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Sensing the Stress: the Role of the Stress-activated p38/Hog1 MAPK Signalling Pathway in Human Pathogenic Fungus *Cryptococcus neoformans*

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Abstract

All living organisms use numerous signal-transduction pathways to sense and respond to their environments and thereby survive and proliferate in a range of biological niches. Molecular dissection of these signalling networks has increased our understanding of these communication processes and provides a platform for therapeutic intervention when these pathways malfunction in disease states, including infection. Owing to the expanding availability of sequenced genomes, a wealth of genetic and molecular tools and the conservation of signalling networks, members of the fungal kingdom serve as excellent model systems for more complex, multicellular organisms. Here, we employed *Cryptococcus neoformans* as a model system to understand how fungal-signalling circuits operate at the molecular level to sense and respond to a plethora of environmental stresses, including osmoticshock, UV, high temperature, oxidative stress and toxic drugs/metabolites. The stress-activated p38/Hog1 MAPK pathway is structurally conserved in many organisms as diverse as yeast and mammals, but its regulation is uniquely specialized in a majority of clinical *Cryptococcus neoformans* serotype A and D strains to control differentiation and virulence factor regulation. *C. neoformans* Hog1 MAPK is controlled by Pbs2 MAPK kinase (MAPKK). The Pbs2-Hog1 MAPK cascade is controlled by the fungal "two-component" system that is composed of a response regulator, Ssk1, and multiple sensor kinases, including two-component.like (Tco) 1 and Tco2. Tco1 and Tco2 play shared and distinct roles in stress responses and drug sensitivity through the Hog1 MAPK system. Furthermore, each sensor kinase mediates unique cellular functions for virulence and morphological differentiation. We also identified and characterized the Ssk2 MAPKKK upstream of the MAPKK Pbs2 and the MAPK Hog1 in *C. neoformans*. The *SSK2* gene was identified as a potential component responsible for differential Hog1 regulation between the

serotype D sibling f1 strains B3501 and B3502 through comparative analysis of their meiotic map with the meiotic segregation of Hog1-dependent sensitivity to the fungicide fludioxonil. Ssk2 is the only polymorphic component in the Hog1 MAPK module, including two coding sequence changes between the *SSK2* alleles in B3501 and B3502 strains. To further support this finding, the *SSK2* allele exchange completely swapped Hog1-related phenotypes between B3501 and B3502 strains. In the serotype A strain H99, disruption of the *SSK2* gene dramatically enhanced capsule biosynthesis and mating efficiency, similar to *pbs2* and *hog1* mutations. Furthermore, *ssk2*, *pbs2*, and *hog1* mutants are all hypersensitive to a variety of stresses and completely resistant to fludioxonil. Taken together, these findings indicate that Ssk2 is the critical interface protein connecting the two-component system and the Pbs2-Hog1 pathway in *C. neoformans*.

Results

Proposed model for the functional connection of the Pbs2-Hog1 MAPK pathway of *C. neoformans* is the following (Fig. 1). Under normal conditions, the Hog1 MAPK is constitutively phosphorylated (inactive form) via the Pbs2 MAPKK and an unknown MAPKKK. Phosphorylated Hog1 MAPK functions to repress capsule and melanin production and sexual development under normal growth conditions. Because the Tco1 and Tco2 sensor kinases do not significantly affect the constitutive phosphorylation of Hog1, another sensor or internal signaling enables Ssk1 to trigger constitutively phosphorylate the Hog1 MAPK. In response to diverse stresses or exposure to the antifungal drug

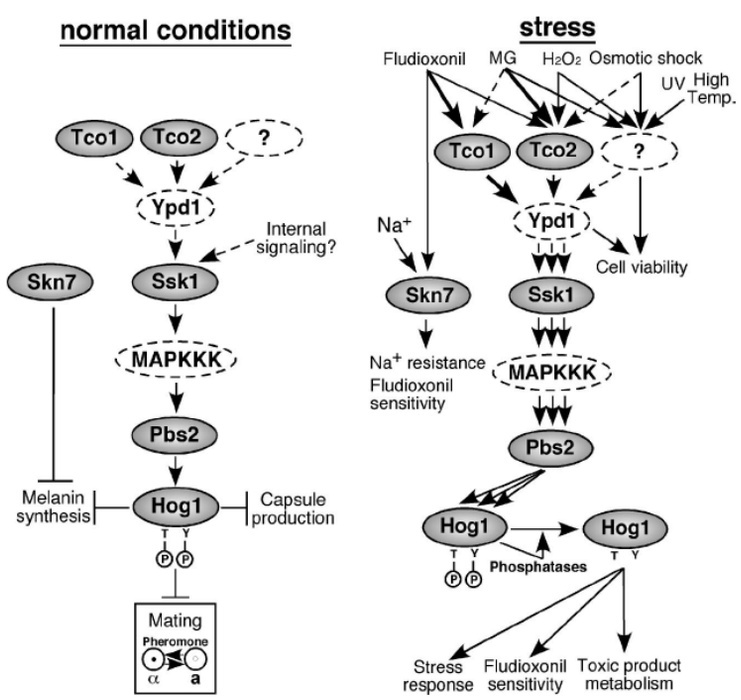

Fig. 1. Proposed model for the functional connection of the Pbs2-Hog1 MAPK pathway with the fungal two-component system *in C. neoformans*.

fludioxonil or the toxic metabolic by-product MG, a variety of sensor kinases, including Tco1 and Tco2, or sensor-independent internal signaling further activates Ssk1, which in turn activates a Hog1-specific phosphatase to dephosphorylate and activate the Hog1 MAPK (references 1-4).

References

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