Review

Strategies for acquiring the phospholipid metabolite inositol in pathogenic bacteria, fungi and protozoa: making it and taking it

Todd B. Reynolds

Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA

myo-Inositol (inositol) is an essential nutrient that is used for building phosphatidylinositol and its derivatives in eukaryotes and even in some eubacteria such as the mycobacteria. As a consequence, fungal, protozoan and mycobacterial pathogens must be able to acquire inositol in order to proliferate and cause infection in their hosts. There are two primary mechanisms for acquiring inositol. One is to synthesize inositol from glucose 6-phosphate using two sequentially acting enzymes: inositol-3-phosphate synthase (Inos1p) converts glucose 6-phosphate to inositol 3-phosphate, and then inositol monophosphatase (IMPase) dephosphorylates inositol 3-phosphate to generate inositol. The other mechanism is to import inositol from the environment via inositol transporters. Inositol is readily abundant in the bloodstream of mammalian hosts, providing a source from which many pathogens could potentially import inositol. However, despite this abundance of inositol in the host, some pathogens such as the bacterium Mycobacterium tuberculosis and the protist parasite Trypanosoma brucei must be able to make inositol de novo in order to cause disease (M. tuberculosis) or even grow (T. brucei). Other pathogens such as the fungus Candida albicans are equally adept at causing disease by importing inositol or by making it de novo. The role of inositol acquisition in the biology and pathogenesis of the parasite Leishmania and the fungus Cryptococcus are being explored as well. The specific strategies used by these pathogens to acquire inositol while in the host are discussed in relation to each pathogen’s unique metabolic requirements.

Introduction

In order to cause an infection, microbes must be able to live and proliferate inside the host. This necessitates the acquisition of essential nutrients by the pathogen. The pathogen must either obtain these nutrients from the host or synthesize the nutrients de novo from more basic compounds. The biosynthesis of phospholipids is an area that has received considerable attention in terms both of the insight it may give us regarding host–pathogen interactions and the possibility that an understanding of phospholipid metabolism may help in the identification of new drug targets (Vial et al., 2003). Phospholipids are complex molecules that are essential for membrane integrity and intracellular signalling. The biosynthesis of these compounds requires a complex ordered set of biochemical steps involving a number of enzymes (Greenberg & Lopes, 1996; van Meer et al., 2008; Vance, 2003; Vial et al., 2003). An area of particular interest is understanding how pathogens acquire basic molecules such as myo-inositol, serine, choline or ethanolamine for the synthesis of phospholipids (Vial et al., 2003). This review will focus on mechanisms used by diverse pathogens to obtain myo-inositol for the synthesis of the phospholipid phosphatidylinositol (PI).

In the last several years a number of studies have been performed to elucidate the roles that proteins involved in myo-inositol acquisition play in controlling virulence and/or viability in a variety of different pathogens (Chen et al., 2008; Martin & Smith, 2005, 2006a; Movahedzadeh et al., 2004). This review will cover the fascinating repertoire of mechanisms used by these different pathogens to acquire myo-inositol.

Inositol

myo-Inositol is a precursor for making PI, which is essential in all eukaryotes, including pathogenic fungi and protozoa, as well as in a small, but very significant group of eubacterial pathogens that includes the mycobacteria (Michell, 2008). In order to synthesize PI the pathogen must obtain myo-inositol. There are two main mechanisms for acquiring this molecule: synthesizing it de novo from glucose 6-phosphate or importing it from the host (Majumder et al., 1997; Michell, 2008; Drew et al., 1995; Einicker-Lamas et al., 2000, 2007; Jin & Seyfang, 2003).

myo-Inositol is a polyol that is characterized as a six-carbon ring where each carbon is hydroxylated. A number of
isomers are biologically active, but myo-inositol is the most common (Majumder et al., 1997; Michell, 2008). For the purposes of this review, myo-inositol will be referred to simply as inositol.

In eukaryotes inositol is used to make PI, which serves as a structural component of the membrane, but also as a precursor for several other very important lipid molecules including sphingolipids, ceramides and glycosylphosphatidylinositol (GPI) anchors (Michell, 2008). GPI anchors are used to attach a number of proteins to the plasma membrane and are essential in eukaryotic microbes (Ferguson et al., 1994; Orlean & Menon, 2007). Certain GPI-anchored proteins play a special role in the virulence of both fungal and protozoan pathogens, serving as adhesins and/or variable epitopes to evade the immune system (Ferguson et al., 1994; Sundstrom, 2002). PI is also the precursor for a wide variety of membrane-bound and non-membrane-bound phosphorylated inositol signal-transduction molecules (Michell, 2008; Strahl & Thorner, 2007). In addition, inositol serves as a ‘compatible osmolyte’ in some metazoan cell-types such as kidney and brain cells (Burg, 1997; Fisher et al., 2002).

Mycobacteria are one of the few groups of eubacteria where PI is found in the membrane. PI serves as a precursor for the generation of more complex glycolipids that compose the outer cell wall of mycobacteria (Michell, 2008; Nigou et al., 2003). In addition, the mycobacteria, along with other actinomycetes, use inositol to generate a thiol compound called mycothiol that serves many of the same functions as glutathione in other organisms, including detoxification and protecting the cell from oxidative damage (reviewed by Newton & Fahey, 2002).

Most eubacteria besides the Actinomycetes do not utilize inositol in their lipid membranes or as a redox molecule. However, a number of bacteria such as Bacillus subtilis (Yoshida et al., 1997, 2008), Aerobacter aerogenes (Berman & Magasanik, 1966), Rhizobium leguminosarum bv. viciae (Fry et al., 2001), Sinorhizobium meliloti (Galbraith et al., 1998), Corynebacterium glutamicum (Krings et al., 2006) and Lactobacillus casei (Yebra et al., 2007) can utilize inositol as a carbon source. Even some microbial eukaryotes (such as Cryptococcus species) have been shown to use inositol as a carbon source (Healy et al., 1977).

**De novo biosynthesis of inositol**

The de novo generation of inositol occurs by a universal mechanism that is conserved from eubacteria to archaea to eukaryotes (Majumder et al., 1997). It depends on the tandem action of two enzymes. The first enzyme is inositol-3-phosphate synthase, which was first cloned from Saccharomyces cerevisiae and was called Ino1p (Donahue & Henry, 1981), a name which will be used throughout this review. This enzyme, which converts glucose 6-phosphate to inositol 3-phosphate via a cyclization reaction, constitutes the rate-limiting step in de novo synthesis. NAD⁺ serves as both a hydrogen acceptor and donor so no net NADH is formed in this reaction (Majumder et al., 1997). The second enzyme in inositol synthesis is an inositol monophosphatase (IMPase), which dephosphorylates inositol 3-phosphate to create inositol (Michell, 2008). In S. cerevisiae, two redundant phosphatases, ScInm1p and ScInm2p, serve this purpose (Lopez et al., 1999). The inositol synthesized by the sequential action of these enzymes can then be used to generate inositol-containing compounds such as PI or mycothiol that, in turn, can be used to make more complex molecules, depending on the organism.

**Imported inositol**

The alternative mechanism for obtaining inositol is to import it from the environment via inositol transporters. The two main types of transporters found in both metazoa and microbes are Na⁺- or H⁺-linked cotransporters, which both rely on ion gradients to transport inositol into cells against concentration gradients. In humans, SMIT1 and SMIT2 encode Na⁺/inositol symporters (Coady et al., 2002; Kwon et al., 1992), and HMIT encodes a H⁺/inositol symporter (Uldry et al., 2001). A few inositol transporters have been characterized in human pathogens. For example, Trypanosoma cruzi has at least two different transporters based on a biochemical analysis of import activities, although neither transporter has actually been cloned. One transporter activity is Na⁺-linked and the other is not (Einicker-Lamas et al., 2000, 2007). In contrast to the case in T. cruzi, Leishmania donovani and Candida albicans have only one inositol transporter each, and in both cases the transporter is a H⁺-linked symporter (Drew et al., 1995; Jin & Seyfang, 2003). Inositol transporter activity has also been identified in the bacterial pathogen Mycobacterium tuberculosis as well as the environmental species Mycobacterium smegmatis. Although putative transporter genes have been identified bioinformatically in both M. tuberculosis and M. smegmatis, these genes have not yet been experimentally linked to transport activities by gene disruptions or transport assays (Movahedzadeh et al., 2004; Newton et al., 2006).

**Mycobacterium species**

In the pathogenic bacterium Mycobacterium tuberculosis, disruption of the inositol phosphate synthase gene MtINO1 ablated the ability of the pathogen to cause disease in a mouse model of infection (Movahedzadeh et al., 2004). The ability of M. tuberculosis to grow within macrophages is essential to virulence. Experiments done in tissue culture macrophages revealed that the Mtino1Δ mutant was killed more efficiently by macrophages than the wild-type strain (Movahedzadeh et al., 2004). Thus, these results indicate that M. tuberculosis needs to generate inositol de novo in order to survive in a macrophage and cause disease, and cannot compensate by importing inositol from the host. This is not because M. tuberculosis lacks the ability to
import inositol altogether. It may be because its ability to import inositol is not efficient. The \( \text{Mtino1D} \) mutant can only grow at wild-type rates on medium containing high inositol concentrations (\( \geq 77 \text{ mM} \), Fig. 1a). It grows poorly in medium containing 10 mM inositol and not at all in medium with 1 mM inositol (Movahedzadeh et al., 2004). The concentration of inositol in human serum is 61.0 \( \pm \) 12.4 \( \mu \text{M} \) and in rats it is 20–100 \( \mu \text{M} \) (Isaacks et al., 1997; Kouzuma et al., 2001; Palmano et al., 1977), which suggests that there is an insufficient concentration of inositol to support growth of the \( \text{Mtino1D} \) mutant while it is in the host.

The 77 mM inositol concentration required to support growth of the \( \text{Mtino1D} \) mutant is very high when compared to other organisms. For example, an \( S. \text{cerevisiae Scino1D} \) mutant can grow on 10 \( \mu \text{M} \) inositol (Graves & Henry, 2000), and an \( \text{Msino1D} \) mutant of the environmental mycobacterium \( M. \text{smegmatis} \) can grow on 1 mM inositol (Fig. 1a) (Alderwick et al., 2007; Haites et al., 2005). The fact that the \( \text{Mtino1D} \) mutant requires such high concentrations of inositol suggests that it does not carry a true inositol transporter, but rather a sugar transporter that can also transport inositol at low efficiency. This question can be addressed when this transporter is cloned and characterized.

The reasons why the \( \text{Mtino1D} \) mutant is not virulent in mice and loses viability in macrophages are not clear. This is in part because there are conflicting data on which downstream inositol-containing molecules are affected by inositol depletion and what role these molecules have in virulence. There are two main types of molecules synthesized from inositol that affect growth and/or virulence characteristics. One molecule is PI, which is used to make inositol-containing lipoglycans such as phosphatidylinositol mannoside (PIM), lipomannan (LM) and lipoarabinomannan (LAM) that localize to the waxy envelope. The other molecule is mycothiol, which is used for redox chemistry (Alderwick et al., 2007; Nigou et al., 2003; Rawat & Av-Gay, 2007).

PI and PIM are required for viability, and LAM modulates the immune response in a manner required for virulence. LAM blocks maturation of the phagosome, which is important for \( M. \text{tuberculosis} \) to survive in the macrophage (reviewed by Briken et al., 2004).
experience a drop in these lipoglycans (Fig. 1b) (Movahedzadeh et al., 2004). The mutant also did not suffer a loss of viability. Similar results were observed for an ino1Δ mutant made in the environmental mycobacterium M. smegmatis as long as it was not actively growing. The Msino1Δ mutant did not exhibit a decrease in viability or lipoglycan levels when incubated in inositol-free media at a high density, where it was not replicating (i.e. stationary phase). However, an opposite result was observed when the Msino1Δ mutant was diluted to a low density (i.e. exponential phase) in inositol-free medium. In exponential phase the Msino1Δ mutant lost viability over time, and the levels of cell wall lipoglycans decreased (Fig. 1c) (Haites et al., 2005). It is not clear if the Mtino1Δ mutant will lose viability if it is incubated in inositol-free medium at a lower density (Movahedzadeh et al., 2004). If an Mtino1Δ mutant loses viability and lipoglycan production during exponential growth, as seen in the Msino1Δ mutant, it may experience a decrease in lipoglycans during infection, which may contribute to its loss of viability and virulence.

Alternatively, it is also possible that ino1Δ mutants constructed in M. smegmatis and M. tuberculosis exhibit fundamental differences in how they respond to inositol deprivation. The Mtino1Δ mutant may simply arrest growth in inositol-free media, but remain viable, regardless of the concentration to which it is diluted. M. smegmatis and M. tuberculosis differ in a number of substantial ways. While M. tuberculosis is an intracellular parasite, M. smegmatis is an environmental saprophyte. In addition, M. smegmatis grows much faster than M. tuberculosis (doubling time of 4 h for M. smegmatis as opposed to 15–22 h for M. tuberculosis; Bhatt et al., 1998; James et al., 2000). In addition, the Msino1Δ mutant grows as well as wild-type in much lower concentrations of inositol (1 mM inositol for Msino1Δ as opposed to 77 mM for Mtino1Δ: Haites et al., 2005). The effect that the loss of de novo inositol synthesis has on viability and lipoglycan production in M. tuberculosis with regard to virulence and viability in macrophages and mice is not yet understood and further work in this area is needed.

Mycothiol levels were clearly affected by inositol deprivation in the Mtino1Δ mutant (Movahedzadeh et al., 2004). This mutant exhibited a decrease in the level of mycothiol in inositol-free medium (Fig. 1b), and it was proposed that a drop in mycothiol might make Mtino1Δ mutants more susceptible to the oxidative damage that occurs after the cells are phagocytosed by macrophages (Movahedzadeh et al., 2004). However, a role for mycothiol in virulence is not clear. Some viable mycothiol biosynthetic mutants (mshB and mshD) are more sensitive than wild-type M. tuberculosis strains to oxidative damage, and do not grow well in macrophages, presumably because of a lowered resistance to oxidative damage (Newton et al., 2008; Rengarajan et al., 2005). In contrast to these results, another mycothiol mutant called msha, which lacks detectable mycothiol, is almost fully virulent in SCID and C57Bl/6 mice, suggesting that mycothiol is not required for virulence (Vilchêze et al., 2008).

The actual molecular basis for the Mtino1Δ mutant’s decrease in virulence is still unclear and much work remains to be done in order to fully understand it.

**Trypanosoma brucei**

*Trypanosoma brucei* is another microbe that must be able to generate inositol *de novo* in order to survive. Paradoxically, *T. brucei* is able to efficiently import inositol from the surrounding environment, but if it cannot synthesize inositol *de novo* it loses viability (Martin & Smith, 2006b).

The inability to obtain a homozygous *TbINO1* disruption in *T. brucei* suggested that this gene is essential. This was shown to be the case using a strain carrying a conditionally expressed allele of *TbINO1*. The two copies of *TbINO1* were disrupted only after the cell was transformed with a plasmid carrying *TbINO1* on a tetracycline-inducible promoter. The resulting strain (*INO1- myc*<sup>T</sup>) grew only when tetracycline was present in the medium (and *TbINO1* was transcribed). When tetracycline was absent the *INO1- myc*<sup>T</sup> strain failed to grow, indicating that *TbINO1* is essential (Martin & Smith, 2006b).

Imported inositol is used in synthesis of GPls at only about 5% the rate of *de novo*-generated inositol (Martin & Smith, 2006b). Metabolic labelling experiments revealed that exogenous [<sup>3</sup>H]inositol was only poorly incorporated into glycolipid precursors (A and C) that are used for synthesizing the GPI anchors (Nagamune et al., 2000) used to attach essential proteins like variable surface glycoprotein (VSG) to the plasma membrane (Sheader et al., 2005). Since [<sup>3</sup>H]inositol is very efficiently incorporated into PI in the membrane, the failure to incorporate label into VSG is not simply a matter of inadequate inositol import. In contrast, when [<sup>3</sup>H]glucose is used in labelling experiments, the GPI anchor precursors (glycolipids A and C) are very efficiently labelled and incorporated into mature GPI-anchored VSGs. Thus, [<sup>3</sup>H]glucose is first converted to glucose 6-phosphate, which Tblno1p then converts to inositol 3-phosphate, which is dephosphorylated to inositol that is incorporated efficiently into GPI anchors.

How can such a contradiction be explained? A model put forth by the authors suggests that there are two pools of phosphatidylinositol synthase (PIS) that tend to use inositol derived from the two different main sources: import and *de novo* synthesis. One pool of PIS is localized to the Golgi (Martin & Smith, 2006a) and uses imported inositol to generate the majority of bulk PI in the cell. The other PIS is localized to the endoplasmic reticulum (ER) (Martin & Smith, 2006a), the site where an inositol monophosphatase (IMPase) is localized (reported as unpublished results by Martin & Smith, 2006a). The authors propose that the inositol 3-phosphate synthesized
by TbIno1p is dephosphorylated at the ER by IMPase, and as a result the majority of inositol fed into the ER-localized PIS comes from inositol 3-phosphate. This ER-localized PIS is proposed to be the source of PI used for GPI anchor assembly, which also occurs in the ER. If this model is correct, then it would explain why T. brucei requires TbINO1 despite its ability to import inositol. It was suggested by the authors that this mechanism may ensure that T. brucei always has a ready supply of inositol for the generation of VSGs. This model is summarized in Fig. 2.

The inositol transporter for T. brucei has not been investigated. However, work has been done on the inositol transport characteristics of the closely related parasite T. cruzi. T. cruzi possesses two inositol transport activities. One is Na\(^+\)-dependent and affects transport at high concentrations of inositol (5–10 μM), and the other is Na\(^+\)-independent and depends on a Na\(^+\) gradient established across the plasma membrane. Addition of the Na\(^+\) ATPase inhibitor furosemide partially inhibits both of these activities. Furosemide does inhibit the Na\(^+\)-dependent activity more than the ‘Na\(^+\)-independent activity’ (Einicker-Lamas et al., 2000, 2007), but this result calls the Na\(^+\)-independence of the second activity into question. Inositol transport is energy dependent, as the addition of energy poisons such as dinitrophenol, azide and KCN also inhibit it. High concentrations of hexoses, glucose and mannose do not diminish inositol import, indicating some specificity for inositol, although this specificity has not been exhaustively examined.

A number of intriguing questions remain for understanding inositol acquisition in Trypanosoma species. It remains to be seen if T. cruzi, like T. brucei, requires its INO1 homologue for viability. Furthermore, it is not known for either of these parasites if inositol transport is required for viability or virulence. If most de novo-synthesized inositol is used for GPI biosynthesis, will a block in inositol transport compromise bulk PI biosynthesis and affect virulence or viability? Answering this question will require identification of the inositol transporter genes in these parasites and analysis of their function using gene deletion approaches.

**Leishmania**

A related kinetoplastid parasite, *Leishmania mexicana*, may share with T. brucei a requirement for de novo inositol biosynthesis for full wild-type growth and virulence. The Lmino1\(\Delta\) mutant, which is an inositol auxotroph, exhibited poor growth even in the presence of exogenous inositol up to 2 mM (Ilg, 2002). In liquid cultures, growing as promastigotes for over 120 h, the Lmino1\(\Delta\) mutant reached a density of only 25% that of wild-type. The Lmino1\(\Delta\) mutant was also avirulent in a mouse infection model. Genetic reconstitution experiments revealed that the Lmino1\(\Delta::\) LmINO1 reintegrate strain remained avirulent and grew to no greater density than the Lmino1\(\Delta\) mutant in the presence of inositol, even though inositol prototrophy was restored. The reason why virulence and optimal growth were not reconstituted is not clear. It is possible that the reconstituted LmINO1 gene is poorly expressed compared to the wild-type gene. It is also possible that the avirulence and poor growth phenotypes are genetically unlinked to the inositol auxotrophy observed in the Lmino1\(\Delta\) mutant. While these results are not conclusive, they suggest the possibility that L. mexicana, like T. brucei, may be unable to utilize exogenous inositol efficiently for GPI synthesis. If this is the case, then utilization of exogenous inositol appears to be more efficient in L. mexicana than T. brucei, since L. mexicana exhibits some limited growth. This is an area for further investigation.

![Fig. 2. The eukaryotic parasite Trypanosoma brucei is able to import inositol from the environment or synthesize it de novo. However, according to the current model, inositol imported from the environment is utilized primarily in bulk phosphatidylinositol (red and yellow phospholipid) production via a phosphatidylinositol synthase (PIS) localized to the Golgi complex. Inositol synthesized de novo is primarily used to generate phosphatidylinositol that is used for production of glycosylphosphatidylinositol (GPIs). The de novo-synthesized inositol is believed to be utilized mostly for GPI production because the IMPase that dephosphorylates inositol 3-phosphate to inositol is localized to the ER, where GPI synthesis occurs. Mutants lacking TbINO1 are inviable because of diminished GPI production.](image)
similar to that of the sugar transporter family (Drew et al., 1995; Jin & Seyfang, 2003; Seyfang & Landfear, 2000). MIT may be necessary for full virulence, as a disruption of the *L. donovani* MIT gene resulted in a mutant that grew at a reduced rate. However, its virulence has not been analysed in an animal model (Mongan et al., 2004).

Despite its sequence similarity to sugar transporters, MIT is very specific for inositol and does not appear to recognize sugar molecules of similar structure such as glucose, galactose, mannose, fucose, xylose and others (Mongan et al., 2004). It has been suggested that it may be possible to exploit this specificity to design toxic analogues of inositol as antibiotics, since the specificities of the *Leishmania* MIT and the human Na\(^+\)/inositol transporters SMIT1 and SMIT2 are different. For example, SMIT1 recognizes fucose (Hager et al., 1995), and SMIT2 recognizes glucose and xylose (Coady et al., 2002). However, a complete one-to-one comparison of substrate specificities for the human H\(^+\)/inositol transporter (HMIT; Uldry et al., 2001) and *L. donovani* MIT has not been performed. Nonetheless, an exciting possibility is that if there is a difference between the specificities of MIT and HMIT, then it may be possible to create toxic analogues of inositol that will specifically be taken up by *Leishmania* MIT and not by human inositol transporters.

### Other parasites

A potential contrast to the above-mentioned parasites may exist among the apicomplexan parasites *Toxoplasma gondii* and *Cryptosporidium parvum*. BLAST searches against the whole-genome sequences of these parasites at EUPATH (http://eupathdb.org/eupathdb/) failed to reveal a homologue for Ino1p. It is possible that *T. gondii* and *Cryptosporidium* do have Ino1p homologues that were missed in the BLAST search algorithm, or they may have functional homologues that differ in primary sequence. However, if indeed these pathogens cannot make inositol *de novo*, then they must have mechanisms for acquiring inositol from the host. This would make inositol transporters essential for these pathogens, which would be an interesting contrast to the case of *Trypanosoma brucei*, where *de novo* synthesis is essential.

### Candida albicans

*Candida albicans* appears to be much more versatile than the above pathogens. During a bloodstream infection it is able to cause infection with equal efficiency whether it imports inositol or makes it *de novo* (Chen et al., 2008). A *C. albicans* Caino1Δ/Caino1Δ mutant is an inositol auxotroph. However, unlike *T. brucei*, *L. mexicana* or *M. tuberculosis*, *C. albicans* Caino1Δ/Caino1Δ mutants are as virulent as wild-type in a mouse model of systemic infection. These data initially suggested two possibilities: (1) *C. albicans* needs to transport inositol rather than make it *de novo* to support an infection; (2) *C. albicans* can synthesize or transport inositol with equal efficiency to support an infection.

*C. albicans* possesses a high-affinity inositol transporter that, like the *L. donovani* transporter, is dependent on a proton gradient across the membrane (Jin & Seyfang, 2003). Biochemical analysis of the transport kinetics suggests that there is only one transporter that is specific for myo-inositol, and it will not recognize glucose, galactose, mannose, fructose, fucose, arabinose and xylose. Based on homology to the *S. cerevisiae* inositol transporters ScItr1p and ScItr2p (Nikawa et al., 1991), the *C. albicans* transporter was identified and cloned, and the gene was disrupted (Chen et al., 2008). The Cain1Δ/Cain1Δ mutant lacked inositol transport activity compared to the wild-type strain, indicating that there is only one transporter, as the previous kinetic studies had suggested (Jin & Seyfang, 2003). Despite the lack of inositol transport, the Cain1Δ/Cain1Δ mutant is fully virulent in a mouse model of systemic infection.

In order to determine if Calno1p and Caltr1p provide the only two routes for acquiring inositol from the host, a conditional double mutant between Caino1Δ and Cain1Δ was constructed. In *C. albicans* the MET3 promoter (\(P_{\text{MET3}}\)) can be used as a conditional promoter because \(P_{\text{MET3}}\) is strongly activated in the absence of sulfur-containing amino acids such as cysteine and methionine (Cys/Met), but in the presence of Cys/Met in the medium \(P_{\text{MET3}}\) is transcriptionally repressed (Care et al., 1999). A conditional double mutant was constructed by disrupting both copies of CaINO1 (Caino1Δ/Caino1Δ) and one copy of CaITR1 (Caitr1Δ). The promoter of the remaining wild-type copy of CaITR1 was replaced on the chromosome with the \(P_{\text{MET3}}\) promoter. The resulting strain grew at a wild-type rate in the absence of Cys/Met in the medium, but did not grow at all in the presence of Cys/Met. These data indicated that *C. albicans* has no way to obtain inositol *in vitro* aside from *de novo* synthesis through Calno1p or import via Caltr1p. This appears to be true for *C. albicans* in the host as well, since in a mouse model for systemic infection, the conditional double mutant was avirulent and the mice showed no symptoms of infection, which is consistent with the hypothesis that the strains could not survive due to a lack of inositol. The mouse bloodstream contains sufficient Cys/Met to shut off the \(P_{\text{MET3}}\) promoter (Chen et al., 2008; Rodaki et al., 2006). Even a strain that contained homozygous mutations for Caino1 and was heterozygous for Caltr1 was attenuated for virulence compared to the wild-type, although it was more virulent than the conditional double mutant.
These data indicate that *C. albicans*, unlike *M. tuberculosis* and *T. brucei*, is able to acquire inositol by importing it from the host or by synthesizing it *de novo* (Fig. 3), and either mechanism is sufficient to cause a wild-type infection. The reasons for these differences in strategies are unclear, but one large difference between *M. tuberculosis* and *T. brucei* and *C. albicans* is that *C. albicans* is a commensal organism. It is normally found in the gut, oral and/or vaginal tracts of humans (Calderone, 2002). Its versatility may help it grow in many different host environments. However, this hypothesis has yet to be tested, as these mutants have not been examined in the context of vaginal, oral or gut infection models. In addition, even in the mouse bloodstream has yet to be tested, as these mutants have not been normally found in the gut, oral and/or vaginal tracts of humans (Calderone, 2002). Its versatility may help it grow in many different host environments. However, this hypothesis has yet to be tested, as these mutants have not been examined in the context of vaginal, oral or gut infection models. In addition, even in the mouse bloodstream has yet to be tested, as these mutants have not been.

How and when CaIno1p and CaIno4p are expressed in the host will be best understood when regulators of these genes are identified. Although *S. cerevisiae* is often a useful guide for exploring such questions in *C. albicans*, in this case there appears to be transcriptional rewiring between these two yeasts regarding the regulation of their respective Ino1p homologues. Thus, CaIno1p regulation is an area of research where much remains to be learned.

The *S. cerevisiae* transcriptional regulators of ScITR1 and ScINO1 are well known. Two proteins, ScIno2p and ScINO1, form a heterodimeric transcriptional activator of ScITR1 and ScINO1 as well as other phospholipid biosynthetic genes. The protein ScOpi1p acts as a repressor of these same targets by binding to ScIno2p (reviewed by Chen *et al.*, 2007; Greenberg & Lopes, 1996). Recent studies have revealed that the *C. albicans* sequence homologues of ScIno2p and ScIno4p (CaIno2p and CaIno4p) do not appear to regulate CaINO1, and may regulate ribosomal genes (Hoppen *et al.*, 2007). It was even suggested that CaINO2 and CaINO4 are essential, although these studies did not conclusively demonstrate this. In addition, CaOpi1p (a sequence homologue of ScOpi1p) does not repress transcription of CaINO1. A Caopi1pΔ/Caopi1pΔ mutant represses CaINO1 in response to exogenous inositol levels in the same manner as a wild-type strain (Y. L. Chen & T. B. Reynolds, unpublished data). This is despite the fact that CaOpi1p can complement a Scopi1Δ mutant for regulation of ScINO1 when expressed heterologously in *S. cerevisiae* (Heyken *et al.*, 2003). Thus, the actual regulators of CaINO1, CaITR1 and other *C. albicans* phospholipid biosynthetic genes remain to be identified. Identification of basic regulatory components for phospholipid biosynthesis in *C. albicans* will aid in understanding the regulation of CaIno1p and CaIno4p during an infection.

It is not known why *C. albicans* mutants lose viability when deprived of inositol, but it is very likely due to the pleiotropic loss of PI and its downstream products, including GPI anchors (Doering & Schekman, 1996), membrane phosphoinositides (Strahl & Thorner, 2007) and sphingolipids (Dickson & Lester, 1999). However, it remains to be determined precisely what defects cause the loss of viability during infection.

**Cryptococcus neoformans**

*Cryptococcus neoformans* is a fungal pathogen that causes a fatal meningoencephalitis especially in immunocompromised patients such as those with acquired immune deficiency syndrome (AIDS) (Casadevall & Perfect, 1998). *Cryptococcus* species such as *C. neoformans* and *C. gattii* are believed to enter the host through the inhalation of spores or desiccated yeast cells that are produced in the environment. The known environmental niches of *C. neoformans* and *C. gattii* are pigeon guano and eucalyptus trees, respectively (Casadevall & Perfect, 1998; Idnurm *et al.*, 2005). This led to the hypothesis that *Cryptococcus* species might sporulate in association with plants or trees, and it was recently demonstrated that substances extracted from plants can stimulate mating and sporulation in both *C. neoformans* and *C. gattii* (Xue *et al.*, 2007). Interestingly, the substance with the strongest effect on mating and sporulation was inositol, which is abundant in plants. If spore formation by *Cryptococcus* species is a major source of infectious propagules for humans, then inositol may be an environmental cue that promotes human infections by inducing mating and sporulation.

The mechanism by which inositol promotes mating and sporulation in *Cryptococcus* species is not known, but other...
observations suggest that Cryptococcus species need to acquire a relatively large amount of inositol. C. neoformans not only generates inositol through a functional inositol-3-phosphate synthase (CnIno1p), it also catabolizes inositol as a sole carbon source via an inositol oxygenase (IOase) activity (Kanter et al., 2003; Molina et al., 1999). Although some Cryptococcus species repress their IOase activity in the presence of glucose as a carbon source (Kanter et al., 2003), C. neoformans expresses IOase activity even when glucose is present in the medium, and the IOase activity increases when inositol is the sole carbon source (Molina et al., 1999). The CnIno1p activity of C. neoformans is much higher than that of S. cerevisiae. For example, in C. neoformans the CnIno1p activity is about sevenfold greater than that of S. cerevisiae when each strain is grown in medium containing glucose, and lacking inositol. When excess inositol is added to the medium (75 μM), the ScIno1p activity is reduced to undetectable levels in S. cerevisiae, but in C. neoformans the CnIno1p activity level is similar to that of S. cerevisiae in the absence of inositol. Only when inositol is used as the sole carbon source is CnIno1p activity reduced to undetectable levels (Molina et al., 1999). Bioinformatic analysis predicts that C. neoformans has approximately seven inositol transporters (Xue et al., 2007). Altogether these data suggest that C. neoformans has a strong requirement for inositol.

The reasons why C. neoformans has such a strong requirement for inositol are unclear. In plants inositol is converted by an IOase activity to glucuronic acid, which feeds into both the pentose phosphate pathway and the UDP-glucuronic acid pathway for cell wall biosynthesis (Loewus et al., 1962; Seitz et al., 2000; Tenhaken & Thulke, 1996). In C. neoformans UDP-glucuronic acid is important for manufacturing an important virulence factor, the capsule (Bar-Peled et al., 2004; Griffith et al., 2004; Moyrand & Janbon, 2004). It has been postulated that the IOase activity in C. neoformans is supplying glucuronic acid used to make UDP-glucuronic acid for capsule biosynthesis (Kanter et al., 2003). However, this does not appear to be the case, as two different studies have shown that mutants carrying disruptions of the UGD1 gene, which encodes the UDP-glucose dehydrogenase that converts UDP-glucose to UDP-glucuronic acid, completely lack UDP-glucuronic acid and capsule synthesis (Griffith et al., 2004; Moyrand & Janbon, 2004). This indicates that in C. neoformans the IOase does not supply UDP-glucuronic acid for capsule biosynthesis. It is possible that IOase does generate glucuronic acid for the pentose phosphate pathway (Hankes et al., 1969; Kanter et al., 2003; Prabhu et al., 2005). Whether CnIno1p, IOase or the many inositol transporter homologues in C. neoformans play a role in virulence in humans is unclear. The role that inositol plays in stimulating mating in C. neoformans and its virulence as a dikaryon in plants (Xue et al., 2007) is also not understood.

C. neoformans has a tropism for the central nervous system, which is rich in inositol (Fisher et al., 2002; Heitman et al., 2006). Tropism for the brain could be driven by the strong requirement for inositol (Molina et al., 1999). It has also been suggested that brain tropism is driven by the abundance of catecholamines in the brain, which can serve as precursors for biosynthesis of melanin, another virulence factor (Eisenman et al., 2007; Polacheck et al., 1990). These factors are not necessarily mutually exclusive. Much work remains to be done to understand if there is a connection between Cryptococcus inositol metabolism and brain tropism.

Prospectus

The broad range of mechanisms by which pathogens synthesize phospholipids during an infection, and the surprising strategies that pathogens use to acquire inositol for PI synthesis while in the host, demonstrate that this area of research will reveal many fascinating aspects of host–pathogen biology. It is interesting that two very different pathogens, Trypanosoma brucei and Mycobacterium tuberculosis, one an extracellular eukaryotic parasite and the other an intracellular prokaryotic parasite, both require inositol biosynthesis for survival and pathogenesis, respectively. The reasons for this requirement, however, are quite different. In contrast, the fungal pathogen Candida albicans requires neither synthesis nor import exclusively, but can survive using either. These studies raise many questions about inositol acquisition in the host. Is the requirement for de novo inositol biosynthesis found in other mycobacterial pathogens such as M. leprae and M. ulcerans? Do other parasites besides T. brucei require de novo inositol biosynthesis for viability or virulence? Do the apicomplexan parasites Toxoplasma gondii and Cryptosporidium parvum carry functional INO1 homologues? Do other fungal pathogens besides C. albicans exhibit versatility in their acquisition of inositol?

Another area for future inquiry is whether any of the enzymes involved in inositol biosynthesis or import may be useful drug targets. For example, could the requirement for the Ino1p enzyme be exploited to generate novel antimicrobials for T. brucei or M. tuberculosis? This enzyme is admittedly not ideal because a homologue exists in the host. However, it may be possible to identify a drug that exhibits specificity for the parasite or bacterial enzyme.

Calno1p clearly cannot serve as a drug target in C. albicans. However, there are other enzymes in this fungal pathogen involved in phospholipid biosynthesis that may be more promising. For example, the phosphatidylycerine synthase (CaCHO1) of C. albicans is unique to fungi and not found in humans (Braun et al., 2005). This has been suggested as a useful drug target. Similarly, enzymes involved in phosphatidylcholine biosynthesis have been suggested as potential antimicrobial drug targets in Plasmodium falciparum (Witola et al., 2008). Phospholipid biosynthesis in these pathogens appears to be a promising area for exploration.
Acknowledgements

I would like to thank Dr Neil Stoker, Dr Terry Smith, Dr Joseph Heitman, Dr Chaoyang Xue and especially Dr Pamela Small for their insightful comments and helpful discussions regarding this manuscript. This work was supported by grants AHA 0765366B and NIH-1R03AI071863-01A1.

References

Alderwick, L. J., Birch, H. L., Mishra, A. K., Eggeling, L. & Besra, G. S. (2007). Structure, function and biosynthesis of the Mycobacterium tuberculosis cell wall: arabinogalactan and lipoarabinomannan assembled with a view to discovering new drug targets. Biochem Soc Trans 35, 1325–1328.

Bar-Peled, M., Griffith, C. L., Ory, J. J. & Doering, T. L. (2004). Biosynthesis of UDP-GlcA, a key metabolite for capsular polysaccharide synthesis in the pathogenic fungus Cryptococcus neoformans. Biochem J 381, 131–136.

Berman, T. & Magasanik, B. (1966). The pathway of myo-inositol degradation in Aerobacter aerogenes. Dehydrogenation and dehydration. J Biol Chem 241, 800–806.

Bhatt, A., Green, R., Coles, R., Condon, M. & Connell, N. D. (1998). A mutant of Mycobacterium smegmatis defective in dipeptide transport. J Bacteriol 180, 6773–6777.

Braun, B. R., van Het Hoog, M., d'Enfert, C., Martchenko, M., Dungan, J., Kuo, A., Inglis, D. O., Uhl, M. A., Hugues, H. & other authors (2008). A human-curated annotation of the Candida albicans genome. PLoS Genet 4, 36–57.

Briken, V., Porcelli, S. A., Besra, G. S. & Kremer, L. (2004). Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. Mol Microbiol 53, 391–403.

Burg, M. B. (1997). Renal osmoregulatory transport of compatible organic osmolytes. Curr Opin Nephrol Hypertens 6, 430–433.

Calderone, R. A. (2002). Candida and Candidiasis. Washington, DC: American Society for Microbiology.

Care, R. S., Trevethick, J., Binley, K. M. & Sudbery, P. E. (1999). The MET3 promoter: a new tool for Candida albicans molecular genetics. Mol Microbiol 34, 792–798.

Casadevall, A. & Perfect, J. R. (1998). Cryptococcus neoformans. Washington, DC: American Society for Microbiology.

Chen, M., Hancock, L. C. & Lopes, J. M. (2007). Transcriptional regulation of yeast phospholipid biosynthetic genes. Biochim Biophys Acta 1771, 310–321.

Chen, Y. L., Kaufman, S. & Reynolds, T. B. (2008). Candida albicans uses multiple mechanisms to acquire the essential metabolite inositol during infection. Infect Immun 76, 2793–2801.

Coady, M. J., Wallendorf, B., Gagnon, D. G. & Lapointe, J. Y. (2002). Identification of a novel Na+/inositol cotransporter. J Biol Chem 277, 35219–35224.

Dickson, R. C. & Lester, R. L. (1999). Yeast sphingolipids. Biochim Biophys Acta 1426, 347–357.

Doering, T. L. & Schekman, R. (1996). GPI anchor attachment is required for Gas1p transport from the endoplasmic reticulum in COP II vesicles. EMBO J 15, 182–191.

Donahue, T. F. & Henry, S. A. (1981). myo-Inositol-1-phosphate synthase. Characteristics of the enzyme and identification of its structural gene in yeast. J Biol Chem 256, 7077–7085.

Drew, M. E., Langford, C. K., Klamo, E. M., Russell, D. G., Kavanaugh, M. P. & Landfear, S. M. (1995). Functional expression of a myo-inositol/H+ symporter from Leishmania donovani. Mol Cell Biol 15, 5508–5515.

Einicker-Lamas, M., Almeida, A. C., Todorov, A. G., de Castro, S. L., Caruso-Neves, C. & Oliveira, M. M. (2000). Characterization of the myo-inositol transport system in Trypanosoma cruzi. Eur J Biochem 267, 2533–2537.

Einicker-Lamas, M., Nascimento, M. T., Masuda, C. A., Oliveira, M. M. & Caruso-Neves, C. (2007). Trypanosoma cruzi epimastigotes: regulation of myo-inositol transport by effectors of protein kinases A and C. Exp Parasitol 117, 171–177.

Eisenman, H. C., Mues, M., Weber, S. E., Frases, S., Chaskes, S., Gerfen, G. & Casadevall, A. (2007). Cryptococcus neoformans laccase catalyses melanin synthesis from both D- and L-DOPA. Microbiology 153, 3954–3962.

Ferguson, M. A., Brimacombe, J. S., Cottaz, S., Field, R. A., Güther, L. S., Homans, S. W., McConville, M. J., Mehliert, A., Milne, K. G. & other authors (1994). Glycosyl-phosphatidylinositol molecules of the parasite and the host. Parasitology 108 (Suppl), 845–554.

Fisher, S. K., Novak, J. E. & Agranoff, B. W. (2002). Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. J Neurochem 82, 736–754.

Fry, J., Wood, M. & Poole, P. S. (2001). Investigation of myo-inositol catabolism in Rhizobium leguminosarum bv. viciae and its effect on nodulation competitiveness. Mol Plant Microbe Interact 14, 1016–1025.

Galbraith, M. P., Feng, S. F., Borneman, J., Triplett, E. W., de Bruijn, F. J. & Rossbach, S. (1998). A functional myo-inositol catabolism pathway is essential for rhizopine utilization by Sinorhizobium meliloti. Microbiology 144, 2915–2924.

Graves, J. A. & Henry, S. A. (2000). Regulation of the yeast INO1 gene. The products of the INO2, INO4 and OPII regulatory genes are not required for repression in response to inositol. Genetics 154, 1485–1495.

Greenberg, M. L. & Lopes, J. M. (1996). Genetic regulation of phospholipid biosynthesis in Saccharomyces cerevisiae. Microbiol Rev 60, 1–20.

Griffith, C. L., Klutts, J. S., Zhang, L., Lavery, S. B. & Doering, T. L. (2004). UDP-glucose dehydrogenase plays multiple roles in the biology of the pathogenic fungus Cryptococcus neoformans. J Biol Chem 279, 51669–51676.

Hager, K., Hazama, A., Kwon, H. M., Loo, D. D., Handler, J. S. & Wright, E. M. (1995). Kinetics and specificity of the renal Na+/inositol cotransporter expressed in Xenopus oocytes. J Membr Biol 143, 103–113.

Hailes, R. E., Morita, Y. S., McConville, M. J. & Billman-Jacobe, H. (2005). Function of phosphatidylinositol in mycobacteria. J Biol Chem 280, 10981–10987.

Hankes, L. V., Pollitzer, W. M., Touster, O. & Anderson, L. (1989). myo-Inositol catabolism in human pentosurics: the predominant role of the glucuronate-xylulose-pentose phosphate pathway. Ann N Y Acad Sci 165, 564–576.

Healy, M. E., Dillavou, C. L. & Taylor, G. E. (1977). -inositol catabolism in human pentosurics: the predominant role of the glucuronate-xylulose-pentose phosphate pathway. Ann N Y Acad Sci 165, 564–576.

Healy, M. E., Dillavou, C. L. & Taylor, G. E. (1977). -inositol catabolism in human pentosurics: the predominant role of the glucuronate-xylulose-pentose phosphate pathway. Ann N Y Acad Sci 165, 564–576.

Healy, M. E., Dillavou, C. L. & Taylor, G. E. (1977). -inositol catabolism in human pentosurics: the predominant role of the glucuronate-xylulose-pentose phosphate pathway. Ann N Y Acad Sci 165, 564–576.

Healy, M. E., Dillavou, C. L. & Taylor, G. E. (1977). -inositol catabolism in human pentosurics: the predominant role of the glucuronate-xylulose-pentose phosphate pathway. Ann N Y Acad Sci 165, 564–576.
activated by a heterodimeric transcription factor related to Ino2 and Ino4 from S. cerevisiae. Mol Genet Genomics 278, 317–330.

Idnurm, A., Bahn, Y. S., Nielsen, K., Lin, X., Fraser, J. A. & Heitman, J. (2005). Deciphering the model pathogenic fungus Cryptococcus neoformans. Nat Rev Microbiol 3, 753–764.

Ilg, T. (2002). Generation of myo-inositol-auxotrophic Leishmania mexicana mutants by targeted replacement of the myo-inositol-1-phosphate synthase gene. Mol Biochem Parasitol 120, 151–156.

Isaacks, R. E., Bender, A. S., Kim, C. Y. & Norenberg, M. D. (1997). Effect of osmolality and myo-inositol deprivation on the transport properties of myo-inositol in primary astrocyte cultures. Neurochem Res 22, 1461–1469.

James, B. W., Williams, A. & Marsh, P. D. (2000). The physiology and pathogenicity of Mycobacterium tuberculosis grown under controlled conditions in a defined medium. J Appl Microbiol 88, 669–677.

Jin, J. H. & Seyfang, A. (2003). High-affinity myo-inositol transport in Candida albicans: substrate specificity and pharmacology. Microbiology 149, 3371–3381.

Kanter, U., Becker, M., Friauf, E. & Tenhaken, R. (2003). Purification, characterization and functional cloning of inositol oxygenase from Cryptococcus. Yeast 20, 1317–1329.

Kouzuma, T., Takahashi, M., Endoh, T., Kaneko, R., Ura, N., Shimamoto, K. & Watanabe, N. (2001). A high capacity myo-inositol synthase. FEBS Lett 513, 133–141.

Kwong, K. M., Yamauchi, A., Uchida, S., Preston, A. S., Garcia-Perez, A., Burg, M. B. & Handler, J. S. (1999). Cloning of the cDNA for a Na+/myo-inositol cotransporter, a hypertonicity stress protein. J Biol Chem 274, 6297–6301.

Loewus, F. A., Kelly, S. & Neufeld, E. F. (1962). Metabolism of myo-inositol in plants: conversion to pectin, hemicellulose, D-xylene, and sugar acids. Proc Natl Acad Sci U S A 48, 421–425.

Lopez, F., Leube, M., Gil-Mascarell, R., Navarro-Avino, J. P. & Serrano, R. (1999). The yeast inositol monophosphatase is a lithium- and sodium-sensitive enzyme encoded by a non-essential gene pair. Mol Microbiol 31, 1255–1264.

Majumder, A. L., Johnson, M. D. & Henry, S. A. (1997). Biogenesis of the initial steps of mycothiol biosynthesis. J Biol Chem 272, 19895–19901.

Martin, K. L. & Smith, T. K. (2006a). The glycosylphosphatidylinositol (GPI) biosynthetic pathway of bloodstream-form Trypanosoma brucei is dependent on the de novo synthesis of inositol. Mol Microbiol 61, 89–105.

Martin, K. L. & Smith, T. K. (2006b). Phosphatidylinositol synthesis is essential in bloodstream form Trypanosoma brucei. Biochem J 396, 287–295.

Michel, R. H. (2008). Inositol derivatives: evolution and functions. Nat Rev Mol Cell Biol 9, 151–161.

Molina, Y., Ramos, S. E., Douglass, T. & Klig, L. S. (1999). Inositol synthesis and catabolism in Cryptococcus neoformans. Yeast 15, 1657–1667.

Mongan, T. P., Ganapasm, S., Hobbs, S. B. & Seyfang, A. (2004). Substrate specificity of the Leishmania donovani myo-inositol transporter: critical role of inositol C-2, C-3 and C-5 hydroxyl groups. Mol Biochem Parasitol 135, 133–141.

Movahedzadeh, F., Smith, D. A., Norman, R. A., Dinadayala, P., Murray-Rust, J., Russell, D. G., Kendall, S. L., Rison, S. C. & McAlister, M. S. & others authors (2004). The Mycobacterium tuberculosis ino1 gene is essential for growth and virulence. Mol Microbiol 51, 1003–1014.

Moyrand, F. & Janbon, G. (2004). UGD1, encoding the Cryptococcus neoformans UDP-glucose dehydrogenase, is essential for growth at 37 °C and for capsule biosynthesis. Eukaryot Cell 3, 1601–1608.

Nagamune, K., Nozaki, T., Maeda, Y., Ohishi, K., Fukuma, T., Hara, T., Schwarz, R. T., Sutterlin, C., Brun, R. & others authors (2000). Critical roles of glycosylphosphatidylinositol for Trypanosoma brucei. Proc Natl Acad Sci U S A 97, 10336–10341.

Newton, G. L. & Fahey, R. C. (2002). Mycothiol biochemistry. Arch Microbiol 178, 388–394.

Newton, G. L., Ta, P., Bzymek, K. P. & Fahey, R. C. (2006). Biochemistry of the initial steps of mycothiol biosynthesis. J Biol Chem 281, 33910–33920.

Newton, G. L., Buchmeier, N. & Fahey, R. C. (2008). Biosynthesis and functions of mycothiol, the unique protective thiol of Actinobacteria. Microbiol Mol Biol Rev 72, 471–494.

Nigou, J., Gilleron, M. & Puzo, G. (2003). Lipoarabinomannans: from structure to biosynthesis. Biochimie 85, 153–166.

Nikawa, J., Tsukagoshi, Y. & Yamashita, S. (1991). Isolation and characterization of two distinct myo-inositol transporter genes of Saccharomyces cerevisiae. J Biol Chem 266, 11184–11191.

Orlean, P. & Menon, A. K. (2007). The yeasts as lipid transporters: implications for lipid trafficking in mammalian cells. Curr Opin Lipidol 18, 197–203.

Palomo, K. S., Whiting, P. H. & Hawthorne, J. N. (1977). Free and lipid myo-inositol in tissues from rats with acute and less severe streptozotocin-induced diabetes. Biochem J 167, 229–235.

Polacheck, I., Platt, Y. & Aronovitch, J. (1990). Catecholamines and virulence of Cryptococcus neoformans. Infect Immun 58, 2919–2922.

Prabhu, K. S., Arner, R. J., Vunta, H. & Reddy, C. C. (2005). Up-regulation of human myo-inositol oxygenase by hyperosmotic stress in renal proximal tubular epithelial cells. J Biol Chem 280, 19895–19901.

Ratwat, M. & Av-Gay, Y. (2007). Mycothiol-dependent proteins in actinomycetes. FEMS Microbiol Rev 31, 278–292.

Rex, D. S., Bloom, B. R. & Rubin, E. J. (2005). Genome-wide requirements for Mycobacterium tuberculosis adaptation and survival in macrophages. Proc Natl Acad Sci U S A 102, 8327–8332.

Rodaki, A., Young, T. & Brown, A. J. (2006). Effects of depleting the essential central metabolic enzyme fructose-1,6-bisphosphate aldolase on the growth and viability of Candida albicans: implications for antifungal drug target discovery. Eukaryot Cell 5, 1371–1377.

Seitz, B., Klos, C., Wurm, M. & Tenhaken, R. (2000). Matrix polysaccharide precursors in Arabidopsis cell walls are synthesized by alternate pathways with organ-specific expression patterns. Plant J 21, 537–546.

Seyfang, A. & Landfear, S. M. (2000). Conserved cytoplasmic sequence motifs are important for transport function of the Leishmania inositol/H+ symporter. J Biol Chem 275, 5687–5693.

Shadeer, K., Vaughan, S., Minchin, J., Hughes, K., Gull, K. & Rudenko, G. (2005). Variant surface glycoprotein RNA interference triggers a precytokinesis cell cycle arrest in African trypanosomes. Proc Natl Acad Sci U S A 102, 8716–8721.

Strahl, T. & Thorner, J. (2007). Synthesis and function of membrane phosphoinositides in budding yeast, Saccharomyces cerevisiae. Biochim Biophys Acta 1771, 353–404.
Sundstrom, P. (2002). Adhesion in Candida spp. Cell Microbiol 4, 461–469.

Tenhaken, R. & Thulke, O. (1996). Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. Plant Physiol 112, 1127–1134.

Uldry, M., Ibberson, M., Horisberger, J. D., Chatton, J. Y., Riederer, B. M. & Thorens, B. (2001). Identification of a mammalian H\textsuperscript{+}-myo-inositol symporter expressed predominantly in the brain. EMBO J 20, 4467–4477.

Vance, J. E. (2003). Molecular and cell biology of phosphatidylserine and phosphatidylethanolamine metabolism. Prog Nucleic Acid Res Mol Biol 75, 69–111.

van Meer, G., Voelker, D. R. & Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9, 112–124.

Vial, H. J., Eldin, P., Tielen, A. G. & van Hellemond, J. J. (2003). Phospholipids in parasitic protozoa. Mol Biochem Parasitol 126, 143–154.

Vilchêze, C., Av-Gay, Y., Attarian, R., Liu, Z., Hazbón, M. H., Colangeli, R., Chen, B., Liu, W., Alland, D. & other authors (2008). Mycothiol biosynthesis is essential for ethionamide susceptibility in Mycobacterium tuberculosis. Mol Microbiol 69, 1316–1329.

Witola, W. H., El Bissati, K., Pessi, G., Xie, C., Roepe, P. D. & Ben Mamoun, C. (2008). Disruption of the Plasmodium falciparum PPMT gene results in a complete loss of phosphatidylcholine biosynthesis via the serine-decarboxylase-phosphoethanolamine-methyltransferase pathway and severe growth and survival defects. J Biol Chem 283, 27636–27643.

Xue, C., Tada, Y., Dong, X. & Heitman, J. (2007). The human fungal pathogen Cryptococcus can complete its sexual cycle during a pathogenic association with plants. Cell Host Microbe 1, 263–273.

Yebra, M. J., Zuniga, M., Beaufils, S., Perez-Martinez, G., Deutscher, J. & Monedero, V. (2007). Identification of a gene cluster enabling Lactobacillus casei BL23 to utilize myo-inositol. Appl Environ Microbiol 73, 3850–3858.

Yoshida, K. I., Aoyama, D., Ishio, I., Shibayama, T. & Fujita, Y. (1997). Organization and transcription of the myo-inositol operon, iol, of Bacillus subtilis. J Bacteriol 179, 4591–4598.

Yoshida, K., Yamaguchi, M., Morinaga, T., Kinohara, M., Ikeuchi, M., Ashida, H. & Fujita, Y. (2008). myo-Inositol catabolism in Bacillus subtilis. J Biol Chem 283, 10415–10424.