The *Phycomyces madA* gene encodes a blue-light photoreceptor for phototropism and other light responses

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Phycomyces blakesleeanus **is a filamentous zygomycete fungus that produces striking elongated single cells that extend up to 10 cm into the air, with each such sporangiophore supporting a sphere containing the spores for dispersal. This organism has served as a model for the detection of environmental signals as diverse as light, chemicals, touch, wind, gravity, and adjacent objects. In particular, sporangiophore growth is regulated by light, and it exhibits phototropism by bending toward near-UV and blue wavelengths and away from far-UV wavelengths in a manner that is physiologically similar to plant phototropic responses. The *Phycomyces madA* mutants were first isolated more than 40 years ago, and they exhibit reduced sensitivity to light. Here, we identify two (duplicated) homologs in the White Collar 1 family of blue-light photoreceptors in *Phycomyces*. We describe that the *madA* mutant strains contain point mutations in one of these genes and that these mutations cosegregate with a defect in phototropism after genetic crosses. Thus, the phototropic responses of fungi through *madA* and plants through phototropin rely on diverse proteins; however, these proteins share a conserved flavin-binding domain for photon detection.**

light, oxygen, or voltage domain | Max Delbrück | photosensor | White Collar 1 | evolution

The most elaborate forms of life on earth required the acquisition of fundamental sensory traits during their evolution to be successful, particularly in terrestrial settings. These environmental signals include gravity, light, touch, and chemicals. With the explosion of research in biochemical molecular biology, signaling by small molecules continues to be studied extensively in diverse organisms. However, questions about how organisms sense other equally important environmental stimuli, such as light, remain to be elucidated at the molecular level.

Phycomyces blakesleeanus is a model zygomycete fungus that grows as a mycelium mass of filamentous hyphal cells. Under the correct environmental cues, the mycelium produces aerial unbranched hyphae of up to 10 cm in length, with each such sporangiophore supporting a sphere that contains the spores for dispersal. Sporangiophore growth is regulated by light (Fig. 1*A*) and exhibits phototropism by bending toward near-UV and blue wavelengths and away from far-UV wavelengths in a manner that is physiologically similar to the phototropic responses of plants (1). The fungus can sense light, chemicals, touch, gravity, and even adjacent objects without contact, and it responds by changing the direction and speed of sporangiophore growth. Also, light induces the synthesis of the pigment β-carotene and regulates the development of sporangiophores from the mycelium (1–3). Phototropism of the sporangiophore has been particularly well examined. It is a blue near-UV response with a very low threshold, similar to that of the human eye (4); the *Phycomyces* sporangiophore can sense blue light over an intensity range from 10^{-9} to 10^{2} W/m^2. To manage this enormous intensity range, *Phycomyces* has mechanisms of light and dark adaptation and at least two photoreceptor systems that are optimized to operate in different intensity ranges (5, 6). The action spectra for phototropism suggested the presence of a flavin-based chromophore (7–9), and substitution by roseoflavin in the photoreceptor of a *Phycomyces* flavin auxotroph demonstrated that the major photoreceptor system(s) contains a flavin molecule (10). However, the nature of the photoreceptors has remained elusive.

Phycomyces mutants with defective phototropism were isolated in the 1960s in the laboratory of Nobel Laureate Max Delbrück, with the aim of identifying the components of the photosensing transduction pathway (11). The phototropic mutants were later named *mad* mutants to honor Max Delbrück. Genetic analysis identified 10 *mad* genes (*madA–madJ*). The pleiotropy of mutations that affect more than one photoresponse suggested that *Phycomyces* phototransduction relies on a combinatorial array of gene products (2). Mutants in the *madA*, *madB*, and *madC* genes have defective phototropism but their sporangiophores react normally to gravity and other environmental signals (Fig. 1*A*). Also, strains with mutations in the *madA* and *madB* gene are also defective in other photoresponses. Detailed characterization of the photoresponses in these strains suggests that the products of the *madA*, *madB*, and *madC* genes represent the components of a major photoreceptor(s) complex, although the triple mutant does exhibit some residual photosensitivity (2, 4).

Most of our understanding of fungal photosensors is based on the ascomycete *Neurospora crassa*. The White Collar (WC)-1 photoreceptor has been characterized extensively and is associated with FAD, presumably through the light, oxygen, or voltage (LOV) domain (12–14), and it functions with WC-2 (15, 16). LOV is a flavin-binding domain present in proteins that sense light, oxygen, and voltage (17), including phototropin, a photoreceptor for plant phototropism (18, 19). WC-1 and WC-2 interact through PAS (Per/Arnt/Sim) domains that are present in both proteins to form a complex that binds the promoter of light-inducible genes to direct light responses, including the induction of conidiation, production of carotenoids, regulation of the circadian clock, and the orientation of the sexual fruiting

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Abbreviations: LOV, light, oxygen, or voltage; WC, White Collar; MAP, mitogen-activated protein.

Data deposition: The nucleotide sequences reported in this paper have been deposited in the GenBank database [accession nos. DQ229145 (*madA*) and DQ229146 (*wcoA*)].

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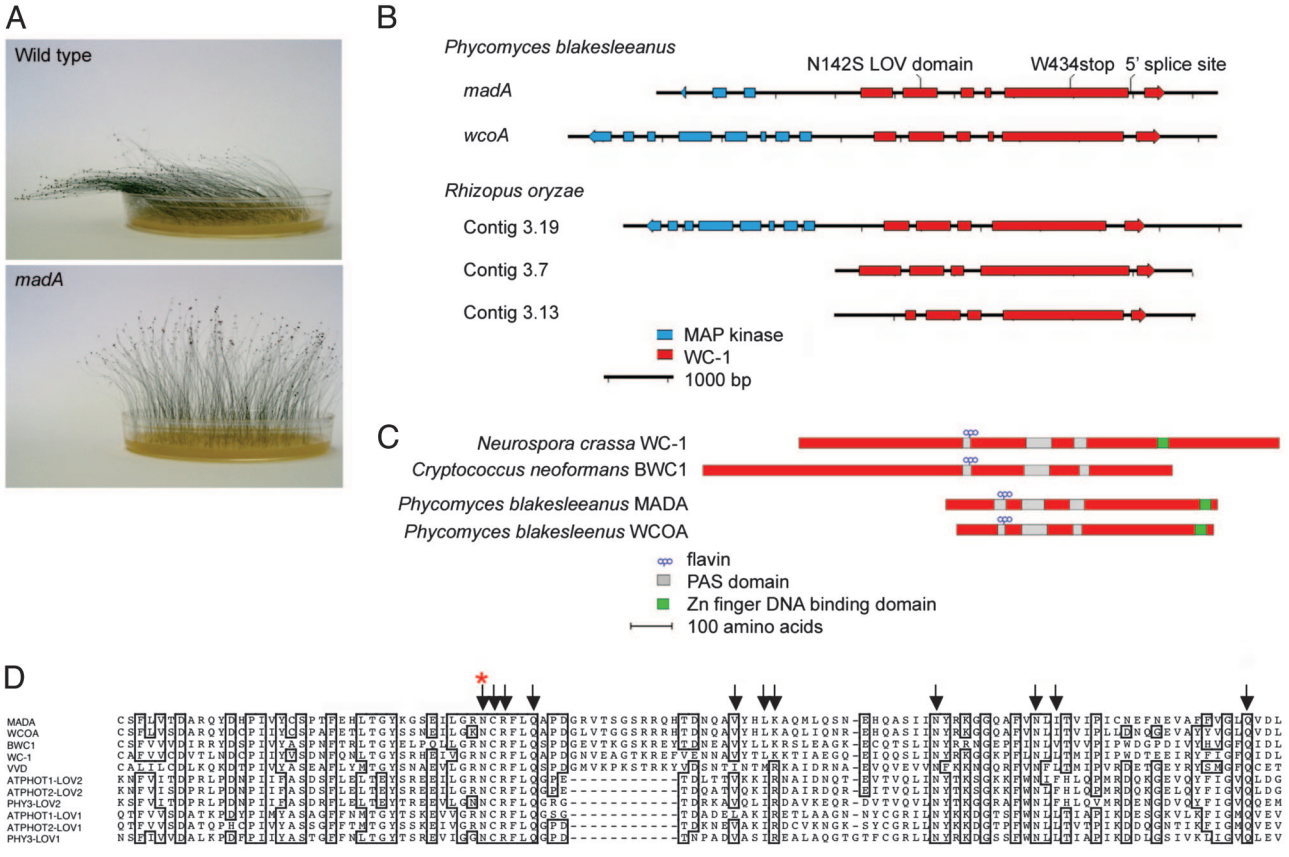


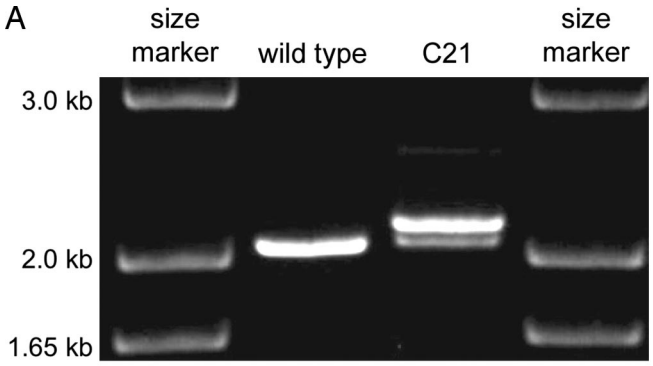
Fig. 1. WC-1 homologs are required for blue-light responses in diverse fungi, including the zygomycete *P. blakesleeanus*. (*A*) Phototropic bending of sporangiophores toward white light (illuminated from the left) occurs in WT *Phycomyces* and is reduced in the *madA* mutant strains. (*B*) Alignment of the conserved intron–exon gene structures of *wc-1* homologs from the zygomycetes *P. blakesleeanus* and *R. oryzae*. A MAP kinase gene lies upstream of both *Phycomyces* genes and one of the *Rhizopus* genes. Mutations identified in *madA* mutant strains of *Phycomyces* are shown, including an amino acid substitution within the LOV domain that is predicted to perturb the flavin chromophore interaction, a premature stop codon, and a splicing mutation. (*C*) Alignment of domain structure in the putative blue-light receptors from the ascomycete *N. crassa*, the basidiomycete *C. neoformans*, and the two homologs from *Phycomyces*. (*D*) Alignment of LOV domains from the *Phycomyces madA* and *wcoA* gene products with other photoreceptor proteins of fungi (*C. neoformans* BWC1 and *N. crassa* WC-1 and VVD) and plants (*A. thaliana* ATPHOT phototropins 1 and 2 and *Adiantum capillus-veneris* PHY3). Arrows indicate residues that are important for flavin interaction. *, Asparagine residue mutated in strain C47.

structures (20–22). Whereas other candidate photoreceptors have emerged from fungal genome-sequencing projects (i.e., opsins, phytochromes, and cryptochromes), the WC-1/WC-2 system is conserved in divergent fungi. Recently a modified WC-1/WC-2 system was identified as required for responses to UV and blue light in the basidiomycetes *Cryptococcus neoformans* and *Coprinus cinereus* (23–25). Therefore, the photosystem predates the ascomycete/basidiomycete divergence. We hypothesized that it might be even more ancient and that the zygomycete fungi, like *Phycomyces*, would contain a WC-1 photosensor. Here, we identify two *wc-1* genes in *Phycomyces*, show that one of them bears mutations in a set of *madA* mutants, and establish (by genetic crosses and linkage analysis) that these mutations cause defects in phototropism.

Results

Zygomycete Fungi Contain Duplicated Copies of a WC-1 Gene. Homologs of the fungal blue-light receptor WC-1 gene were sought by amplification from *Phycomyces* genomic DNA by using PCR with degenerate oligonucleotide primers. The primers were designed based on conserved regions within the ascomycete and basidiomycete WC-1 proteins. Segments of two genes similar to *wc-1* were amplified. The complete genes were isolated by screening gene libraries and inverse PCR, and intron–exon positions were confirmed by sequencing cDNA clones from RT-PCR products (Fig. 1*B*). The two *Phycomyces* genes share limited identity at the DNA sequence level, with the longest stretch of 321 bp being 73% identical. The two predicted proteins are similar in size (624 and 660 aa), are 58% identical, and have three predicted PAS domains, the first of which is a putative flavin-binding LOV domain, and a zinc-finger DNA-binding domain (Fig. 1*C*). The proteins have nuclear localization signals at their C termini and are predicted to be nuclear-localized based on analysis with PSORT II software (26). The genes each have five introns, and their positions are conserved (Fig. 1*B*). Based on the nucleotide identity and conserved intron–exon boundaries, we propose that an ancestral gene was duplicated. This conclusion is supported also by the finding that a gene encoding a Fus3/Kss1 family mitogen-activated protein (MAP) kinase is located upstream of both genes. This gene is also divergent at the nucleotide level but shares common intron–exon boundaries. After the discovery of the *Phycomyces* wc-1 genes, the genome sequence for another zygomycete (*Rhizopus oryzae*) was released (Broad Institute, Cambridge, MA). Three *wc-1* genes are present within the *R. oryzae* genome, and all three of them are predicted to share intron–exon boundaries with the *Phycomyces* genes. However, only one of the three genes has a MAP kinase gene-associated upstream (Fig. 1*B*).

***Phycomyces madA* Phototropism Mutants Contain Mutations in One of the *wc-1* Genes.** To determine whether either of the two *Phycomyces wc-1* genes is required for light-sensing, the genes were



B

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Wild type AGAAAGgtaatatacatttatagaatctgtacactacatttttt
C21 a     AGAAAGATAATATACATTTATAGAATCTGTACACTACATTTTTTT
C21 b     AGAAAGATAATATACATTTATAGAATCTgtacactacatttttt
C21 c     AGAAAGATAATATACATTTATAGAATCTGTACACTACATTTTTT

Wild type ttctttggtttttatctagtacacattgaattcctttgtacgcca
C21 a     TTCTTTGGTTTTTATCTAGTACACATTGAATTCCTTTGTACGCCA
C21 b     ttctttggtttttatctagtacacattgaattcctttgtacgcca
C21 c     TTCTTTGGTTTTTATCTAgtacacattgaattcctttgtacgcca

Wild type ttcttaattttaatttttaattttttttaaaaaaaaaatagCAAAA
C21 a     TTCTTAATTTTAATTTTTAATTTTTTTTAAAAAAAAAATAGCAAAA
C21 b     ttcttaattttaatttttaattttttttaaaaaaaaaatagCAAAA
C21 c     ttcttaattttaatttttaattttttttaaaaaaaaaatagCAAAA
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Fig. 2. Aberrant cDNA splicing of the *madA* gene in the light-sensing mutant C21. (*A*) The cDNAs for the *madA* gene from the WT strain NRRL1555 and *madA* mutant strain C21 were amplified by PCR, separated by agarose gel electrophoresis, and visualized after staining with ethidium bromide. Size markers are a 1-kb DNA ladder. Two transcripts were observed with cDNA obtained from strain C21. (*B*) Nucleotide sequence of a single transcript cloned from WT and three different transcripts (a, b, and c) cloned from strain C21. The G → A mutation in strain C21 is shown in bold. Coding nucleotides are shown in uppercase, and intron nucleotides are shown in gray lowercase font. For C21 transcripts a and b, the zinc-finger domain will be deleted by introduction of premature stop codons (underlined in C21 a). For transcript c, an additional 19 aa will be added to the protein, in frame with the zinc-finger DNA-binding domain.

sequenced from a collection of *Phycomyces* strains that contain *madA*, *madB*, and *madC* mutations. These strains have been well studied, and they include original strains from Delbrück's laboratory (e.g., C21 and C47). Mutations were found in one of the *wc-1* genes in the following investigated *madA* mutant strains: A893/A895, C21, and C47 (Fig. 1*B*).

In strains A893 and A895, a premature stop codon is caused by a TG**G** → TG**A** mutation (Trp-434). This mutation will result in a protein that is 227 aa shorter than WT and that lacks the zinc-finger domain.

In strain C47, an A**A**C → A**G**C mutation causes an asparagine (Asn-142)-to-serine substitution. This amino acid is highly conserved within LOV domains and mediates hydrogen bonding with the flavin chromophore and water (27) (Fig. 1*D*). Mutation of this residue to aspartic acid in the LOV1 or LOV2 domains of the oat phototropin protein nph1 reduces FMN affinity to 5% and 20%, respectively (28). The equivalent mutation has been introduced into the *N. crassa* WC-1 protein and expressed in place of WT WC-1 (29). The resulting strains are less sensitive to light, comparable with strains in which the adjacent cysteine residue (which forms a light-induced covalent linkage with FAD) has been mutated.

In strain C21 a G|**G**TAATA to G|**A**TAATA substitution in the final intron blocks splicing (|), resulting in mRNAs with frameshifts and premature stop codons (Fig. 2). In Northern blot analyses of strain C21 (see Fig. 4), transcription of the *madA* gene appears to be WT. Therefore, the splicing mutation in C21 was confirmed after PCR amplification and sequence analysis of corresponding cDNAs from the WT and the *madA* strain C21 (Fig. 2). PCR of the *madA* strain C21 generated two DNA species that were both longer than the cDNA from WT. The sequence of the cDNAs showed that they contained DNA derived from intron 5; the longer cDNA contained the entire intron 5, two types of shorter cDNAs were cloned that contained either 22 or 57 nt from intron 5, suggesting that secondary splicing sites have been activated within this intron (Fig. 2).

The three examined *madA* strains have mutations in the same gene that is similar to *wc-1*. Therefore, we hypothesize that the *madA* phenotype is caused by mutations in this gene, and we propose that it be named *madA* (we name the second *wc-1* gene *wcoA*, for *w*hite *c*ollar *o*ne *A*).

Mutations in *madA* Cosegregate with Impaired Phototropic Response in a Genetic Analysis. To test the hypothesis that the *wc-1* gene corresponds to the *madA* locus, we performed genetic crosses between *madA* strains (C21 or C47, mating type minus) and an isogenic strain A56 (mating type plus). Two strains were coinoculated onto agar medium, underwent fusion, and formed a line of zygospores between the inoculation points. *Phycomyces* zygospores exhibit dormancy, and thus, they were isolated onto wet filter paper and observed weekly; 3–4 months later, the zygospores germinated to form sporangia from which the meiotic progeny were isolated. From the C21 × A56 cross, 17 progeny from six zygospores were analyzed for both phototropic response and the sequence of the *wc-1* allele. The G → A mutation cosegregated with all 13 progeny exhibiting reduced phototropism (data not shown). The C47 mutant allele introduces a PvuII restriction-enzyme site, thus allowing facile genetic analysis. Progeny (50, from 21 independent zygospores) from the C47 × A56 cross demonstrate 100% linkage between the reduced light sensitivity in 34 of the progeny and the PvuII polymorphism (see

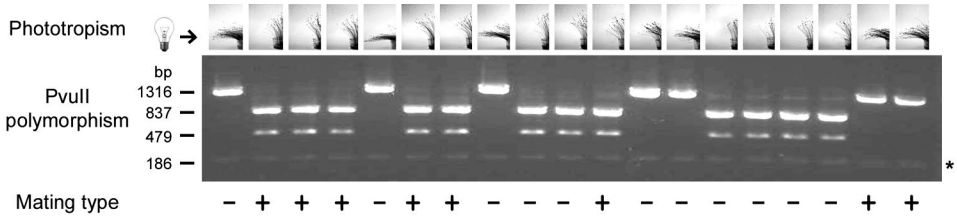

Fig. 3. Genetic evidence that the N142S mutation is linked to impaired light-sensing in *Phycomyces madA* strain C47. Progeny (19) from crosses of strains C47 × A56 were examined for phototropic responses, and the edges of the Petri dishes were photographed (equivalent to the left third of Fig. 1*A*). The *madA* gene was amplified by PCR and cleaved with the restriction enzyme PvuII to produce two (WT) or three (mutant) fragments. *, The smallest fragment of 186 bp is faint. The parents C47 (phototropism mutant, mating type minus (−), three PvuII fragments) and A56 (phototropism WT, mating type plus (+), two PvuII fragments) are not shown. The PvuII site introduced by the mutation cosegregates with reduced phototropic response in progeny from the cross. Mating type represents an independent locus to demonstrate meiotic recombination has occurred in the progeny.

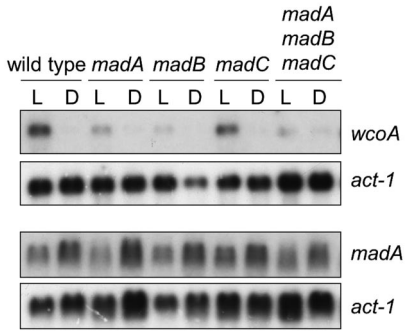

Fig. 4. Light-dependent and independent transcript regulation of the *Phycomyces wc-1* genes. RNAs from mycelia that were exposed to blue light for 30 min (L) or maintained in the dark (D) were hybridized with probes for the *madA*, *wcoA*, or *act-1* (actin) genes. The analyzed strains are as follows: WT, NRRL1555; *madA*, C21; *madB*, C111; *madC*, L1; and *madA madB madC*, L72. The *madA* transcript is still present in strain C21 because the *madA* mutation causes aberrant transcription events. The *wcoA* gene is induced by light dependent on the *madA* and *madB* genes, whereas *madA* levels are reduced slightly by exposure to light independent of *mad* mutant status. The light-induction experiments were performed three times, except on the triple mutant, which was examined twice.

Fig. 3 for a subset of this analysis). Thus, meiotic genetic-segregation analysis supports the conclusion that the *wc-1* gene corresponds to the *madA* locus.

Transcript Abundance of *madA* and *wcoA* Is Influenced by Light and Other *mad* Mutations. Because *wc-1* is light-regulated in *N. crassa*, we investigated the regulation of the *Phycomyces madA* and *wcoA* genes by blue light. In three independent experiments, blue light promoted the accumulation of *wcoA* mRNA, whereas the expression of *madA* was repressed slightly by light (Fig. 4). The full photoinduction of *wcoA* required the product of the *madA* and *madB* genes but not the *madC* gene. There was still some residual light induction in the triple *madA madB madC* mutant, perhaps mediated by the *wcoA* gene product. The *madC* mutant strains have defective phototropism of the sporangiophore, but mycelial photoresponses involving carotenogenesis and morphogenesis are normal (11). Thus, our observation confirms that the *madC* gene product is specific for sporangiophore photoresponses (30). Interestingly, the *madA* probe detected two mRNA species of similar size in the mycelia of the WT (and *mad* mutants), which may result from different transcription start sites.

Discussion

For decades, *Phycomyces* was a model species for the study of blue-light responses in diverse organisms. During the late 1960s, the first deliberately created mutants with impaired responses to light in any organism were generated in *Phycomyces*. However, it was not until the 1990s that the first blue-light receptors were cloned, not from *Phycomyces* but from the plant *Arabidopsis thaliana* and a divergent fungus *N. crassa*. The first blue-light receptor (the cryptochrome HY4 of *A. thaliana*) showed remarkable similarity to the photolyase proteins that use light to repair thymine dimer-induced damage of DNA after UV irradiation (31). Like photolyase, the photon-capturing ability of HY4 is mediated by interaction with a flavin (FAD) chromophore. A second class of blue-light receptors that also interact with flavins (FAD or FMN), but through a very different domain than in the cryptochromes, was identified subsequently. The light-sensing proteins WC-1 of *N. crassa* and nph1/phot1 of *A. thaliana* share a conserved LOV domain found in proteins that sense light, oxygen, or voltage (14, 32). The importance of this domain in photon detection is highlighted also by the recent analysis of a second blue-light receptor mediating sensitivity to light in *N. crassa* (33, 34). VIVID is a small (186-aa) protein, most of which comprises a LOV domain that interacts with either FAD or FMN. Here, we report that the *Phycomyces madA* mutants contain nucleotide substitutions that are predicted to impair the function of a related LOV domain protein.

Two homologs of the *wc-1* gene were identified from *Phycomyces* by using a degenerate primer/PCR cloning approach. That two genes are present is in marked contrast to other fungal species; current genome sequence evidence shows that all other fungi examined have no such gene (the hemiascomycetes, like *Saccharomyces cerevisiae*) or one such gene, as in the basidiomycetes or other ascomycetes (see the Fungal Genome Initiative, Broad Institute). The exception is a close relative of *Phycomyces*, the zygomycete *R. oryzae*, whose sequence was made available during these studies (Fig. 1*B*). Conserved intron–exon boundaries and conserved synteny with an upstream gene demonstrate that the zygomycete *wc-1* genes arose by means of gene duplication. Gene-duplication events in fungi (as shown best in the ascomycete fungus *S. cerevisiae*) can be either ancient or generated recently during laboratory culture (35, 36). The divergence in nucleotide sequence between the *Phycomyces* genes indicates an ancient duplication for the two *wc-1* homologs. This duplication event may have provided an opportunity to diversify function, tissue specificity, chromophore binding, sensitivity to light intensity, or regulation of target genes. A second evolutionary consideration is that the zygomycete WC-1 proteins contain a zinc-finger DNA-binding domain, which is present in the ascomycete but absent from the basidiomycete, WC-1 proteins (Fig. 1*C*) (23). The zygomycetes are considered to be basal to the ascomycetes and basidiomycetes within the fungal kingdom. The most parsimonious explanation for the current structures of the WC-1 proteins is that the form containing the zinc-finger DNA-binding domain is ancestral and the domain has been lost in the basidiomycete lineage.

One of the two *Phycomyces wc-1* genes corresponds to the *madA* locus. Mutations at this locus result in a severe loss of light sensitivity; the threshold for phototropism is 10,000-fold higher than that of the WT (8, 11). Mutations at *madA* also impair the mycelial carotenogenesis and morphogenesis photoresponses (30). One additional phenotype of *madA* is a reduction in flavin content, leading to a hypothesis that the reduced photoresponses in *madA* strains could be attributable to an impairment in chromophore availability to the photoreceptor(s) (37). The phenotype of the *madA* mutants, together with the data presented here on (*i*) the sequence of the *madA* gene from WT strain and three independent *madA* mutants, (*ii*) the nature of these *madA* mutations that affect the flavin-interacting domain and the zinc-finger DNA-binding domain, and (*iii*) genetic evidence, indicate that the MADA protein acts as a photoreceptor/transcription factor for phototropism and other light responses in *Phycomyces*.

The sequences of the proteins required for phototropism in *Phycomyces* and plants differ considerably. However, MADA and the photoreceptors for plant phototropism, the phototropins, both have flavin-binding LOV domains (Fig. 1*D*). One LOV domain is present in MADA, whereas two are present in phototropins (18, 19). The fungi use a LOV domain coupled to a zinc-finger DNA-binding domain as a direct means to alter transcription. The plant phototropins employ two adjacent LOV domains coupled to a serine/threonine kinase domain to alter protein phosphorylation. Because the similarity between MADA and phototropins is limited to the LOV domain, we conclude that the use of similar chromophore-binding domains in the photoreceptors for plant and fungal phototropism results from convergent evolution, probably mediated through domain shuffling, and that the downstream signal-transduction events are likely to be very different.

An important question for future consideration is how the light signal is transmitted from MADA to affect phototropism of *Phycomyces*. Because the stem of a plant is a multicellular structure, phototropic responses can be explained by differing growth rates in individual cells that are influenced by a gradient of light or a secondary signal throughout the tissue. The *A. thaliana* phot1 phototropin is predominantly a plasma membrane associated protein, capable of changing the phosphorylation state of ion channels or, possibly, auxin receptors (38). In contrast, the *Phycomyces* sporangiophore is a unicellular structure containing thousands of nuclei, and MADA is predicted to function as a nuclear localized transcription factor. Thus, the physical mechanisms of signal transduction during phototropism is likely to be very different between plants and fungi. Research has demonstrated that the sporangiophore acts as a magnifying lens to focus light (39). It was generally thought that the *Phycomyces* photoreceptor for phototropism was bound to the plasma membrane to provide the asymmetry of light perception that allows the unidirectional growth of the sporangiophore (40, 41). However, there is experimental evidence by laser microillumination that the receptor lies between the central axis of the sporangiophore and the cell wall (42), which is consistent with a nuclear (or cytoplasmic) localization, as supported by our findings on MADA. Altered transcription from those nuclei receiving the highest input from light MADA must enable sporangiophore bending; however, the controls of intracellular polarity remain a mystery that may be resolved by the identification of the genes mutated in other *mad* strains.

There are many active areas of investigation for *Phycomyces* photobiology, including the nature of the other *mad* mutations (*madB-madJ*) and the function of the second *wc-1* gene. WCOA may be active as a mycelial photoreceptor or active under specific light treatments. The observation (Fig. 4) that the *wcoA* transcript accumulates after 30 min of exposure to blue light in the mycelium supports this latter hypothesis, and it is tempting to speculate that this protein is required for photoadaptation or high-intensity photoresponses. The *madA* strains show reduced photoadaptation (43). The *madA*-dependent induction of *wcoA* is similar to the situation of the *N. crassa vivid* gene, which encodes a LOV-domain protein that mediates photoadaptation and is also light regulated through the WC-1 photoreceptor (33, 34). The other *madB* and *madC* strains could have mutations in *wc-2* homologs or in fungal blue-light receptors, such as cryptochromes or G protein-coupled receptors (44). The *madD–madJ* mutants are predicted to correspond to genes encoding products controlling signaling events downstream of light detection and/or to regulate the function of the photoreceptors themselves (2, 45). A completed *Phycomyces* genome sequence could be used to develop microarrays and high-resolution genetic maps to facilitate positional cloning of the other *mad* genes, further elucidating how the fungus responses to light, as well as enable discovery of the molecular basis by which other environmental signals are perceived.

Here, 40 years after their first isolation, we have identified the mutations in Max Delbrück's *madA* strains of *Phycomyces*, and thus, we demonstrate that this zygomycete shares a common photoreceptor with its basidiomycete and ascomycete counterparts. The identification of the molecular nature of the *madA* mutations begins the molecular characterization of phototropism and other photoresponses in *Phycomyces*. Also, plants and fungi employ a conserved flavin-binding domain in their photoreceptors that are responsible for phototropism. Although exposure of plants and fungi to light can mediate similar responses (such as phototropism), it can also have very different effects. This common LOV domain implies that photochemical and photophysiological studies (which in the past may have appeared to be species- or phenotype-specific) are of broader significance across species and even kingdoms. The prescient comment that Max Delbrück made in 1976, ". . . *Phycomyces* is the most intelligent primitive eukaryote and as such capable of giving access to the problems in biology that will be central in the biology of the next decades. . . . I do not expect to make great discoveries, but if I continue to do the spade work my successors may do so" (46), demonstrates his extraordinary vision to provide a research legacy that lasts to the present time.

Materials and Methods

Fungal Strains. Unless indicated otherwise, the *mad* mutants used in this study have been isolated in the WT *P. blakesleeanus* strain NRRL1555 (mating type minus) (11). The mutants were generated by nitrosoguanidine or ICR-170 chemical mutagenesis, and they include the following strains: *madA* (C21, C47, A893, and A895), *madB* (C109, C111, and C112), and *madC* [B2, B3, A202, C59 (NRRL1554 background), and L1 (mixed genetic background with UBC21)], as reported (11, 47–49). The strains contain single mutations, with the exception of C21 (*madA pde-1*) (50) and L72, which is a *madA madB madC* triple mutant (49). Strain A56 is a mating type plus strain isogenic to NRRL1555 (51). Strains were grown in yeast extract/peptone/2% glucose medium or in minimal agar (52) to generate mycelium for DNA or RNA extraction.

DNA Manipulations. Genomic DNA was extracted from freeze-dried mycelium by using an alkyltrimethylammonium bromide extraction buffer method (53). The degenerate oligonucleotide primers JOHE12844 (5′-AAYTGYCGYTTYYTBCARKC-HCC-3′) and JOHE12849 (5′-YTGRCGVARYTCRWAYT-GCC-3′) were used to amplify fragments of the *wc-1* genes from *Phycomyces*, and fragments were cloned into pCR 2.1 TOPO (Invitrogen). The following other primer combinations were used successfully to amplify these genes: JOHE14555 (5′-ATCGATTTMGGTCCCGTCGACTTBTCNTG-3′) and JOHE14558 (5′-GGNTGTTCNACVAAATCVACYTG-3′) or JOHE14557 (5′-CAAGTBGATTTBGTNGAACANCC-3′) and JOHE14562 (5′-CCACATATAGCCAGARTTCTTRCG-GCGRATDG-3′). An alternative approach was a nested PCR experiment with the following primers: WC1F, 5′-TACTCT-WVNWSNGGNTTYGAYATG-3′; and WC1R, 5′-AGGNAC-DATRTCNSWNGGRTG-3′. Then, 1 μl from a 1:50 dilution was used in a PCR with the following primers: WC2F, 5′-GGTAGAAAYTGYMGNTTYYTNCA-3′; and WC2R, 5′-CTGTTCNACNARRTCNAYYTG-3′. The standard degeneracy nomenclature for nucleotides is used (N = A, C, G, T; Y = C, T; R = A, G; B = C, G, T; K = G, T; H = A, C, T; V = A, C, G; M = A, C; W = A, T; D = A, G, T; S = C, G), and primer design took into consideration the codon bias of *Phycomyces* (54). Size-selected EcoRI and BamHI genomic libraries of strain NRRL1555, based on Southern blot analysis data, were constructed in pBluescript II SK(−), transformed into *Escherichia coli* strain DH5α, and screened to isolate fragments containing the *wc-1* homologs. An additional sequence was obtained by inverse PCR. For cDNA analysis, total RNA was reverse transcribed, and the genes were amplified with the following primers: Wc1.1F, 5′-CCTTTTCACGCATAAA-TACT-3′; Wc1.1R, 5′-AAAGTATATGTGATGCAAGG-3′; Wc1.2F, 5′-CACTTACTCATTCGTTAATT-3′; and Wc1.2R, 5′-AGCATTAGCATTAGCATTAG-3′. The genes were then cloned into pGEM T-Easy (Promega) and sequenced.

Northern Blot Analysis. Mycelium was exposed to darkness or 30 min of blue light (2.3×10^3 J/m^2) at the age of 48 h. RNA was extracted, separated by electrophoresis in an agarose–formaldehyde gel, transferred to a nylon membrane, and probed as reported (55) with [^{32}P]dCTP-labeled fragments of the *madA*, *wcoA*, and actin genes.

Mating Assays and Genetic Segregation. Crosses were conducted on 5% V8 juice agar medium (pH 7) between mating type minus and plus strains. Zygospores were removed from the plates with a pair of tweezers and placed on moist filter paper. Zygospores germinated after 3–4 months to form sporangia, from which the meiotic progeny were isolated by mechanical agitation in sterile distilled water and serial dilutions were inoculated onto V8 medium. For the progeny of C21 × A56, a fragment of the *madA* gene was amplified with the primers JOHE12901 (5′-GGTCTCTACTTTCTACCC-3′) and JOHE13508 (5′-GATGTAAAGATCCAAGAAGATGTG-3′) and sequenced with the primer JOHE12901. To map the mutation that is present in strain C47 for 49 progeny, a fragment of the *madA* gene was amplified from genomic DNA by PCR with the primers JOHE13505 (5′-ACAACCTGCCGAAGCCCAGAAACC-3′) and JOHE13506 (5′-CATCACTGGCACAATATCAGAAGG-3′), and it was cut with PvuII restriction enzyme. The WT allele results in two fragments (1,316 and 186 bp), and the mutant allele results in three bands (837, 479, and 186 bp) when resolved by agarose gel electrophoresis (Fig. 3). A 50th progeny (strain A265) was analyzed independently by sequencing the *madA* gene. For all progeny, mating type was assessed by crossing to the NRRL1555 and A56 strains to confirm that meiotic segregation had occurred.

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1. Cerdá-Olmedo, E. & Lipson, E. D. (1987) *Phycomyces* (Cold Spring Harbor Lab. Press, Woodbury, NY).
2. Cerdá-Olmedo, E. (2001) *FEMS Microbiol. Rev.* **25,** 503–512.
3. Bergman, K., Burke, P. V., Cerdá-Olmedo, E., David, C. N., Delbrück, M., Foster, K. W., Goodell, E. W., Heisenberg, M., Meissner, G., Zalokar, M., *et al*. (1969) *Bacteriol. Rev.* **33,** 99–157.
4. Galland, P. (1990) *Photochem. Photobiol.* **52,** 233–248.
5. Galland, P. (1991) *Photochem. Photobiol.* **54,** 1119–1134.
6. Galland, P. & Lipson, E. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 104–108.
7. Curry, G. M. & Green, H. E. (1959) *Proc. Natl. Acad. Sci. USA* **45,** 797–804.
8. Galland, P. (1983) *Photochem. Photobiol.* **37,** 221–228.
9. Galland, P. & Tölle, N. (2003) *Planta* **217,** 971–982.
10. Otto, M. K., Jayaram, M., Hamilton, R. M. & Delbrück, M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 266–269.
11. Bergman, K., Eslava, A. P. & Cerdá-Olmedo, E. (1973) *Mol. Gen. Genet.* **123,** 1–16.
12. He, Q., Cheng, P., Yang, Y., Wang, L., Gardner, K. H. & Liu, Y. (2002) *Science* **297,** 840–843.
13. Froehlich, A. C., Liu, Y., Loros, J. J. & Dunlap, J. C. (2002) *Science* **297,** 815–819.
14. Ballario, P., Vittorioso, P., Magrelli, A., Talora, C., Cabibbo, A. & Macino, G. (1996) *EMBO J.* **15,** 1650–1657.
15. Linden, H. & Macino, G. (1997) *EMBO J.* **16,** 98–109.
16. Crosthwaite, S. K., Dunlap, J. C. & Loros, J. J. (1997) *Science* **276,** 763–769.
17. Crosson, S., Rajagopal, S. & Moffat, K. (2003) *Biochemistry* **42,** 2–10.
18. Briggs, W. R. & Christie, J. M. (2002) *Trends Plant Sci.* **7,** 204–210.
19. Celaya, R. B. & Liscum, E. (2005) *Photochem. Photobiol.* **81,** 73–80.
20. Harding, R. W. & Melles, S. (1983) *Plant Physiol.* **72,** 996–1000.
21. Liu, Y., He, Q. & Cheng, P. (2003) *Cell. Mol. Life Sci.* **60,** 2131–2138.
22. Ballario, P. & Macino, G. (1997) *Trends Microbiol.* **5,** 458–462.
23. Idnurm, A. & Heitman, J. (2005) *PLoS Biol.* **3,** 615–626.
24. Lu, Y.-K., Sun, K.-H. & Shen, W.-C. (2005) *Mol. Microbiol.* **56,** 280–291.
25. Terashima, K., Yuki, K., Muraguchi, H., Akiyama, M. & Kamada, T. (2005) *Genetics* **171,** 101–108.
26. Nakai, K. & Horton, P. (1999) *Trends Biochem. Sci.* **24,** 34–35.
27. Crosson, S. & Moffat, K. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 2995–3000.
28. Salomon, M., Christie, J. M., Knieb, E., Lempert, U. & Briggs, W. R. (2000) *Biochemistry* **39,** 9401–9410.
29. Cheng, P., He, Q., Yang, Y., Wang, L. & Liu, Y. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 5938–5943.
30. Corrochano, L. M. & Cerdá-Olmedo, E. (1992) *Trends Genet.* **8,** 268–274.
31. Ahmad, M. & Cashmore, A. R. (1993) *Nature* **366,** 162–166.
32. Huala, E., Oeller, P. W., Liscum, E., Han, I. S., Larsen, E. & Briggs, W. R. (1997) *Science* **278,** 2120–2123.
33. Heintzen, C., Loros, J. J. & Dunlap, J. C. (2001) *Cell* **104,** 453–464.
34. Schwerdtfeger, C. & Linden, H. (2003) *EMBO J.* **22,** 4846–4855.
35. Koszul, R., Caburet, S., Dujon, B. & Fischer, G. (2004) *EMBO J.* **23,** 234–243.
36. Wolfe, K. H. & Shields, D. C. (1997) *Nature* **387,** 708–713.
37. Hohl, N., Galland, P. & Senger, H. (1992) *Photochem. Photobiol.* **55,** 247–255.
38. Sakamoto, K. & Briggs, W. R. (2002) *Plant Cell* **14,** 1723–1735.
39. Fukshansky, L. (1993) *J. Photochem. Photobiol. B* **19,** 161–186.
40. Jesaitis, A. J. (1974) *J. Gen. Physiol.* **63,** 1–21.
41. Steinhardt, A. R., Popescu, T. & Fukshansky, L. (1989) *Photochem. Photobiol.* **49,** 79–87.
42. Meistrich, M. L., Fork, R. L. & Matricon, J. (1970) *Science* **169,** 370–371.
43. Galland, P. & Russo, V. E. A. (1984) *J. Gen. Physiol.* **84,** 101–118.
44. Tsolakis, G., Moschonas, N. K., Galland, P. & Kotzabasis, K. (2004) *Photochem. Photobiol.* **79,** 360–370.
45. Campuzano, V., Galland, P., Alvarez, M. I. & Eslava, A. P. (1996) *Photochem. Photobiol.* **63,** 686–694.
46. Fischer, E. P. & Lipson, C. (1998) *Thinking About Science: Max Delbrück and the Origins of Molecular Biology* (Norton, New York).
47. Campuzano, V., Galland, P., Senger, H., Alvarez, M. I. & Eslava, A. P. (1994) *Curr. Genet.* **26,** 49–53.
48. Alvarez, M. I., Ootaki, T. & Eslava, A. P. (1983) *Mol. Gen. Genet.* **191,** 507–511.
49. Lipson, E. D., Terasaka, D. T. & Silverstein, P. S. (1980) *Mol. Gen. Genet.* **179,** 155–162.
50. Reddy, V. M., Galland, P. & Lipson, E. D. (1985) *Mol. Gen. Genet.* **201,** 124–125.
51. Alvarez, M. I. & Eslava, A. P. (1983) *Genetics* **105,** 873–879.
52. Cerdá-Olmedo, E. (1987) in *Phycomyces*, eds. Cerdá-Olmedo, E. & Lipson, E. D. (Cold Spring Harbor Lab. Press, Woodbury, NY).
53. Pitkin, J. W., Panaccione, D. G. & Walton, J. D. (1996) *Microbiology* **142,** 1557–1565.
54. Corrochano, L. M. & Ruiz-Albert, J. (2004) *Mycol. Res.* **108,** 858–863.
55. Rodríguez-Romero, J. & Corrochano, L. M. (2004) *Curr. Genet.* **46,** 295–303.