Supporting Information

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SI Text

Cloning the *wcoB cDNA*. We first amplified the 5′ and 3′ ends by 2 PCRs using primers (wcoBR5 and wcoB4F, and wcoBF3 and wcoB3R respectively (supporting information (SI) Table S2). The PCR products were digested with XhoI and ligated to obtain a *wcoB* cDNA that lacked the last exon. The cDNA sequence comprising the 3′ *wcoB* gene was obtained by RACE PCR (1), using the BD Smart RACE cDNA amplification kit (BD Biosciences), oligonucleotide wcoB-RACE3′ (Table S2), and mRNA from wild type mycelia exposed to blue light (30 min, $2.34{\cdot}10^3$ J/m^2). The RACE PCRs yielded a 440-bp DNA segment for the 3′ end of the *wcoB* cDNA, which was cloned and sequenced. The complete *wcoB* cDNA was amplifed using primers wcoB5R and wcoB4F (Table S2).

Bioinformatic Analysis. Protein domains were predicted using the SMART database (2). Protein comparisons and phylogenetic analysis were performed using the program MEGA4 (3). Phylogenetic trees were performed by the Neighbor-Joining method with corrected distances using the JTT model and pairwise gap deletions, and 1000 boostrap replicates. Multiple protein alignments were performed with the program ClustalW included in MEGA4.

Expression of the MADA/MADB Complex in *E. coli*. *Phycomyces* cDNAs for the MADA and MADB proteins were amplified and cloned into the bacterial expression vectors pGEX4T1 (GST-tag) (GE Healthcare) and pET28a (6XHis) (Novagen). DNA fragments encoding the MADA and MADB proteins were inserted into the BamHI-NotI sites of each plasmid. The fusion proteins were expressed in *E. coli* strain Rosetta2 (DE3) pLysS (Novagen) transformed with each or both recombinant plasmids. Proteins were expressed by adding isopropyl-D-galactopyranoside (0.5 mM, final concentration) to cultures at an OD_{600} between 0.4 and 0.5 at 37 °C in LB medium supplemented with ampicillin (100 μg/mL) and kanamycin (30 μg/mL). Expression was carried out in darkness for 4 h at 22 °C. For cell lysis, bacteria were frozen overnight at −20 °C, resuspended in 1X Bugbuster lysis buffer (Novagen), and incubated 20 min at room temperature on a wheel mixer to complete the lysis, or lysed with a French pressure cell press (Thermo Spectronic). The proteins samples were clarified by centrifugation at 120000 × *g* for 20 min at 4 °C. GST fusion proteins were purified with a GST resin (Novagen), stored at 4 °C in gluthathione elution buffer [50 mM Tris·HCl (pH 8.0), 10 mM gluthathione reduced], and used for SDS/PAGE and western hybridization.

SDS/PAGE and Protein Detection. The protein samples were boiled 5 min with 1XSDS loading buffer and proteins were resolved by SDS/PAGE through 7 and 10% gels and visualized by staining with Coomassie brilliant blue G in 40% methanol-10% glacial acetic acid. Gels were destained and scanned. The amounts of purified proteins were determined by a Bradford assay (Bio-Rad), and equal amounts of protein were loaded on gels. Proteins were transferred onto a nitrocellulose membrane (Bio-Rad) by protein gel blotting. The membrane was blocked to remove nonspecific binding using 8% skimmed milk powder in TBS-T (10 mM Tris·HCl, pH 7.5, 150 mM NaCl, and 0.1% [vol/vol] Triton X-100). An anti-MADA polyclonal antibody and an anti-MADB polyclonal antibody were used to 1:500 and 1:10000 dilution to detect MADA and MADB, respectively. Secondary antibody was used at 1:5000 dilutions and detected using anti-mouse or anti-rabbit antibody conjugated to horseradish peroxidase (Promega). To visualize a loading control, blots were stained with ponceau S (0.1% [wt/vol] in 1% [vol/vol] acetic acid). The data shown are representative of at least 3 independent experiments.

Antibodies Against MADA and MADB. Monospecific antibodies against MADA and MADB were produced by Pacific Inmunology after injection of synthetic peptides conjugated to carrier protein (MADA: ADDVAMSDMTEEEV, amino acids 545–558; MADB: VHDEDEETNEQQKRV, amino acids 286–300) to New Zealand White Rabbits. The antibodies were purified from production bleeds with affinity columns before storage and use. Specific binding to MADA and MADB was observed with protein extracts from *E. coli* strains that expressed either protein, but not in protein extracts obtained from *E. coli* strains that carried control plasmids without *Phycomyces* DNA.

Supplementary Legend to Fig. 1*C*. Accession numbers for LOV and WC-2 proteins. WC-1 and LOV-domain photoreceptors: *Phycomyces blakesleeanus* MADA (ABB77846), *Phycomyces blakesleeanus* WCOA (ABB77844), *Phycomyces blakesleeanus* WCOB (CAQ76857), *Mucor circinelloides* MWC-1A (CAJ13843), *Mucor circinelloides* MWC-1B (CAJ13844), *Mucor circinelloides* MWC-1C (CAJ13845), *Ustilago maydis* WC-1 (XP_400795), *Cryptococcus neoformans* BWC1 (AAT73612), *Coprinus cinereus* DST-1 (BAD99145), *Tuber borchii* WC-1 (CAE01390), *Magnaporthe grisea* WC-1 (XP_360995), *Trichoderma atroviride* BLR-1 (AAU14171), *Neurospora crassa* WC-1 (CAA63964), *Podospora anserina* WC-1 (CAD60767), *Aspergillus nidulans* LREA (AAP47230), *Neurospora crassa* VVD (CAF06140), *Bacillus subtillis* YTVA (BAF91488), *Adiantum capillus-veneris* PHY3 LOV2 (BAA36192), *Arabidopsis thaliana* PHOT1-LOV2 (O48963.1); WC-2 proteins: *Phycomyces blakesleeanus* MADB (CAQ76858), *Phycomyces blakesleeanus* WCTB (CAQ76859), *Phycomyces blakesleeanus* WCTC (CAQ77079), *Phycomyces blakesleeanus* WCTD (CAQ76860), *Aspergillus nidulans* LREB (AAP47576.1), *Bipolaris oryzae* BLR2 (BAF47401.1), *Neurospora crassa* WC-2 (CAE81996.1), *Trichoderma atroviride* BLR2 (AAU14172.1), *Hypocrea jecorina* WC-2 (AAV80186.1).

Supplementary Legend to Fig. 3*C*. We used the *mad* strains C21 and C47 (*madA*), C111 (*madB*), L1 (*madC*), C149 (*madD*), C110 (*madE*), A329 (*madF*), C307 (*madG*), L83 (*madH*), L153 (*madI*), A909 (*madJ*), L51 (*madA madB*), and L72 (*madA madB madC*).

1. Scotto-Lavino E, Du G, Frohman MA (2006) 3′ end cDNA amplification using classic RACE. *Nat Protoc* 1:2742–2745.
2. Marchler-Bauer A, Bryant SH (2004) CD-Search: Protein domain annotations on the fly. *Nucleic Acids Res* 32:W327–331.
3. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.
4. Crosson S, Moffat K (2001) Structure of a flavin-binding plant photoreceptor domain: Insights into light-mediated signal transduction. *Proc Natl Acad Sci USA* 98:2995–3000.
5. Idnurm A, et al. (2006) The *Phycomyces madA* gene encodes a blue-light photoreceptor for phototropism and other light responses. *Proc Natl Acad Sci USA* 103:4546–4551.

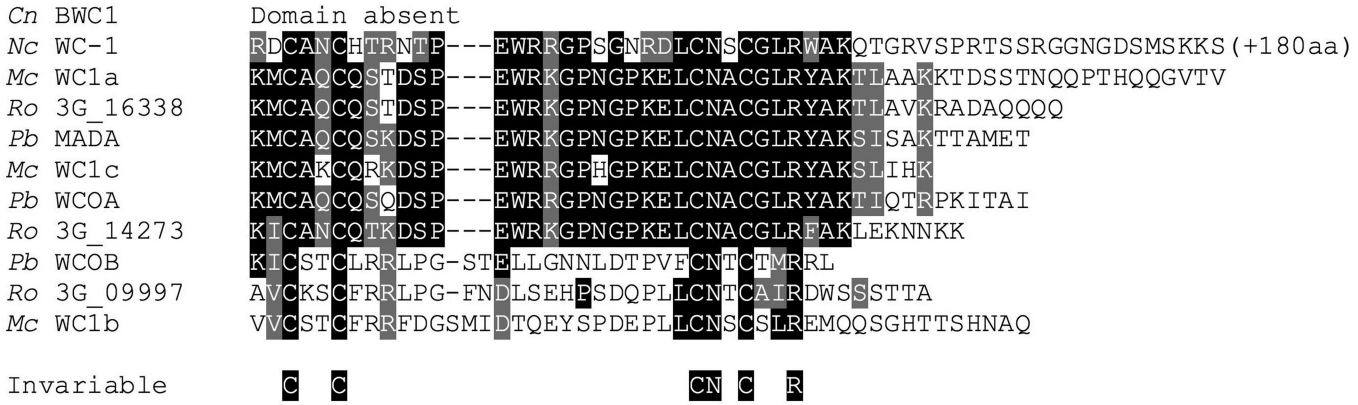


Fig. S1. A comparison of the zinc finger in the WC-1 proteins in *Neurospora crassa* (Nc), *Mucor circinelloides* (Mc), *Rhizopus oryzae* (Ro), *Cryptococcus neoformans* (Cn), and *Phycomyces blakesleeanus* (Pb). The zinc-finger is not present in the *Cryptococcus* protein, but a modified noncanonical version of the zinc-finger may be present in *Phycomyces* WCOB, *Mucor* WC1b, and the *Rhizopus* WC-1 protein 09997 as suggested by the conservation of a relevant set of cysteines. Amino acids that are conserved in all of the proteins are boxed in black, whereas less conserved amino acids are boxed in gray.

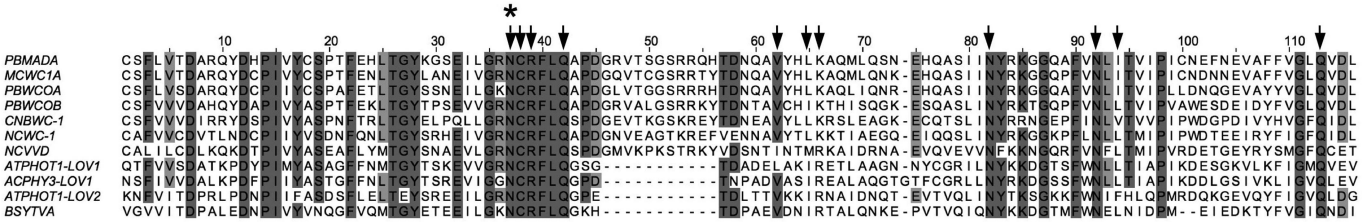


Fig. S2. Alignment of LOV domains. The proteins used in the alignments are PBMADA (*Phycomyces blakesleeanus* MADA, ABB77846), PBWCOA (*Phycomyces blakesleeanus* WCOA, ABB77844), PBWCOB (*Phycomyces blakesleeanus* WCOB, CAQ76857), MCWC1A (*Mucor circinelloides* MWC-1A, CAJ13844), CNBWC-1 (*Cryptococcus neoformans*, AAT73612), NCWC-1 (*Neurospora crassa* WC-1, CAA63964), NCVVD (*Neurospora crassa* VVD, CAF06140), ATPHOT1-LOV1 (*Arabidopsis thaliana* PHOT1-LOV1, O48963.1), ATPHOT1-LOV2 (*Arabidopsis thaliana* PHOT1-LOV2, O48963.1), ACPHY3-LOV1 (*Adiantum capillus-veneris* PHY3, BAA36192), BSYTVA (*Bacillus subtillis* YTVA, BAF91488). Arrows indicate residues that are important for flavin interaction in the PHY3 LOV2 domain (4). The asterisk indicates the asparagine residue mutated to serine in *madA* strain C47 (5).

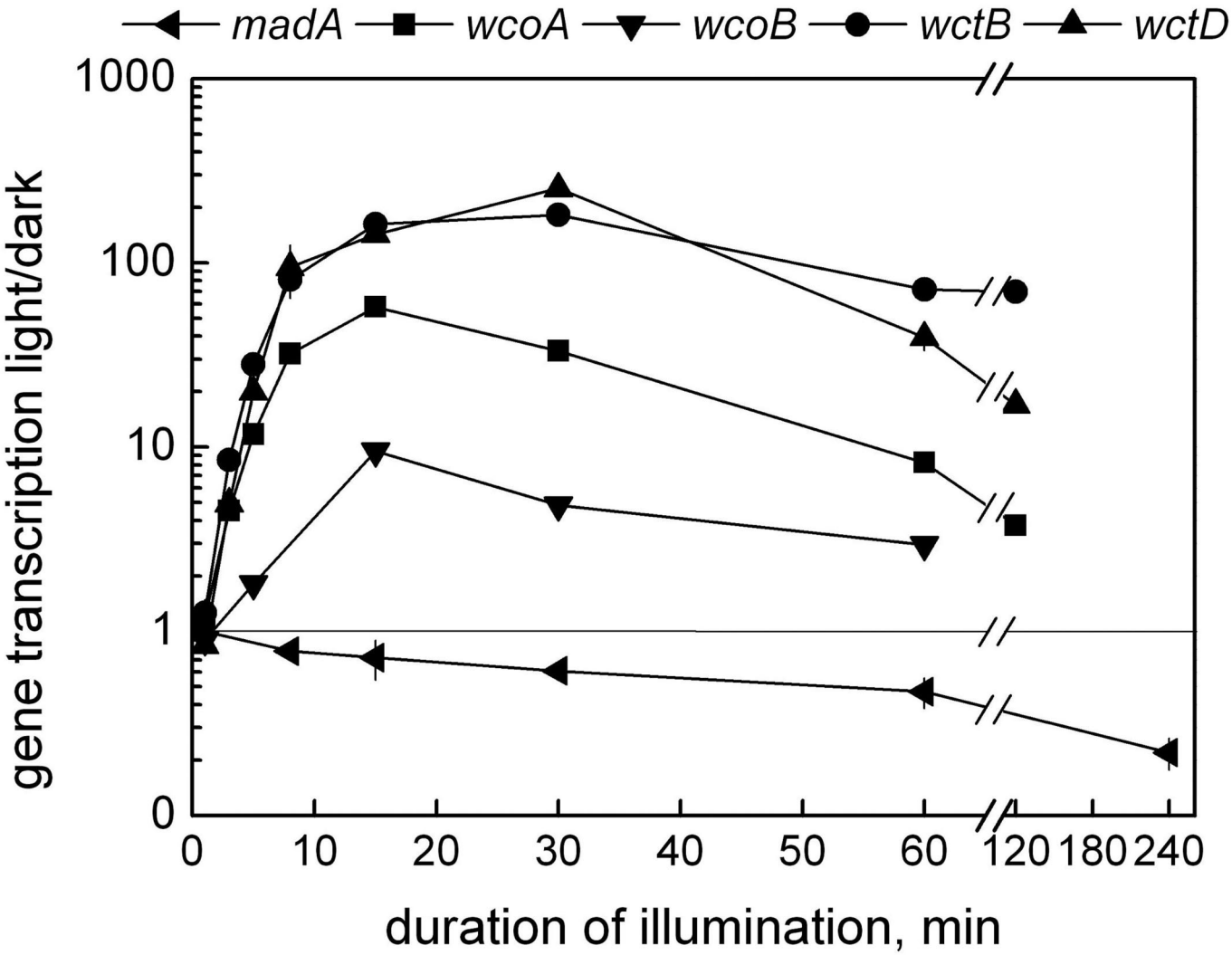
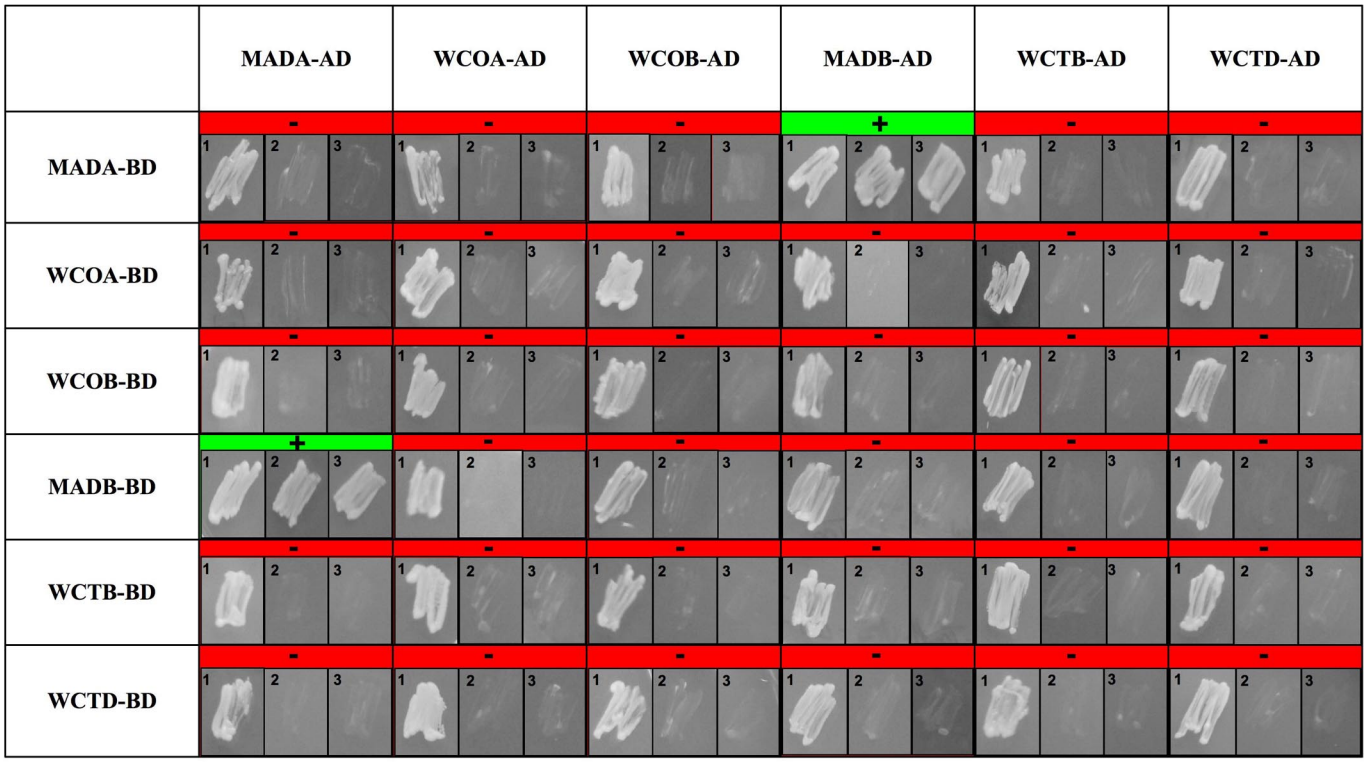


Fig. S3. Photoactivation of gene expression in the wild type after light exposures of different duration. Total RNAs were isolated from mycelia exposed to blue light for the times indicated, or kept in the dark. The amount of mRNA for each gene was assayed by quantitative RT-PCR. Each fluorescent signal was first normalized to the corresponding actin (*act-1*) signal to correct for loading errors and then was normalized to the signal obtained in the dark. The plots show the average and standard error of the mean of the relative photoactivation in 2–6 independent experiments.

+ POSITIVE INTERACTIONS. Cells grew on minimal media without uracil or histidine+10mM 3AT and became blue in a β-galactosidase filter assay .
- NEGATIVE INTERACTIONS. Cells did not grow on media lacking uracil or histidine + 10mM 3AT.



1 Synthetic Complete Medium without leucine and tryptophan (SC - Leu - Trp)
2 Synthetic Complete Medium without leucine, tryptophan and uracil (SC - Leu - Trp - Ura)
3 Synthetic Complete Medium without leucine, tryptophan and histidine + 10mM 3-Aminotriazole (SC - Leu - Trp - His + 10mM 3AT)

Fig. S4. MADA and MADB interact in yeast 2-hybrid assays. The coding regions of the *wc-1* genes *madA*, *wcoA*, and *wcoB*, and the *wc-2* genes *madB*, *wctB*, and *wctD* were fused adjacent to the AD and BD segments of *S. cerevisiae GAL4*. Plasmids were cotransformed into a *S. cerevisiae* strain in that the *GAL4* UAS regulates *URA3*, *HIS3*, and *lacZ* genes. Growth of strains in the absence of uracil or histidine and increased β-galactosidase activitiy as shown by a filter assay indicate protein–protein interactions. The gene *wctC* was not included because of our inability to isolate the corresponding cDNA or detect the *wctC* mRNA by PCR.

Table S1. Strains of *Phycomyces blakesleeanus* used in this work

Strain*	Genotype†	Origin‡
NRRL1555	(−)	
UBC21	(+)	
A56	(+)	A32 X NRRL1555
C21	*madA7 pde-1* (−)	NRRL1555, NTG
C47	*madA35* (−)	NRRL1555, NTG
A893	*madA403* (−)	NRRL1555, NTG
A896	*madA403 nicA101* (+)	A893 X C247
C109	*madB101* (−)	NRRL1555, NTG
C111	*madB103* (−)	NRRL1555, NTG
C112	*madB104* (−)	NRRL1555, NTG
C114	*madB106* (−)	NRRL1555, NTG
A457	*madB106 nicA101* (+)	A178 X S102
A458	*madB106 nicA101* (+)	A178 X S102
A459	*madB106 nicA101* (−)	A178 X S102
A178	*madB106* (+)	A56 X C114
A179	*madB106* (+)	A56 X C114
A269	*madB103* (+)	A56 X C111
A271	*madB103* (−)	A56 X C111
A273	*madB104* (+)	A56 X C112
A276	*madB104* (−)	A56 X C112
A754	*madB104 nicB401* (−)	A532 X C112
A820	*madB106 uraA* (+)	A520 X C169
A821	*madB106 uraA* (+)	A520 X C169
L1	*madC119* (−)	C264 X C148
A202	*madC469* (−)	A56 X B24
B2	*madC452* (−)	NRRL1555, ICR170
A429	*madC452 nicA101* (+)	A146 X S102
A905	*madC406* (−)	NRRL1555, ICR170
A34	*madD120 furA401* (+)	B71 X C149
A279	*madD120* (−)	A56 X C149
B27	*madD471* (−)	NRRL1555, ICR 170
C68	*madD59* (−)	NRRL1555, NTG
C149	*madD120* (−)	NRRL1555, NTG
A767	*madE102 nicB401* (−)	A532 X C110
C110	*madE102* (−)	NRRL1555, NTG
L157	*madE720* (−)	NRRL1555, NTG
L163	*madE726* (−)	NRRL1555, NTG
A327	*madF48 nicA101* (+)	A56 X C316
L161	*madF724* (−)	NRRL1555, NTG
A329	*madF48* (−)	A56 X C316
A281	*madG131* (+)	A56 X C307
C288	*madG131* (+)	C107 X C264
C307	*madG131* (−)	C288 X NRRL1555
L83	*madH703* (−)	NRRL1555, NTG
L85	*madH705* (−)	NRRL1555, NTG
L151	*madI714* (−)	NRRL1555, NTG
L153	*madI716* (−)	NRRL1555, NTG
A914	*madI712, lysA401* (−)	A637 X A845
A909	*madJ407* (−)	NRRL1555, NTG
A917	*madJ407 purA51* (−)	A543 X A909
L52	*madA7 madC119* (−)	L2 x C21
L51	*madA7 madB103* (−)	C303 X C21
L72	*madA7 madB103 madC119* (−)	L2 x L51
A818	*uraA* (+)	A520 X C169
A819	*uraA* (+)	A520 X C169

*Strains with prefix A come from Prof. Arturo P. Eslava (Universidad de Salamanca, Spain); C, from the late Prof. M. Delbrück (California Institute of Technology, Pasadena, CA, U.S.A.); L, from Prof. E. D. Lipson (Department of Physics, Syracuse University, Syracuse, NY, U.S.A.); S, from Prof. Cerdá-Olmedo (Universidad de Sevilla, Spain).

†Mutations labelled *mad* affect the phototropism of the sporangiophore; *pde*, cAMP phosphodiesterase; *car* , beta-carotene biosynthesis; *nic*, biosynthesis of nicotinic acid; *ura*, biosynthesis of uracil; *fur*, resistance to 5-fluorouracil; *lys*, biosynthesis of lysine. The mating type is indicated by the symbol (–) or (+).

‡The standard wild type NRRL1555 was obtained originally from the Northern Regional Research Laboratory, USDA, Peoria, IL, USA. A56 is the (+) isogenic strain of NRRL1555. Mutants were obtained previously by N-methyl-N′-nitro-N-nitrosoguanidine mutagenesis (NTG) or by 2-methoxy-6-chloro-9-[3-(ethyl-2-chloro-ethyl) aminopropylamino] acridine-2HCl mutagenesis (ICR170).

Table S2. Oligonucleotides used in this work

Name	Sequence (5′-3′)	Position*
Primers for *wctA* cloning		
WC2 1R	CCARCGNARNCCRCANGCRTTRCA (aa CNACGLRW)	
WC2 1F	TTCMSNMRNMGNAARAAYTGG (aa FRRRKNW)	
Primers for amplification for subsequent sequencing *wc* genes in *mad* mutants		
madAF	CGTTAATTCATCATGGATTCCTTCTCATCTTACC	−12 to + 22
madAR	GCCTTGCATTATGTTTCCATTGCAGTGGTTT	+2569 to + 2539
wcoAF	CATATATTACAATGCCTCAGTACTGTGACATATC	−11 to + 23
wcoAR	GGATTAGATTTAGATGGCTGTGATTTTAGGCC	+2385 to + 2354
wcoBF	CTCTTACTGTGTGACTTCTCGCATATT	−238 to −212
wcoBR	GAGTATCCAAGTTATTTCCAAGCAA	+3049 to + 3025
madBF	GGCAAATCCGTAGGCTCACAACA	−237 to −215
madBR	CGCGATAGCGATTATACACTATGGG	+1403 to + 1379
wctBF	CTGCACTTCCTCTTGCCTATTCC	−78 to −56
wctBR	GGTTCTTACCTCAGTGTAATG	+1548 to + 1528
wctCF	GAACAATTGGCACGCTTAGTTCGTA	−500 to −476
wctCR	GGTATTGATGAGGGAGGACGACTA	+1430 to + 1407
wctDF	ACATACCTCTTGTACATTC	−68 to −50
wctDR	AGGGATCACTAGGATCTCAC	+1510 to + 1491
Primers for ORF confirmation		
wctB-1F	TCTTGCCTATTCCCAAAGAC	
wctB-1R	TGACTTGCCAAATCGTATAC	
wctD-1F	CATTTACATACCTCTTGTACATTC	
wctD-1R	GAATTGGGATCACTAGGATC	
wctA-1F	TCCTACAACTTGTCTTTCAC	
wctA-1R	ATCCAACTATACGTGTGTAC	
wcoB-R5	CACAGTGGTTATGTCAGAGG	
wcoB-4F	GTACGCCATGAATCCCTTTG	
wcoB-F3	TTGGCATGAGCTGCTACTTG	
wcoB-3R	TGTCCATAGGTTCCACACTC	
wcoB-RACE3	GCCGGGCTGATGCAGTGCCTGTTCTGGG	
wcoB-5R	CTACAAGCGCCGCATAGTAC	
Primers for *madB* genotyping		
JOHE18108	CTTCATACGATATGCTTGAG	
JOHE18109	TTGCTTGTCTTTGCCCAACG	
Primers for quantitative PCR		
act-11	AGGCTGTCCTTTCCCTTTACG	
act-12	GACACCATCACCGGAATCG	
hspA1	CGAATTCTCGCACACTCTCTTG	
hspA2	CTGTCCGTCAACATCGTCAAA	
madA1	AGTGAAGATGTGGTCGGCTACTC	
madA2	TGTCAAGAACGTAACGGAAGATTG	
madB1	GCCATTTCTTCAAAGGATGTTTCT	
madB2	TGTGTCCATCATGCGAGTAGACT	
wcoA1	GAAGGAGCCTCGATTTACCAATATAT	
wcoA2	TTGGCGGACCAGGTCAAA	
wcoB1	GCAACTTCCTTTCGCTCAAGA	
wcoB2	CATGGCTCCACTGTTCTGTTGT	
wctB1	GCTATTAGGTTACCAGCCCTCAGA	
wctB2	CCTCGCCGTCGTGAATG	
wctC1	TGCAGGACCATCAATACACACA	
wctC2	GGATTCGTGGAGATTGTCTTGAA	
wctD1	CGGCCACTACCCACATGAG	
wctD2	ACGTCCAGATCGTCGATATGC	

*Position from the initiation ATG