RESEARCH ARTICLE

The Fungal Pathogen *Aspergillus fumigatus* Regulates Growth, Metabolism, and Stress Resistance in Response to Light

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ABSTRACT **Light is a pervasive environmental factor that regulates development, stress resistance, and even virulence in numerous fungal species. Though much research has focused on signaling pathways in *Aspergillus fumigatus*, an understanding of how this pathogen responds to light is lacking. In this report, we demonstrate that the fungus does indeed respond to both blue and red portions of the visible spectrum. Included in the *A. fumigatus* light response is a reduction in conidial germination rates, increased hyphal pigmentation, enhanced resistance to acute ultraviolet and oxidative stresses, and an increased susceptibility to cell wall perturbation. By performing gene deletion analyses, we have found that the predicted blue light receptor LreA and red light receptor FphA play unique and overlapping roles in regulating the described photoresponsive behaviors of *A. fumigatus*. However, our data also indicate that the photobiology of this fungus is complex and likely involves input from additional photosensory pathways beyond those analyzed here. Finally, whole-genome microarray analysis has revealed that *A. fumigatus* broadly regulates a variety of metabolic genes in response to light, including those involved in respiration, amino acid metabolism, and metal homeostasis. Together, these data demonstrate the importance of the photic environment on the physiology of *A. fumigatus* and provide a basis for future studies into this unexplored area of its biology.**

IMPORTANCE **Considerable effort has been taken to understand how the mold pathogen *Aspergillus fumigatus* senses its environment to facilitate growth within the immunocompromised host. Interestingly, it was shown that the deletion of a blue light photoreceptor in two divergent fungal pathogens, *Cryptococcus neoformans* and *Fusarium oxysporum*, leads to an attenuation of virulence in their respective animal infection models. This suggests that light signaling pathways are conservatively involved in the regulation of fungal pathogenesis. However, an understanding of whether and how *A. fumigatus* responds to light is lacking. Here we demonstrate that this organism coordinates broad aspects of its physiology with the photic environment, including pathways known to be involved in virulence, such as carbohydrate metabolism and oxidative stress resistance. Moreover, the photoresponse of *A. fumigatus* differs in notable ways from the well-studied model *Aspergillus nidulans*. Therefore, this work should represent a general advancement in both photobiology and *A. fumigatus* research communities.**

Received 22 February 2013 **Accepted** 26 February 2013 **Published** 26 March 2013

Citation 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):e00142-13. doi:10.1128/mBio.00142-13.

Editor Joseph Heitman, Duke University

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Species from essentially all branches of life gain important environmental information through the perception of light. Though vertebrate vision serves as the most dramatic example, microorganisms have also evolved photosensory systems that are capable of detecting both quantitative and qualitative changes in light. Information about the photic environment can then be used as an indicator for both time and space to drive adaptive and developmental decisions. In fungi, for example, the presence of light may signal the soil/air interface for optimal spore dispersal, high temperature, and the presence of genotoxic ultraviolet (UV) radiation (1, 2). In some fungal species, the light signal serves to cue the organism's internal timekeeping system, the circadian clock, to anticipate predictable daily environmental fluctuations (3–6).

Although the influence of light on fungal development has been reported for many species, the molecular basis for photoreception is best characterized in the model ascomycete *Neurospora crassa*. In this mold, light promotes asexual development (conidiation), protoperithecial formation, the direction of ascospore release, and carotenoid pigment production (7, 8). All of these photoresponses appear to be mediated by blue light only, which is detected by the White Collar-1 (WC-1) photoreceptor. WC-1 is a Zn finger transcription factor that contains a specialized PAS (Per-Ant-Sim) domain, called the LOV domain (light-oxygen-voltage), which binds flavin adenine dinucleotide (FAD) as the photon-absorbing chromophore (9, 10). WC-1 interacts with another Zn finger protein, WC-2, to form a transcriptional regulator called the White Collar complex (WCC), which either directly or indirectly influences the expression of nearly 6.0% of the *N. crassa* genome after exposure to light (8, 9, 11–13). *N. crassa* expresses additional putative photoreceptor genes, including an opsin, two phytochromes, a cryptochrome, and the small LOV domain pro-

tein called VVD; however, only deletion of the *wc-1* or *wc-2* gene leads to an inability of the organism to respond to light (12, 14–18). With orthologs found in all major fungal divisions, including the *Chytridiomycetes*, the white collar proteins are the most evolutionarily conserved photoreceptors in the Mycota (19, 20).

Beyond environmental adaptation, there are intriguing reports suggesting that photoreception pathways may also influence the virulence potential of pathogenic fungal species. Deletion of the *wc-1* ortholog in both the basidiomycete yeast *Cryptococcus neoformans* and the ascomycete mold *Fusarium oxysporum* results in an attenuation of virulence in their respective murine infection models (21, 22). Taken at face value, these data suggest that these organisms are capable of detecting light *in vivo*, through WC-1, to induce cellular pathways involved in virulence. Alternatively, the WCC may be regulating virulence genes in the dark. In either case, the WCC, and potentially other photosensory systems, may represent novel virulence-associated pathways across a variety of fungal species.

Among fungi with a WC-1 ortholog is *Aspergillus fumigatus*, which has emerged as the predominant mold pathogen of immunocompromised patients. Even with the use of antifungal agents, the mortality rates associated with invasive aspergillosis remain around 50%, thus making the development of novel treatment strategies imperative (23, 24). Current data suggest that the pathogenic potential of *A. fumigatus* is a polygenic trait derived from its evolution as a competitive member of the compost ecosystem. Stresses within this niche to which the fungus must respond are similar to those found in the mammalian lung, including high temperatures, nutritional limitation, hypoxia, and oxidative stress (25–27). Given the data from *C. neoformans* and *F. oxysporum*, it is tempting to speculate that light also serves as an important environmental factor that drives the expression of virulence determinants in this pathogen. However, despite the intensive studies that have been undertaken with this organism over the past several decades, a detailed description of a photoresponse in *A. fumigatus* is lacking.

In contrast to *A. fumigatus*, *Aspergillus nidulans* is a rarely pathogenic member of the aspergilli and has recently emerged as a model for fungal photobiology, along with *N. crassa*. In *A. nidulans*, light induces asexual sporulation while concurrently downregulating sexual development and secondary metabolism (28). Unlike *N. crassa*, *A. nidulans* overtly responds to both blue and red portions of the visible spectrum through a variety of photoreceptor proteins. For blue light sensing, *A. nidulans* utilizes not only a WC-1 homolog, called LreA, but also a photolyase/cryptochrome, CryA, that also binds to FAD (29). For the detection of red light, *A. nidulans* employs a phytochrome, called FphA, that binds the bilin tetrapyrole as a chromophore. Unlike WC-1/LreA, phytochromes are not transcription factors but instead are presumed to regulate the activity of interacting proteins through a conserved kinase domain (30). Interestingly, the blue and red light sensory pathways interact both genetically and physically in *A. nidulans*, as LreA and FphA form a photoreceptor complex that also contains LreB (the homolog of *N. crassa* WC-2) and the developmental regulator Velvet A (VeA) (31, 32). Therefore, the molecular basis for light perception is considerably more complex in *A. nidulans* than in *N. crassa*, involving the integration of qualitatively distinct light inputs via multiple photosensory pathways.

Notably, the quality of the light response appears to vary considerably between fungal species, even between those of the same genus. For example, light serves as a repressor of sexual development in *Aspergillus oryzae*, opposite to that of *A. nidulans* (33). As such, photoreceptor components may be conserved from species to species yet are connected into signaling pathways differently such that photobiological characterizations in one organism may offer little predictive value for another organism.

We have undertaken a detailed investigation of the photobiology of *A. fumigatus* and have found that it is indeed a light-responsive organism. Blue and red wavelengths of light display independent and synergistic influences on growth rate, germination kinetics, hyphal pigmentation, cell wall homeostasis, and acute UV and oxidative stress responses. In addition, whole-genome microarray analysis has revealed a broad influence of light on central metabolism. The blue and red light responses, in part, are dependent on the WC-1/LreA homolog and a phytochrome, respectively; however, the data clearly implicate the involvement of additional photoreceptors that participate in a complex photosystem. Taken together, this work provides new insight into *A. fumigatus* growth and developmental regulation and represents new avenue of research for this important pathogen.

RESULTS

The *A. fumigatus* genome contains orthologs of conserved blue and red light photoreceptors. We first sought to identify putative photoreceptor genes in *A. fumigatus*. Essentially all fungi with a characterized photoresponse can sense blue light through a conserved GATA transcription factor, the first member of which is the *N. crassa* WC-1 (20). BLAST analysis of the *A. fumigatus* (Af293) genome with the *A. nidulans* WC-1 ortholog LreA revealed a single ortholog (AFUA_3G05780), the predicted sequence of which is 851 residues and displays 52% identity to *A. nidulans* LreA and 45% identity to WC-1 of *N. crassa*. The *A. fumigatus* LreA is predicted to contain all conserved functional domains of the WC-1/LreA orthologs, including a LOV domain for chromophore binding, two additional PAS domains, and a C-terminal nuclear localization signal (NLS) motif followed by a Zn finger DNA binding domain (Fig. 1A). Notably, both *Aspergillus* LreA proteins lack the N- and C-terminal polyglutamine (poly-Q) stretches found in strain WC-1; however, the poly-Q regions appear not to be essential for WC-1 functionality (B. Wang, J. J. Loros, and J. C. Dunlap, unpublished data).

Putative red light-sensing phytochromes can be found in the genomes of both ascomycetes and basidiomycetes; however, only FphA of *A. nidulans* has been shown to function in a fungal biological response to red light (30, 34). The *A. fumigatus* genome contains two putative FphA orthologs AFUA_4G02900 and AFUA_6G09260. Both proteins contain all the predicted features for a functional phytochrome, including the N-terminal sensory region (composed of PAS, GAF [cGMP-specific phosphodiesterases, adenylyl cyclases, and FhlA], and PHY [phytochrome] domains) for bilin binding and the C-terminal output domains (composed of the histidine kinase, ATPase, and response regulatory domains) for signal transmission (Fig. 1A and data not shown) (1). Because AFUA_4G02900 demonstrates 56.3% identity to *A. nidulans* FphA (versus 39.5% identity for AFUA_6G09260), we will refer to this protein as *A. fumigatus* FphA for continuity. Consequently, we will refer to the second paralog as FphB.

In *N. crassa*, the *wc-1* message is increased upon exposure to light (35). To determine whether such regulation is conserved in

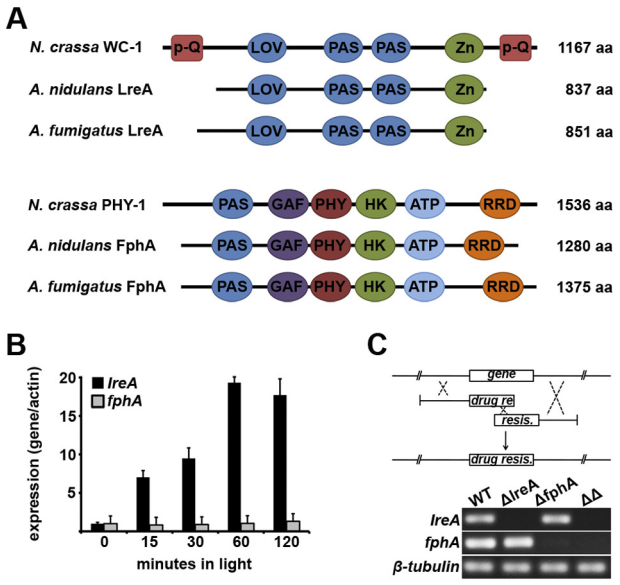

FIG 1 Characterization of the LreA and FphA photoreceptors in *A. fumigatus*. (A) Cartoon depiction of the domain architectures for the WC-1/LreA and phytochrome proteins in *N. crassa*, *A. nidulans*, and *A. fumigatus*. The *A. fumigatus* models are based upon the predicted protein sequences found in GenBank. Relative differences in protein length (aa, amino acids) and domain separation are depicted but are not shown to scale. Domain functions are described in the text. p-Q, polyglutamine stretch; Zn, zinc finger domain; HK, histidine kinase; ATP, ATPase; RRD, response regulator domain. (B) qRT-PCR analysis of *lreA* and *fphA* from a representative time course experiment. Bars reflect the $2^{-\Delta\Delta CT}$ values relative to the 0-min time point (plus standard deviations [SD] [error bars] of 3 technical replicates). (C) (Top) Split-marker deletion strategy of *lreA* and *fphA*. The *lreA* and *fphA* genes were replaced with *hph* (hygromycin resistance) for the single deletion mutants. The *fphA* gene was replaced with *bleR* (phleomycin/bleomycin resistance) in the Δ*lreA* background to generate the double mutant. (Bottom) RT-PCR demonstrating the expected loss of transcript(s) in the respective deletion strains.

A. fumigatus, we grew the wild-type (WT) *A. fumigatus* strain Af293 in constant darkness for 48 h and then harvested samples 0, 15, 30, 60, or 120 min after transfer to white light, as depicted in Fig. S5 in the supplemental material. Quantitative reverse transcription-PCR (qRT-PCR) revealed that *lreA* transcript was indeed potently induced following illumination, thereby demonstrating that *A. fumigatus* can respond to light at the level of gene expression (Fig. 1B). In contrast to *lreA*, neither *fphA* nor *fphB* demonstrated a light-dependent induction in *A. fumigatus* (Fig. 1B and data not shown), which is consistent with the phytochrome orthologs (*phy-1*/*phy-2*) in *N. crassa* (15). Notably, the transcriptional regulation of *lreA* and *fphA* genes in *A. nidulans* has not been specifically reported, and neither one of these two genes were among the light-induced genes detected by microarray (36).

The *lreA* and *fphA* genes were chosen for our initial functional analyses in *A. fumigatus* on the basis of their close homology to known functional photoreceptors in other species. The corresponding open reading frames were replaced with genes for either hygromycin or phleomycin resistance in the Af293 background (Fig. 1C). Furthermore, WT copies of *lreA* and *fphA* genes were introduced into their respective deletion strain to confirm their involvement in any observed mutant phenotypes (see Fig. S1 in the supplemental material). In addition to the single deletion mutants, an *lreA fphA* double deletion mutant was also generated to discern functional interactions between the two genes. The isogenic WT, deletion, and reconstituted (R') strains were then analyzed for various photobiological behaviors.

Light has a weak inhibitory influence on asexual development and growth rate in *A. fumigatus*. We began by comparing colonial characteristics of *A. fumigatus* cultures grown in either constant darkness or constant illumination. In stark contrast to *A. nidulans*, which requires light to induce conidiation (37), *A. fumigatus* efficiently produced conidia irrespective of the photic environment. In fact, quantitation actually revealed a small but significant reduction in total conidia on light-grown *A. fumigatus* plates (see Fig. S2 in the supplemental material). Conidiation appeared unaffected in the Δ*lreA*, Δ*fphA*, and Δ*lreA* Δ*fphA* mutants, suggesting that LreA and FphA are not major regulators of asexual development in *A. fumigatus* (data not shown). In summary, the requirement for light in establishing asexual developmental competence appears not to be conserved between *A. fumigatus* and *A. nidulans*.

The radial growth rate of *A. fumigatus* was also slightly reduced in the presence of white light, relative to the fungus grown in the dark (see Fig. S2 in the supplemental material). Treatment with monochromatic blue or red light was unable to affect growth rate to the extent of white light or dichromatic treatment with red and blue light. Moreover, both the Δ*lreA* and Δ*fphA* mutants demonstrated a partial derepression of growth rate in light (Fig. S3), suggesting that LreA and FphA are directly involved in the response and function in an interdependent manner.

Light induces hyphal pigmentation in an *lreA*-dependent manner. The most obvious effect of light on *A. fumigatus* was the regulation of hyphal pigmentation. Colonies grown in the presence of constant white or blue light were drastically more pigmented than those cultures kept in constant darkness. Colonies treated with red light alone, however, were indistinguishable from cultures grown in the dark, suggesting a specific influence of blue light on this phenotype (Fig. 2A). Strikingly, when the fungus was cultured in an alternating 12-h light/12-h dark photocycle, corresponding rings of pigmented and nonpigmented hyphae were formed (Fig. 2A). This indicates that the subapical (older) hyphal regions were no longer responsive to light but were instead terminally differentiated to be pigmented or nonpigmented based on the light environment when those regions represented the apical growth front. The widths of the pigmented bands (light phase) were notably smaller than the widths of the nonpigmented bands (dark phase), which can likely be attributed to reduced concentration of pigments at the edge of the zone due to diffusion and/or reduced growth rate of the fungus during the light phase. In addition, *A. fumigatus* may undergo photoadaptation, a negative-feedback response in which light ultimately leads to the repression of light-induced genes (38, 39). If photoadaptation does take place, *A. fumigatus* may not produce hyphal pigments during the entire light phase.

The ability of white or blue light to induce hyphal pigmentation was lost in either the Δ*lreA* or Δ*lreA* Δ*fphA* mutants (Fig. 2B). The reconstituted *lreA* mutant displayed a WT pigmentation phenotype, confirming that the mutant defect was specifically due to loss of *lreA* (see Fig. S1 in the supplemental material). In contrast to the Δ*lreA* mutant, the Δ*fphA* mutant was indistinguishable from WT with regards to the photopigmentation phenotype. This is consistent with the finding that red light alone was unable to

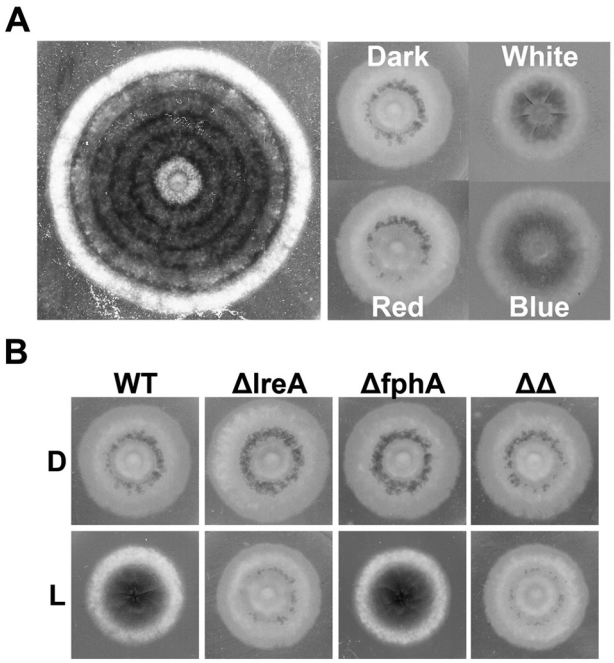

FIG 2 *A. fumigatus* photopigmentation response. (A) (Left) *A. fumigatus* Af293 grown in an alternating 12-h dark/12-h white light environment for 6 days. (Right) Af293 after 72 h of constant darkness or constant illumination conditions, as indicated. (B) WT or deletion mutants (Δ*lreA*, Δ*fphA*, and Δ*lreA* Δ*fphA* [ΔΔ] mutants) after 72 h of constant darkness [D] or constant white light illumination [L]. All experiments were performed on GMM plates and incubated at 37°C. All pictures are scans of the plate bottoms shown as gray-scale images.

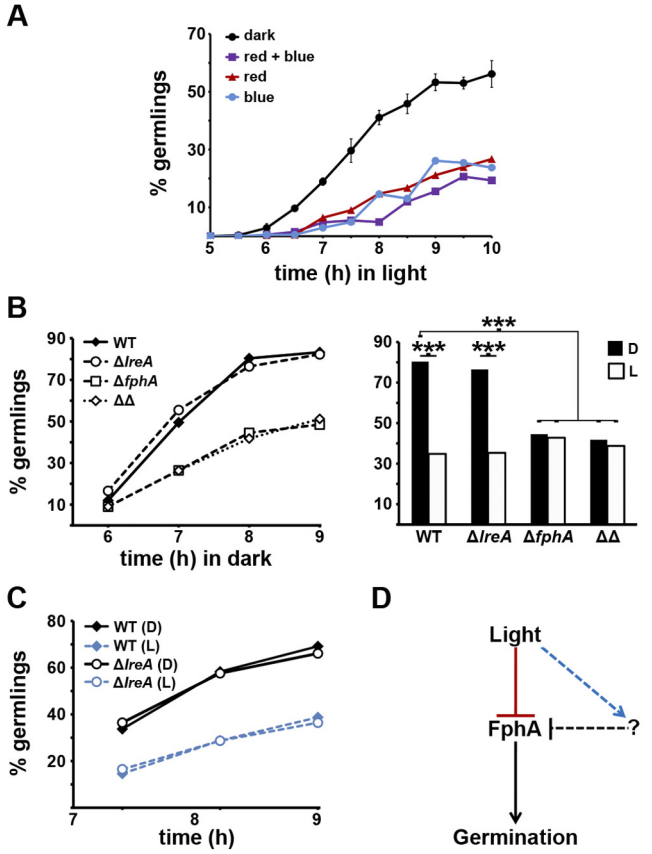

FIG 3 Light regulation of conidial germination in *A. fumigatus*. (A) Germination rates of Af293 conidia under constant illumination conditions. Each light condition was performed at different times, and each light condition was run concurrently with a dark time course experiment. The averages of the three dark time course experiments are shown (±SD). (B) (Left) Germination rates of WT or mutant strains in constant darkness. (Right) Percentage of germination after 8 h in constant darkness [D] or constant red plus blue light illumination [L]. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (C) Germination rates of the WT versus Δ*lreA* mutant in constant darkness versus constant blue light illumination. Abbreviations: D, constant darkness; L, constant blue light illumination. (D) Proposed model for light-regulated germination. Red light inhibits FphA directly, whereas blue light inhibits FphA via an unknown blue light sensor. All germination experiments were performed in liquid GMM and incubated at 37°C.

induce pigmentation and demonstrates that the defect in the Δ*lreA* Δ*fphA* mutant can be attributed solely to the loss of *lreA*. Along with the conserved sequence and light-induced regulation of *lreA* described above, these data demonstrate that *lreA* encodes a bona fide blue light receptor in *A. fumigatus* that is capable of regulating downstream phenotypes.

Light inhibits germination of *A. fumigatus* conidiospores by affecting the FphA phytochrome. In addition to the reduced rates of hyphal extension described above, light also had a strong negative impact on the kinetics of conidial germination (Fig. 3A). For example, the percentage of germinated *A. fumigatus* Af293 conidia when irradiated with dichromatic blue/red light was approximately one-third that of dark-grown cultures at 10 h postinoculation. Surprisingly, both monochromatic blue and red light were capable of inhibiting germination to the same extent as dichromatic treatment or white light. This result, in combination with the data showing that blue and red light interact synergistically to inhibit growth rate, demonstrates that *A. fumigatus* is capable of responding to the red portion of the visible spectrum.

The basic model for a light response consists of light activation of a photoreceptor, which then acts upon a downstream target. Therefore, we hypothesized that the inhibition of germination was due to a repressing action of LreA or FphA following their activation by blue or red light, respectively. Accordingly, we anticipated that deletion of *lreA* or *fphA* would lead to an unresponsive mutant in which the corresponding light spectrum would no longer inhibit germination. Contrary to this prediction, however, the Δ*fphA* and Δ*lreA* Δ*fphA* mutants displayed reduced germination kinetics in the dark and were not further inhibited by light (Fig. 3B). Additionally, the germination kinetics of dark-grown Δ*fphA* and Δ*lreA* Δ*fphA* mutants were comparable to those of the WT and Δ*lreA* mutant in the light (Fig. 3B). Taken together, these data are consistent with a model in which the phytochrome promotes germination in the dark, with light impinging on this activity (Fig. 3D).

To reconcile the finding that blue light alone could inhibit germination, we hypothesized that activated LreA could directly or indirectly inhibit FphA-dependent germination; if this hypothesis is correct, then blue light would no longer be able to inhibit germination in the Δ*lreA* background. Contrary to this prediction, blue light still inhibited the germination of the Δ*lreA* mutant (Fig. 3C), suggesting that LreA is not the photoreceptor involved in this blue light response. Therefore, it is likely that an additional and unidentified photoreceptor is involved in inhibition of germi-

nation by blue light, as indicated in the model presented in Fig. 3D.

A reduction in conidial germination by light has not been reported for *N. crassa*, for which the photoresponse is well characterized; however, it is not clear whether a germination phenotype has actually been rigorously tested. Therefore, we analyzed germination rates of *N. crassa* in the dark versus white light and observed identical kinetics under both conditions (see Fig. S4 in the supplemental material). Similarly, an *N. crassa* Δ*phy-1* Δ*phy-2* deletion mutant (15) demonstrated no germination defect compared to the WT, indicating that the influences of light and phytochromes on conidiospore germination are not conserved in *A. fumigatus* and *N. crassa*.

Whole-genome transcriptional analysis reveals broad regulation of metabolism and stress response genes by light in *A. fumigatus*. To gain a more comprehensive insight into light-regulated processes in *A. fumigatus*, a whole-genome microarray analysis was performed. Total RNA from each of the five time points shown in Fig. S5 in the supplemental material (0, 15, 30, 60, and 120 min after transfer to light) was labeled by reverse transcription and then competitively hybridized against a pooled reference. The Af293 oligonucleotide arrays contained four independent probes each of 9,628 genes, representing approximately 99.9% of the genome. A gene was considered light regulated if it met the three following criteria. (i) It demonstrated at least a 1.5-fold intensity difference (either up or down) relative to the dark (0 min) time point. (ii) The change in expression was consistent for at least two consecutive light time points, except if the change initially occurred at 120 min. (iii) The first two criteria were met on at least two independent probes from the array. Based on these criteria, 250 genes were identified as light-regulated genes in *A. fumigatus*, which was approximately 2.60% of all genes detected in the experiment.

Of the 250 light-regulated genes, 102 were photoinduced (see Table S1 in the supplemental material). The majority of such genes demonstrated an early onset of induction, with 65 genes showing increased expression by 15 min after light transfer and an additional 24 genes showing increased expression by 30 min. Most of these "early light-induced" genes (58 of 89) demonstrated either full or partial repression by the 120-min time point, suggesting that *A. fumigatus* had become photoadapted. The remaining light-induced genes (13 of 102) demonstrated induction beginning 60 or 120 min after light transfer.

The photoresponse of *N. crassa* is mediated through a hierarchical transcriptional cascade in which the light-activated WCC promotes the early expression of additional transcription factors, which then regulate their own subset of genes in the light regulon (e.g., late-light-responsive genes) (12). In addition to the blue light receptor gene, *lreA*, we identified two additional transcription factors in the *A. fumigatus* light-induced set, one annotated as an NF-X1 transcription factor (AFUA_7G04710) and another as a CP2 transcription factor (AFUA_3G11170); both demonstrated early light induction (within 30 min). A role for these proteins in regulating downstream light-responsive genes seems likely and will be the subject of future investigations.

The gene demonstrating the greatest fold induction on the array was AFUA_3G01430, a putative member of the TspO/MBR (tryptophan-rich sensory protein/mammalian peripheral-type benzodiazepine receptor) family (see Table S1 in the supplemental material). qRT-PCR confirmed an early light induction of the gene, which peaked at around 20-fold by 60 min after transfer to light (see Fig. S4 in the supplemental material). Interestingly, AFUA_3G01430 may belong to a conserved photoresponsive gene family, as its orthologs are potently light induced in both *N. crassa* and *A. nidulans* (12, 36). While not functionally characterized in fungi, in bacteria, these proteins are proposed light/oxygen-regulated porphyrin efflux pumps involved in negatively regulating carotenogenesis and photosynthesis (40). Therefore, the TspO/MBR orthologs may have important roles in the fungal photoresponse.

The second-most highly light-induced gene was a predicted photolyase (AFUA_1G01600, annotated as *phr1* in *A. fumigatus*), a member of a class of enzymes that absorb photons in the blue/near-UV spectrum and use the energy to catalyze the repair of cyclobutane adducts (e.g., pyrimidine dimers) caused by UV; the ortholog is induced in both *N. crassa* and *A. nidulans* (12, 36). In addition to the *lreA* photoreceptor gene, an opsin-related gene (AFUA_7G01430) was also induced by light on the array, which is consistent with the array data from *A. nidulans* (36). In summary, we have identified several light-induced genes on our array whose orthologs are similarly regulated in other organisms, thereby lending confidence that the array data provide an accurate look at light-mediated changes in the *A. fumigatus* transcriptome. Functional term enrichment of the light-induced genes revealed that this set was significantly enriched for genes involved in UV resistance, cholesterol homeostasis, phospholipid metabolism, glycogen metabolism, and amino acid catabolism, among others (see Table S2A in the supplemental material).

In contrast to what has been reported in *N. crassa*, *A. nidulans*, and *Trichoderma atroviride* (12, 36, 41), we identified more light-repressed genes in *A. fumigatus* (148 total) than light-induced genes (see Table S3 in the supplemental material). Term enrichment analysis demonstrated that downregulated gene categories were largely metabolic, including gluconeogenesis, tricarboxylic acid (TCA) cycle, electron transport/respiration, amino acid biosynthesis, and cholesterol/steroid metabolism (Table S2B). Several genes from each category were confirmed by qRT-PCR analysis (Fig. S5).

Visible light promotes *A. fumigatus* resistance to UV irradiation and oxidative stress. In addition to the photolyase gene described above, a gene encoding a putative mismatch repair enzyme (AFUA_3G14260) was also upregulated in the array experiment and confirmed by qRT-PCR (Fig. 4A). On the basis of the light induction of its orthologs in *N. crassa* and *A. nidulans*, we also tested the expression of a UV endonuclease encoded by *uvdE-1* (AFUA_6G10900) and found that it was also induced by light in *A. fumigatus*, albeit weakly. Interestingly, the light induction of *uvdE* was lost in the Δ*lreA* mutants, and the induction of *phr1* was lost in both the Δ*lreA* and Δ*fphA* mutants (Fig. 4A). From these data, we hypothesized that the exposure to visible light would promote resistance of *A. fumigatus* to UV irradiation and that this light-induced resistance phenotype would be lost in the *lreA* or *fphA* photoreceptor mutants. To test this, dilutions of conidia were spread across a series of plates and incubated overnight in constant darkness. The plates were then either exposed to visible light or kept in the dark for 2 h immediately prior to UV insult. Following UV irradiation, plates from both pretreatment groups (i.e., kept in the dark versus exposed to light) were incubated together, and colonies arising after 2 days of incubation were counted and compared to no-UV controls (Fig. 4B). Consistent

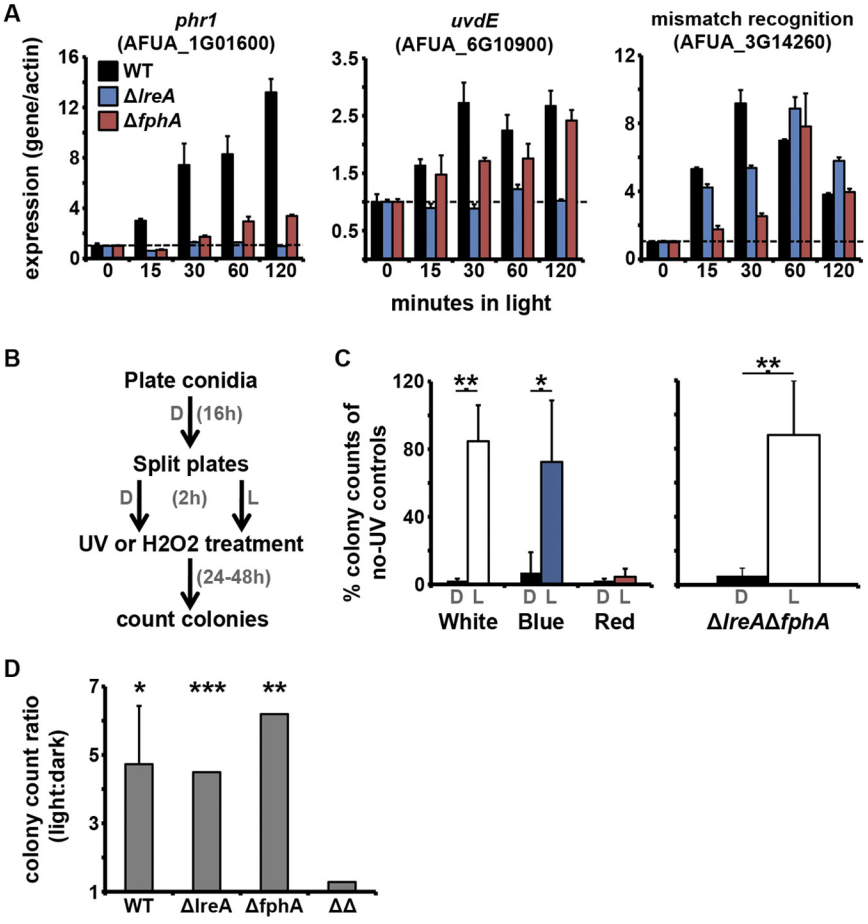

FIG 4 Light induction of stress resistance in *A. fumigatus*. (A) qRT-PCR analysis of DNA repair genes in a representative time course experiment. Bars reflect the $2^{-\Delta\Delta CT}$ values, relative to the 0-min time point for that strain (plus SD of 3 technical replicates). (B) Schematic of the UV or H_2O_2 stress resistance assays performed. All cultures were incubated at 37°C on GMM plates. (C) (Left) Comparison of white, blue, and red light treatment regimens in the UV stress assay using strain Af293.(Right) The UV stress assay, using white light, with the Δ*lreA* Δ*fphA* mutant. (D) Comparison of WT versus the mutant strains in the H_2O_2 assay. Each of the mutants strains was tested separately, and each strain was tested with its own WT control. The average of the three WT experiments is shown (plus SD). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

with the first hypothesis, WT plates that were exposed to light prior to UV treatment gave rise to significantly more colonies than those kept in the dark. This was observed when either white or blue light was used, but not red light (Fig. 4C). Surprisingly, and in contrast to our second hypothesis, the Δ*lreA*, Δ*fphA*, and Δ*lreA* Δ*fphA* mutants all demonstrated light-enhanced resistance to the UV treatment to the same extent as the WT (Fig. 4C and data not shown). This suggests that additional photoreceptors may be functioning to promote UV resistance in response to light.

In addition to direct DNA damage, the deleterious effects of UV arise through the formation of intracellular reactive oxygen intermediates. Accordingly, we hypothesized that visible light might also serve as an anticipatory signal for oxidative stress and promote resistance to an oxidizing agent. This was tested experimentally in a manner similar to the UV experiments, except that the treatment consisted of overlaying plates with either hydrogen peroxide or water as a control (Fig. 4B). As predicted, those plates preexposed to white light yielded more colonies than those kept in the dark. This resistance phenotype persisted as robustly as the WT phenotype in both the Δ*lreA* and Δ*fphA* mutants but was lost in the Δ*lreA* Δ*fphA* double mutant (Fig. 4D). Therefore, LreA and FphA appear to function in a redundant or synergistic fashion to promote the resistance to oxidative stress.

FphA promotes cell wall homeostasis in the presence of light. Several genes encoding cell wall-associated proteins, including four glycosylphosphatidylinositol (GPI)-anchored proteins, demonstrated light repression in the microarray experiment, suggesting that cell wall homeostasis is modulated by light (see Table S3 in the supplemental material). To test this prediction phenotypically, we took advantage of the fact that alterations in cell wall integrity typically manifest as changes in sensitivity to agents that perturb the cell wall. *A. fumigatus* conidia were spot plated onto medium containing various concentrations of Congo red, a dye that promotes osmolysis by binding to polysaccharide fibrils (e.g., glucan and chitin) of the cell wall. Interestingly, the fungus was more susceptible to the dye when the plates were incubated in white light than when the plates were incubated in the dark (Fig. 5A). Blue light alone was capable of enhancing susceptibility, but not to the same extent as white light; conversely, plates exposed to red light were indistinguishable from those kept in the dark. Osmotic stabilization of the medium with 1.2 M sorbitol was able to ameliorate the enhanced sensitivity in white light, indicating that the sensitivity to Congo red was, in fact, due to cell wall perturbation (Fig. S6).

The Δ*lreA*, Δ*fphA*, and Δ*lreA* Δ*fphA* mutants were also tested in this assay. In the dark, the susceptibility of each of the mutants was identical to that of the WT strain (see Fig. S6 in the supplemental material). In the light, however, the Δ*fphA* and Δ*lreA fphA* mutants were even more susceptible to Congo red than the WT was, despite the finding that red light did not impact the susceptibility of the organism. The reconstituted Δ*fphA* strain displayed a WT-like susceptibility profile, confirming that the enhanced defect was due to loss of the *fphA* gene (Fig. 5B). In contrast, the phenotype of the Δ*lreA* mutant was similar to the WT phenotype, suggesting that the phenotype of the Δ*lreA* Δ*fphA* mutant was due to loss of the *fphA* gene, specifically.

DISCUSSION

In this study, we have demonstrated that *A. fumigatus* regulates broad aspects of its physiology in response to visible light. Additionally, many of the phenotypic and transcriptional light outputs of *A. fumigatus* differ from those of well-described photoresponsive species, such as *A. nidulans* and *N. crassa*. As such, this work

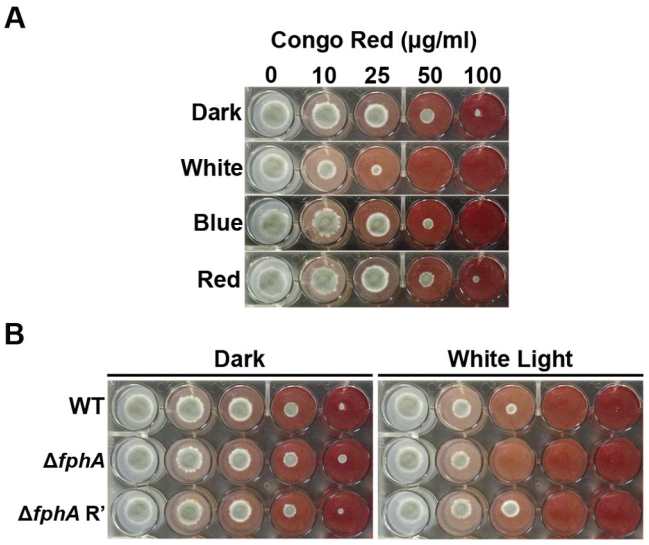

FIG 5 Influence of light on cell wall homeostasis. (A) Susceptibility profiles of WT *A. fumigatus* to Congo red under the indicated light conditions. (B) Comparison of WT, Δ*fphA*, and Δ*fphA* R' strains grown in the dark versus white light. All pictures were taken after 48-h incubation at 37°C.

adds depth to the understanding of how conserved photosensory pathways have evolved differently in species with distinct environmental niches and lifestyles. Moreover, the presence or absence of light has considerable impact on the metabolic and stress-resistant state of *A. fumigatus*, thereby making photoperception an intriguing candidate for a virulence-associated process in this important pathogen.

Light is a major regulator of asexual development in species across the fungal kingdom, including both ascomycete and zygomycete molds (28, 33, 42–45). Despite being a light-responsive organism, however, we found that *A. fumigatus* produced asexual spores prodigiously in both the light and dark. While this would suggest that the influence of light on conidiation is not conserved in *A. fumigatus*, it was recently shown that an Af293 mutant strain (called Af293^{FL} [FL for fluffy]) displays severe conidiation defects unless exposed to light during the initial 18 h of culturing (46). This, therefore, suggests that a light-dependent conidiation pathway does indeed exist in *A. fumigatus* and the Af293^{FL} mutation affects a gene in a parallel developmental pathway that promotes conidiation in the dark. With the exception of a single conidial hydrophobin (AFUA_5G09580), we did not observe the light induction of other known *Aspergillus* conidiation genes on our array or by qRT-PCR (data not shown); this included a transcriptional regulator of conidiation genes, *brlA*, which is induced by light in *A. nidulans* (36). The lack of concordance with regard to the light regulation of conidiation genes is perhaps surprising; however, an important consideration is that our light induction time courses were performed in submerged/shaking culture, a condition that is not typically conducive for asexual development in *A. fumigatus* and other molds. The difference in culture conditions may have also contributed to the considerably smaller number of light-regulated genes we report for *A. fumigatus* (250 genes) by microarray versus the 533 genes reported in *A. nidulans* (36). Despite the difference in absolute number however, a term enrichment analysis of upregulated genes from the *A. nidulans* array revealed that similar gene categories were induced (see Table S2C in the supplemental material).

A major interpretation of the cumulative data is that light serves as a stress signal for *A. fumigatus*. This was evidenced initially as a generalized growth inhibition of light-grown cultures, primarily at the level of conidial germination. Germination involves the irreversible activation of respiratory metabolism and the polarization machinery, thereby breaking metabolic dormancy and committing the fungus to vegetative growth (47). Therefore, if light serves as a signal for a stress environment (e.g., UV, elevated temperatures), delaying germination by some hours may be advantageous for the fungus by increasing the likelihood that growth is initiated under more favorable conditions. The mechanisms by which growth and germination rates are attenuated by light are currently unclear but could, in part, be explained by the light repression of genes involved in respiratory metabolism and protein synthesis observed by microarray.

Our data implicate a central role for the phytochrome, FphA, in the germination response. More specifically, FphA appears to promote germination in the dark and is then inhibited by red or blue light, the latter in an LreA-independent manner. While both a dark function for FphA and the dispensability of LreA in this response were surprising to us, the exact same roles for blue and red light, and for FphA and LreA, in the germination of *A. nidulans* were recently reported (48). This demonstrates that not only is the influence of light on germination conserved between the two aspergilli but also that an unknown blue light receptor is likely inhibiting FphA-mediated germination in both organisms.

Beyond the inhibition of growth, the detection of visible light by *A. fumigatus* also led to the upregulation of pathways that directly promote protection against the harmful effects of UV. The most obvious response was the production of hyphal pigments, which may protect the cell by either directly absorbing UV light or by scavenging UV-generated reactive oxygen intermediates (49). This response was dependent upon LreA, which fits with the finding that only blue light was able to induce the response. Although photopigmentation has not previously been seen in an *Aspergillus* species, it constitutes a light response observed in many ascomycete molds, including *N. crassa*, *Fusarium fujikuroi*, *Fusarium oxysporum*, and *Cercospora kikuchii*, as well as in the model zygomycete *Phycomyces blakesleeanus* (22, 35, 50–53). Moreover, the LreA/WC-1 ortholog appears to be involved in, although not necessarily essential for, the photo-pigmentation response in all species in which it has been tested.

We also observed light induction of several DNA repair genes, including a photolyase and a UV endonuclease. Importantly, the pigmentation and transcriptional responses correlated with a light-induced protection of *A. fumigatus* against a lethal dose of UV. Similar to photopigmentation, this protective phenotype was elicited by white and monochromatic blue light, but not by red light. An interesting aspect of the data, then, was the involvement of the LreA and FphA photoreceptors in this response. For example, the light induction of the photolyase encoded by *phr1* was lost in the Δ*lreA* mutant and attenuated in the Δ*fphA* mutant. This suggests that FphA is required for the full activity of LreA in inducing the expression of *phr1*, despite the fact that red light alone is unable to induce UV protection. Even more surprising is the fact that the light-mediated UV resistance phenotype of *A. fumigatus* persisted even in the Δ*lreA* Δ*fphA* mutant. This differs from both *C. neoformans* and *F. oxysporum*, in which loss of the *wc-1*/*lreA*

ortholog alone leads to a hypersensitivity to UV (21, 22). This suggests that an additional photoreceptor(s) may be operative in *A. fumigatus* capable of upregulating UV stress resistance pathways. Along these lines, the light induction of the putative mismatch repair gene persisted in the Δ*lreA* Δ*fphA* mutant. An alternative explanation is that such genes are upregulated after DNA damage rather than by signal transduction (i.e., downstream in a photoreceptor pathway); however, the early induction of the mismatch repair gene (15 min after exposure to light) is perhaps indicative of a genuine light response. Indeed, these data, together with the LreA-independent effects of blue light on germination, support the presence of additional functional photoreceptors.

Perhaps the most likely candidate for an additional blue light receptor in *A. fumigatus* is the aforementioned Phr1. The Phr1 ortholog in *A. nidulans*, CryA, not only displays the requisite DNA repair activity of a photolyase, but its deletion also leads to a deregulation of sexual developmental genes and an increase in cleistothecial development (29). This indicates that CryA is serving dual roles as a photolyase and cryptochrome, the latter of which are bona fide blue light receptors that typically lack DNA repair capability. Phr1 represents the only protein resembling a photolyase or cryptochrome by sequence analysis in the *A. fumigatus* genome, suggesting that it too may have both repair and signaling capabilities. Thus, even though the light induction of *A. fumigatus phr1* is lost or reduced in the Δ*lreA* and Δ*fphA* backgrounds, there may still be Phr1-mediated signaling to additional repair genes that then lead to the resistance phenotype.

We also observed the induction of an opsin-like gene on the array and confirmed this by qRT-PCR. Genes of this family are widespread across fungal species and are even present in the *Saccharomycotina*, which lack a known light response or any other photoreceptor ortholog (20). However, the proteins encoded by these genes are not conventional rhodopsins, as they lack a crucial lysine residue required for retinal binding (54). The ortholog in *A. nidulans* has been deleted, but no phenotype has been discovered (R. Fischer, personal communication); therefore, it is unclear what role, if any, these opsin-related proteins are playing in a fungal light response.

Last, we have noted the presence of a second putative phytochrome in the genome of *A. fumigatus*, which we have called *fphB*. This is in contrast to *A. nidulans*, in which *fphA* represents the sole phytochrome-encoding gene. Because all expected phytochrome domains are predicted in its sequence and because transcript levels can be detected, it is possible that the FphB protein displays light signaling capability in *A. fumigatus*. However, because the Δ*fphA* mutant displays clear phenotypes that are similar to those described for the *fphA* mutant of *A. nidulans* (48), we predict that FphB may be redundant to FphA. The roles for Phr1 and FphB in the *A. fumigatus* photoresponse are under investigation.

Also predicted from the array was an effect of light on *A. fumigatus* cell wall homeostasis, which manifested as an enhanced sensitivity to Congo red in light. It is currently unclear whether the loss of cell wall integrity is due to direct deleterious effects of light on cell wall components or is an indirect result of transcriptional changes in light (as seen on the array) or both. However, cell wall defects are known to translate into reduced conidiation and growth rates in *A. fumigatus* (55, 56), so the influence of light on the wall may, in part, explain the light-dependent reductions in conidiation and growth rate we observed. We have identified FphA as an important regulator of cell wall homeostasis in the light, which was unexpected given the apparent inability of red light to influence Congo red sensitivity. This finding then adds to the UV and oxidative stress data in which FphA plays a central role in a red light-independent response.

We also found that light can promote resistance of *A. fumigatus* against exogenous oxidative stress. However, we did not observe a corresponding light induction of obvious oxidative stress-related genes, such as catalase, superoxide dismutase, or glutathione peroxidase; such genes were light induced in both *N. crassa* and *A. nidulans* (12, 36). Instead, the physiological response of *A. fumigatus* may be explained by the light-mediated change in the metabolic state of the cell, as discerned by microarray. For example, genes involved in mitochondrial function/respiration as well as heavy metal (e.g., iron) transport were highly enriched among the light-repressed genes. As both the redox activity of the mitochondria and iron-mediated Fenton chemistry are major sources of endogenous oxidative stress for the cell (57), the downregulation of these gene categories may provide an oxidant-protected cellular state in *A. fumigatus*. Indeed, the downregulation of these pathways has been associated with oxidative stress resistance in several bacterial species (58, 59). However, it should be noted that the relationship between heavy metal/respiratory homeostasis and oxidative stress sensitivity is complicated; iron and copper are essential cofactors for reactive oxygen species (ROS) detoxifying enzymes, like catalase and superoxide dismutase, respectively, while yeast cells with mitochondrial dysfunction (e.g., petite cells) display enhanced sensitivity to oxidative stress (60). Therefore, at most, the metabolic data provide hypotheses for future experimental validation. Unlike the UV resistance phenotype, the light-induced resistance to oxidative stress was lost in the Δ*lreA* Δ*fphA* mutant, suggesting that LreA and FphA regulate this stress response in a redundant or synergistic fashion.

In addition to the metal homeostatic and respiratory changes just described, the light environment has broad impacts on *A. fumigatus* metabolism. Complementing the reduction in respiration is a generalized induction of genes involved in glycogen metabolism, amino acid catabolism, fatty acid metabolism, cholesterol homeostasis, sugar/glucose uptake, and glycolysis. Conversely, there was a concurrent drop in the metabolism of various amino acids and protein synthesis, in general. Despite these transcriptional regulatory differences, we did not observe a difference in radial growth rate on different carbon sources between cultures grown in constant darkness versus constant light (data not shown). Thus, beyond the stress resistance implications proposed above, the adaptive consequence of these broad metabolic shifts by light are currently unclear. A more in-depth analysis of metabolism and the photic environment, as well as the relative contributions of the various photoreceptor pathways, will be the focus of ongoing and future investigations. In the meantime, these data underscore that the photic environment should be considered an environmental variable in *A. fumigatus* experiments, particularly for those involving stress response, metabolic and transcriptional outputs, which are commonplace for work with this organism.

Perhaps most importantly, it is well-known that many of the cellular processes that are light regulated contribute to *A. fumigatus* virulence. As examples, loss of hyphal pigmentation, reduced germination kinetics, and defects in cell wall homeostasis are all associated with attenuated virulence phenotypes in murine models of invasive aspergillosis (49, 56). Similarly, carbohydrate and nitrogen metabolism and iron acquisition through siderophores,

shown here to be light-regulated processes, are also known virulence determinants in this fungus (26, 61). Looking forward, then, it will be interesting to determine whether perception of the light environment by *A. fumigatus* will prove important not only for adaptation to its ecological niche, but also for adaptation and growth within the immunocompromised host.

MATERIALS AND METHODS

Growth conditions and light treatment. All *A. fumigatus* strains were maintained on glucose minimal medium (GMM), which contains 1% glucose and ammonium tartrate as a nitrogen source, as previously described (62). Cultures for the light induction RNA time courses were incubated in liquid YG (2% glucose, 0.5% yeast extract). All incubators were placed in a dark room, and "dark" samples were isolated with all lights off with the aid of infrared goggles.

For cultures treated with white light, plates or flasks (as indicated) were irradiated under cool fluorescent light bulbs (General Electric F20T12-CW) emitting light over a broad spectrum from 400 to 700 nm; the total light intensity was ~40 μmol/photons/m^2/s. For blue and red light treatments, plates were incubated in an E-30LED growth chamber equipped with blue and red light-emitting diodes (Percival Scientific, Inc., Perry, IA). All incubations were performed at 37°C.

***A. fumigatus* and *N. crassa* strains used in this study.** *Aspergillus fumigatus* strain Af293 was used as the WT organism. Targeted gene deletions were accomplished using the split-marker method (63), as depicted in Fig. 1C and described below. All PCRs were performed with iProof (Bio-Rad) unless otherwise noted, and all primer sequences are listed in Table S4 in the supplemental material. Putative knockout and reconstituted strains were initially identified by a genomic PCR screen and subsequently confirmed by RT-PCR analysis.

(i) Deletion of *lreA*. The *lreA* left arm (LA) was amplified from Af293 genomic DNA (gDNA) with primers 1 and 2, and the first two-thirds of the hygromycin resistance cassette was amplified from pAN7-1 using primers 3 and 4; the two products were then combined by overlap PCR using primers 1 and 4 to yield the left deletion construct. The second two-thirds of the hygromycin cassette was amplified from pAN7-1 using primers 5 and 6, and the *lreA* right arm (RA) was amplified from gDNA using primers 7 and 8; the two products were combined by overlap PCR using primers 5 and 8 to yield the right deletion construct. Ten-microgram amounts of both the left and right deletion constructs were used for cotransformation into Af293 protoplasts, and hygromycin-resistant colonies were selected for screening.

(ii) Deletion of *fphA*. For the single deletion mutant, the *fphA* LA was amplified from gDNA using primers 9 and 10 and was combined with the first two-thirds of the hygromycin cassette using primers 9 and 4 to yield the left deletion construct. The *fphA* RA was amplified with primers 15 and 16 and was subsequently combined with the second two-thirds of the hygromycin cassette using primers 5 and 16 to create the right deletion construct. Ten-microgram amounts of the left and right deletion constructs were cotransformed into Af293 protoplasts, and hygromycin-resistant colonies were selected for screening.

For the double deletion mutant, the first two-thirds of the phleomycin resistance construct was amplified from plasmid pBC-phleo using primers 11 and 12; this was subsequently combined with the *fphA* LA using primers 9 and 12 to form the left deletion construct. The second two-thirds of the phleo cassette was amplified using primers 13 and 14 and combined with the *fphA* RA using primers 13 and 16 to form the right deletion construct. The left and right deletion constructs were cotransformed into Δ*lreA* mutant protoplasts, and phleomycin constructs were selected for screening.

(iii) Complementation of Δ*lreA* and Δ*fphA*. A 3.7-kb region of the *lreA* locus, consisting of 700 bp of the native promoter through the translation stop, was amplified with primers 17 and 18 and cloned into the plasmid pSC-B-amp/kan (Strataclone) to form the *lreA* R′ plasmid. Twenty micrograms of linearized *lreA* R′ plasmid (HindIII) and 2 μg of the pBC-phleo construct were cotransformed into Δ*lreA* protoplasts, and phleomycin-resistant colonies were selected for screening.

A 5-kb region of the *fphA* locus, consisting of 850 bp of the native promoter through the translational stop, was amplified with primers 19 and 20 and was cloned into plasmid pSC-B-amp/kan (Strataclone) to generate the *fphA* R′ plasmid. Twenty micrograms of PCR-amplified *fphA* R′ plasmid insert and 2 μg of the pBC-phleo construct were cotransformed into Δ*fphA* protoplasts, and phleomycin-resistant colonies were selected for screening.

For *N. crassa* experiments, strains OR74A and 324-8 were used as the WT strains. The Δ*phy-1* Δ*phy-2* mutant was previously generated by Froehlich et al. (15).

Quantitation of growth rate and conidiation. For radial growth rate studies, 2 μl of a 5×10^6 ml^{-1} conidial suspension of the indicated strains was point inoculated onto the center of a GMM plate and incubated at 37°C under the indicated light conditions. At each 24-h time point, the plates were removed from the incubator and briefly exposed to a low-intensity red light (standard safe light) so the hyphal growth front could be marked accurately. The experiment was performed three times (each in triplicate), and the mean 24-to 72-h growth rates for each of the groups were compared statistically by Student's *t* test.

To quantify total conidia produced by the Af293 strain, 1×10^4 conidia were point inoculated onto GMM plates and incubated for 4 days at 37°C in the indicated light environment. Following incubation, total conidia were harvested by swabbing conidia into sterile water (3 times) and enumerating with a hemacytometer. Data are expressed as total conidia divided by the colony area (cm^2). The experiment was performed in triplicate, and conidial counts from the dark-grown and illuminated groups were compared by Student's *t* test.

Analysis of conidial germination. For the germination assays, 5.0×10^5 conidia ml^{-1} were inoculated into liquid GMM, and 3-ml aliquots were dispensed into 10-mm petri plates containing a single sterile coverslip. The plates were incubated at 37°C under the described light conditions and removed from the incubator at the indicated time points. A minimum of 200 conidia were scored for the presence or absence of a germ tube using a bright-field microscope. The statistical differences for the values for groups at the 8-h time point were determined pairwise by the chi-square test.

For the *N. crassa* germination experiments, frozen conidia were inoculated onto a minimal medium slant (64) and incubated for 1 week at 30°C in constant darkness to generate conidia. Conidia were suspended in sterile water and filtered through Miracloth (Calbiochem) to eliminate hyphal debris. The conidia were then inoculated into Bird medium containing 2% glucose and aliquoted into petri plates as described above for the *A. fumigatus* experiments. Incubations were performed at 30°C under the indicated light environments.

RNA isolation and quantitative RT-PCR. Following culturing as described above, mycelial tissue was harvested by brief vacuum filtration followed by freezing in liquid nitrogen. Total RNA was isolated from the frozen tissue by crushing the tissue with a glass rod and processing the powder with the RNeasy kit (Qiagen). Two micrograms of RNA was used for DNase treatment (Roche) based on the manufacturer's guidelines, and 8 μl of the DNase-treated RNA was used for first-strand cDNA synthesis (Superscript III; Invitrogen). For quantitative RT-PCR analysis, 1 μl of 1/5 or 1/10 diluted cDNA was used in a 20-μl PCR mixture (iTaq fast SYBR green master mix; Bio-Rad) detected by an ABI 7500 real-time system. Expression values for all analyzed genes were calculated using the $2^{-\Delta\Delta CT}$ method, using actin or tubulin as a reference.

Analysis of light-induced genes by microarray. For each of the five time points analyzed (0, 15, 30, 60, and 120 min), 15 μg of DNase-treated RNA was used for reverse transcription (Superscript II; Gibco) in the presence of aminoallyl-dUTP (catalog no. A-0410; Sigma) from 1-μg aliquots of poly(A) RNA. The dyes Alexa Fluor 555 and Alexa Fluor 647 (catalog no. A-32755; Invitrogen) were coupled to the pooled reference and time point cDNA, respectively, in the presence of 7.5 mg/ml sodium

bicarbonate buffer. The cDNA was subsequently cleaned by using the Illustra Cyscribe GFX purification kit (catalog no. 27-9606-02; Amersham/GE).

Slides (Af293 CAT-40K arrays; MYcroarray, Ann Arbor, MI) were prehybridized in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate (SDS), and 1.0% bovine serum albumin (BSA) for 1 h at 42°C, washed, and spun dried. Following slide prehybridization, labeled cDNA was resuspended in 36 μl of hybridization solution (25% formamide, 5× SSC, 0.1% SDS, 0.1 μg/μliter single-stranded DNA [ssDNA], 0.2 μg/μliter tRNA), and the suspension was heated at 95°C for 5 min and subsequently transferred into the space between microarray slide and LifterSlip cover glass (Erie Scientific, Portsmouth, NH). Hybridization was carried out for 16 h at 42°C in a Boekel InSlide Out hybridization oven. A GenePix 4000 scanner was used to acquire images, and GenePix Pro 7 software was used to quantify hybridization signals.

Data processing was performed using BRB-Array Tools version 4.2.1 developed by Richard Simon and Amy Peng Lam. BRB-Array Tools is offered without support by the Biometric Research Branch of the National Cancer Institute. Raw intensity data were background subtracted, filtered to remove weak or missing spots, and normalized using an intensity-dependent lowess method.

Stress resistance assays. For the UV stress experiments, conidial dilutions of the indicated strains were spread plated onto GMM plates and incubated for ~16 h in constant darkness. The plates were then either kept in darkness or transferred to the indicated light condition for an additional 2 h. The plates were then irradiated with 7 J/m^2 by placing the plates in a UV Stratalinker 1800 (Stratagene). Nonirradiated plates served as the no-treatment controls. Following treatment, all plates were incubated at 37°C in a standard incubator (not light controlled), and colonies were counted following 24 to 48 h of incubation.

The H_2O_2 experiments were performed as described above for the UV stress experiments, except the treatment consisted of overlaying the plates with 0.007 to 0.008% H_2O_2 for 10 min at room temperature. The H_2O_2 was then decanted, and the plates were washed by overlaying them in water for 5 min (repeated 3 times). No-treatment groups were treated the same way, except water was used for the initial 10-min treatment. Both the treatment and washes were performed under ambient light conditions. The plates were then incubated and scored as described for the UV experiments.

For the Congo red susceptibility assay, 2 μl of a 5.0 × 10^6 conidial suspension were point inoculated onto GMM medium containing the indicated concentrations of Congo red. All pictures shown were taken after 48-h incubation at 37°C in the indicated light environment.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00142-13/-/DCSupplemental.

Figure S1, JPG file, 0.3 MB.
Figure S2, JPG file, 0.3 MB.
Figure S3, JPG file, 0.2 MB.
Figure S4, JPG file, 0.2 MB.
Figure S5, JPG file, 0.4 MB.
Figure S6, JPG file, 1 MB.
Table S1, PDF file, 0.1 MB.
Table S2, PDF file, 0.1 MB.
Table S3, PDF file, 0.1 MB.
Table S4, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We thank Judith Rhodes at the University of Cincinnati for the generous gift of the Af293 strain and Robb Cramer at the Geisel School of Medicine, Dartmouth College, for critical reading of the manuscript and helpful suggestions. We are also grateful to Mike Whitfield at the Geisel School of Medicine for the use of the array analysis software. We thank the Fungal Genetics Stock Center at the University of Missouri for providing strains.

This work was supported by grants from National Institute of General Medical Sciences of the National Institutes of Health to J.J.L. (grant R01 GM083336) and J.C.D. (grants R01 GM34985 and P01 GM68087).

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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