

MINIREVIEWS

Mitochondria and Fungal Pathogenesis: Drug Tolerance, Virulence, and Potential for Antifungal Therapy[∇]

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Recently, mitochondria have been identified as important contributors to the virulence and drug tolerance of human fungal pathogens. In different scenarios, either hypo- or hypervirulence can result from changes in mitochondrial function. Similarly, specific mitochondrial mutations lead to either sensitivity or resistance to antifungal drugs. Here, we provide a synthesis of this emerging field, proposing that mitochondrial function in membrane lipid homeostasis is the common denominator underlying the observed effects of mitochondria in drug tolerance (both sensitivity and resistance). We discuss how the contrasting effects of mitochondrial dysfunction on fungal drug tolerance and virulence could be explained and the potential for targeting mitochondrial factors for future antifungal drug development.

Although it has been studied quite extensively in the model yeast *Saccharomyces cerevisiae*, mitochondrial function has remained understudied in human fungal pathogens. A possible reason is that several pathogenic fungi, such as *Candida albicans* and *Cryptococcus neoformans*, are so-called “petite-negative” yeasts, i.e., they cannot survive mitochondrial genome loss, which is a classic and extensively used tool for studying mitochondrial function in *S. cerevisiae*. Recent work from several laboratories has revealed that mitochondria have a fundamental role as a control point in the cellular networks impacted by antifungal drugs, as well as a prominent role in fungal virulence. These studies suggest that the functions of mitochondria in these pathways are complex. With antifungal drugs that target cell membranes, such as the azoles and the polyenes, both resistance and sensitivity of mitochondrial mutants have been reported (9, 10, 18, 22, 45, 75, 85, 86). In contrast, only sensitivity has been observed with agents that target the cell wall, which includes the echinocandin class of antifungal drugs (3, 15, 17, 22, 38, 90). A similarly complex picture is observed in regard to virulence of mitochondrial mutants, with both hypo- and hypervirulence of *Candida* spp. mitochondrial mutants observed in animal infection models (1, 4, 10, 19, 25, 64). These studies pose several questions. What is the biochemical basis for the impact of mitochondria on drug tolerance? When (and why) does a change to mitochondrial function lead to hypo- versus hypervirulence? Would mitochondrial factors be useful targets for antifungal drug development? In this review, we will consider the molecular and cellular mechanisms behind the observed drug sensitivity and virulence phenotypes of mitochondrial mutants, mostly discussing *Candida* spp., for which the most is known, and mentioning the other pathogens where appropriate. We will also

draw on the model fungus *S. cerevisiae*, because mitochondrial functions have been extensively studied in this yeast and there is reasonable evidence that the mechanisms of mitochondrial involvement in drug resistance are conserved with pathogenic fungi. Finally, we will discuss the potential for targeting mitochondrial factors for the development of future antifungal therapies.

MITOCHONDRIA AND PLASMA MEMBRANE-TARGETING DRUGS

Mitochondrial roles in azole tolerance. The azole drugs target fungal membrane biogenesis by inhibiting the ergosterol biosynthesis enzyme Erg11, which is a cytochrome P450 enzyme lanosterol 14- α -demethylase. Studies in *Candida glabrata*, *C. albicans*, and *S. cerevisiae* have shown that mitochondrial dysfunction can lead to both resistance and susceptibility to the azoles. We will first consider activation of the drug resistance pathway upon mitochondrial dysfunction and then the examples of azole sensitivity of mitochondrial mutants.

The molecular mechanism of drug resistance induced by mitochondrial dysfunction has been studied in *C. glabrata* and *S. cerevisiae*. Compromised mitochondrial function leads to the activation of homologous transcription factors Pdr3 of *S. cerevisiae* (ScPdr3) and Pdr1 of *C. glabrata* (CgPdr1), which then results in upregulation of their target genes encoding efflux pumps, such as ScPdr5 or CgCdr1 and CgCdr2 (9, 33, 35, 70, 75, 84, 85, 89; reviewed in references 60 and 79) (Fig. 1). Both *C. glabrata* and *S. cerevisiae* can live without mitochondrial DNA (mtDNA), and the drug-resistant mutants most commonly lost their mitochondrial genome (33, 35, 45, 75, 84, 89). In fact, mtDNA loss appears to be a key activating mutation for the drug resistance pathway. Loss of mtDNA and associated drug resistance is relatively common for *in vitro* cultures of both *S. cerevisiae* (about 2% of cells) (89) and *C. glabrata* (frequencies of 2×10^{-4} to 4×10^{-4}) (75). However, very few reports of clinical azole resistance in *C. glabrata* are

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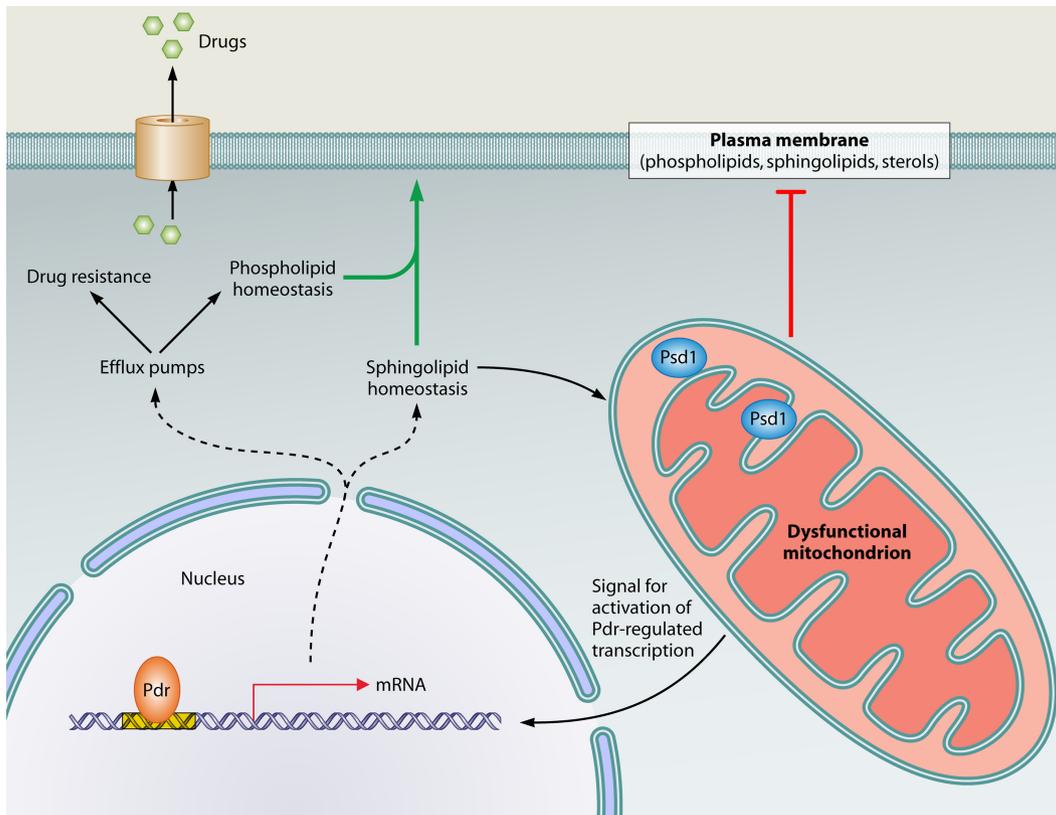


FIG. 1. Pathways linking mitochondrial dysfunction to the activation of multidrug resistance genes. In dysfunctional mitochondria, changes in the membrane association characteristics of the mitochondrial inner membrane protein Psd1 create a signal for activation of the transcription factors *ScPdr3* and *CgPdr1* (indicated by Pdr in the figure). This results in upregulation of genes encoding efflux pumps (which mediate drug resistance and have additional roles in phospholipid homeostasis), as well as genes required for sphingolipid metabolism. Dysfunctional mitochondria have an effect on membrane lipid homeostasis (depicted by the red “T”). This could be compensated by activation of phospholipid and sphingolipid homeostasis genes via the Pdr pathway (green arrow). Additionally, sphingolipid biosynthesis intermediates can feed into the synthesis of phosphatidylethanolamine by nonmitochondrial pathways, thereby compensating for aberrant lipid composition of mitochondrial membranes in cells with dysfunctional mitochondria.

linked to mitochondrial dysfunction (8, 24), and thus, the relevance of this mechanism in the clinic remains to be studied. Loss of the mitochondrial genome as the key activator of the drug resistance pathway also has to be considered in the context of other major pathogens, such as *Cryptococcus* spp. or *C. albicans*, that are unable to survive without mtDNA (16, 83). Consequently, the specific mechanisms behind the role of mitochondria in hyperresistance to azoles might be different in these various pathogens. One study reports that there is a relationship between upregulation of the efflux pump gene *MDR1*, with its resulting higher azole resistance in *C. albicans*, and uncoupled mitochondrial oxidative phosphorylation (18). However, the mutant was less susceptible to some azole drugs (fluconazole and voriconazole) but not others (itraconazole and ketoconazole), and the MIC was still in the susceptible range according to CLSI recommendations (18). A study in *C. neoformans* also reports higher azole resistance related to respiratory deficiency, which was reversible and, thus, not very likely to be due to mtDNA loss (68). How mitochondrial dysfunction leads to azole resistance in the petite-negative yeasts *C. albicans* and *C. neoformans* requires further investigation.

The relationship between mitochondrial dysfunction and drug resistance is strong, but the mechanistic details, par-

ticularly the trigger for activation of drug resistance by mitochondrial dysfunction and why these two pathways are linked, is far from understood. Screening of *S. cerevisiae* mutants indicates that respiratory deficiency *per se* is not enough to cause drug resistance (89), and thus, another mechanism of mitochondrial dysfunction related to mtDNA loss must be involved. Other mitochondrial mutations can cause drug resistance in *C. glabrata* and *S. cerevisiae*; for example, deletion of the mitochondrial inner membrane translocase gene *ScOXAI* (35) that is required for the assembly of mitochondrially encoded subunits of the respiratory complexes into the inner membrane (7, 26, 37, 43). *OXAI* is not essential for mtDNA stability, but reports differ as to whether and to what extent the *oxa1Δ* mutant is affected in mitochondrial genome maintenance, perhaps depending on the yeast strain background (20, 58, 60, 63). In any case, activation of the drug resistance pathway by deletion of *OXAI* appears to be distinct from that which occurs in cells that have lost mtDNA (35, 89). Another example is the inactivation of the phosphatidylglycerol synthase gene *CgPGS1* in *C. glabrata*, which is necessary for synthesis of the phospholipids phosphatidylglycerol and cardiolipin in mitochondria (3). *pgs1Δ* mutants of *S. cerevisiae* tend to lose mtDNA (90), but activation of the drug resistance genes of

the *C. glabrata* *pgs1* Δ mutant was complemented by reintroduction of a wild-type copy of *PGS1* (3), and it is therefore unlikely to have been due to mtDNA loss (which is expected to be irreversible).

The common denominator in mitochondrial mutations that cause drug resistance is a change to the protein and/or lipid structure of mitochondrial membranes, as demonstrated by the following evidence. Several subunits of the respiratory complexes located in the inner mitochondrial membrane are encoded on the mitochondrial genome. After mtDNA loss, the complexes are not correctly assembled. Moreover, the levels of cardiolipin and phosphatidylglycerol, which are synthesized in mitochondria and are specific to mitochondrial membranes, are altered in the absence of mtDNA, probably because of the interdependency of cardiolipin synthesis and respiration (30). In the *S. cerevisiae* *oxa1* Δ mutants, respiratory complexes in the inner membrane are inappropriately assembled (7, 43) and the mutants display lower levels of another important phospholipid in mitochondrial membranes, phosphatidylethanolamine (63). In *pgs1* Δ mutants of *C. glabrata*, both phosphatidylglycerol and cardiolipin are absent from mitochondrial membranes (3). Taken together, these data suggest that changes to the structure of mitochondrial membranes could be the trigger for activation of the drug resistance pathway. Recent studies from the Moye-Rowley laboratory suggested that changed mitochondrial membranes in *S. cerevisiae* cells lacking mtDNA lead to diminished membrane association of Psd1, the phosphatidylserine decarboxylase in the mitochondrial inner membrane (33). This in turn leads to activation of the multidrug resistance pathway (33) (Fig. 1). A similar scenario might be operating in *C. glabrata* and *C. albicans* (33, 70). The exact mechanism is not clear, but because the catalytic activity of ScPsd1 was dispensable for activation of drug resistance, it was proposed that the release of a fraction of the protein from the inner membrane into the intermembrane space could permit interactions with another molecule, which then constitutes the signal for activation of the Pdr factors (33, 70) (Fig. 1). Interestingly, in *oxa1* Δ mutants, ScPsd1 is not properly inserted into the inner membrane (63) and deletion of the mitochondrial protease ScYme1 suppresses both the Psd1 maturation defects and multidrug resistance of *oxa1* Δ mutants (63, 89), thus supporting a role for changes in ScPsd1 membrane localization in triggering multidrug resistance.

Why do fungi activate the Pdr pathway upon mitochondrial dysfunction? The available evidence points to a link between mitochondrial control over membrane structure and lipid homeostasis and the role of the Pdr pathway in membrane biogenesis (Fig. 1). As has recently been reviewed by Shahi and Moye-Rowley (79), the Pdr transcription factors control the expression of genes required for the homeostasis of two key lipid components of membranes, sphingolipids and phospholipids, and consistently, activation of the Pdr pathway by mitochondrial dysfunction in *S. cerevisiae* leads to increased expression of genes encoding enzymes of sphingolipid biosynthesis (Fig. 1). Mitochondrial function is important for membrane lipid structure, and thus, activation of lipid homeostasis by the multidrug resistance pathway could serve to compensate for changes to membrane structure/composition upon mitochondrial dysfunction (Fig. 1). This model is supported by several observations. Mitochondria are the predominant cellu-

lar site for synthesis of the ubiquitous membrane phospholipid phosphatidylethanolamine in a reaction catalyzed by Psd1 (6, 11, 78, 88) (Fig. 2). Phosphatidylethanolamine also serves as the precursor for synthesis of another major membrane phospholipid, phosphatidylcholine, in the endoplasmic reticulum (ER) (6, 88) (Fig. 2). Sphingolipid homeostasis is disturbed in cells that have lost the mitochondrial genome (34). Additionally, two cytochrome P450 enzymes in the biosynthetic pathway of the membrane lipid ergosterol require heme as a cofactor (21). Heme is synthesized in mitochondria, and it has been shown that mitochondrial mutants have an altered cellular sterol composition (9, 28, 29).

It is also tempting to speculate that activation of the multidrug resistance pathway might result from an attempt to compensate not only for problems in general membrane lipid homeostasis but also, more specifically, for defective mitochondrial membrane structure in mitochondrial mutants. Cardiolipin, which is dramatically reduced in mitochondrial membranes of mutants which activate the drug resistance pathway, such as cells lacking mtDNA and *pgs1* Δ cells, has critical roles in mitochondrial biogenesis. For example, cardiolipin is required for the activity of the respiratory chain complexes in the inner membrane and protein import into mitochondria (44). It has to be stressed here that mitochondria are essential organelles, and even in cells with no mtDNA, mitochondrial function must be maintained. Cardiolipin defects in mitochondrial membranes can be compensated for by phosphatidylethanolamine, with the two phospholipids having overlapping roles (31). Intermediates in sphingolipid biosynthesis can be used as precursors for phosphatidylethanolamine biosynthesis by nonmitochondrial pathways (78), and this could provide a further link between regulation of sphingolipid biosynthesis by the drug resistance pathway and its activation by mitochondrial dysfunction (Fig. 1).

In contrast to the above-described azole resistance, some mitochondrial mutations in *C. glabrata* and *S. cerevisiae* lead to increased susceptibility to azoles (22, 38, 86). This further corroborates the point that only very specific mitochondrial mutations and defined changes to mitochondrial physiology lead to activation of the multidrug resistance pathway. In other words, a decrease in mitochondrial function is not sufficient to trigger the changes leading to resistance to antifungal compounds, but rather, specific molecular mechanisms are involved. The reason for decreased azole resistance of some mitochondrial mutants is not fully understood, but it could be related to changes to the sterol content of membranes in the absence of proper mitochondrial function (9, 28, 29). Other mechanisms are also possible, such as fitness defects that are typical for mitochondrial mutants. Determining the molecular mechanism behind increased susceptibility to azoles of cells with dysfunctional mitochondria is key to understanding why some mitochondrial mutants are resistant to azoles while others are sensitive. Data from *C. glabrata* suggest that the answer to this question will not come down to whether or not the specific mitochondrial mutations activate the Pdr-dependent multidrug resistance pathway, as the azole hypersensitive mitochondrial mutant overexpressed the CgPdr1 target, the *CDR1* efflux pump (86).

Mitochondria and polyene drugs. Polyene drugs (e.g., amphotericin B and nystatin) disrupt membrane integrity by binding to sterols and forming pores. Mitochondrial mutants of *C.*

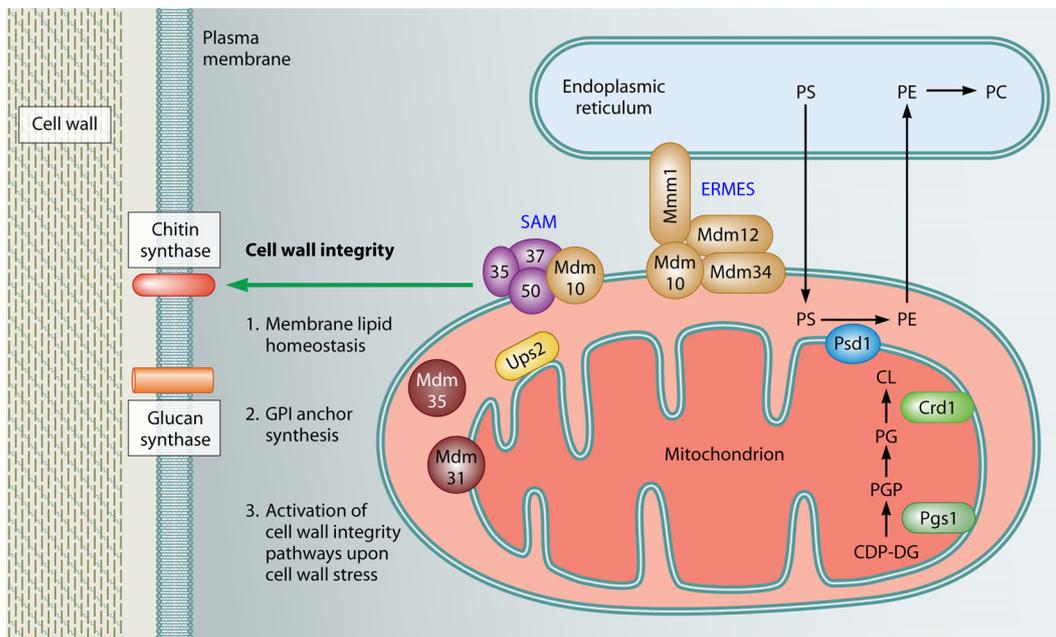


FIG. 2. Roles of mitochondria in cell wall integrity and resistance to the echinocandin antifungal drugs. Mitochondrial proteins affecting phospholipid homeostasis (Sam37, Mdm10, Mdm35, Mdm31, Ups2, Pgs1, and Psd1) have prominent roles in cell wall integrity. Sam37 and Mdm10 are subunits of the SAM and ERMES complexes, which are necessary for phospholipid trafficking between the ER and the mitochondria. Psd1, Pgs1, and Crd1 are enzymes required for the synthesis of phospholipids PE, PG, and CL. The conversion of PGP to PG is catalyzed by the phosphatidylglycerol phosphatase Gep4, which is not shown in the figure for simplicity's sake. Mitochondrial phospholipid biosynthesis could (1) affect the activity of wall synthesis enzymes in the plasma membrane, (2) impinge on synthesis of the GPI anchor, and (3) be required for the activation of pathways necessary for responding to cell wall stress. PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PGP, phosphatidylglycerol phosphate; CDP-DG, CDP-diacylglycerol; PG, phosphatidylglycerol; CL, cardiolipin.

glabrata are more sensitive to amphotericin B (9, 86). Decreased susceptibility to this drug was also observed for *C. albicans* treated with tetracycline; this was attributed to the effects of tetracycline on the function of mitochondria (66). Tetracycline also increased the sensitivity to amphotericin B of *C. neoformans* and *Aspergillus fumigatus* (66), implying that mitochondrial roles in polyene tolerance are broadly conserved in pathogenic fungi. Moreover, a large study by Hillenmeyer et al. that included 1,144 chemical genomic assays in *S. cerevisiae* found a significant enrichment in the GO (Gene Ontology) terms “mitochondrial inheritance,” “mitochondrial localization,” and “mitochondrial distribution” among genes required for wild-type fitness in the presence of nystatin (38). However, two reports in *C. albicans* suggest that mitochondrial mutants display higher resistance to amphotericin B (28, 29), while one report finds no difference (18).

The link between mitochondria and polyene treatment is again lipids. It has been proposed that the increased susceptibility of mitochondrial mutants to polyenes is due to changes in sterol levels (9, 28, 29), which could be due to the requirement for heme as a cofactor for the ergosterol biosynthesis enzymes Erg5 and Erg11.

MITOCHONDRIA AND CELL WALL-TARGETING DRUGS

The echinocandin antifungal drugs inhibit β -1,3 glucan synthase, the enzyme responsible for synthesis of the main glucan component of the yeast cell wall (23). Mitochondrial mutants of *C. albicans*, *Candida parapsilosis*, and *S. cerevisiae* are sen-

sitive to the echinocandins (15, 17, 22, 38, 76). Mitochondrial dysfunction leads to cell wall defects in *C. glabrata* (3, 10). While sensitivity to the echinocandins has not been tested directly yet, the close relationship between *C. glabrata* and *S. cerevisiae* predicts that mitochondria will be required for echinocandin tolerance in *C. glabrata* also.

Which mitochondrial function is the most critical for cell wall integrity and tolerance of the echinocandin drugs? A recent screen of a collection of *S. cerevisiae* mitochondrial mutants in our laboratory suggests that respiratory deficiency does play a role, as mutants that are respiratory deficient are hypersensitive to the echinocandin drug caspofungin (22). A similar conclusion can be drawn from the large study by Hillenmeyer et al. (38). Moreover, in *C. parapsilosis*, treatment with inhibitors of the mitochondrial respiratory pathways resulted in lowering of the MIC of caspofungin (15), also suggesting a role for mitochondrial respiration in cell wall integrity and echinocandin tolerance. However, the extent of caspofungin hypersensitivity did not correlate with respiratory deficiency, but again, the strongest link appeared to be the function of mitochondria in lipid homeostasis (22). The mitochondrial mutants most sensitive to caspofungin were those with impaired mitochondrial biosynthesis of the phospholipids phosphatidylethanolamine and cardiolipin (22, 49, 67, 82) (Fig. 2). Consistently, deletion of the phosphatidylglycerol synthase gene *PGS1* leads to cell wall defects in *S. cerevisiae* and *C. glabrata* (3, 90, 91) and to caspofungin sensitivity in *S. cerevisiae* (76). Moreover, the *C. albicans* mutants with deletions of the genes for the phospholipid-biosynthetic enzymes phosphatidylserine synthase

(*CHO1*) and phosphatidylserine decarboxylase (*PSD1* and *PSD2*) have defective cell walls and dysfunctional mitochondria and are sensitive to caspofungin (17). Additionally, the *C. albicans* mutant lacking the mitochondrial outer membrane SAM (Sorting and Assembly Machinery) complex subunit Sam37 is sensitive to caspofungin (22). The SAM complex functions in the assembly of outer membrane proteins (14), and at the cellular level, it is required for both mitochondrial phospholipid biosynthesis and cell wall integrity (22).

What aspect of cell wall integrity is affected by mitochondrial dysfunction? Three possibilities can be envisaged and are depicted in Fig. 2. Impairment of mitochondrial functions could lead to changes in the lipid composition of the plasma membrane, which could in turn have an impact on the activity of biosynthesis enzymes located in the plasma membrane, such as glucan synthase and chitin synthase. In support of this mechanism, the *S. cerevisiae pgs1Δ* mutant, cells lacking mtDNA, and the *C. albicans* mutant with a mutation in the posttranscriptional regulator Ccr4 that coordinates the network linking cell wall integrity to mitochondria and phospholipid homeostasis all have lower levels of cell wall β -glucans (22, 90). Also, in the *S. cerevisiae pgs1Δ* mutant, the steady-state protein level of the glucan synthase catalytic subunit Fks1 decreases and its activity is lower (91). Another mechanism linking mitochondrial phospholipid biosynthesis and cell wall integrity is the requirement for phosphatidylethanolamine in synthesis of the glycosylphosphatidylinositol (GPI) anchor (5, 41), with a large number of cell wall proteins and cell wall biosynthesis enzymes being GPI anchored (47, 71) (Fig. 2). Finally, studies with the *S. cerevisiae pgs1Δ* mutant suggest that mitochondrial phospholipid biosynthesis is required for activation of the PKC-dependent cell wall integrity pathway (91), although the exact mechanism is not clear. Of note, links have been reported between cell wall integrity signaling and lipid metabolism. For example, the kinases Pkh1 and Pkh2, which are activated by the long-chain base phytosphingosine (an intermediate in sphingolipid biosynthesis), have roles in cell wall integrity by acting on the central PKC-dependent cell wall integrity pathway, as well as via a parallel pathway involving the Ypk1 and Ypk2 kinases (13, 27, 42, 52, 73, 77; reviewed in reference 51). In this context, it is interesting that work from the Moye-Rowley laboratory in *S. cerevisiae* linked mitochondrial dysfunction and consequent activation of the Pdr pathway to regulation of the expression of genes encoding enzymes of sphingolipid biosynthesis, as well as the membrane transporter gene *RSB1* that is required for tolerance of long-chain bases (34, 48, 69, 79) (Fig. 1). Whether and how mitochondrial dysfunction, sphingolipid metabolism and signaling, and cell wall integrity are functionally interconnected is unclear at this stage, but it is certainly worth exploring. Moreover, an important goal for the future is to use directed biochemical experiments to determine exactly how membrane lipids and mitochondria affect the activity of the wall biosynthesis enzymes in the plasma membrane. In addition, we need a large-scale study of the effects of mitochondrial dysfunction on cell wall carbohydrate and protein composition, particularly in pathogens such as *C. albicans* and *C. glabrata*, using diverse mitochondrial mutants and the “-omics” approaches of systems biology.

MITOCHONDRIA AND VIRULENCE OF HUMAN FUNGAL PATHOGENS

In addition to being required for antifungal drug tolerance, mitochondria have also been associated with the ability of fungal pathogens to cause disease. This link is supported by direct assays of mitochondrial mutants in animal infection models, and it can also be inferred indirectly from the requirement for mitochondrial function in cellular pathways associated with virulence. We will first consider the direct assays and then the indirect evidence.

The majority of the direct assays support a positive correlation between mitochondrial function and virulence of human fungal pathogens. Loss of mitochondrial function in *C. glabrata* cripples virulence (10). Moreover, in *C. albicans*, inactivation of the mitochondrial protein Goa1, putative subunits of the respiratory complex I, or factors required for mitochondrial biogenesis and morphology all result in attenuated virulence in the mouse model of systemic candidiasis (1, 4, 64). Moreover, deletion of the mitochondrial superoxide dismutase gene *SOD2* in *C. neoformans* reduced virulence in the mouse model (62). The attenuation of virulence of strains with dysfunctional mitochondria is probably due to a combination of reduced fitness, metabolic changes, and sensitivity to oxidative stress, which is linked to higher production of reactive oxygen species (ROS) because of defective respiration. In this context, it has been shown that inactivation of the mitochondrial alternative oxidase in *A. fumigatus* causes an increase in ROS and enhanced killing of the fungus by macrophages (54). Additional support for a positive role for mitochondrial function in virulence comes from studies of the Vancouver Island and North American outbreaks of hypervirulent *Cryptococcus gattii*. It was proposed that the hypervirulence was associated with more efficient mitochondrial function, which occurred via a change in mitochondrial morphology toward more tubular organelles (12, 53). The effects of tubular mitochondria on fungal survival in macrophages were proposed as the mechanism, but the molecular basis of this remains to be determined (53).

The idea that mitochondrial function is necessary for virulence is challenged by a recent report of more virulent mitochondrial mutants of *C. glabrata* (25). These were clinical isolates that lost mtDNA in the host. Surprisingly, inducing mtDNA loss in the parental clinical isolate *in vitro* by ethidium bromide mutagenesis gave the opposite result: the mutant was not more virulent than the wild type and even showed slightly reduced fungal burdens in the kidneys (25). This raises the question of whether the clinical mitochondrial mutants acquired other mutations unrelated to mitochondrial dysfunction that rendered them more virulent. It is also possible that pressures operating on respiration-deficient mutants in the host are different from those in an *in vitro* environment, activating compensatory pathways specifically in the host upon loss of mitochondrial function which then contribute to virulence.

Indirect evidence for a role of mitochondria in the survival of fungal pathogens within the host includes a requirement for mitochondrial function for metabolic pathways necessary for virulence (such as the glyoxylate cycle and gluconeogenesis) (2, 72), the activation of genes required for mitochondrial respiration during colonization of the central nervous system by *C. neoformans* (80), and the involvement of mitochondria in sur-

vival under oxidative stress in *C. albicans*, *C. neoformans*, *A. fumigatus*, and *Paracoccidioides brasiliensis* (1, 54, 56, 62), as well as the role of mitochondria in fungal morphogenesis (1, 46, 56, 57, 87). With respect to morphogenesis, *C. albicans* mutants lacking the mitochondrial protein Goa1, as well as those with inactivation of the NADH dehydrogenase Ndh51 or subunits of the mitochondrial pyruvate dehydrogenase complex, all display a defect in switching from an ovoid yeast to a filamentous cell morphology, which is a key aspect of virulence (1, 46, 57, 87). There is, however, a report that inhibiting respiration by using antimycin A induces filamentous growth in *C. albicans* (61). Whether this discrepancy is due to differences in strain backgrounds of *C. albicans* or the nature of the mitochondrial mutations remains to be determined. In the dimorphic pathogen *P. brasiliensis*, inhibition of mitochondrial respiration blocks the switch from filamentous to yeast morphology that is important for virulence (56). In conclusion, the available data from both direct and indirect studies support the notion that wild-type mitochondrial function is required for virulence.

TARGETING MITOCHONDRIAL FACTORS FOR DRUG DISCOVERY

Would mitochondrial functions/factors represent promising targets for antifungal therapy? We would argue that they would, as inactivating mitochondrial factors is likely to substantially cripple virulence for multiple reasons, such as effects on fitness, metabolism, and oxidative stress responses. While the resistance to azole drugs that is associated with some mitochondrial mutations is a concern, it has to be noted that resistance is only seen with specific mutations, and several mitochondrial mutants are known which are sensitive to the azoles (22, 38, 86). Also, azole resistance due to mitochondrial mutations is rarely observed in the clinic (8, 24). Moreover, even the drug-resistant mitochondrial mutants of *C. glabrata* and *C. albicans* were crippled for virulence in animal models, probably due to a substantial fitness defect (10, 18, 19).

Which mitochondrial factors could be promising drug targets? Some predictions can be made based on studies in the model yeast *S. cerevisiae* and in the pathogen *C. albicans*. In *S. cerevisiae*, a deletion collection covering all nonessential open reading frames has been screened for genes required for mitochondrial respiration (so-called “*pet* genes,” because they give rise to mitochondrial or “petite” mutants) (58). Based on three independent screens, a core set of 129 genes encoding mitochondrial proteins have been identified that are likely to be required for mitochondrial respiration regardless of the strain background (58). Petite mutants of fungal pathogens can be predicted to have reduced virulence in animals due their reduced fitness, as well as other defects discussed above. Therefore, the identified *pet* genes from *S. cerevisiae* can be the starting point for creating mitochondrial mutants in human fungal pathogens and testing their requirement for virulence. The functions of the *pet* genes should be tested directly in several human fungal pathogens, as inferring the function of a gene based on knowledge from the model yeast system can be complicated by profound changes to the organization and regulation of cellular pathways (50, 55). In the context of mitochondrial function, *S. cerevisiae* has a strikingly different respi-

ratory chain than the *Candida* species: it does not have the respiratory complex I or alternative respiratory pathways (36, 40, 59, 74, 81). Also, not all mitochondrial mutants will have the same effect on virulence, as exemplified by the study of Becker et al. (4). Among 11 mitochondrial mutants tested, 10 of which were not viable *in vitro*, some were essential for virulence in mice while some were only attenuated (4).

One group of *S. cerevisiae* *pet* genes that we would like to discuss in greater detail encompasses a set of factors required for maintenance of the mitochondrial genome. In the most recent screen of the *S. cerevisiae* deletion collection, 162 mutants were identified which tend to lose mtDNA (58). While these mtDNA maintenance genes are not essential for viability in *S. cerevisiae*, they are likely to be essential for viability in petite-negative pathogens, such as *C. albicans* or *C. neoformans* (4, 58, 83), and could thus represent potentially attractive drug targets. This idea is supported by a recently published screen of *C. albicans* strains (4): seven mitochondrial genes were identified which are essential in *C. albicans* but not in *S. cerevisiae*. For all of them, the likely reason for the essential function in *C. albicans* is an effect on mtDNA stability: four genes encode mitochondrial ribosomal proteins, with mitochondrial translation being required for mtDNA maintenance (20); the other three are *MGM101*, the mitochondrial RNA polymerase gene *RPO41*, and the ERMES (ER-mitochondria-encounter structure) subunit gene *MMM1*, all of which affect mtDNA stability in *S. cerevisiae* (32, 39, 58).

CONCLUDING REMARKS

While recent studies have highlighted the importance of mitochondrial function for tolerance of antifungal drugs and for virulence, our understanding of the roles of this organelle in human fungal pathogens is still limited. Functions in lipid homeostasis are likely to be at the heart of the involvement of mitochondria in tolerance of antifungal drugs, but the exact molecular mechanisms are not fully understood. Biochemical experiments in mitochondrial mutants addressing lipid composition, activities of plasma membrane enzymes, and the structure of the cell wall should answer some of the outstanding questions. Also important is to test the virulence of many more mutants affected in diverse mitochondrial functions. This could produce a list of potentially attractive targets for future antifungal drug development. It is of interest that a large number of mitochondrial proteins are likely to be essential for viability in petite-negative pathogens, such as *C. albicans* and *C. neoformans*. An important point is that several of these mitochondrial factors do not have close orthologs in humans; for example, see reference 65. Collectively, these observations indicate that studies into how mitochondria contribute to the fitness, pathogenicity, and drug tolerance of human fungal pathogens are worthwhile.

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