Light Sensing in *Aspergillus fumigatus* Highlights the Case for Establishing New Models for Fungal Photobiology

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ABSTRACT Microbes inhabit diverse environmental locations, and many species need to shift their physiology between different niches. To do this effectively requires the accurate sensing of and response to the environment. For pathogens, exposure to light is one major change between a free-living saprophyte lifestyle and causation of disease within the host. However, how light may act as a signal to influence pathogenesis, on the side of either the host or the pathogen, is poorly understood. Research during the last 2 decades has uncovered aspects about the machinery for light sensing in a small number of fungi. Now, Fuller et al. have initiated studies into the role that light and two photosensor homologs play in the behavior of the ubiquitous fungal pathogen *Aspergillus fumigatus* [K. K. Fuller, C. S. Ringelberg, J. J. Loros, and J. C. Dunlap, mBio 4(2):e00142-13, 2013, doi:10.1128/mBio.00142-13]. Light represses the germination of *A. fumigatus* spores and enhances resistance to ultraviolet light, oxidative stresses, and cell wall perturbations. The phenotypes of the strains with mutations in the LreA and FphA homologs revealed that these sensors control some, but not all, responses to light. Furthermore, interactions occur between blue and red light signaling pathways, as has been described for a related saprophytic species, *Aspergillus nidulans*. Genome-wide transcript analyses found that about 2.6% of genes increase or decrease their transcript levels in response to light. This use of *A. fumigatus* establishes common elements between model filamentous species and pathogenic species, underscoring the benefits of extending photobiology to new species of fungi.

Pick up most textbooks or general review articles that discuss signal transduction in fungi and the "usual suspects" will be featured: G protein-coupled receptors, small GTPases, cascades of kinases, and the small molecules and proteins that modulate these core components. However, one of the signaling pathways best studied from the perspective of fungal evolution is actually light sensing, specifically detection of blue wavelengths by a pair of conserved transcription factors (1, 2). The ability to sense light has not been maintained in all species; i.e., some fungi are now blind, most notably the yeasts, including the model *Saccharomyces cerevisiae* and the human pathogen *Candida albicans*. The *white-collar* (*wc-1*, *wc-2*) genes required for responses to blue light were identified in the filamentous fungus *Neurospora crassa*, and homologs are found in other fungal species. The effects of light on fungi still remain largely to be elucidated. For instance, it has only recently been appreciated that other photosensors operate in fungi, such as phytochromes for red light sensing and cryptochromes for blue light sensing in *Aspergillus nidulans* (3, 4). Research using species other than the model filamentous fungi promises to advance our understanding of how fungi use the daily signal of light from the sun.

Fuller et al. investigated the effects of light on the pathogen *Aspergillus fumigatus* (8). This filamentous species is a worldwide saprophyte and a clinical problem in immunodeficient patients, in whom the fungus establishes disease after inhalation of asexual conidiospores (9). One hypothesis is that the stresses encountered by the fungus in nature have selected for the ability to grow within a weakened host. The altered light regime between a saprophytic environment and that within the human host is a potential cue for the fungus. Analysis of other signal transduction pathways for sensing the differing conditions outside and within the host have revealed components for successful adaptation, such as to oxygen levels (10). However, there has been no characterization of the response of *A. fumigatus* to light.

One reason for this lack of research is the absence of a dramatic effect of light on *A. fumigatus* in culture, such as a change in sporulation. This is in contrast to laboratory strains of its relative *A. nidulans*, which, for experimental convenience, carry the *velvet* (*veA*) mutation that suppresses the effects of light and promotes asexual sporulation (3). This careful characterization of *A. fumigatus* indicates that light and two putative photosensors have many effects on the fungus (8). Light causes changes in growth rate, hyphal pigmentation, conidiospore germination, and resistance to ultraviolet irradiation, oxidative, and cell wall stresses. About 2.6% of genes have higher or lower transcript levels in response to light, as estimated from microarray analysis.

Light of both blue and red wavelengths affects *A. fumigatus*. The genome was searched for candidate photosensors, and Fuller et al. mutated two genes in *A. nidulans* that have characterized roles of perceiving blue (*lreA*, the *wc-1* homolog) and red (*fphA*, encoding phytochrome) wavelengths. The loss of these genes abolished a subset of the responses to light but not all of them. For instance, the protective role of light to subsequent UV exposure was unaffected in *fphA* and *lreA* single mutants and in *fphA lreA* double mutants. The other photosensors or light responses in the absence of the two characterized photosensors are worth further investigation.

Light sensing may be involved in fungal virulence. Analysis of *wc* mutants of *Cryptococcus neoformans* revealed a contribution to disease causation (5). *Fusarium oxysporum* also requires the *wc-1*

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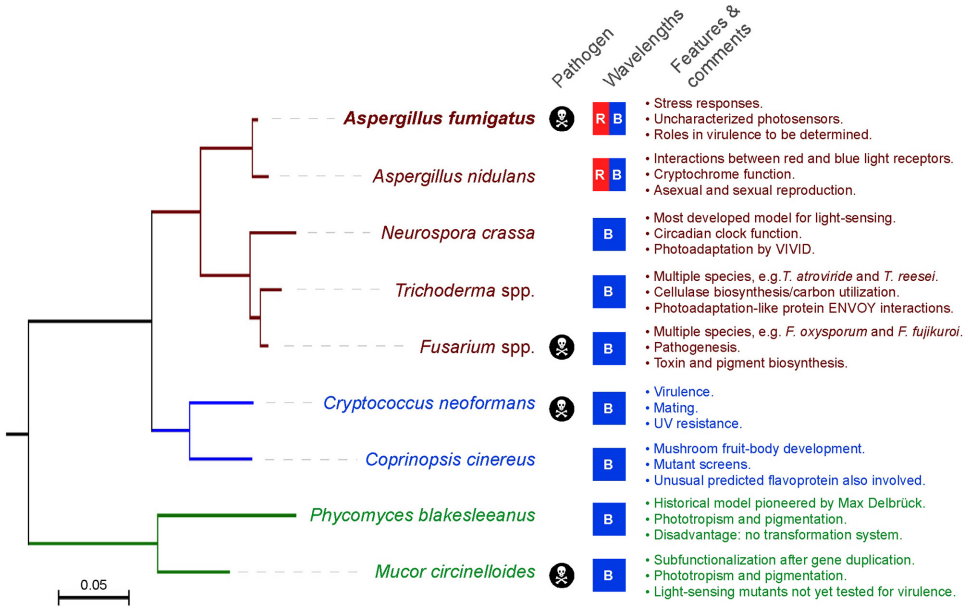

FIG 1 Fungi that have emerged or are emerging as models for research on sensing and response to light. The phylogeny (partial 18S rDNA) divides the species into the *Ascomycota* (red), *Basidiomycota* (blue), and *Mucoromycotina* (green). All nine species have saprophytic growth capabilities, and some can also cause disease. Wavelengths with a characterized response are red (R) or blue (B). Useful features in photobiology research or behavior modified by light for individual species are listed. Common research directions in these species can provide a better understanding of how light influences fungal biology.

homolog for virulence; curiously, the decreased virulence of the *wc-1* mutant is in an animal disease model and not on its normal plant host (6). Recently, *wc-1* has been implicated in maize disease caused by *Cercospora zeae-maydis* by reducing the tropism to the stomata used for gas exchange on the leaf surface (7). The mechanisms by which *wc-1* function affects pathogenesis are not established, nor have roles for other photosensors in pathogenesis been assessed.

A prediction based on the effects of light and the light-regulated genes is that light and the two photosensors will contribute to the ability of *A. fumigatus* to cause disease. First, spore germination is inhibited by light: the conditions in the lung would support germination. Second, mutation of the *lreA* and *fphA* genes reduces oxidative stress resistance and cell wall stress resistance, properties important for hyphal growth within the host. These findings provide possible explanations for how light sensing impacts pathogenesis that could also be explored in other pathogenic fungi. A key future experiment for *A. fumigatus* is to test the function in the pathogenesis of the *lreA* and *fphA* genes.

A comparison of *A. fumigatus* and *A. nidulans*, in which the effects of blue and red light and the corresponding photosensors have already been investigated, can help clarify the evolution of photosensing. In particular, the two species have active red light responses, which is thus far uncharacterized in other fungi despite the presence of phytochrome homologs in their genomes. In the two *Aspergillus* species, phytochrome regulates the inhibition of conidiospore germination (11). *A. nidulans* exhibits physical and genetic interactions between the blue and red light signaling components, with a large photosensory complex formed that includes the LreB protein acting in blue light responses, the FphA phytochrome, and the VeA velvet protein (12). In *A. fumigatus*, there is a genetic interaction between the two pathways, so a similar complex may also function in this species. Exposure of *A. nidulans* to light alters transcript levels of about 5% of the genes in the species (13). Fuller et al. commented that there is little overlap between the light-regulated genes identified in *A. nidulans* and the 2.6% that they identified in *A. fumigatus*, with the caveat that the two experiments used different culture conditions. A side-by-side comparison of the wild-type and photosensor mutant strains of the two species exposed to light and dark would be a powerful approach toward understanding conservation and divergence in the transcriptional responses to light. Thus, the use of *A. fumigatus* can establish how common overlapping regulation is within the *Aspergillus* genus or *Eurotiomycetes* class.

While *N. crassa* has led the research in light sensing in fungi, especially the study of how the WC-1/WC-2 complex is integrated into the circadian clock, other fungi have also emerged in the last decade as models for research on the responses to light (Fig. 1). Here, Fuller et al. demonstrated how rapidly a new species can provide information about light sensing. This is facilitated by the available genome sequence data, which can be used for bioinformatic identification of photosensor homologs, the design of gene replacement constructs, and expression profiling using microarrays or RNA sequencing. The one drawback for *A. fumigatus* is that the tools of classical genetics that are available for *A. nidulans* (14) are still in development for *A. fumigatus* (15). This limits the ability to assemble strains, through crossing, with a suite of genetic manipulations.

There are open questions about how fungi sense and respond to light for which the development of new species for research would be ideal. These questions include how photosensors are distributed and function in different species (e.g., those taxa with little research), what role photoperception plays in virulence (e.g., in plant pathogens), what is the central oscillator in the circadian clocks of species without a homolog of the *N. crassa frequency* gene, whether circadian time influences disease, and how the sig-

nal transduction pathways from light cross talk with pathways signaling other environmental conditions. Future analysis of *A. fumigatus* will continue to provide insight into these matters, particularly with respect to the role of light sensing in pathogenesis.

REFERENCES

1. **Corrochano LM, Garre V.** 2010. Photobiology in the Zygomycota: multiple photoreceptor genes for complex responses to light. Fungal Genet. Biol. **47**:893–899.
2. **Sanz C, Rodríguez-Romero J, Idnurm A, Christie JM, Heitman J, Corrochano LM, Eslava AP.** 2009. *Phycomyces* MADB interacts with MADA to form the primary photoreceptor complex for fungal phototropism. Proc. Natl. Acad. Sci. U. S. A. **106**:7095–7100.
3. **Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, Frankenberg-Dinkel N, Fischer R.** 2005. The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr. Biol. **15**: 1833–1838.
4. **Bayram Ö, Biesemann C, Krappmann S, Galland P, Braus GH.** 2008. More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. Mol. Biol. Cell **19**:3254–3262.
5. **Idnurm A, Heitman J.** 2005. Light controls growth and development via a conserved pathway in the fungal kingdom. PLoS Biol. **3**:615–626.
6. **Ruiz-Roldán MC, Garre V, Guarro J, Mariné M, Roncero MIG.** 2008. Role of the white collar 1 photoreceptor in carotenogenesis, UV resistance, hydrophobicity, and virulence of *Fusarium oxysporum*. Eukaryot. Cell **7**:1227–1230.
7. **Kim H, Ridenour JB, Dunkle LD, Bluhm BH.** 2011. Regulation of stomatal tropism and infection by light in *Cercospora zeae-maydis*: evidence for coordinated host-pathogen responses to photoperiod? PLoS Pathog. **7**:e1002113. http://dx.doi.org/10.1371/journal.ppat.1002113.
8. **Fuller KK, Ringelberg CS, Loros JJ, Dunlap JC.** 2013. The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. mBio **4**(2):e00142-00113. http://dx.doi.org/10.1128/mBio.00142-13.
9. **Latgé J-P, Steinbach WJ (ed).** 2009. *Aspergillus fumigatus* and aspergillosis. ASM Press, Washington, DC.
10. **Grahl N, Puttikamonkul S, Macdonald JM, Gamcsik MP, Ngo LY, Hohl TM, Cramer RA.** 2011. In vivo hypoxia and a fungal alcohol dehydrogenase influence the pathogenesis of invasive pulmonary aspergillosis. PLoS Pathog. **7**:e1002145. http://dx.doi.org/10.1371/journal.ppat.1002145.
11. **Röhrig J, Kastner C, Fischer R.** 6 February 2013. Light inhibits spore germination through phytochrome in *Aspergillus nidulans*. Curr. Genet. doi:10.1007/s00294-013-0387-9.
12. **Purschwitz J, Müller S, Kastner C, Schöser M, Haas H, Espeso EA, Atoui A, Calvo AM, Fischer R.** 2008. Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. Curr. Biol. **18**: 255–259.
13. **Ruger-Herreros C, Rodríguez-Romero J, Fernández-Barranco R, Olmedo M, Fischer R, Corrochano LM, Canovas D.** 2011. Regulation of conidiation by light in *Aspergillus nidulans*. Genetics **188**:809–822.
14. **Todd RB, Davis MA, Hynes MJ.** 2007. Genetic manipulation of *Aspergillus nidulans*: meiotic progeny for genetic analysis and strain construction. Nat. Protoc. **2**:811–821.
15. **Sugui JA, Losada L, Wang W, Varga J, Ngamskulrungroj P, Abu-Asab M, Chang YC, O'Gorman CM, Wickes BL, Nierman WC, Dyer PS, Kwon-Chung KJ.** 2011. Identification and characterization of an *Aspergillus fumigatus* "supermater" pair. mBio **2**(6):e00234-00211. http://dx.doi.org/10.1128/mBio.00234-11.

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