MINIREVIEW

Sterol biosynthesis and sterol uptake in the fungal pathogen *Pneumocystis carinii*

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Abstract

Members of the fungal genus *Pneumocystis* colonize healthy mammalian hosts without causing apparent disease, but colonization in immunocompromised hosts may result in a potentially fatal pneumonia known as *Pneumocystis* pneumonia. Although *Pneumocystis* are fungi, this genus has characteristics that make it atypical among other fungi. *Pneumocystis* do not appear to synthesize the major fungal sterol, ergosterol, and biochemical analyses have shown that they utilize cholesterol rather than ergosterol as the bulk sterol. *Pneumocystis carinii* appears to scavenge exogenous sterols, including cholesterol, from its mammalian host. As a result, it has long been held that their ability to scavenge cholesterol from their hosts, and their inability to undergo sterol biosynthesis, makes them resistant to antifungal drugs that target ergosterol or ergosterol biosynthesis. However, genome scans and *in vitro* assays indicate the presence of sterol biosynthetic genes within the *P. carinii* genome, and targeted inhibition of these enzymes resulted in reduced viability of *P. carinii*, suggesting that these enzymes are functional within the organism. Heterologous expression of *P. carinii* sterol genes, along with biochemical analyses of the lipid content of *P. carinii* cellular membranes, have provided an insight into sterol biosynthesis and the sterol-scavenging mechanisms used by these fungi.

Introduction

Members of the genus *Pneumocystis* are opportunistic fungi capable of causing a lethal pneumonia in mammalian hosts. *Pneumocystis* colonization of immunocompetent hosts appears to have minimal clinical consequences, but colonization in hosts with debilitated or compromised immune systems may result in the development of *Pneumocystis* pneumonia (PCP). Before the AIDS epidemic in the early 1980s, PCP was a rare occurrence seen only in malnourished children, transplant recipients, cancer patients and those with immune deficiencies (Gajdusek, 1957). Since the advent of HIV/AIDS, the number of cases and deaths due to PCP has drastically increased, and although highly active antiretroviral therapy helps to prevent HIV replication, by allowing reconstitution of the immune system, many patients develop AIDS, and PCP is one of the most common AIDS-defining illnesses (Grabar *et al.*, 2008).

Despite the fungal nature of *Pneumocystis*, drugs used for the treatment of PCP include pentamidine, atovaquone and combinations of either trimethoprim and sulfamethoxazole (TMP-SMX) or clindamycin and primaquine (Hughes *et al.*, 1974, 1991; Girard *et al.*, 1987; Black *et al.*, 1991), which are typically used to treat bacterial and protozoal infections. *Pneumocystis* are resistant to many standard antifungal drugs that target either enzymes involved in sterol biosynthesis or ergosterol, the end product of the sterol biosynthesis in fungal cells. Resistance to these drugs has been attributed in part to the lack of detectable ergosterol within the membranes of *Pneumocystis*. It has been hypothesized that *Pneumocystis* scavenges cholesterol from its mammalian host and incorporates it into its cellular membranes, making cholesterol rather than ergosterol the bulk sterol of *Pneumocystis* (Worsham *et al.*, 2003). The inability of *Pneumocystis carinii* to synthesize ergosterol, the substitution of cholesterol as the bulk sterol, combined with the lack of efficacy of standard antifungal drugs that target the sterol pathway, would seem to indicate that *de novo* sterol synthesis does not occur within *P. carinii*. Yet, the presence of several putative ergosterol biosynthetic genes in the *P. carinii* genome (Cushion & Smulian, 2001) and the presence of non-host-derived sterols within the membranes of *P. carinii* (Kaneshiro *et al.*, 1996, 1999; Kaneshiro & Wyder, 2000;

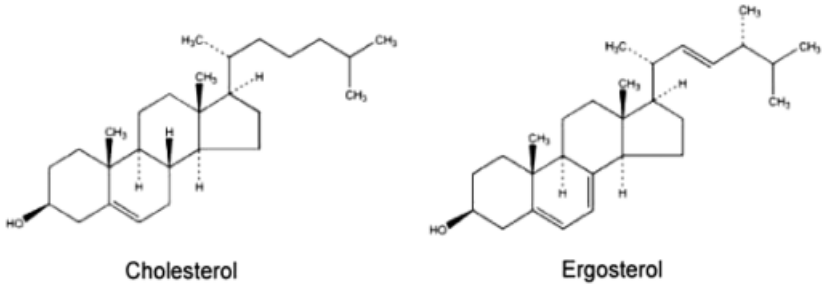

Fig. 1. The molecular structure of cholesterol and ergosterol.

Giner *et al.*, 2002) seem to indicate the existence of a functional sterol pathway. The steps involved in ergosterol and cholesterol synthesis have been determined for both fungi and mammals, respectively, but the complete sterol pathway of *P. carinii* has not been determined.

Sterol biosynthesis

Sterols are vital components of all eukaryotic cell membranes, and are essential for cell growth and viability. Ergosterol, the major sterol found in fungal cell membranes, functions in the same capacity as cholesterol, the major sterol found in mammalian cell membranes (Henneberry & Sturley, 2005). Sterols have many roles in eukaryotic membranes including establishing appropriate membrane fluidity (Lees *et al.*, 1979), regulating membrane-bound enzymes (Cobon & Haslam, 1973) and maintaining membrane permeability (Bard *et al.*, 1978). The sterol biosynthetic pathway in fungi and mammals is strikingly similar, but differences in the later steps of both pathways result in two structurally different molecules. Both ergosterol and cholesterol (Fig. 1) have a $-$OH group on C-3 of the sterol ring and a double bond at C-5 of the ring. However, the synthesis of ergosterol has three additional steps, resulting in two additional double bonds at C-7 and C-22 and a methyl group at C-24 of the ergosterol side chain. These structural differences make cholesterol and ergosterol remarkably suited for fulfilling both the cellular and the membrane requirements of the organism in which they are the most abundant sterol (Henriksen *et al.*, 2006).

All enzymes of the postsqualene or committed sterol pathway are conserved between mammals and fungal organisms until after the formation of zymosterol (Figs 2 and 3). After the formation of lanosterol, the ergosterol pathway proceeds in a linear fashion toward the production of ergosterol (Fig. 2), but the cholesterol pathway proceeds to cholesterol through either one of two routes: (1) through zymosterol or (2) through lathosterol (Fig. 3). These divergent routes to sterol production result in sterols that are uniquely suited for mammalian and fungal cells. In mammalian cell membranes, cholesterol is arranged in a bilayer

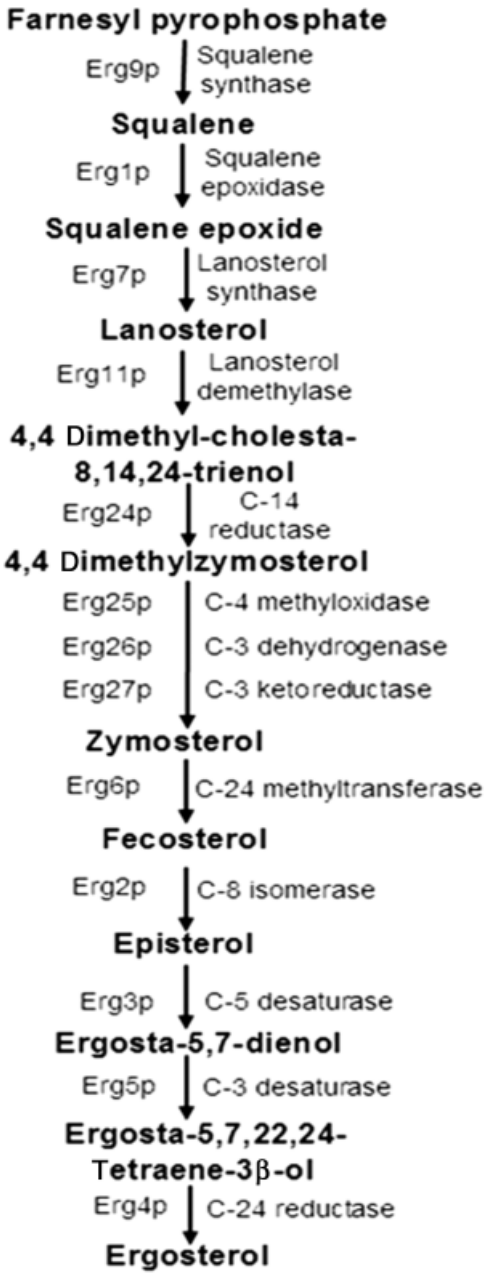

Fig. 2. The committed ergosterol biosynthetic pathway.

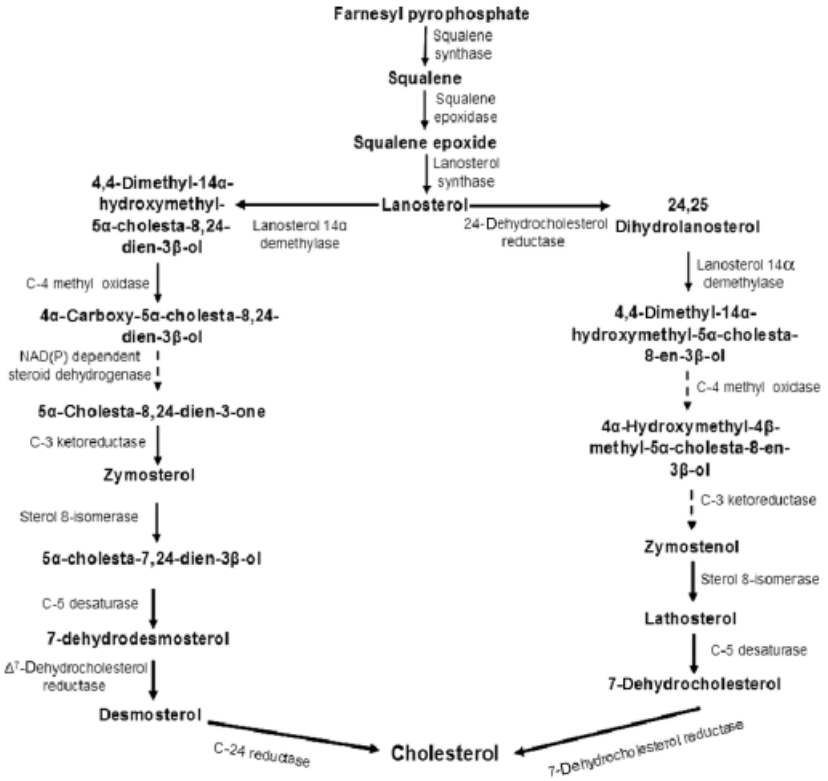

Fig. 3. The committed cholesterol biosynthetic pathway.

conformation, allowing external forces to be distributed more efficiently (Hildenbrand & Bayerl, 2005), while in fungal cell membranes, ergosterol is arranged in a monolayer conformation, causing the membrane to be more rigid and less flexible than mammalian cell membranes (Hildenbrand & Bayerl, 2005). These differences may be attributed to the lack of a cell wall in mammalian cells and the presence of one in fungal cells. The cell wall is located outside the cell membrane and provides structural integrity and protection from external forces. Mammalian cells lack a cell wall; therefore, the cell membrane establishes structural integrity and protection from external forces. Consequently, mammalian cell membranes are more flexible than fungal cell membranes, and the divergence of the sterol pathways contributes to the nature of these two membranes. In ergosterol, two additional double bonds formed by the actions of the C-5 desaturase and C-22 desaturase enzymes (Arthington *et al.*, 1991; Skaggs *et al.*, 1996) contribute to the rigidity of fungal cell membranes, whereas the cholesterol molecule lacks these additional modifications, allowing the mammalian cell membrane more flexibility to protect it from outside forces (Hildenbrand & Bayerl, 2005).

Data from several studies point toward the existence of a *de novo* sterol pathway in *P. carinii* (Florin-Christensen *et al.*, 1994; Kaneshiro *et al.*, 1994b; Giner *et al.*, 2001, 2002). Incubation of *P. carinii* with radiolabeled sterol precursors such as acetate, mevalonate, squalene, HMG-CoA and isopentenyl diphosphate resulted in the synthesis of radiolabeled sterols in *P. carinii*, and suggested that sterol synthesis occurs through the acetate–mevalonate pathway (Florin-Christensen *et al.*, 1994; Kaneshiro *et al.*, 1994b; Ellis *et al.*, 1996; Sul & Kaneshiro, 2001). It is thought that this pathway leads to the formation of rarely detected C_{28} and $C_{29}\Delta^7$ sterols such as fungisterol and stigmast-7-en-3β-ol (Florin-Christensen *et al.*, 1994), which have only been found in *Trypanosoma cruzi* (Liendo *et al.*, 1999) and plant pathogenic rust fungi of the class *Uredinales* (Weete, 1989). In addition to these rare sterols, the organism appears to synthesize its own unique sterols, including [(24*Z*)-ethylidenelanost-8-en-3β-ol] (pneumocysterol) (Florin-Christensen *et al.*, 1994; Kaneshiro *et al.*, 1994a, 1999; Urbina *et al.*, 1997), which may be necessary for survival. Because these sterols are synthesized *de novo* by the organism despite its ability to scavenge available sterols, these sterols have been called 'metabolic sterols' (Haughan & Goad, 1991; Kaneshiro *et al.*, 1994a), and because these sterols appear to be unique to *Pneumocystis*, they may not only provide excellent drug targets against the organism (Haughan & Goad, 1991), but they may have potential as possible markers for the detection of PCP (Kaneshiro *et al.*, 1999).

Cholesterol accounts for up to 81% of the total sterols isolated from *Pneumocystis* obtained from rat lungs, and it has been postulated that most, if not all, the cholesterol is scavenged from the host (Giner *et al.*, 2002; Worsham *et al.*,

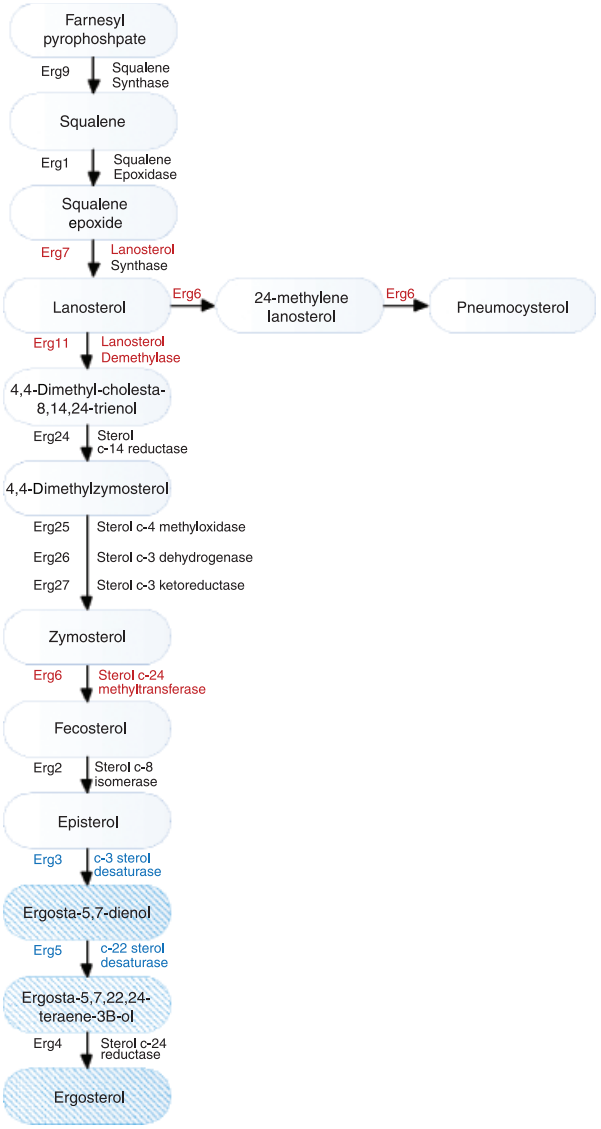

Fig. 4. Putative *Pneumocystis carinii* sterol pathway. A putative sterol biosynthetic pathway indicating genes that have been cloned and functionally characterized (red), and genes that have not been detected in analyses (blue) is represented. Other genes listed include those where either genomic or cDNA sequences have been identified by the *Pneumocystis* genome project (http://pgp.cchmc.org), but the genes have not been characterized. However, the sterol products of these reactions have been identified in previous analyses. Sterol products that have not been identified to date are indicated by hatch markings. Note: Two postlanosterol pathways are proposed for *P. carinii*, one leading to the formation of pneumocyterol and another leading to the formation of episterol.

2003). Conversely, one report speculates that *P. carinii* may synthesize cholesterol through a *de novo* pathway (Zhou *et al.*, 2002), but to date, there is no evidence to suggest that the organism contains all of the genes necessary to synthesize either cholesterol or ergosterol. Despite the lack of detectable ergosterol in *Pneumocystis* membranes, genes involved in sterol synthesis have been identified within its genome, and many of these genes have been proven functional based on targeted inhibition of these enzymes and the subsequent reduction in the viability of *P. carinii* (Kaneshiro *et al.*, 2000). Figure 4 outlines the putative sterol biosynthetic pathway of *P. carinii* based on our current knowledge, and Table 1 lists *P. carinii* sterol enzymes and identifies the reaction products that have been detected in the membranes of the fungus. These putative *P. carinii* sterol enzyme genes were identified based on sequence similarity to other known fungal sterol enzymes; however, functional analyses are necessary to determine their function. To date, only three of these genes, ERG7 (lanosterol synthase), ERG11 (lanosterol 14α demethylase) and ERG6 (sterol C-24 methyl transferase), have been the subject of research investigations.

Lanosterol synthase (Erg7)

The activity of lanosterol synthase or Erg7 results in the conversion of the last acyclic sterol precursor into lanosterol, the first cyclic sterol intermediate of the sterol pathway. In *Saccharomyces cerevisiae*, loss of lanosterol synthase function results in a nonviable phenotype; similarly, inhibition of the *P. carinii* enzyme has been shown to reduce the viability of *P. carinii in vitro* (Kaneshiro *et al.*, 2000). *Saccharomyces cerevisiae* Erg7 localizes to lipid particles, and when expressed in an *S. cerevisiae ERG7* null mutant, homologs of Erg7 from the plant pathogen *Arabidopsis thaliana* and the parasite *T. cruzi* localized to lipid particles in an *S. cerevisiae ERG7* mutant (Milla *et al.*, 2002a, b). Lipid particles are thought to derive from the endoplasmic reticulum (ER), where neutral lipids accumulate within the lipid bilayer and bud off into the cytoplasm after reaching a certain size (Athenstaedt *et al.*, 1999). Lipid particles are surrounded by a monolayer membrane that contains 16 proteins, all of which function in lipid metabolism (Athenstaedt *et al.*, 1999). Consequently, several roles have been ascribed to lipid particles including lipid metabolism and storage (Athenstaedt *et al.*, 1999).

Pneumocystis carinii ERG7 was cloned and expressed in an *S. cerevisiae ERG7* mutant strain by two independent laboratories. While both studies concluded that *P. carinii ERG7* complemented the *ERG7* null mutant yeast and retained residues of the squalene cyclase domain that are necessary for the catalytic ability of the enzyme, the two studies differ in their conclusions regarding *P. carinii* Erg7 localization. In one study, localization of *P. carinii* Erg7 was inconclusive, but the same group speculated that the *P. carinii* enzyme did not localize to lipid particles in yeast (Milla *et al.*, 2002b). These observations were based largely on the lack of activity of *P. carinii* Erg7 in isolated lipid particle fractions and the lack of a band corresponding to *P. carinii* Erg7 in a Coomasie-stained gel containing lipid particle proteins (Milla *et al.*, 2002b). In the second study,

Table 1. *Pneumocystis carinii* sterol genes and reaction products

Gene name	Expected product	Product detected
Erg7 lanosterol synthase	Lanosterol	Yes
Erg11 lanosterol demethylase	4,4-Dimethyl-cholesta-8,14,24-trienol	No
Erg 24 C-14 reductase	4,4-Dimethylzymosterol	Yes
*Erg 25 C-4 methyloxidase	4-Methylzymosterol-4-carboxylic acid	*
*Erg 26 C-3 dehydrogenase	3-Keto-4-methylzymosterol	*
*Erg 27 C-3 ketoreductase	*Zymosterol	Yes
Erg 6 C-24 methyltransferase	Fecosterol	Yes
Erg 2 C-8 isomerase	Episterol	Yes
Erg 3 C-5 desaturase	Ergosta-5,7,24(28)-trienol	ND
Erg 5 C-22 desaturase	Ergosta-5,7,22,24(28)-tetraenol	ND
Erg 4 C-24 reductase	Ergosterol	ND

Reaction products that have been identified among *Pneumocystis carinii* sterols are indicated.
*Enzymes involved in C-4 demethylation (Erg25-27) repeat their activity 2 ×. The products of the reactions may be negligible. Zymosterol is the end product of C-4 demethylation.
ND, not detected.

we showed that the *P. carinii* enzyme localizes to lipid particles in yeast using Western blotting and fluorescent microscopy (Joffrion *et al.*, 2010). In addition, using fluorescent microscopy, we identified putative lipid particles in *P. carinii*, and localization of the *P. carinii* enzyme to putative lipid particles in its native organism was demonstrated. The differences between these two studies were likely due to the sensitivities of the techniques used. Our studies utilized a polyclonal *P. carinii* Erg7 antiserum to detect the presence of the enzyme in yeast lipid particles and putative lipid particles in *P. carinii* (Joffrion *et al.*, 2010), while the previous study relied on detection of the protein in a stained polyacrylamide gel (Milla *et al.*, 2002b), which was neither specific nor sensitive. A dual localization has been noted for several proteins within lipid particles and the ER (Natter *et al.*, 2005), and loss of activity was demonstrated upon separation of the ER from lipid particles suggesting a potential interaction between these two cellular compartments (Leber *et al.*, 1998). The observed lack of activity of *P. carinii* Erg7 in yeast lipid particles (Milla *et al.*, 2002b) may have been due to the separation of these two compartments, and while it was demonstrated that lanosterol was produced in the *ERG7* mutant yeast containing the *P. carinii* enzyme (Joffrion *et al.*, 2010), it was not determined whether lanosterol was produced predominantly in lipid particle fractions.

Lanosterol 14α demethylase (Erg11)

ERG11 encodes lanosterol C-14 demethylase, a cytochrome P450 enzyme. Inhibition of Erg11 in yeast is lethal unless a second mutation occurs in the gene encoding Erg3 or C-5 sterol desaturase (Taylor *et al.*, 1983). Inhibition of Erg3 is not required for *ERG11* mutants of *C. albicans* (Sanglard *et al.*, 2003), but deletion or inhibition of Erg3 has been associated with resistance to azole antifungals that target Erg11 (Kelly *et al.*, 1995; Sanglard *et al.*, 2003). We have not been able to amplify the gene encoding Erg3 using degenerate primers, and it has been observed both *in vitro* and *in vivo* that six commonly used imidazoles are ineffective against *P. carinii* (Bartlett *et al.*, 1994). However, the resistance of *P. carinii* to azoles may be unrelated to the apparent lack of *ERG3* as a separate *in vitro* study utilizing sterol biosynthesis inhibitors indicated that two proprietary imidazoles produced by GlaxoSmithKline (GR 40317A and GR 42539X) were effective against *P. carinii*, whereas the commonly prescribed imidazoles, such as fluconazole, remained ineffective (Kaneshiro *et al.*, 2000). These data suggest that *P. carinii* Erg11 may still be a viable drug target, and that newer drugs targeting the gene may reduce the viability of *Pneumocystis*. Sequence analysis comparing the translated ORF of *P. carinii* Erg11 with fungal Erg11 homologs revealed the presence of amino acid substitutions at positions 113 and 125 of the highly conserved substrate recognition site (Morales *et al.*, 2003). These substitutions are also found in a fluconazole-resistant *C. albicans* strain (Asai *et al.*, 1999). Functional analysis of *P. carinii ERG11* expressed in an *S. cerevisiae ERG11* mutant revealed that in order to achieve a 50% reduction in growth, *P. carinii* Erg11 required a 2.2-fold higher dose of voriconazole and a 3.5-fold higher dose of fluconazole than *S. cerevisiae* Erg11 expressed under similar conditions (Morales *et al.*, 2003). Based on these data, the group concluded that *P. carinii* Erg11 is intrinsically resistant to azole antifungals (Morales *et al.*, 2003).

Sterol methyltransferase (Erg6)

ERG6 encodes the enzyme sterol C-24 methyltransferase that catalyzes methylation of carbon 24 of the sterol side chain in fungi. NMR analysis of HPLC isolated sterols

revealed the structures of 43 *P. carinii* sterols, and of these, 32 contained a methyl group on C-24 of the sterol side chain, indicating that Erg6 is a highly active enzyme in *P. carinii* (Giner *et al.*, 2002). The high activity of *P. carinii* Erg6, the ability of drugs targeting the enzyme to decrease the viability of *P. carinii in vitro*, and the fact that mammals do not alkylate the C-4 position of sterols have lead to the idea that Erg6 may be a novel anti-*Pneumocystis* drug target (Kaneshiro *et al.*, 2000; Kaneshiro, 2002; Zhou *et al.*, 2002). *Pneumocystis carini ERG6* was cloned and expressed in *Escherichia coli*, and was shown to use lanosterol and 24-methylenelanosterol as preferred substrates, which is unlike other fungi, where zymosterol is the Erg6 substrate (Kaneshiro *et al.*, 2002). Consequently, it was speculated that lanosterol to 24-methylenelanosterol is the major post-lanosterol pathway in *P. carinii*. This would indicate that lanosterol demethylation by Erg11 occurs after C-24 alkylation by Erg6 in *P. carinii*, and that substrates for *P. carinii* Erg11 are 24-alkylsterols and not lanosterol (Kaneshiro *et al.*, 2002). This is not entirely unlikely, as this alternate pathway has been observed in a fluconazole-resistant *C. albicans* strain (Asai *et al.*, 1999).

Sterol uptake in *P. carinii*

Several enzymes of the postsqualene ergosterol biosynthetic pathway require molecular oxygen, making ergosterol biosynthesis an oxygen-dependent process. When grown under aerobic conditions, *S. cerevisiae* is able to synthesize sterols, and is unable to acquire exogenous sterols, a phenomenon known as aerobic sterol exclusion (Andreasen & Stier, 1953). Under anaerobic conditions, the activity of the postsqualene sterol pathway is decreased, and as a consequence, sterol scavenging becomes the major mechanism for obtaining sterols (Andreasen & Stier, 1953). While *S. cerevisiae* is only able to take up exogenous sterols during anaerobic growth, some filamentous fungi such as *Aspergillus fumigatus* are able to take up sterols under aerobic conditions (Xiong *et al.*, 2005). The molecular mechanisms behind aerobic sterol exclusion have not been elucidated, but heme has been implicated in the process. Cells are able to sense oxygen availability through the levels of heme, which is produced in an oxygen-dependent mechanism. Heme stimulates transcription through the Hap1 transcriptional activator, and both heme and Hap1 are involved in aerobic ergosterol biosynthesis. Hap1 is responsible for aerobic induction and anaerobic repression of *ROX1* (Ushinsky & Keng, 1994), a well-known repressor of hypoxic genes, which is activated upon expression of Hap1 in a heme-dependent mechanism (Keng, 1992). Many genes involved in the later steps of ergosterol biosynthesis require molecular oxygen for catalysis, and as a result, these enzymes are down-regulated as the supply of oxygen declines. Likewise, because heme production is dependent on the supply of oxygen, heme-mediated Rox1 repression of hypoxic genes declines as oxygen levels decrease, resulting in an increased expression of nearly all Rox1 repressed genes (Kwast *et al.*, 1997). The upregulation of hypoxic genes and decreased activity of ergosterol biosynthetic genes results in exogenous sterol uptake.

Many genes involved in cholesterol biosynthesis have homologs in ergosterol biosynthesis, and while many of these have been identified within the *P. carinii* genome, *P. carinii* does not appear to encode all of the genes necessary to synthesize cholesterol through a *de novo* pathway (e.g. C-5 desaturase). Thus, it is unlikely that *P. carinii* is able to synthesize cholesterol, and most, if not all, of the cholesterol found within the membranes of *P. carinii* was scavenged from host cells by *P. carinii*. The ability of *P. carinii* to scavenge lipids was confirmed after incubation of *P. carinii* with the fluorescent fatty acid analog Bodipy-C_{12}. Fluorescent microscopy and fluorimetry indicated that *P. carinii* readily scavenged Bodipy-C_{12} from the medium and incorporated the fatty acid uniformly in all morphological forms of *P. carinii* (Furlong *et al.*, 1997). Uptake of Bodipy-C_{12} by *P. carinii* occurred rapidly, and peaked 1 h postincubation (Furlong *et al.*, 1997), suggesting that *P. carinii* uses rapid and robust sterol-scavenging mechanisms. A separate study utilizing *in vitro* radiolabeling revealed that incorporation of radiolabeled squalene into sterols occurred predominantly in noncholesterol sterol fractions, whereas the relative specific activity of the crude cholesterol fraction was 20-fold less than those of the other sterol fractions, indicating that cholesterol was not synthesized by *P. carinii* under these conditions (Worsham *et al.*, 2003). The ability of *P. carinii* to scavenge sterols from alveolar cells was shown using *P. carinii* attached to A549 alveolar epithelial cells. In this study, *P. carinii*-associated fluorescence was observed after an overnight incubation with Bodipy-C_{12} labeled A549 cells (Furlong *et al.*, 1997), and cellular fluorescence was fivefold higher in *P. carinii* organisms attached to A549 cells compared with nonadherent *P. carinii*, suggesting that attachment facilitated lipid transfer (Furlong *et al.*, 1997). In addition to the presence of cholesterol within the membranes of *P. carinii*, several plant sterols have been biochemically detected in *P. carinii* including campesterol, β-sitosterol, brassicasterol and stigmasterol (Giner *et al.*, 2002). It has been proposed that plant sterols were not synthesized by *P. carinii*, but were originally a part of the host diet that was incorporated into the lung, and subsequently scavenged by *P. carinii* and then incorporated into *P. carinii* cellular membranes (Giner *et al.*, 2002).

While cholesterol and plant sterols are incorporated unchanged into *P. carinii* membranes, experimental data provided by two separate studies suggest that the pathogen can remodel host-derived sterols. An early study looking at the fate of scavenged fluorescent lipids revealed that although the majority of scavenged lipids were incorporated

unchanged into *P. carinii* membranes, detection of the fluorescent label could be found in other lipid classes, including neutral lipids and phospholipids, suggesting the ability of *P. carinii* to modify scavenged lipids into complex lipid classes (Furlong *et al.*, 1997). An analysis of sterols within *P. carinii* revealed the presence of sterols that cannot be synthesized *de novo* by either *P. carinii* or mammalian cells. *Pneumocystis carinii* contains a number of Δ^5 alkylated C-24 sterols (Giner *et al.*, 2002), but mammals are unable to alkylate the C-24 position of the sterol nucleus, and the lack of triene sterols in *P. carinii* (Giner *et al.*, 2002) suggests that the organism is not able to destaurate C-5. The lack of the gene encoding C-5 desaturase has led to the belief that these Δ^5 alkylated sterols were first scavenged from the host and subsequently modified by *P. carinii* Erg6 (Giner *et al.*, 2002).

The presence of large amounts of cholesterol within the membranes of *P. carinii* suggests that cholesterol uptake may be a constitutive process in *P. carinii*. In yeast, two genes have been implicated in sterol uptake during anaerobiosis: UPC2 and SUT1. Under anaerobic conditions, Upc2 binds to and induces the expression of anaerobically expressed genes (Abramova *et al.*, 2001) and is also involved in sterol uptake (Shianna *et al.*, 2001). SUT1 expression is increased 9.6-fold under anaerobic conditions (Shianna *et al.*, 2001), and overexpression of SUT1 results in a 2.6-fold increase in sterol uptake under aerobic conditions (Bourot & Karst, 1995). Sut1, however, is unable to mediate sterol uptake unless both Dan1 and Aus1 are functionally expressed (Alimardani *et al.*, 2004). AUS1 encodes a member of the ATP-binding-cassette family of transporters that is necessary for sterol uptake and that requires ATP to facilitate the uptake (Wilcox *et al.*, 2002). Dan1 is a cell wall mannoprotein that was shown to be upregulated in response to SUT1 overexpression, and thus has been identified as a hypoxia-regulated gene (Alimardani *et al.*, 2004). Currently, there is no information regarding *P. carinii* sterol uptake under anaerobic conditions, and homologs of UPC2, AUS1, DAN1 have not been detected within the genome of *P. carinii*. Consequently, the mechanism of sterol uptake and the genes involved in sterol uptake in *P. carinii* are unknown.

Conclusions

Pentamidine, atovaquone, and combinations of trimethoprim and sulfamethoxazole, and clindamycin and primaquine have successfully reduced the number of deaths attributed to PCP infection. However, many patients are unable to tolerate these drugs, and evidence is accumulating that *Pneumocystis jirovecii*, the *Pneumocystis* spp. that infects humans, may be evolving resistance to sulfamethoxazole and atovaquone (Costa *et al.*, 2001). It has become increasingly obvious that new drugs must be identified. The essential nature of sterols in eukaryotic organisms makes the ergosterol pathway an attractive drug target for antifungal therapy. The abundance of cholesterol found in isolated fractions of *P. carinii* sterols and the presence of sterol biosynthetic genes within the *P. carinii* genome, in addition to the unique sterols found in *P. carinii*, together indicate that while the sterol pathway of *P. carinii* may have similarities to other fungi, it also involves deviations from the typical sterol pathway found in other fungal species. Although the lack of ergosterol may make *Pneumocystis* (spp.) resistant to polyene antifungal drugs that target ergosterol, studies have shown that *P. carinii* are susceptible to drugs targeting sterol enzymes (Contini *et al.*, 1994; Kaneshiro *et al.*, 1994b, 2000). The *P. carinii* C-24 methyltransferase sterol enzyme has been proposed to be a novel anti-*Pneumocystis* drug target due to the lack of the enzyme in the mammalian sterol pathway (Kaneshiro *et al.*, 1994b) and the fact that *P. carinii* contains a large variety of 24-alklyated sterols (Giner *et al.*, 2002). Additionally, despite the presence of lanosterol synthase in mammalian cells, *P. carinii* and mammalian enzymes have varying sensitivities to drugs that target the enzyme (Hinshaw *et al.*, 2003). Thus, the presence of a functional sterol pathway in *Pneumocystis* suggests that novel anti-*Pneumocystis* drug targets may exist; however, a better understanding of the *Pneumocystis* sterol pathway and its sterol-scavenging abilities is necessary for adequate drug design.

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