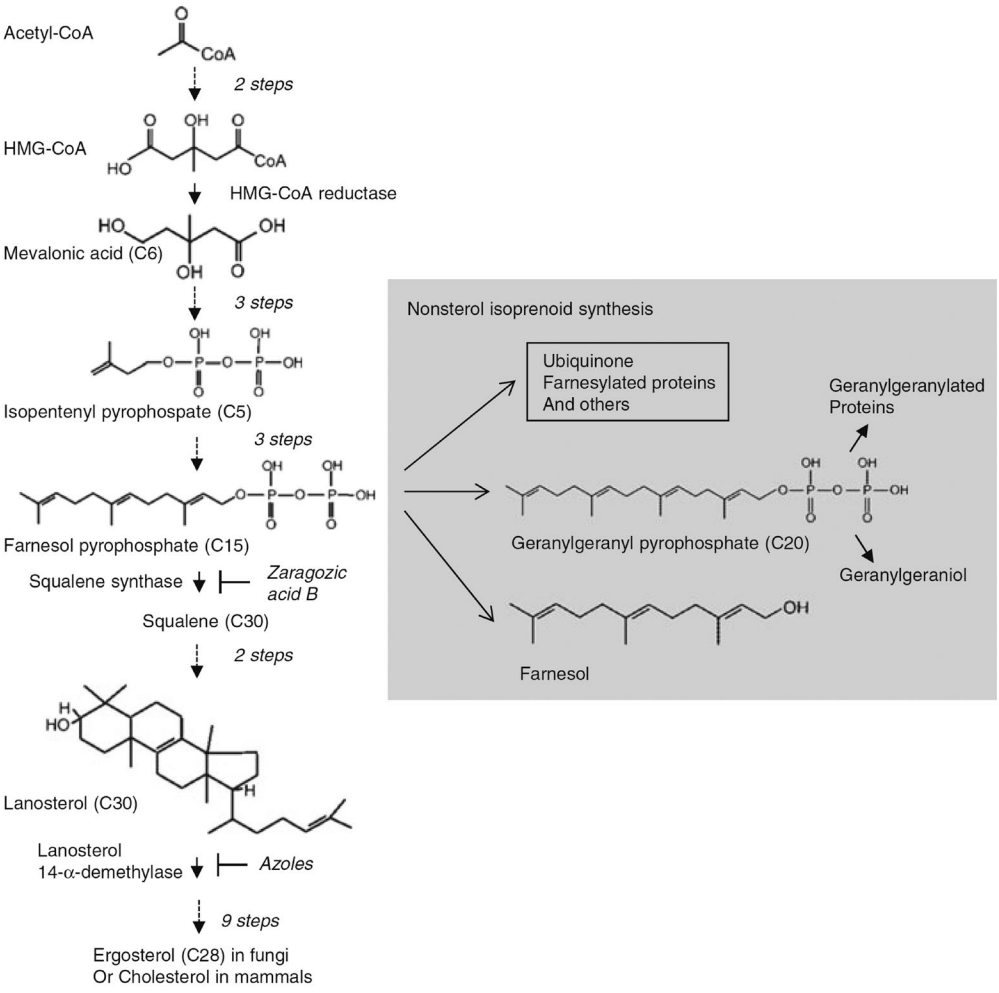

Fig. 3 The biosynthetic pathway for the synthesis of sterols and nonsterol isoprenoids. The molecules and enzymes discussed in the text are indicated. Dashed arrows represent multienzyme steps, with the number of reactions indicated in parentheses to the right of these arrows. The enzymes inhibited by zaragozic acid B, and the azoles (fluconazole, clotrimazole, ketoconazole or miconazole) are shown.

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