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FUNGAL PHYSIOLOGY

Red light plugs into MAPK pathway

A classical mutant screen and genetic analyses powered by next-generation sequencing reveal that *Aspergillus nidulans* phytochrome-dependent red light sensing is transmitted via the high-osmolarity-glycerol mitogen-activated protein kinase cascade.

Alexander Idnurm and Yong-Sun Bahn

Most of our understanding of how eukaryotic organisms sense and respond to their environments has been obtained from studying fungi; however, we still do not fully understand how signals are transmitted or the interactions between different stimuli and pathways. In this issue of *Nature Microbiology*[1], Yu *et al.* unexpectedly show that red/far-red light-sensing phytochrome in the model filamentous fungus *A. nidulans* is an upstream component of a stress-sensing mitogen-activated protein kinase (MAPK) pathway.

Light is a major signalling cue that influences fungal physiology, including sexual and asexual differentiation, secondary metabolite synthesis, circadian rhythm and disease outcomes in pathogenic species, processes that are also regulated by many other environmental signals. Responses to blue light, which is sensed using the so-called white collar complex, are the best studied in fungi. Although other wavelengths of light are clearly important for fungal biology, relatively little is known about how signals are transduced and how they relate to other pathways. Phytochromes are a family of proteins that can detect red and far-red wavelengths and signal through a histidine kinase domain. In fungi, phytochrome functions have only been characterized in *Aspergillus* species[2–4], in which the phytochrome FphA and the blue-light sensing complex physically interact. How red light signals are translated into different physiological outcomes was unknown. To identify the signalling pathway activated by *A. nidulans* phytochrome, Yu *et al.* devised a genetic screen for 'blind' mutants, that is, ones that grew poorly in the light, and through next-generation sequencing of blind and wild-type progeny identified mutations in the MAPK SakA (also known as HogA), as well as in genes encoding the upstream MAPK kinase PbsB and MAPKK kinase SskB (known as Hog1, Pbs2 and Ssk2, respectively, in *Saccharomyces cerevisiae*).

The high osmolarity glycerol (HOG) pathway is best known as a stress-activated MAPK module (Fig. 1). The human p38 MAPK — which is orthologous to Hog1 — plays important roles in stress and immune responses[5]. In fungi, Hog1 is activated by a multi-component phosphorelay system (consisting of hybrid sensor histidine kinases, phosphotransfer proteins and response regulators), which is responsive to a plethora of environmental stresses, such as osmotic shock, oxidative stress, UV irradiation and high temperature. In addition, the HOG pathway is critical for developmental and differentiation processes in a number of fungal species. Activated Hog1 phosphorylates other downstream kinases and a number of transcription factors. In *S. cerevisiae*, Hog1 can also be recruited to target promoter sites. Given these pleiotropic roles, it is no surprise that the HOG pathway plays pivotal roles in controlling the virulence of most pathogenic fungi[5].

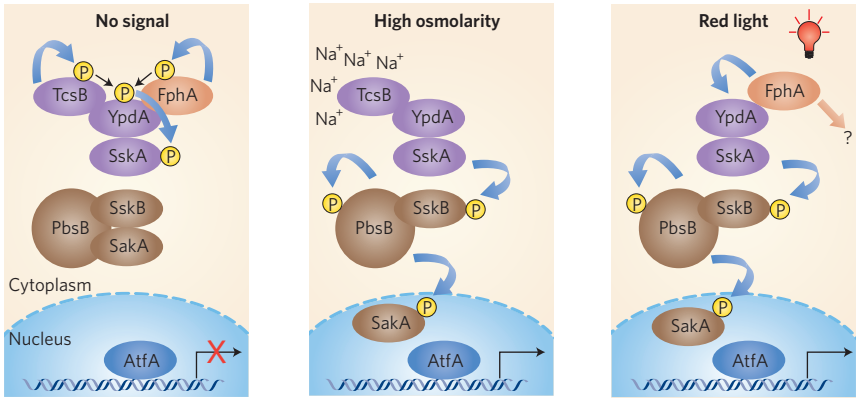

Figure 1 | Revised model of the Hog1 MAPK pathway in *Aspergillus nidulans*. High osmolarity and red light are recognized by histidine kinases TcsB and FphA, respectively (middle and right). Under isotonic or dark conditions (no signal), the histidine kinases are likely to be autophosphorylated and their phosphate groups are relayed to the YpdA phosphotransfer protein and the SskA response regulator. Phosphorylated SskA does not interact with the MAPK kinase kinase SskB. In response to high osmolarity or red light, the phosphorelay system (purple) becomes dephosphorylated to activate a series of kinases (brown), resulting in nuclear translocation of the MAPK SakA and its activation of bZIP-transcription factors, such as AtfA, that regulate target gene transcription. The subcellular localization of these events remains to be fully defined, as FphA is also found in the nucleus under certain conditions. Other targets of FphA are unknown, as indicated by the question mark.

Through co-immunoprecipitation and bimolecular fluorescence complementation, Yu *et al.* showed that FphA interacts with the YpdA phosphotransfer protein, and phosphorylated SakA translocates to the nucleus in response to light in an FphA-dependent manner, underscoring the role of phytochrome as an input sensor for this pathway (Fig. 1). The HOG pathway has an alternative input sensor, the stress-sensing histidine kinase TscB, which also acts via YpdA (Fig. 1). Yu *et al.* show that the activities of the two histidine kinases are distinct. For instance, phosphorylated SakA accumulates in the nucleus of wild-type cells in response to either light or a salt stress, and although deletion of *fphA* abolishes SakA nuclear translocation in response to light, SakA is still responsive to salt stress.

The identification of FphA as an upstream activator of the HOG pathway raises new questions. First, how widespread

is this phytochrome-induced signalling pathway in fungi? Phytochrome-encoding genes have been mutated in *Cryptococcus neoformans*, *Neurospora crassa* and *A. fumigatus*, yet phenotypes similar to those of the *A. nidulans* HOG mutants have not been reported in these species[4,6–8]. However, there is suggestive evidence of a common link between phytochrome, the HOG pathway and an interaction with the blue-light sensing system. For instance, exposing *Trichoderma atroviride* to light increases tolerance to osmotic stress through the HOG pathway. This is not a blue-light dependent effect, yet it does lead to the induction of genes encoding the blue-light sensors[9]. In *N. crassa*, blue light regulates transcription of the *sskB* homologue[10]. Uncovering these interconnections will be an exciting direction for future research.

A second question is how the HOG pathway discriminates different incoming signals to activate different sets of downstream genes. Although there are multiple sensor histidine kinases in most fungi, all of their signals appear to be relayed through a single phosphotransfer protein, converge on an SskA (known as Ssk1 in *S. cerevisiae*)-type response regulator and subsequently activate the Hog1 MAPK module (Fig. 1). If the HOG pathway reads all incoming signals as identical, it should activate the same set of genes regardless of the kind of stress. However, Hog1 is differentially phosphorylated in response to different stresses, and some HOG-dependent genes are regulated by specific stresses. Future transcriptomic analysis after exposure to white, blue or red light could identify light-induced, HOG-dependent genes. Although there may be a certain level of cross-talk between stresses — for example, Yu *et al.* demonstrate that osmotic stress can activate some light-regulated genes — there could be adaptors or scaffold proteins that allow stress-specific cellular responses.

In conclusion, the link between phytochrome and the HOG pathway comes as an exciting find. Yu *et al.* highlight the role of classical mutant screens in making major new discoveries. Their finding of phytochrome signalling via the HOG pathway paves the way to analyse the extent of this signalling system in fungi and beyond, and the intricacies of light and stress signalling. ❐

Alexander Idnurm is in the School of BioSciences, the University of Melbourne, VIC 3010, Australia. Yong-Sun Bahn is in the Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea.
e-mail: alexander.idnurm@unimelb.edu.au; ysbahn@yonsei.ac.kr

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