Cryptococcus neoformans, a fungus under stress

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Cryptococcus neoformans is a human fungal pathogen that survives exposure to stresses during growth in the human host, including oxidative and nitrosative stress, high temperature, hypoxia, and nutrient deprivation. There have been many genes implicated in resistance to individual stresses. Notably, the catalases do not have the expected role in resistance to external oxidative stress, but specific peroxidases appear to be critical for resistance to both oxidative and nitrosative stresses. Signal transduction through the *HOG1* and calcineurin/calmodulin pathways has been implicated in the stress response. Microarray and proteomic analyses have indicated that the common responses to stress are induction of metabolic and oxidative stress genes, and repression of genes encoding translational machinery.

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Introduction

The ubiquitous environmental fungus, *Cryptococcus neoformans*, can cause morbid meningioencephalitis in the mammalian host [1]. The disease progresses after inhalation into the lung, followed by evasion of the innate immune system, replication within phagocytes and tissues, and systemic dissemination. Stressors include those that impede growth, such as temperature, pH, anoxia and nutrient deprivation, and those that are potentially toxic, such as reactive oxidative, nitrosative and chlorinating species. It is unlikely that the stress response pathways were developed specifically for survival in a mammalian host, but are the result of stress that the fungus encounters in its primary ecological niche. How *C. neoformans* is able to survive the diverse stressors it encounters within the host is the focus of research over the last three years.

Substantial progress has been made in the field of stress response, but the regulation and mechanisms behind the overall stress responses are not fully understood. Research has continued to utilize studies of individual genes and pathways, but the recent sequencing of the genome [2•] has led to the development of *C. neoformans*-specific microarrays and the application of proteomic techniques. These research strategies have contributed to the field by providing genome-wide data that has led to hypotheses about global responses, such as translation repression, metabolic changes, or cell wall rearrangements. This review will discuss the genes that are important for stress resistance, the signal transduction pathways that have been implicated in stress response, and the trends revealed by genome-wide studies (see Figure 1).

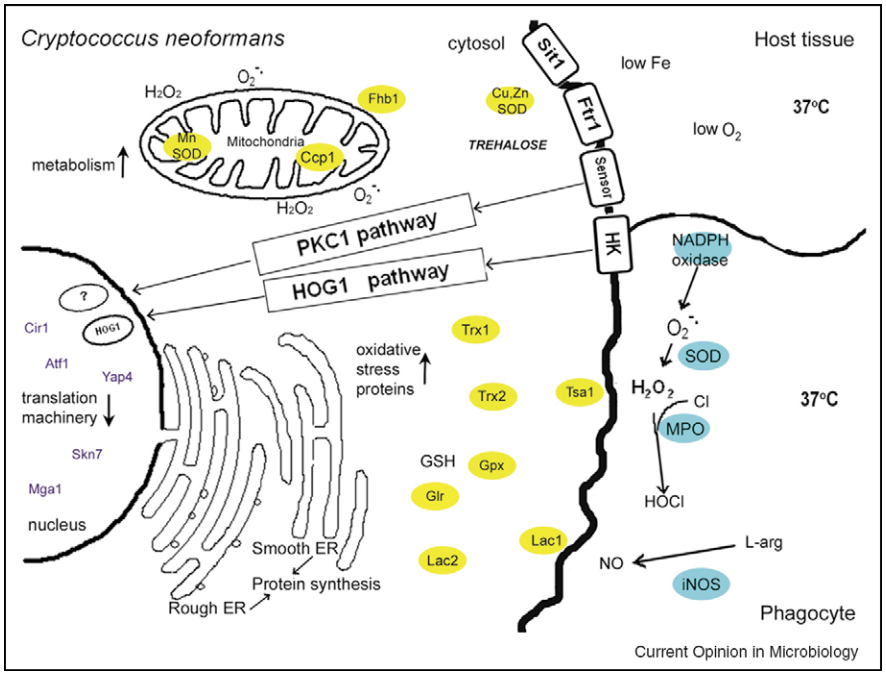
Increased temperature is likely the first stressor that *C. neoformans* encounters after it gains entry to the host

Two diverse temperature resistance mechanisms employed by *C. neoformans* include the apparent protective properties of the sugar, trehalose, and the mechanistic link between antioxidant protection and the adaptation to growth at human body temperature.

Accumulation of the disaccharide trehalose in *Saccharomyces cerevisiae* has been shown to prevent denaturation of important proteins as well as to aid heat-shock protein chaperones in re-naturation by preventing aggregation of denatured proteins [3]. In *C. neoformans*, null mutants of trehalose-6-phosphate synthase (*TPS1*) and trehalose-6-phosphate phosphatase (*TPS2*) revealed they are both required for growth at 37 °C, but the protective mechanism of trehalose remains to be elucidated. As expected, these T^s mutants were avirulent in mammalian infection models, however the *tps1*Δ strain also had attenuated virulence in a *C. elegans* model at 25 °C, suggesting that trehalose biosynthesis has a role in virulence independent from its role in growth at 37 °C [4].

Recent data implies that higher temperatures may stimulate the rate of mitochondrial respiration, leading to an increase in production of superoxide. Mitochondrial manganese superoxide dismutase (SOD), which promotes the rapid degradation of superoxide into hydrogen peroxide and O_2, has been suggested to augment adaptation of *C. neoformans* to human host body temperature [5] by regulating steady-state concentrations of oxygen

Figure 1



A schematic of the stresses that *C. neoformans* encounters in the human host, and the genes and pathways involved in the stress responses. The *C. neoformans* proteins are defined in the text.

radicals in the mitochondria and contributing to the integrity of the electron transport chain.

Resistance to reactive oxidative and nitrosative species is important for successful colonization of mammalian hosts

C. neoformans is phagocytosed by alveolar macrophages during the initial stages of infection, and must protect itself from reactive nitrogen and oxygen intermediates. Activated alveolar macrophages can produce up to 14 mM hydrogen peroxide and 57 μM nitric oxide (NO) [6], however *C. neoformans* can prevent macrophage activation to avoid exposure to these reactive molecules. The inhibition of activation is mediated by the extracellular polysaccharide capsule [7]. *C. neoformans* does not appear to be unusually resistant to reactive oxidative and nitrosative stresses, compared to other fungi. However, resistance is clearly important for survival in mammalian hosts, since impairment of several resistance pathways reduces survival in macrophages and attenuates virulence. Furthermore, these pathways are upregulated by oxidative and nitrosative stresses.

Cu, Zn superoxide dismutase has been shown to be important for resistance to oxygen radicals generated by treatment with the electron donor epinephrine, and for growth in macrophages [8]. Unexpectedly, catalases do not seem to play a major role in detoxification of exogenous reactive molecules [9••], since deletion of the entire family of catalases has no effect on resistance to oxidative stress or virulence. Instead, several peroxidases have critical roles in resistance to oxidative stress and, in some cases, overlap with resistance to nitrosative stress.

The glutathione system, which ultimately depends on the pools of reduced glutathione (GSH) maintained by glutaredoxin, is critical for resistance to oxidative and nitrosative stress. Glutathione peroxidase (GPX) removes hydrogen peroxide, and as expected, gpx null mutants are sensitive to peroxides [10]. Another protein in the glutathione system that impacts resistance to nitrosative, but not oxidative, stress is glutathione reductase [11]. The precise role of Glr in nitrosative stress is puzzling because in other systems, Glr has been shown to destroy alkyl peroxides and remove glutathione from protein thiols [12].

Laccases are important for production of the pigment melanin, which is a free radical scavenger and thus plays a protective role in stress resistance. Laccase also sequesters iron during infection, which interferes with the oxidative burst of phagocytes. Laccase expression decreases as fungal burden increases [13], although the mechanism of regulation of laccase during *C. neoformans* infection has yet to be fully described. *In vivo* regulation of laccase proceeds, at least in part, via the co-activator Ssa1, a member of the Hsp70 family [14].