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FULL PAPER

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Isolation of *madA* homologs in *Pilobolus crystallinus*

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Abstract *Pilobolus crystallinus* shows unique photoresponses at various growing stages. cDNAs for putative photoreceptors were cloned from this fungus. Three genes named *pcmada1*, *pcmada2*, and *pcmada3* were identified from the PCR fragments, and amplified with degenerated primers for the LOV domain, which is conserved in many blue-light receptors. Deduced amino acid sequences for PCMADA1, PCMADA2, and PCMADA3 had one light-oxygen-voltage (LOV)-sensing and two PER-ARNT-SIM (PAS) domains. A zinc finger DNA-binding motif was conserved in the C-terminals of PCMADA1 and PCMADA3. However, PCMADA2 lacked the zinc finger motif. Expression of *pcmada1* was suppressed by blue light whereas that of *pcmada3* was promoted by blue-light irradiation.

Key words Blue light · Mucorales · Photoreceptor · Phototropism

Introduction

As the mucoralean fungus *Pilobolus* shows various photoresponses, it has been used for studies of photomorphogenesis (Jacob 1959; Kubo and Mihara 1986), circadian rhythm (Uebelmesser 1954; Bruce et al. 1960), and phototropism (Page and Curry 1966; Kubo and Mihara 1996a). Blue light induces the formation of a trophocyst, from which a sporangiophore develops (Page 1956; Jacob 1959). The initiation of sporangiophore development is suppressed by continuous light. A dark treatment given after trophocyst formation induces the initiation of sporangiophore development, and the dark treatment can be substituted by a low-temperature treatment (Jacob 1961; Kubo and Mihara 1986). Light induces the formation of a sporangium at the top of a sporangiophore (Page 1962). Projection of the sporangium is controlled by circadian rhythm (Uebelmesser 1954; Bruce et al. 1960).

Phototropism of the sporangiophore is one of the most sensitive photoresponses of *Pilobolus* and has been extensively studied (Jacob 1964; Page and Curry 1966; Kubo and Mihara 1996a). Young and mature sporangiophores show typical positive phototropism to blue light and negative phototropism from ultraviolet-B (UV-B) light. Although the fluence-response curves for both blue and UV-B light show first and second positive curves (Kubo and Mihara 1988), the sensitivity of a sporangiophore to light is affected by preirradiation (Kubo and Mihara 1989). The lag period for the second positive curve was extended when the fluence rate was changed after the start of irradiation, indicating an adaptive process is involved in the phototropism of *P. crystallinus* (Kubo and Mihara 1996b). These diverse photoresponses cannot be explained by a simple photoreceptor system. Such unique phototropism and photomorphogenesis of *P. crystallinus* are suitable subjects for future photobiological studies. However, no molecular biological research on photoreceptors and signal transduction has been carried out in *P. crystallinus*.

In other fungi, plants, and stramenopiles (Takahashi et al. 2007), various blue-light photoreceptors have been heretofore identified. Many of them are flavoproteins having a light-oxygen-voltage (LOV)-sensing domain that is a subgroup of the PER-ARNT-SIM (PAS) domain. WHITE COLLAR-1 (WC-1), the first identified fungal photoreceptor, was cloned from a blind mutant of *Neurospora crassa* (Ballario et al. 1996). WC-1 contains one LOV domain, two PAS domains, and one zinc finger domain. Another blue-light photoreceptor, VIVID, was isolated from a mutant of *N. crassa* that shows an increased accumulation of carotenoids (Heintzen et al. 2001). VIVID contains only one LOV domain and is considered to be involved in photoadaptation of blue-light responses (Schwerdtfeger and Linden 2003). In Zygomycetes, the *madA* gene of *Phycomyces blakesleeanus* was recently identified as the blue-light receptor for phototropism by using degenerate oligonucleotide primers designed from the LOV domain sequences (Idnurm et al. 2006). Three *wc-1* homologs are also identified in

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Mucor circinelloides (Silva et al. 2006). Here, I cloned *madA* homologs from *P. crystallinus* and preliminarily analyzed their expression under different light conditions.

Materials and methods

Pilobolus crystallinus (F.H. Wiggers) Tode, strain NBRC 8561, was obtained from the NITE Biological Resource Center, Kazusa, Japan. Sporangiospores were inoculated in MYC medium (malt extract 1%, yeast extract 0.2%, casamino acids 0.2%) and cultured at 22°C under light or in darkness. White light (7.2 W/m^2) was obtained from fluorescent tubes (FL20SD; Toshiba, Tokyo, Japan). Red (2.1 W/m^2) and blue light (0.5 W/m^2) was obtained from fluorescent tubes (FL40SD-SDL; Toshiba) combined with Acrylite plates no. 102 and no. 313 (Mitsubishi Rayon, Tokyo, Japan), respectively. Dim red light was used for the isolation of RNA and photobiological treatments in a dark room.

Genomic DNA was isolated with the modified CTAB method (Hayakawa 1997). Mycelium (200 mg) grown in liquid medium was washed with distilled water and drained thoroughly, then ground in liquid N_2 and extracted with extraction buffer [100 mM Tris-HCl (pH 8.0), 20 mM ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl, 2% CTAB] at 65°C. After extraction twice with chloroform: isoamyl alcohol (24:1), DNA was precipitated with 1% CTAB buffer [50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% CTAB]. The pellet was dissolved with 1 M CsCl and DNA was harvested by adding 100% ethanol. The DNA pellet was washed with 70% ethanol, dried, resuspended in sterilized MilliQ water, and used for polymerase chain reaction (PCR). Total RNA was isolated by the standard phenol method. Mycelium ground in liquid N_2 was extracted with extraction buffer [100 mM Tris-HCl (pH 9.0), 100 mM NaCl, 1% sodium dodecyl sulfate (SDS)] and phenol equilibrated with 1 M Tris-HCl (pH 9.0). After extraction with phenol:chloroform:isoamyl alcohol (25:24:1) three times, RNA was precipitated with ethanol and 250 mM NaCl. The pellet was dissolved with RNase-free water and RNA was precipitated by LiCl. After ethanol precipitation, the pellet was washed with 70% ethanol, dried, resuspended in RNase-free water, and used for reverse transcription-PCR (RT-PCR).

PCR was performed with the degenerated oligo primers: MadFwd (5′-AAYTGYMGNTTYYTNCARKCNCC-3′) (Idnurm et al. 2006), MadRev (5′-GCYTGNCCNCCYT TNCKRTARTT-3′), Mad2Fwd (5′-TTYGARNNNYTNA CNGGNTA-3′), and Mad2Rev (5′-TTYGARNNNYTN ACNGGNTA-3′) to amplify fragments of *madA* ortholog in *P. crystallinus* (N = A, C, G, T; Y = C, T; R = A, G; K = G, T; M = A, C). Amplified fragments were cloned into pGEM T-Easy (Promega, Madison, WI, USA) or Bluescript SK^+ (Stratagene, La Jolla, CA, USA), and the sequences were determined.

For 3′-and 5′-RACE (rapid amplification of cDNA ends), first-strand cDNA was synthesized by M-MuLV reverse transcriptase (Takara, Shiga, Japan) with a RACE primer (5′-AAGACTTCTCTCCGGTTTTTTTTTTTTTT-3′) for 3′-RACE and a gene-specific primer for 5′-RACE. For 5′-RACE, the 3′-end of the cDNA was polyadenylated with terminal deoxynucleotidyl transferase (Promega). PCR was carried out with a gene-specific primer and adapter primer (5′-AGCAAGACTTCTCTCCGGTT-3′). Amplified fragments were cloned into pGEM T-Easy and the sequences were determined. Sequence data were analyzed with GENETYX-MAC ver. 9.0 software (Software Development, Tokyo, Japan). Alignment and phylogenetic analysis of LOV domain sequences were performed with ClustalW ver. 1.83 (Thompson et al. 1994) and PHYML (Guindon et al. 2005), respectively. Conserved 116 amino acids in the LOV domains shown in Fig. 1 were used for phylogenetic analysis. Bootstrap cycle was 100. The phylogenetic tree was displayed with TREEVIEW ver 1.6.6 (Page 1996).

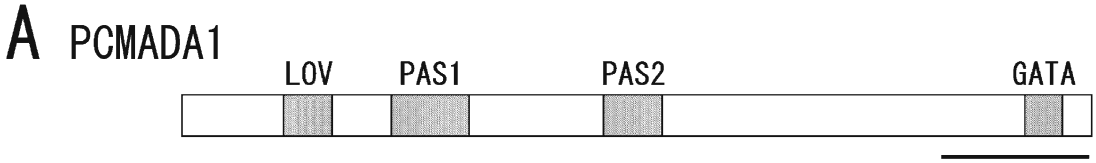
For semiquantitative RT-PCR, first-strand cDNA was synthesized by M-MuLV reverse transcriptase (Takara) with an oligo-$d(T)_{15}$ primer. The same amount of total RNA was used for template. PCR product was loaded on an agarose gel, electrophoresed, and stained with ethidium bromide. The density of the band was quantified by a densitometer (Densitograph; Atto, Tokyo, Japan). All the steps were done quantitatively. The nucleotide sequences of the primers used for sequencing and RT-PCR are shown in Table 1.

Table 1. Primer sequence

Primer name	Sequence
PCMAD1-1Fwd	5′-GCACAGATGTTGCAGAACAA-3′
PCMAD1-1Rev	5′-TTCAGATGGAACACTGCCTG-3′
PCMAD1-2Fwd	5′-AACACCAGGCCAGTATTATC-3′
PCMAD1-2Rev	5′-TTGTCTGTGTATGTACGGCG-3′
PCMAD1-3Fwd	5′-CATCTGCTGGAACATGATCC-3′
PCMAD1-3Rev	5′-GCATCGTGTGATGGAGATTC-3′
PCMAD1-4Fwd	5′-GTGCACTGGAACTGGTCAAG-3′
PCMAD1-4Rev	5′-GATCGGGATGACAGTAATTA-3′
PCMAD2-1Fwd	5′′-CAAGACACATATGGTACAGG-3′
PCMAD2-1Rev	5′-TTTCTCCTGGATCCTATAGC-3′
PCMAD2-2Fwd	5′-TAAGGAATCCCAGTCTAGTA-3′
PCMAD2-2Rev	5′-GATCCTATAGCCACATGACC-3′
PCMAD2-3Fwd	5′-CAGTGGTTATATGTGGATAG-3′
PCMAD2-3Rev	5′-GTACTGGCCACCATGACTTC-3′
PCMAD2-4FWD	5′-TGATCTTACCCGTGCATTGG-3′
PCMAD2-4Rev	5′-ATGACTGTTAACAGATTGAC-3′
PCMAD3-1Fwd	5′-AGTCACTTGTGGTTCACGTA-3′
PCMAD3-1Rev	5′-GCTTGATGTTCTTTTCCTTG-3′
PCMAD3-2Fwd	5′-CAATCAAGGAAAAGAACATC-3′
PCMAD3-2Rev	5′-TACGTGAACCACAAGTGACT-3′
PCMAD3-3Fwd	5′-CGATGCTAAACAGTATGACA-3′
PCMAD3-3Rev	5′-CCTTTAATATGGTATACGGC-3′
PCMAD3-4Fwd	5′-CCCATCAAGGAGAAATAGAT-3′
PCMAD3-4Rev	5′-GTATACCGGTCTTTCTCTGC-3′
GPDFwd	5′-TTATTCCTCTTGATTACATG-3′
GPDRev	5′-TGTAGCCCAAAATGCCCTTC-3′

Results

Degenerated primers designed for the LOV domain amplified several PCR fragments. Main fragments were cloned into a cloning plasmid and sequenced. Two MADA homo-

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(a) LOV domain
Pc  70-104 FLVTDARQYDCPIVYCSPTFEHLTGYHANEIVGRN
Mc  90-124 ---------------------N----L--------
Pb 108-142 ----------H---------------KGS------

(b) PAS domain 1
Pc 258-328 QQEWNKLLLDQSEDFIHVLSLKGFFLYSSRSSSHLLEHDPEELVGHPLSSICHPSDIVPVMREVKEAASHP
Mc 278-348 ----------------------------C-----------------------------------I------T
Pb 297-367 ---------EH----------------C-----D------------S----------------I----G-S

    PAS domain 2
Pc 140-194 KEHQASIINYRKGGQPFVNLITVIPITNDNNEVAFFVGLQVDLVEQPNAILEKMK
Mc 160-214 --------------------------C----------------------------

(c) GATA-type zinc finger domain
Pc 576-601 CAQCQRVDSPEWRKGPNGPKELCNAC
Mc 595-620 -----ST-------------------
Pb 618-643 -----ST-------------------
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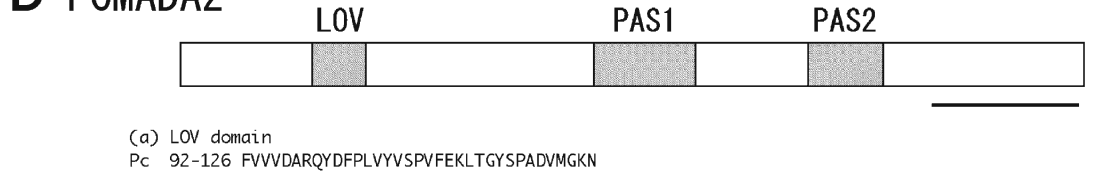


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(a) LOV domain
Pc  92-126 FVVVDARQYDFPLVYVSPVFEKLTGYSPADVMGKN
Mc  73-107 ------K--------A--M--R----A-SE-I-R-
Pb 138-172 ------H---A-I--A--T-------T-SE-V-R-

(b) PAS domain 1
Pc 280-350 RRSLHELLLDESPDFIHVLTIKGIFLYGSESTKQVLEYDPNELVGKSLKAICHPSDITTVMRELKQSSNDS
Mc 311-374        ---Q----------------C-D--A-L---E-S----------------------------H-
Pb 341-404        ---Q---------V------C--A-RPL--------------E------------------S-A

    PAS domain 2
Pc 427-479 LSVDGLLLYISWTCANILGSSPNEIIGTSLYQLMRSNRTTDLTRALAEVREGK
Mc 455-507 --A------V---V--V---P-S----------L---------------K---
Pb 491-543 --L------A---------LP-SQV------------------------K---
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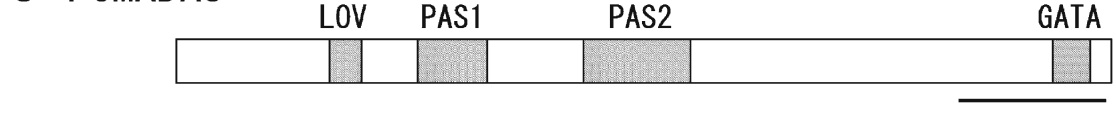


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(a) LOV domain
Pc 106-128 IIYCSPAFERLTGYTNKEIVGKN
Mc  54-76  V---------------N------
Pb  95-117 I--------T----SSN--L---

(b) PAS domain 1
Pc 283-353 QKEWNKTLLDQCTDFIHVLSLKGLFLYVSNSSESMLEYSPHELIGDSLSSICHPSDIIPVMREIKEASNDP
Mc 241-311 -----QL----SC----------V--------A-----E-E--L-N--------------------TSN-
Pb 284-347        I---SD-----V----F---C-GAAKNL---E-ED-L-K----------V---------AASG

    PAS domain 2
Pc 164-218 KEHQASIINYRKDGQPFVNLVTVIPLWDENNTIELFVGLQVDLVEQPNSIIEKMK
Mc 112-166 ------------G------------ILGD-GQVDY--------------------
Pb 153-207 R-----------G--A----I-----L-NQGEVAYY--------------M----

(c) GATA-type zinc finger domain
Pc 601-626 CANCQRKDSPEWRKGPNGPKELCNAC
Mc 560-585 --K----------R--H---------
Pb 582-607 --Q--SQ------R------------
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Fig. 1. Amino acid sequences of *A*, PCMADA1, *B*, PCMADA2, and *C*, PCMADA3: the LOV domain (**a**), the PAS domain (**b**), and the GATA-type Zn finger domain (**c**). Amino acid sequences were analyzed with ScanProsite (http://ca.expasy.org/prosite/). Pc, *Pilobolus crystallinus*; Mc, *Mucor circinelloides*; Pb, *Phycomyces blakesleeanus*; -, the same amino acid residue as that in *Pilobolus crystallinus*. *Bars* indicate the length of 100 amino acids

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                                                       ***  *
PCMADA1        68 CSFLVTDARQYDCPIVYCSPTFEHLTGYHANEIVGRNCRFLQAPDGQVTCGSRRTYTDN 126
PCMADA2        90 ---V-V------F-L--V--V--K----SPADVM-K------S---H-AI----K---- 148
PCMADA3        92 -A-V-S--K---M--I----A--R----TNK----K------S---K-------QH--- 150
LOV consensus     —F——D——P—PI—AS—F——T-Y——E—G-NCRFLQG——————————————T—

                    *  **              *         * *                  *
PCMADA1       127 QAVFHLKAQMLQNKEHQASIINYRKGGQPFVNLITVIPITNDNNEVAFFVGLQVDLV 183
PCMADA2       149 TT-Y-I-TH-V-G--S-S-------T-------L-----SW-SD-IDY-M------- 205
PCMADA3       151 ---Y-I-G-IN-G------------D-------V----LWDE--TIEL--------- 207
LOV consensus     ————IR——————————————NY-K-G—FWN—————————D—G———IG-O————
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Fig. 2. Comparison of amino acid sequences for the LOV domains among PCMADA1, PCMADA2, and PCMADA3. LOV consensus shows conserved amino acids in most of the LOV domains (Crosson and Moffat 2001). *Stars* show conserved amino acid residues that are thought to be necessary for binding with FAD. -, the same amino acid residue as that in PCMADA1

logs were identified from PCR fragments amplified by the MadFwd-MadRev primer set. Another MADA homolog was identified from the Mad2Fwd-MadRev primer set. These cDNAs were named *pcmada1* (DDBJ/GenBank/EMBL accession no. FJ536284), *pcmada2* (FJ536285), and *pcmada3* (FJ536286). The amino acid sequence deduced from the *pcmada1* cDNA showed high homology with those of MADA of *Phycomyces blakesleeanus* and MCWC-1A of *Mucor circinelloides*. Sixty-eight percent of amino acid residues were conserved between PCMADA1 and MADA and 83% between PCMADA1 and MCWC-1A. The amino acid sequence of PCMADA2 was similar to MCWC-1B of *M. circinelloides* (63% identity) and WCOB of *P. blakesleeanus* (59% identity). PCMADA3 was similar to MCWC-1C of *M. circinelloides* (62% identity) and WCOA of *P. blakesleeanus* (54% identity).

PCMADA1, PCMADA2, and PCMADA3 each have one LOV domain and two PAS domains; the latter is proposed to function in protein–protein interactions (Taylor and Zhulin 1999). Figure 1 shows the amino acid sequences of LOV and PAS domains in MADA homologs. The LOV domain binds a flavin chromophore and forms a photoadduct by blue light (Crosson and Moffat 2001). Most of the amino acid residues necessary for the formation of the photoadduct are conserved in PCMADA1, PCMADA2, and PCMADA3 (Fig. 2).

Figure 3 shows a phylogenetic tree for the LOV domain of MADA homologs. All the MADA homologs of Zygomycetes formed one clade (Fig. 3). I named MADA, MCWC-1A, and PCMADA1 as group 1, WCOB, MCWC-1B, and PCMADA2 as group 2, and WCOA, MCWC-1B, and PCMADA3 as group 3.

Zinc finger DNA-binding motifs are conserved in the C-terminals of PCMADA1 and PCMADA3 but are lacking in PCMADA2 (see Fig. 1). All three genes are predicted to be localized in the nucleus by the WoLF PSORT program (Horton et al. 2007).

Irradiation affects the expression of several *madA* homologs in *P. blakesleeanus* and *M. circinelloides* (Idnurm et al. 2006; Silva et al. 2006). Expression of *madA* homologs of *P. crystallinus* was thus examined in mycelia grown under light or dark conditions by semiquantitative RT-PCR. The expression of *pcmada1* was suppressed under continuous light. The expression of *pcmada2* was almost the same between light- and dark-grown mycelia whereas that of *pcmada3* decreased in continuous darkness (Fig. 4). The expression of *pcmada1* was not significantly changed by the irradiation for 10 min but was reduced by irradiation for 24 h (Fig. 5). On the other hand, the expression of *pcmada3* increased within 10 min when irradiated with white light and blue light. The expression of *pcmada3* after irradiation for 24 h was lower than that after irradiation for 10 min.

Discussion

I could isolate cDNAs encoding putative photoreceptor proteins from *Pilobolus crystallinus* with degenerated primers for the LOV domain and identified them as the three MADA homologs. Three MADA homologs were also conserved in *M. circinelloides* and *Phycomyces blakesleeanus*. They are classified into three groups (Fig. 3). As *Rhizopus oryzae* also has three MADA homologs (Idnurm et al. 2006), three MADA homologs seem to be common in Mucorales.

PCMADA1 was similar to MADA and MCWC-1A. All three genes have one LOV domain, two PAS domains, and one zinc finger DNA-binding domain. As MADA and MCWC-1A are the photoreceptors for phototropism, it is reasonable to assume that PCMADA1 is also a photoreceptor for phototropism in *P. crystallinus*, although evidence is still lacking. PCMADA2 and PCMADA3 might also be involved in phototropism because several photoreceptors are involved in the phototropic response of *P. crystallinus* (Kubo and Mihara 1989). On the other hand, PCMADA3 might be a photoreceptor for carotenogenesis, as it is very similar to MCWC-1C, the photoreceptor for carotenogenesis in *M. circinelloides*.

Although nothing about the function of PCMADA2 is known yet, the amino acid sequence of PCMADA2 was similar to PCMADA1 (identity 41%) and PCMADA3

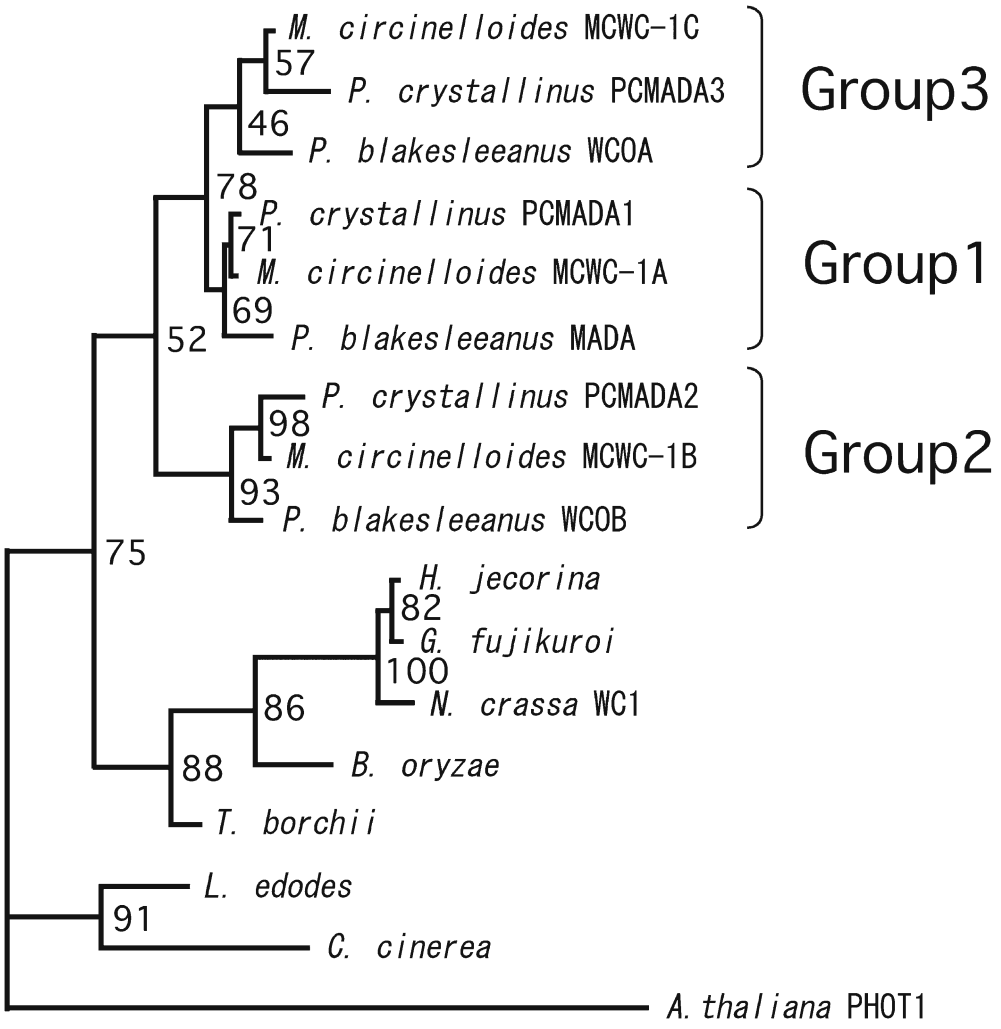

Fig. 3. Phylogenetic tree of the LOV domain of MADA homologs constructed with the maximum-likelihood method; 116 amino acid residues in the LOV domains were used for constructing the tree (TreeBASE study accession no. S2344; Matrix accession no. M4451). Bootstrap values are shown next to the branching. *Coprinopsis cinerea* (DDBJ/GenBank/EMBL accession no. AB195817.1), *Lentinula edodes* (AB279630.1), *Bipolaris oryzae* (AB273633.1), *Tuber borchii* (AJ575416.1), *Hypocrea jecorina* (AY823264.1), *Trichoderma atroviride* (AY628431.1), *Gibberella fujikuroi* (AM778551.1), *Neurospora crassa* WC1 (CAA63964.2), *Mucor circinelloides* MCWC-1A (AM040841.2), *Pilobolus crystallinus* PCMADA1 (FJ536284), *Phycomyces blakesleeanus* MADA (DQ229146.1), *Phycomyces blakesleeanus* WCOA (DQ229145.1), *Pilobolus crystallinus* PCMADA3 (FJ536286), *Mucor circinelloides* MCWC-1C (AM040843.1), *Mucor circinelloides* MCWC-1B (AM040842.1), *Pilobolus crystallinus* PCMADA2 (FJ536285), *Phycomyces blakesleeanus* WCOB (CAQ76857), *Arabidopsis thaliana* PHOT1 (AAC01753)

(identity 42%), and the LOV domain and two PAS domains were conserved; thus, there is a possibility that PCMADA2 might function as a photoreceptor, too. Recently it was shown that the *mcwc-1b* mutant suppressed the phenotype of the *crgA* mutant, which accumulated unusual amounts of carotenes (Silva et al. 2008). MCWC-1B is suggested to act as an activator of carotenogenesis and to be regulated by proteolysis-independent ubiquitylation mediated by CrgA (Silva et al. 2008). Then, PCMADA2 might have a similar function.

The expression of *pcmada3* was enhanced by irradiation. The increase of expression was a quick response, occurring within 10 min, which is comparable to the irradiation periods used for the induction of *wcoA* and *mcwc-1c* (30 min and 5 min, respectively) (Idnurm et al. 2006; Silva et al. 2006). In *pcmada3*, however, a significant level of transcript was detected even in darkness. This feature was quite different from those of *wcoA* and *mcwc-1c* in which expression was not observed in dark-grown mycelia (Idnurm et al. 2006; Silva et al. 2006).

The expression of *pcmada1* was suppressed by irradiation. In contrast with the induction of *pcmada3* in which the increase was observed within 10 min after the start of irradiation, the suppression of *pcmada1* required longer irradiation. A similar phenomenon has been observed in *madA*. Autoregulation does not seem to be involved, at least in *P. blakesleeanus*, because the expression of *madA* is suppressed by light in a *madA* mutant (Idnurm et al. 2006).

It is interesting to note that the expression of the photoreceptor is regulated by another photoreceptor. Although the function of this photoreceptor cascade is not known, one possibility is to capacitate the fungi to respond to a wide range of fluence as shown in the carotenogenesis of *P. blakesleeanus*, which exhibits two separate components with low and high thresholds (Jayaram et al. 1979; Bejarano et al. 1990). VIVID, one of the blue-light receptors of *N. crassa*, shows light-regulated expression, which is dependent on another photoreceptor WC-1. VIVID is suggested to enable the fungus to detect and to adapt to changes in light intensities (Schwerdtfeger and Linden 2003). One of the MADA homologs might have a similar function to VIVID.

Pilobolus crystallinus shows many photoresponses such as phototropism, carotenogenesis, photomorphogenesis (Jacob 1959; Page and Curry 1966), and circadian regulation (Uebelmesser 1954; Bruce et al. 1960). These diverse responses should be regulated with multiple photoreceptors. Isolation and characterization of the mutants lacking

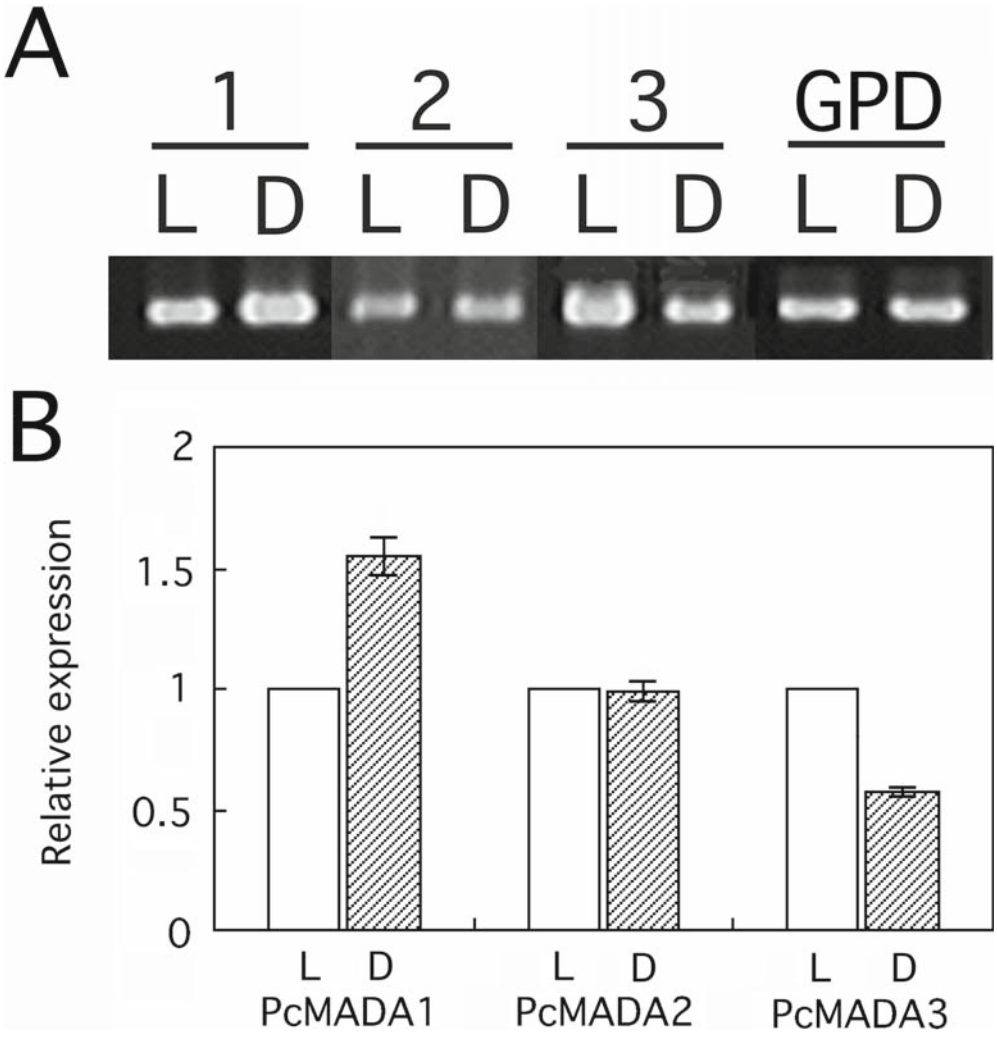

Fig. 4. Expression of *pcmada1*, *pcmada2*, and *pcmada3*. **A** Expression of *pcmada1* (*1*), *pcmada2* (*2*), and *pcmada3* (*3*) are semiquantitatively demonstrated. Mycelia were grown under white light (*L*) or in darkness (*D*) for 15 days. Polymerase chain reaction (PCR) cycles were 27, 36, 30, and 24 for *pcmada1*, *pcmada2*, *pcmada3*, and *gpd* (glyceraldehyde-3-phosphate dehydrogenase) (*GPD*), respectively. **B** Levels of expression relative to that of *gpd* in darkness (*D*) are shown relative to the level under white light (*L*). Each value is the average and SE from 5, 4, and 7 PCR reactions, respectively, for *pcmada1*, *pcmada2*, and *pcmada3*

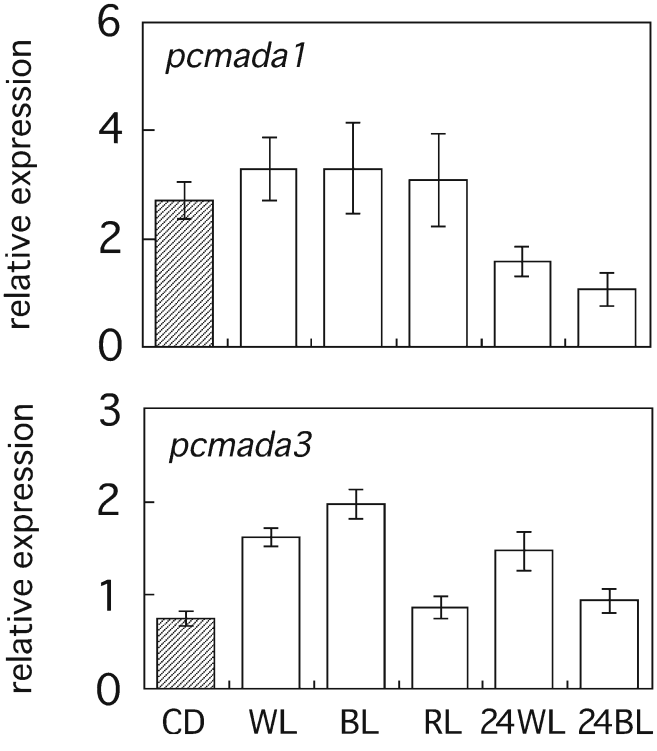

Fig. 5. Effect of light on the expression of *pcmada1* and *pcmada3*. Level of expression relative to that of gpd is shown relative to that in continuous white light. Mycelia grown for 7 days in darkness were irradiated for 10 min with white light (*WL*), blue light (*BL*), or red light (*RL*), or irradiated for 24 h with white light (*24WL*) or blue light (*24BL*). Each value is the average and SE from four to eight PCR reactions

three *madA* homologs is required for elucidation of the function of these putative photoreceptors.

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