

Finding the Sweet Spot: How Human Fungal Pathogens Acquire and Turn the Sugar Inositol against Their Hosts

Chaoyang Xue

Public Health Research Institute, Department of Microbiology, Biochemistry, and Molecular Genetics, Rutgers University, New Jersey Medical School, Newark, New Jersey, USA

ABSTRACT Inositol is an essential nutrient with important structural and signaling functions in eukaryotes. Its role in microbial pathogenesis has been reported in fungi, protozoans, and eubacteria. In a recent article, Porollo et al. [*mBio* 5(6):e01834-14, 2014, doi:10.1128/mBio.01834-14] demonstrated the importance of inositol metabolism in the development and viability of *Pneumocystis* species—obligate fungal pathogens that remain unculturable *in vitro*. To understand their obligate nature, the authors used innovative comparative genomic approaches and discovered that *Pneumocystis* spp. are inositol auxotrophs due to the lack of inositol biosynthetic enzymes and that inositol insufficiency is a contributing factor preventing fungal growth *in vitro*. This work is in accord with other studies suggesting that inositol plays a conserved role in microbial pathogenesis. Inositol uptake and metabolism therefore may represent novel antimicrobial drug targets. Using comparative genomics to analyze metabolic pathways offers a powerful tool to gain new insights into nutrient utilization in microbes, especially obligate pathogens.

Microorganisms quickly adapt to a changing environment by sensing and responding to environmental cues, such as nutrients. Understanding how pathogens sense and utilize nutrients during pathogen-host interactions provides important insights into how to control diseases caused by these microbes. Inositol is a nutrient that plays important structural and signaling roles in both mammalian hosts and fungal pathogens, in particular by serving as a precursor for phosphatidylinositol (PI), which is a key component of cellular membranes and a signaling molecule (Fig. 1). Phosphorylated derivatives of PI and other products of PI metabolism govern many essential cellular processes, including the osmotic stress response, calcium signaling, vesicle trafficking, telomere length, and chromatin remodeling (1, 2). Inositol-dependent cellular pathways are best studied in model organisms such as *Saccharomyces cerevisiae*, but inositol utilization and metabolism remain poorly understood in many pathogens. In a recent article, Porollo et al. reported using comparative genomics to shed light on inositol utilization by *Pneumocystis*, an important obligate human fungal pathogen (3).

There are two main sources by which fungal cells acquire inositol (Fig. 1). One is to synthesize inositol internally. Intracellular glucose can be converted into *myo*-inositol—the most common biologically active inositol isomer—in a *de novo* inositol biosynthetic pathway (4). *myo*-inositol 1-phosphate synthase (Ino1) is the key enzyme in this synthesis, converting glucose 6-phosphate to inositol 1-phosphate. The second step is carried out by inositol monophosphatase (Inm1), which dephosphorylates inositol 1-phosphate to *myo*-inositol. Inositol can also be imported from the extracellular environment via inositol transporters (ITRs). The ITR gene family is part of the sugar transporter superfamily and plays an important role in inositol sensing in fungi, including *S. cerevisiae* (5, 6), *Candida albicans* (4, 7, 8), *Schizosaccharomyces pombe* (9), and *Cryptococcus neoformans* (10, 11). There are two inositol transporters in *S. cerevisiae*, which were first isolated by complementation of a yeast mutant defective in inositol uptake. Itr1 is the major transporter with abundant mRNA levels and is both transcriptionally and posttranslationally repressed by inositol. Itr2 is a minor transporter that is constitutively expressed at a low level (5). A recent study showed that like *S. cerevisiae*, *C. albi-*

cans can generate inositol *de novo* through Ino1 and can also import inositol from the environment through the inositol transporter Itr1. *C. albicans* may utilize these two complementary mechanisms to obtain inositol during host infection (8). In the gut, *C. albicans* is exposed to one gram of inositol per day in the intestine, but as it disseminates fungal cells could salvage inositol from serum and tissues at micromole ranges. Therefore, *C. albicans* has a steady supply of inositol from the host (7). The proton-coupled inositol transporter exhibits high substrate specificity for inositol, and interactions between the transporter and the C-2, C-3, and C-4 hydroxyl groups of *myo*-inositol are critical for substrate recognition and binding (7).

C. neoformans, the most common cause of fungal meningitis, possesses a unique ability to utilize inositol in that it can use inositol as a sole carbon source. *C. neoformans* and its sibling species *Cryptococcus gattii* are environmental microorganisms that are closely associated with several niches, including bird guano and certain plants. Previous studies showed that *Cryptococcus* can utilize the abundant inositol present in its plant niches to complete its sexual cycle (12). Significantly, inositol is also abundant in the mammalian brain (13). The *INO1* and *INM1* gene transcripts were found to be present at high levels in a SAGE library generated from RNAs isolated during brain infection, suggesting a potential role for inositol in the development of meningitis (14). Thus, it is possible that an evolutionary specialization that may have originally promoted survival on plants also serves a role in enabling successful infection of the human host. In accord with this hypothesis, the *ITR* gene family in *C. neoformans* has significantly expanded in comparison to that of other fungi, containing more

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Address correspondence to Chaoyang Xue, xuech@njms.rutgers.edu.

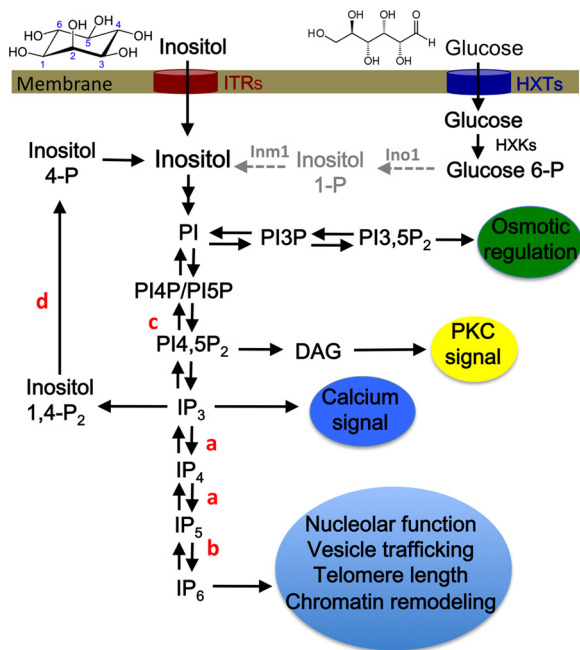


FIG 1 Inositol acquisition, metabolism, and control of cellular function. *myo*-inositol (referred as inositol) can be either imported from the environment via inositol transporters (ITRs) or synthesized intracellularly by converting glucose into inositol. Intracellular inositol is a precursor for producing phosphatidylinositol (PI), which can be utilized to produce additional inositol polyphosphates through PI kinase-mediated phosphorylation. Inositol intermediate metabolites and the enzymes (PI kinases and inositol polyphosphate phosphatases) that modulate their concentration direct diverse cellular processes, including calcium signaling, protein kinase C (PKC) signaling, osmotic stress, vesicle trafficking, nucleolar function, telomere length, chromatin remodeling, etc. The enzymes described in the article by Porollo et al. (3) are presented. Ino1 and Inm1 are two enzymes missing in *Pneumocystis* spp., while four enzymes (a to d) are enriched. (a) Inositol polyphosphate multikinase; (b) inositol pentakisphosphate 2-kinase; (c) phosphoinositide 5-phosphatase; (d) inositol-1,4-bisphosphate 1-phosphatase.

than 10 members. Furthermore, cryptococcal ITRs are required both for inositol uptake and for fungal penetration of the blood-brain barrier and brain infection (10, 11, 15–17). Studies of inositol biosynthesis and catabolism suggest that *C. neoformans* has a larger intracellular inositol pool than *S. cerevisiae* and that the Ino1 enzyme is highly expressed (18). Together, these studies show that inositol is important for *C. neoformans* to complete its sexual cycle and to produce spores for infection initiation, as well as for disease development in the brain.

In their recent article (3), Porollo and coworkers identified interesting features of inositol acquisition and metabolism in fungal pathogens belonging to *Pneumocystis* spp. *Pneumocystis pneumonia* (PCP) is a common opportunistic infection in persons with a compromised immune system, such as HIV infection, and a major cause of death in people with AIDS patients. The major obstacle for studying *Pneumocystis* biology is that these fungi are obligate pathogens that cannot be cultured *in vitro* with continuous passage. The lack of a long-term *in vitro* culture system indicates that some crucial nutrients are missing or are undersupplemented in standard laboratory medium. Earlier studies have determined that *Pneumocystis* lacks the complete ergosterol biosynthetic pathway and has significant underrepresentation of amino acid biosynthetic pathways (22). However, attempts to es-

tablish a long-term *in vitro* culture system for this fungus by supplementing sterol, amino acids, and other nutrients have not been successful.

Porollo and coworkers (3) utilized the available genome information for three *Pneumocystis* species—rodent pathogens *P. carinii* and *P. murina* and human pathogen *P. jirovecii*—to better understand their metabolic circuits and to identify any potential essential nutrients that are not being synthesized in these organisms. To this end, the researchers compared the metabolic pathways of *Pneumocystis* spp. with the phylogenetically related free-living fission yeast *S. pombe*. This analysis confirmed earlier reports that several enzymes involved in steroid biosynthesis and amino acid metabolism in *S. pombe* are missing in *Pneumocystis* spp (22). The defects in steroid biosynthesis and amino acid metabolism are likely related to their obligate nature. However, adding amino acids and sterol to media is not sufficient for long-term growth of *Pneumocystis* cultures *in vitro*. Significantly, the authors found that several enzymes involved in inositol phosphate metabolism are enriched in these species, with an increase in *Pneumocystis*-specific genes involved in inositol metabolism. However, it remains unclear why only certain enzymes are enriched, instead of the whole pathway. It would be interesting to also compare *Pneumocystis* spp. with additional fungal species to determine whether these enzymes are missing only in *S. pombe*. Porollo et al. also found that, as with *S. pombe*, all three *Pneumocystis* genomes lack Ino1 and Inm1, two key enzymes in the *de novo* inositol biosynthetic pathway (Fig. 1). Therefore, *Pneumocystis* spp. are inositol auxotrophs that have to obtain inositol from the host via inositol transporters (ITRs). Interestingly, the *Pneumocystis* spp. infecting rodents have two ITRs (Itr1 and Itr2), but *P. jirovecii* has only Itr1, suggesting that these species may have different inositol requirements. Importantly, the addition of inositol to the culture medium extended the period of *Pneumocystis* survival *in vitro*, confirming that inositol insufficiency is one factor preventing the organism's growth in culture. This study also suggests that inositol may be required for *Pneumocystis* infection; therefore, controlling inositol utilization may be a novel way to treat PCP.

Porollo and coworkers also suggested that inositol may be required for the sexual reproduction of *Pneumocystis* spp., which may occur in the host lung. The role of inositol in sexual reproduction has been reported in other fungi, including *C. neoformans* (12) and *S. pombe*. In *S. pombe*, high concentrations of inositol in the culture medium stimulate mating and sporulation, while a low concentration supports only vegetative growth (9). It was found that inositol regulates the production of pheromone P and the response of cells to pheromones. It is likely that inositol or one of its metabolites is involved in pheromone P secretion and pheromone signaling and thereby influences sexual reproduction (19). Therefore, it is not surprising that inositol metabolism may also play a role in the mating of *Pneumocystis*, further confirming the conserved function of inositol in sexual reproduction.

The significance of the work of Porollo et al. (3) is multifold. First, advances in whole-genome sequencing technology make large-scale comparative genomics possible. The approach used in this study offers a powerful tool that opens a new avenue in analyzing nutrient utilization in fungi, especially obligate pathogens that have been difficult to study. Second, by using comparative genomics, several defective nutrient biosynthetic pathways have been identified in *Pneumocystis* spp., which may lead to a better

understanding of their obligate nature and to the development of a medium that supports their growth *in vitro*. To this end, the observation that the addition of inositol to culture medium improves *Pneumocystis* viability and growth (although it is not sufficient to sustain prolonged fungal growth *in vitro*) is highly significant. Furthermore, earlier studies in *C. albicans* and *C. neoformans* indicate that inositol is required for fungal infection and disease development. In the study by Porollo et al. (3), evidence is provided that this is also likely to be the case in the obligate fungal pathogen *Pneumocystis*. This work suggests that inositol is important for disease development as a signaling molecule and/or as an essential nutrient. These conclusions underscore the clinical significance of studying the inositol transporters in fungi. The activity of inositol transporters present in unicellular eukaryotes, such as fungi (7) and protozoans (20, 21), is proton coupled, which distinguishes them both kinetically and pharmacologically from the sodium-dependent inositol transporter system in humans, and thus could be developed as a potential target for drug treatment. Therefore, further investigation is warranted to better understand the role of inositol in pathogenesis, which could lead to the development of novel drug targets.

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