Regulation of Conidiation by Light in *Aspergillus nidulans*

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ABSTRACT Light regulates several aspects of the biology of many organisms, including the balance between asexual and sexual development in some fungi. To understand how light regulates fungal development at the molecular level we have used *Aspergillus nidulans* as a model. We have performed a genome-wide expression analysis that has allowed us to identify >400 genes upregulated and >100 genes downregulated by light in developmentally competent mycelium. Among the upregulated genes were genes required for the regulation of asexual development, one of the major biological responses to light in *A. nidulans*, which is a pathway controlled by the master regulatory gene *brlA*. The expression of *brlA*, like conidiation, is induced by light. A detailed analysis of *brlA* light regulation revealed increased expression after short exposures with a maximum after 60 min of light followed by photoadaptation with longer light exposures. In addition to *brlA*, genes *flbA–C* and *fluG* are also light regulated, and *flbA–C* are required for the correct light-dependent regulation of the upstream regulator *fluG*. We have found that light induction of *brlA* required the photoreceptor complex composed of a phytochrome FphA, and the white-collar homologs LreA and LreB, and the *fluffy* genes *flbA–C*. We propose that the activation of regulatory genes by light is the key event in the activation of asexual development by light in *A. nidulans*.

MANY organisms encounter cycles of light/darkness during their lives, and the presence of light serves as an environmental signal to regulate different aspects of their biology, even in nonphotosynthetic organisms. In fungi, light has a strong influence on development, regulates metabolic pathways, and may direct the growth of reproductive structures (Corrochano and Galland 2006; Bahn *et al.* 2007; Corrochano and Avalos 2010; Rodriguez-Romero *et al.* 2010). For example, fungi growing in dark or shaded areas use light as a signal to promote vegetative reproduction and to direct the growth of reproductive structures toward open air to facilitate spore dispersal (Corrochano and Galland 2006; Corrochano and Avalos 2010; Rodriguez-Romero *et al.* 2010). An excess of light can be harmful, particularly UV light. Thus, the activation of the biosynthesis of pigments, like carotenoids and the activation of genes for light-dependent DNA repair by light can be considered a protection mechanism from light (Berrocal-Tito *et al.* 1999; Alejandre-Duran *et al.* 2003; Corrochano and Avalos 2010). It appears that the capacity to receive and respond to light improves fungal adaptation and survival in nature.

Photoreceptor proteins sense light through a chromophore, a light-absorbing molecule bound to the protein that provides the sensitivity to a specific range of wavelengths. Several of these proteins have been identified in fungi (Corrochano 2007; Idnurm *et al.* 2010; Rodriguez-Romero *et al.* 2010). The molecular mechanism of fungal photoreception has been investigated in greatest detail in the ascomycete *Neurospora crassa* (Chen *et al.* 2010b). This fungus perceives light through the white-collar protein 1 (WC-1), a zinc-finger protein with a flavin-binding domain (named LOV for light–oxygen–voltage), and a PAS domain (*Drosophila* period, PER–vertebrate aryl hydrocarbon receptor nuclear translocator, ARNT–*Drosophila* single-minded, SIM) for protein–protein interactions (Ballario *et al.* 1996). The LOV domain binds the flavin FAD, allowing WC-1 to act as a photoreceptor for blue light (Froehlich *et al.* 2002; He *et al.*

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2002). The WC-2 protein contains one zinc-finger and two PAS domains (Linden and Macino 1997). WC-2 interacts with WC-1 to form a heterodimeric complex via their PAS domains to form a WC complex (WCC) that binds to the promoters of light-inducible genes to regulate their transcription (Froehlich *et al.* 2002; He and Liu 2005; Belden *et al.* 2007b; Chen *et al.* 2009; Olmedo *et al.* 2010b; Smith *et al.* 2010). Another blue-light photoreceptor in *N. crassa* is VIVID (VVD), a small protein with a LOV domain that is required for the transient activation of gene transcription (photoadaptation) by light (Heintzen *et al.* 2001; Schwerdtfeger and Linden 2003). The transduction of the light signal in WC-1 and VVD requires the formation of a covalent photoadduct between a conserved cysteinyl residue of the LOV domain and the flavin cofactor (Cheng *et al.* 2003; Schwerdtfeger and Linden 2003). Recently, a physical interaction between VVD and the WCC has been shown to be required for the regulation of the activity of the WCC (Chen *et al.* 2010a; Hunt *et al.* 2010; Malzahn *et al.* 2010). Although VVD participates in the photoadaptation of gene expression in *N. crassa*, homologs of VVD are not widely distributed in fungi (Rodriguez-Romero *et al.* 2010). Phytochromes are photoreceptors that sense red and far-red light through a linear tetrapyrrole chromophore (Rockwell *et al.* 2006). Previous characterization of phytochrome mutants did not show any major alteration in light-dependent regulation of gene expression in *N. crassa* (Froehlich *et al.* 2005; Chen *et al.* 2009). Cryptochromes were initially identified as plant blue-light photoreceptors very similar to photolyases, enzymes for blue-light–dependent DNA repair (Lin and Todo 2005). The *N. crassa* cryptochrome binds the chromophores FAD and MTHF, and a strain with a deletion of the cryptochrome gene showed a minor change in circadian clock entrainment (Froehlich *et al.* 2010). *N. crassa* strains with deletions in the cryptochrome CRY-1, the rhodopsin NOP-1, and the phytochrome PHY-2 showed increased light-dependent accumulation of mRNA of light-regulated genes (*con-6* and *con-10*). This observation suggests that these photoreceptors modify the activity of the WCC, presumably through a repressor (Olmedo *et al.* 2010b).

Several orthologs of the *N. crassa* photoreceptors have also been recently described in *Aspergillus nidulans* (Bayram *et al.* 2010; Rodriguez-Romero *et al.* 2010): a phytochrome, FphA, for red-light detection (Blumenstein *et al.* 2005; Purschwitz *et al.* 2008), a homolog of WC-1, LreA, and a homolog of WC-2, LreB, for blue-light detection (Purschwitz *et al.* 2008), and a cryptochrome, CryA, (Bayram *et al.* 2008a). Unlike *N. crassa*, *A. nidulans* can sense red light through the phytochrome to modulate development and other light-dependent processes (Blumenstein *et al.* 2005; Rodriguez-Romero *et al.* 2010). Another interesting aspect in the *A. nidulans* photobiology is that the phytochrome forms a large complex with the *A. nidulans* homologs of WC-1 and WC-2, and the velvet A (VeA) protein, a repressor of light-regulated conidiation and an activator of sexual development (Purschwitz *et al.* 2008). VeA interacts with additional proteins to regulate sexual development and the synthesis of secondary metabolites (Bayram *et al.* 2008b; Sarikaya Bayram *et al.* 2010). In addition, the UV/blue-light sensing cryptochrome is involved in the regulation of sexual development in *A. nidulans* by light (Bayram *et al.* 2008a).

Conidiation, the development of asexual spores, is controlled by a pathway, encompassing BrlA, AbaA, and WetA (see reviews by Adams *et al.* 1998; Yu *et al.* 2006). The first component in the regulatory cascade, BrlA, is necessary and sufficient to drive conidiation (Adams *et al.* 1988). Transcription of the *brlA* gene induces conidiation, and *brlA* itself is controlled by a number of genes, including the *fluffy* genes. Deletion of any of the *fluffy* genes gives a typical fluffy phenotype with cotton-like colonies and reduced levels of *brlA* expression (Adams *et al.* 1998; Yu *et al.* 2006). The *fluffy* genes are: *fluG* and *flbA–E*. *fluG* encodes a protein similar to glutamine synthetase and is responsible for the synthesis of an extracellular factor that induces conidiation (Lee and Adams 1994). FluG works upstream of the *flbA–E* genes (Yu *et al.* 2006). FlbA is a regulator of the protein G activity, which participates in a protein kinase A-dependent pathway promoting filamentous growth and inhibiting conidiation (Yu *et al.* 1996). FlbE interacts with FlbB at the fungal tip and is required for proper activation of FlbB (Garzia *et al.* 2009). FlbB is a bZip transcription factor that activates the transcription of *flbD*, a cMyb-type regulator. Then, both FlbB and FlbD jointly activate the transcription of *brlA* (Garzia *et al.* 2010). FlbC is a putative C2H2 Zn finger protein that constitutes a third path for the regulation of *brlA* expression (Kwon *et al.* 2010). These *fluffy* genes are expressed in vegetative mycelium and are able to respond to intracellular stimuli to induce a coordinated activation of the master regulator *brlA* (Etxebeste *et al.* 2010).

Light regulates the balance between asexual or sexual development in *A. nidulans* (Rodriguez-Romero *et al.* 2010), and it has been shown that light increases the accumulation of *brlA* mRNA (Mooney and Yager 1990). In *N. crassa*, light activates the accumulation of mRNAs for the developmental genes *fluffy* (*fl*) (Belden *et al.* 2007a; Olmedo *et al.* 2010a) and *csp-1* (Chen *et al.* 2009). The FL protein is a transcription factor that is necessary and sufficient to induce conidiation (Bailey and Ebbole 1998; Bailey-Shrode and Ebbole 2004). *fl* is induced during conidiation along with many other genes in *N. crassa* (Greenwald *et al.* 2010). The light-dependent activation of *fl* by the WCC suggested a simple model for the activation of conidiation by light in *N. crassa* with light-activated WCC increasing *fl* transcription and the subsequent accumulation of regulatory FL protein activating the conidiation pathway (Olmedo *et al.* 2010a). This model does not appear to be so simple in *A. nidulans*.

Here we show that the expression of key upstream regulators is induced by light and traced down the path for the light-dependent induction of conidiation in *A. nidulans*.

Materials and Methods

Strains, media, and culture conditions

Strains used in this study are listed in Table 1. Strains were grown in complete or minimal media containing the appropriate supplements (Cove 1966). A total of 1% glucose and 10 mM NH_4NO_3 were used as carbon and nitrogen sources. Conidia were inoculated on the surface of 25 ml of complete liquid medium in a Petri dish. Cultures were grown for 18 hr at 37° in the dark before the light-induction experiments.

Light-induction experiments

Mycelial mats were exposed to light generated by a set of Phillips Master TL-D 36 W/865 white fluorescent bulbs for the indicated times (11 W/m^2). After the exposure to light, mycelia were collected in the dark and immediately frozen in liquid nitrogen. Samples were stored at −80°. Control samples were harvested in complete darkness. All light-induction experiments were performed at 22°. Control samples were kept at the same temperature during the duration of the experiment. Light intensities were measured with a calibrated photodiode.

RNA isolation

Aspergillus mycelia (100–200 mg) were disrupted in 1 ml of TRI reagent (Sigma) with 1.5 g of zirconium beads (0.5-mm diameter) by using two 0.5-min pulses in a cell homogeneizer (FastPrep-24, MP Biomedicals). Cell debris was spinned down by centrifugation. Supernatants were extracted with chloroform and RNA was precipitated with isopropanol. The RNA samples were treated with DNase I (USB) prior to use in RT–PCR experiments. In an alternative method, *Aspergillus* mycelia (100–200 mg) were ground into fine powder in a mortar with a pestle. RNA was isolated from the powder by using the RNeasy plant mini kit (Qiagen) with the RLC buffer.

For the microarray experiments, total RNA was isolated with TRIZOL reagent (Invitrogen). Cells were broken up with acid-washed glass beads in a homogenizer (MM200, Retsch) working at maximum speed (frequency 25/s) for 5 min. Total RNA was further purified using RNeasy spin minicolumns (Qiagen). RNA was subjected to quality control in a 2100 Bioanalyzer (Agilent) using Eukaryote Total RNA as standard before the microarray experiments.

RNA labeling, hybridization, and microarray analysis

The *A. nidulans* DNA (version 2) microarray slides used in this study were obtained from the Pathogen Functional Genomics Resource Center (J. Craig Venter Institute, Rockville, MA). The slides were spotted with 70-mer oligonucleotides corresponding to 11,481 genes. The glass slide arrays have an "in-slide" replicate for each gene.

RNA was isolated from mycelia grown in the dark (control sample) or illuminated with white light for 30 min. Thirty-five micrograms of total RNA was labeled with Cy3 or Cy5 dyes in a reaction containing 5 μg oligo-dT primer, Expand RT mix, and RNAseH (Invitrogen) for 2 hr at 42°. Dye-swap experiments were done using RNA samples pooled from two different experiments. The labeled samples were purified in Microcon YM30 columns according to manufacturer instructions. Hybridizations and washings were performed according to the standard operating procedure of the Institute for Genomic Research (http://pfgrc.jcvi.org/index.php/microarray/protocols.html).

The hybridized slides were scanned with an Axon GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA). The images were further processed with GenePix Pro software (Molecular Devices). Intensities were calculated from Lowess normalized M Log ratios, substracting the background using the following parameters: global, 0.4 smoothing and 10 iterations. Values of low quality were excluded according to a quality filter set to "sum of median $<$ 200," which corresponds to a saturated signal in both channels. Feature background intensities were calculated by using morphological opening and the following parameters: closing to three pixels, opening to two, feature separation. Data have been deposited in ArrayExpress (accesion no. E-MEXP-3218).

Quantitative RT–PCR

The primers employed for quantitative RT–PCR are detailed in supporting information, Table S1. Quantitative RT–PCR experiments were performed using one-step RT–PCR, using 25 μl 1X Power SYBR Green PCR Master mix (Applied Biosystems), 6.25 units MultiScribe Reverse Transcriptase

Table 1 ***A. nidulans*** **strains used in this study**

Strain	Genotype	Source
FGSC4	Wild type	FGSC
BD205	*pyrG89*; *pyroA4*; *veA*$^+$	Herrero-García *et al.* (2011)
SJP1	*pyrG89*; Δ*argB:trpC*Δ*B*; *pyroA4* Δ*fphA*::*argB*; *veA*$^+$	Purschwitz *et al.* (2008)
SJP69	*yA1*, *pyrG89*; Δ*argB:trpC*Δ*B*; *pyroA4*; Δ*lreA*::*argB*; *veA*$^+$	This study
LBV$^+$	*biA*; Δ*lreB*::*argB*; *pyroA4*; *veA*$^+$	Purschwitz *et al.* (2008)
SJP21.3	*pyrG89*; Δ*argB:trpC*Δ*B*; *pyroA4*; Δ*lreA*::*argB*, Δ*lreB*::*argB*, Δ*fphA*::*argB*; *veA*$^+$	Purschwitz *et al.* (2008)
RNJ3.1	*biA1*; Δ*flbA*::*argB*$^+$; *veA1*	Shin *et al.* (2009)
DKA91	Δ*flbA*::*argB*$^+$; *veA*$^+$	RNJ3.1 × BD205, this study
BD215	Δ*flbB*::*pyrG*; *pyrG89*; *pyroA4*; *veA*$^+$	Herrero-García *et al.* (2011)
TNJ14.1	*biA1*; *methG1*; Δ*flbC*::*argB*$^+$; *veA1*	Kwon *et al.* (2010)
DKA82	Δ*flbC*::*argB*$^+$; *veA*$^+$	TNJ14.1 × BD205, this study

FGSC, Fungal Genetics Stock Center.

(Applied Biosystems), 1.25 units RNase Inhibitor (Applied Biosystems), 0.2 μM of each primer, and 100 ng of RNA in a 25-μl reaction in a 7500 Real-Time PCR System (Applied Biosystems) according to the manufacturer's directions. The reaction consisted of 30 min at 48°, 10 min at 95°, and 40 cycles of DNA amplification (15 s at 95° and 1 min at 60°). After each PCR, we performed melting curve analysis to show the specific amplification of single DNA segments and the absence of nonspecific amplified DNA.

Additional quantitative RT–PCR experiments were performed in a LightCycler 480 II (Roche) by using the One-Step SYBR PrimeScript RT–PCR kit (Takara Bio), 0.2 μM of each primer and 50 ng of RNA in a 10-μl reaction. The reaction consisted of 5 min at 42°, followed by 10 s at 95°, and then 40 cycles of DNA amplification (5 s at 95° and 20 s at 60°). After each PCR, we performed melting curve analysis to show the specific amplification of single DNA segments and the absence of nonspecific amplified DNA. Comparisions of both protocols showed consistency and reliability between both methods.

The fluorescent signal obtained for each gene was normalized to the corresponding fluorescent signal obtained with *benA* to correct for sampling errors. In all cases, expression data are shown relative to the wild-type mycelia grown in the dark and are the average of at least three independent biological replicates.

Results

Identification of light-regulated conidiation genes in A. nidulans

Light is a major environmental signal regulating many different biological processes. In *A. nidulans*, light controls asexual and sexual development as well as the production of secondary metabolites. To get a global view of genes regulated during asexual development and of genes involved in other light-regulated biological processes, a genome-wide approach was undertaken. Total RNA was isolated from surface-grown, developmentally competent mycelia of the wild-type strain FGSC4 exposed to white light (11 W/m^2) for 30 min or grown in the dark, labeled, and hybridized to a spotted microarray of *A. nidulans*. After background correction and Lowess M normalization the threshold was set to 2-fold to identify differentially regulated genes under light *vs.* dark conditions (Figure 1A). Under these conditions, 533 out of 10,560 genes were differentially regulated, ~5% of the genome. We observed large differences in the light-dependent induction and repression provoked by light: out of the 533 differentially regulated genes 425 were upregulated, but only 108 were downregulated (Figure 1 and Table S2). The highest upregulated gene, *ccgB* (a homolog of the *clock-controlled gene 1* of *N. crassa*), showed an ~240-fold increase, while the highest downregulated gene, *veA* (a gene required for light regulation of conidiation in *A. nidulans*), showed only a 8.6-fold decrease (Figure 1A and Tables 2 and 3).

We analyzed the distribution of light-regulated genes in the chromosomes to identify putative light-regulated specific regions in the *A. nidulans* genome (Figure 1B). In addition to the visual plot of light-regulated genes, we divided the total number of upregulated genes by the total number of downregulated genes to find out whether an overrepresentation of upregulated genes was present in any of the chromosomes. Chromosome III shows a significant increase in upregulated genes, with a ratio of 5.3 in comparison to an average ratio of 4.06. Although no obvious light-regulated specific regions were identified, a high number of upregulated genes was found close to the telomeric region in chromosome III. Most of these genes belong to the top 50 most upregulated genes, among which is a previously identified cluster encoding conidia-specific mRNA of unknown function (SpoC1) (Gwynne *et al.* 1984; Aramayo *et al.* 1989). Chromosome I also shows a high number of upregulated genes. In contrast, a high proportion of downregulated genes is located on chromosomes VI and VIII.

Some of the upregulated genes were predicted to be involved in biological processes known to be regulated by light, *i.e.*, circadian rhythm and conidiation. Some other genes were predicted to be involved in other processes such as carbon metabolism and transport, redox reactions, or stress responses. Some of these genes are transcription factors and proteins probably implicated in the activation of light-dependent signaling pathways (Table S2 and Table S3). The activation of these genes may trigger the changes in development, stress responses, and secondary metabolism that *A. nidulans* undergoes when living in the light. The gene with the highest level of light induction is *ccgB* (Table 2), a homolog of the *N. crassa ccg-1* gene, a clock-controlled and glucose-repressed gene of unknown function (Arpaia *et al.* 1995; Bell-Pedersen *et al.* 1996). Interestingly, *ccgB* appears to be duplicated in the genome of *A. nidulans* but not in other related species such as *A. fumigatus* and *A. niger*. Both genes, *ccgA* and *ccgB*, are induced by light to a similar extent (219- and 239-fold, respectively). Another light-induced gene that we have identified is *conJ*, the homolog of the light-regulated and conidiation gene *con-10* in *N. crassa* (Roberts *et al.* 1988; Olmedo *et al.* 2010b). The function of *con-10* in *N. crassa* is not known (Springer and Yanofsky 1992; White and Yanofsky 1993). Other interesting upregulated genes are photoreceptors (Table S2) like *cryA* (induced 3.4-fold) that encodes for a UV/blue-light sensing cryptochrome involved in the regulation of sexual development in *A. nidulans* by light (Bayram *et al.* 2008a). In addition, we found that the gene *nopA* predicted to encode an opsin-like protein (AN3361) is induced by light (22.5-fold). This protein is related to opsins, a group of membrane-embedded proteins with seven transmembrane helices that bind to a retinal chromophore (Brown 2004). The function of NopA is still unknown but homologous proteins in other fungi show a photocycle, suggesting that they could work as sensory photoreceptors. However, an *A. nidulans nopA*-deletion

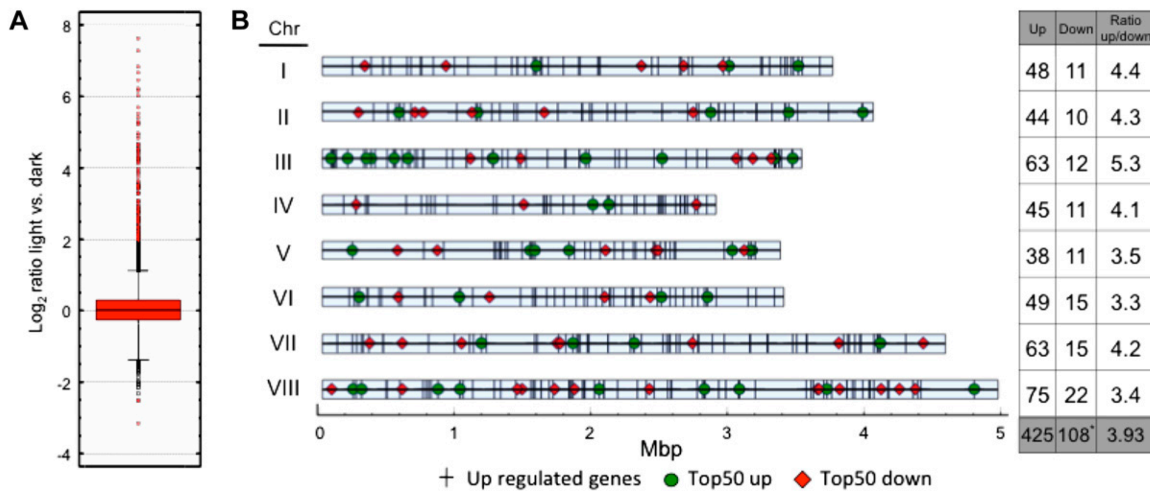

Figure 1 Whole-genome regulation of gene expression by light. (A) Boxplot diagram of genes showing the Log2 ratio of expression in light *vs.* dark conditions. (B) Schematic representation of the location of the light-regulated genes in the *A. nidulans* chromosomes. Each vertical bar represents an upregulated gene (425 genes). The location of the highest 50 upregulated genes are shown by circles. All downregulated genes are depicted with diamonds, with the exception of (AN09511), which has not been allocated in the genome. The table on the right shows the number of up- and downregulated genes in each chromosome and the ratios of up/downregulated genes.

strain did not display any phenotype (J. Rodríguez-Romero and R. Fischer, unpublished results).

Several genes involved in conidiation were found in the list of upregulated genes (*e.g.*, AN8638, *cetJ*; AN5015, *conJ*; etc.; see Table S2). Only one gene involved in the regulation of conidiation (*flbC*) was found in the list of upregulated genes (3.4-fold induction). Further inspection of the data uncovered that some of the conidiation genes (*brlA*, *fluG*, *and flbB*) were also upregulated by light (Table S4). However, the induction of those genes was below the 2-fold threshold.

Most downregulated genes were related to transport (12%), oxidoreductase functions (10%), nuclear components (14%), and nitrogen metabolism (Table 3 and Table S2). The most downregulated gene was *velvet A* (*veA*), a component of the light regulator complex (Purschwitz *et al.* 2008) that has been implicated in the light response in *A. nidulans* (Kafer 1965; Mooney and Yager 1990; Calvo 2008). VeA represses conidiation and promotes sexual development, and therefore, the regulation of VeA affects the balance between asexual and sexual development and the coordination of morphogenesis and secondary metabolism (Calvo 2008).

Light induction of the conidiation gene brlA is fast and transient

Mooney and Yager (1990) reported that *brlA* was activated by light (Mooney and Yager 1990) and we found *brlA* in the

Table 2 Top 15 upregulated genes obtained in the microarray hybridization experiments

Top	Locus	Gene description	Log_2 ratio light *vs.* dark	Fold change	SD
1	AN5056	*ccgB* homolog to *ccg-1* from *N. crassa*	7.90	239.35	1.246
2	AN9285	*ccgA* homolog to *ccg-1* from *N. crassa*	7.78	219.79	0.263
3	AN0045	Solid-state culture expressed protein (Aos23)	7.75	214.97	0.683
4	AN0693	Hypothetical protein	7.26	152.75	0.519
5	AN8339	Hypothetical protein	6.81	112.13	0.670
6	AN4299	Clock controlled and temperature regulated	6.55	93.57	0.158
7	AN7558	Hypothetical protein	6.36	82.03	0.145
8	AN8641	Hypothetical protein	6.27	77.33	0.784
9	AN8638	Conidia enriched transcript (*cetJ*)	6.19	73.06	0.494
10	AN8018	Auxin efflux transporter family protein	6.07	67.37	0.076
11	AN9310	Hypothetical protein	6.07	67.32	0.129
12	AN5004	Hypothetical protein	6.02	65.03	0.410
13	AN5015	Conidiation gene (*conJ*)	5.95	61.82	0.282
14	AN3872	Hypothetical protein	5.93	60.84	0.423
15	AN5764	Hypothetical protein	5.75	53.67	0.122

Average of log_2 of the ratio of light *vs.* dark was used to calculate the fold change. SD is the standard deviations of log_2 of ratio of a swap-dye experiment. The gene descriptions were determined using the *Aspergillus* genome database AspGD (www.aspergillusgenome.org). Hypothetical proteins have unknown function but are conserved proteins.

list of upregulated genes in our microarray hybridization experiments (Table S3). To characterize in detail the regulation by light of the *A. nidulans* conidiation genes, we assayed the response of *brlA* expression to light. Mycelia of *A. nidulans* grown in the dark for 18 hr were exposed to white light (11 W/m^2) for time periods ranging from 1 min to 4 hr. After RNA extraction, the amount of *brlA* mRNA was determined by quantitative RT–PCR. Light activation of *blrA* was quick as a twofold increase of the mRNA amount was already detected after 5 min of illumination. The *brlA* mRNA reached a maximum of a fourfold increase after 30–60 min of light compared to the amount obtained in mycelia kept in the dark (Figure 2A). The accumulation of the *brlA* mRNA was transient and decreased when the exposure time was >60 min. Light-dependent *brlA* mRNA accumulation was not detected in mycelia exposed to light for 4 hr, suggesting that a photoadaptation event reminiscent of the one observed in *N. crassa* and *Phycomyces blakesleeanus* (Schwerdtfeger and Linden 2003; Rodriguez-Romero and Corrochano 2006) also exists in *A. nidulans*. Illumination for periods >4 hr and up to 12 hr did not result in photoinduction of *brlA* (data not shown).

Two overlapping transcripts are produced from the *brlA* gene, where BrlAβ activates the transcription of *brlAα* and subsequently BrlAα activates the conidiation genes (Prade and Timberlake 1993). To check whether any of these transcripts was preferentially regulated upon light exposure, primer sets specific for each transcript were used in the quantitative RT–PCR experiments (Figure 2B). The induction levels of both transcripts were similar at short light-exposure times (Figure 2C). After 30 min of illumination, the α transcript accumulated to slightly higher levels than the β transcript (2.6-fold induction for the α transcript compared to 1.7-fold for the β transcript). Although the differences are not statistically significant, it was still observed after 60 min of light. This observation might be relevant for *brlA* regulation as the expression of *brlAα* is regulated by *brlAβ* (Barton and Prade 2008) and the β transcript predominantly accumulates over the α transcript at long light exposures (Kato *et al.* 2003).

The expression of the brlA upstream regulatory genes is induced by light

Given the fast induction by light of *brlA*, we asked whether the regulation of *brlA* expression by light is direct or whether regulatory genes upstream of *brlA* are additional targets for the light signal. The genes *fluG* and *flbA–C* encode developmental regulators that act upstream of *brlA*, and their deletion reduces the expression of *brlA*, resulting in aconidial, fluffy phenotypes (Adams *et al.* 1992, 1998; Wieser *et al.* 1994). Thus, our next question was whether these regulatory genes were also induced by light. Cultures of the wild-type strain were exposed to white light for different time periods and the expression of *fluG*, *flbA*, *flbB*, and *flbC* was assayed by RT–PCR. Genes *fluG* and *flbA–C* were clearly induced by light as shown by the light-dependent accumulation of the corresponding mRNAs (Figure 3). Maximum mRNA accumulation reached 4- to 6-fold after 60 min of light exposure, compared to mRNA accumulation in mycelia kept in the dark. Interestingly, the light-dependent induction of the *fluffy* genes displayed a similar pattern to the one observed for *brlA* (Figure 2), *i.e.*, a response to short light exposures (the induction was minor but already evident after 1–5 min) and maximum accumulation (4- and 6.5-fold) after 30–60 min of illumination under our conditions (Figure 3).

Light activation of the conidiation genes requires the photoreceptor complex

Light is perceived by a protein complex composed of the phytochrome FphA for red-light reception and the white-collar LreA/LreB proteins for blue-light reception (Purschwitz

Table 3 Top 15 downregulated genes obtained in the microarray hybridization experiments

Top	Locus	Gene description	Log_2 ratio light *vs.* dark	Fold change	SD
1	AN1052	velvetA(*veA*)	−3.11	8.60	0.242
2	AN8647	ALS family protein	−2.65	6.28	0.722
3	AN1008	Putative nitrate transporter (*crnA*)	−2.37	5.17	0.228
4	AN5558	Alkaline protease (*prtA*)	−2.31	4.95	0.255
5	AN3304	GABA transporter, putative	−2.20	4.59	0.611
6	AN0231	Conidiophore-specific phenol oxidase (*ivoB*)	−2.14	4.40	0.074
7	AN8063	Acid phosphatase activity	−1.98	3.94	0.094
8	AN9076	Putative adhesin function	−1.97	3.91	0.751
9	AN2926	60S ribosomal protein Nsa2, putative	−1.95	3.86	0.183
10	AN8539	GNAT family acetyltransferase, putative	−1.92	3.78	0.309
11	AN5353	Hypothetical protein	−1.89	3.70	0.381
12	AN9240	Putative C2H2 finger domain transcription factor	−1.81	3.49	0.829
13	AN0367	Integral membrane protein	−1.80	3.49	0.310
14	AN0190	Subunit of the tRNA splicing endonuclease,	−1.77	3.41	0.117
15	AN1131	Cytosolic Cu/Zn superoxide dismutase, putative	−1.75	3.36	0.763

Average of log_2 of the ratio of light *vs.* dark was used to calculate the fold change. SD is the standard deviations of log_2 of ratio of a swap-dye experiment. The gene descriptions were determined using the *Aspergillus* genome database AspGD (www.aspergillusgenome.org). Hypothetical proteins have unknown function but are conserved proteins.

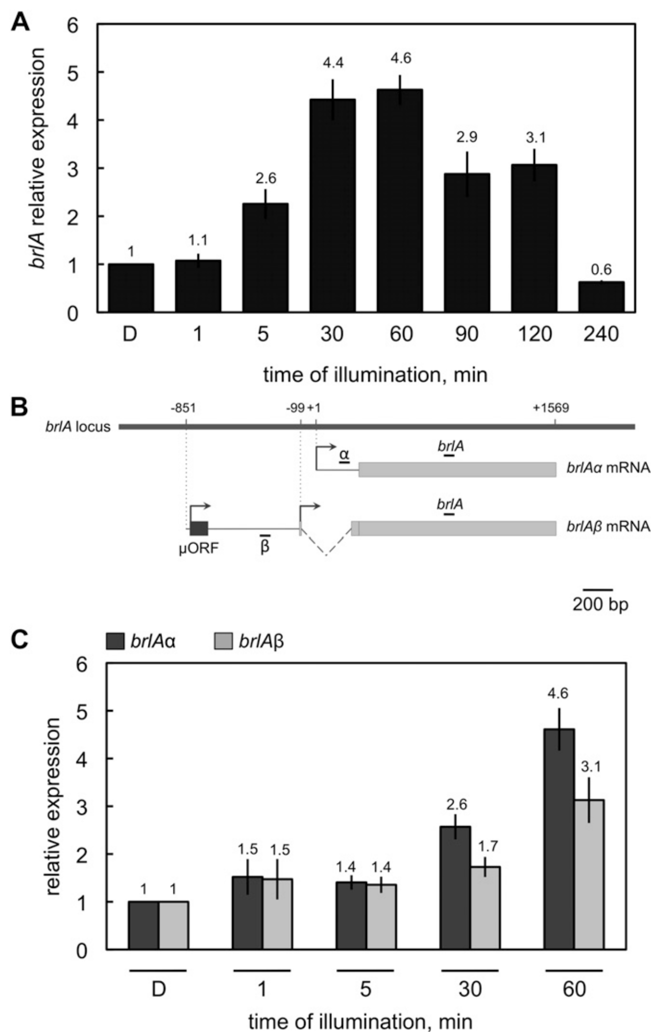

Figure 2 The *brlA* gene is induced by light. Total RNA was isolated from vegetative mycelia of the wild-type strain that had been exposed to white light (11 W/m^2 blue light) for various periods or kept in the dark "D" for 60, 90, 120, or 240 min prior to RNA isolation. Dark samples collected at 60, 90, 120, and 240 min of illumination gave similar values of mRNA accumulation for *brlA*, *brlA*α, and *brlA*β. (A) The *brlA* transcript was amplified with primers that detect both transcripts. Samples were taken at time intervals ranging from 1 min to 4 hr. Maximum induction was observed after light exposures of 30–60 min. (B) Diagram depicting the organization of the *brlA* locus and the position of the primers used to amplify the α, the β. or both transcripts. (C) Light activates the two *brlA* mRNAs, *brlA*α and *brlA*β. The plot shows the average and standard error of the mean of the relative photoactivation values with respect to the dark samples at 60 min in at least three independent experiments.

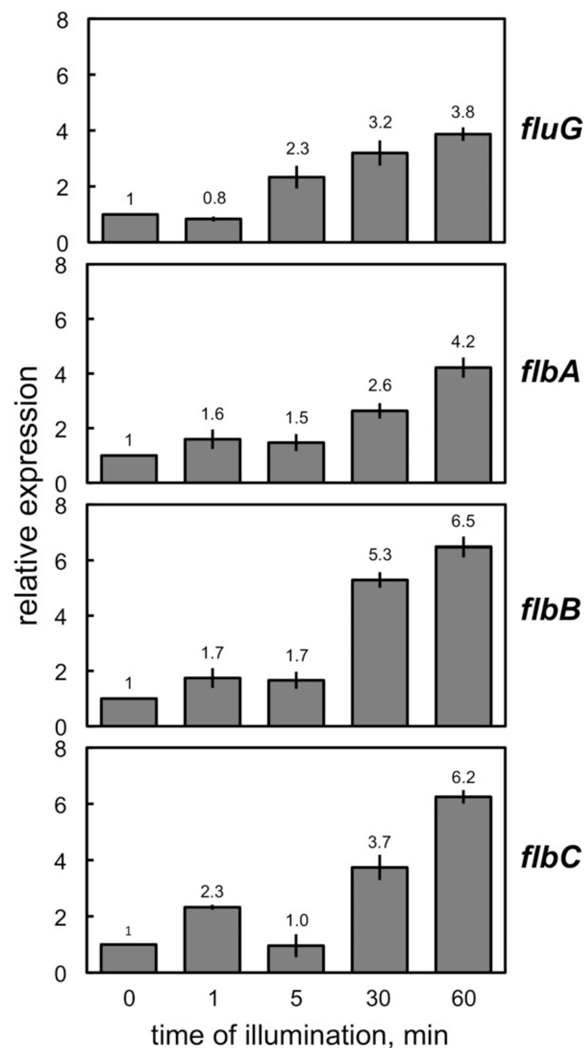

Figure 3 Expression of the *fluffy* genes in response to light. Mycelia of the wild-type strain were exposed to white light for different times and the expression of the genes assayed by quantitative RT–PCR. *fluG* and *flbA–C* gene expression was induced by light. Results are shown as relative expression compared to control samples kept in the dark (0) for 60 min prior to RNA isolation. The plots represent the mean value and standard error of the mean of at least three independent experiments.

et al. 2008). To investigate whether these photoreceptors were required for the light-dependent activation of the conidiation genes, we assayed light-dependent mRNA accumulation in strains carrying single deletions of the photoreceptor genes *fphA*, *lreA*, or *lreB*, and in a triple deletion mutant strain (Δ*fphA* Δ*lreA* Δ*lreB*). The light-dependent accumulation of *brlA* mRNA was not observed in the Δ*fphA* or Δ*lreB* strains, but was only slightly decreased in the Δ*lreA* mutant (Figure 4). These results suggest a role for the phytochrome and the white-collar 2 homolog LreB in *brlA* activation by light but not for the white-collar 1 homolog LreA. We did not detect any light-dependent *brlA* mRNA accumulation in the triple Δ*fphA* Δ*lreA* Δ*lreB* mutant (Figure 4). These results suggest a major role for FphA and LreB in the activation of *brlA* by light.

Deletion of *fphA* did not prevent the light-dependent accumulation of *flbA–C* or *fluG* mRNAs but the light-dependent mRNA accumulation was reduced as compared to the wild-type levels (Figure 5). Deletion of the *wc-1* gene, *lreA*, did not modify the expression by light of any of the genes under investigation with the exception of *flbC*, which showed a 50% reduction in light-dependent mRNA accumulation. Contrary to what happened with *brlA* expression, deletion

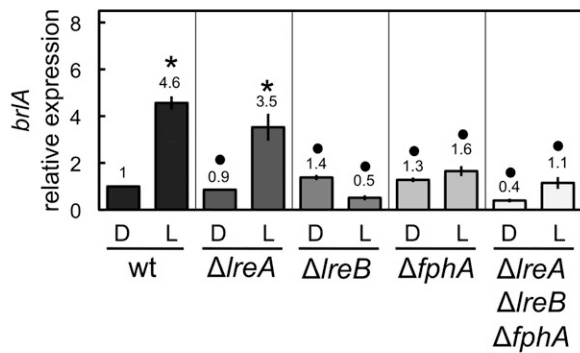

Figure 4 The activation of *brlA* by light requires the photoreceptor complex. Mycelia of the wild-type, Δ*lreA*, Δ*lreB*, Δ*fphA*, or Δ*lreA*Δ*lreB*Δ*fphA* strains were exposed to white light for 60 min or kept in the dark (D) prior to RNA extraction. The amount of *brlA* mRNA was assayed by quantitative RT–PCR. Results are shown as relative to control samples of the wild type kept in the dark. The plot shows the average and standard error of the mean of the relative photoactivation values in at least three independent experiments. Differences between dark and light conditions for the same strain, indicated by * and between different strains under the same conditions by •, are statistically significant according to the Wilcoxon–Mann–Whitney test and a *P* value <0.05.

of the *wc-2* gene *lreB* resulted in derepression of *fluG* and *flbC*. The difference in the expression of *flbA* in the Δ*lreB* strain, although higher than in the wild-type strain, was not statistically significant (Figure 5). A triple mutant strain with deletions in *fphA*, *lreA*, and *lreB* still showed a limited light-dependent accumulation of *flbA–C* mRNAs. This suggests different roles for LreB in the regulation of *brlA* and the *fluffy* genes (*flbA–C* and *fluG*).

Light-dependent induction of brlA requires FlbA, FlbB, and FlbC

The induction of *brlA* and *flbA–C* gene expression required the photoreceptor complex. However, it was not known whether the light induction of *brlA* required the proteins FlbA–C, as these proteins are required for the developmental regulation of *brlA* (Adams *et al.* 1998). Thus, light might activate directly *brlA* expression to activate conidiation; in addition, light may indirectly activate *brlA* by directly activating the expression of *brlA* regulators, *flbA–C*. Therefore, we assayed the light-dependent induction of *brlA* in the wild type and the single deletion mutants of *flbA*, *flbB* or *flbC* (Figure 6). Under dark conditions, expression of *brlA* was slightly lower in the Δ*flbB* and Δ*flbC* strains. When the mycelia was illuminated with white light for 60 min, no significant increase in the expression of *brlA* was detected in any of the deletion mutants, showing that FlbA, FlbB, and FlbC were required for the light induction of *brlA*.

Cross-regulation of light-dependent induction in the fluffy genes

The expression of *fluG* was induced by light. However, the response of *flbA–C* to light exposure was slightly faster than the response of *fluG* (Figure 3). Since the regulatory role of

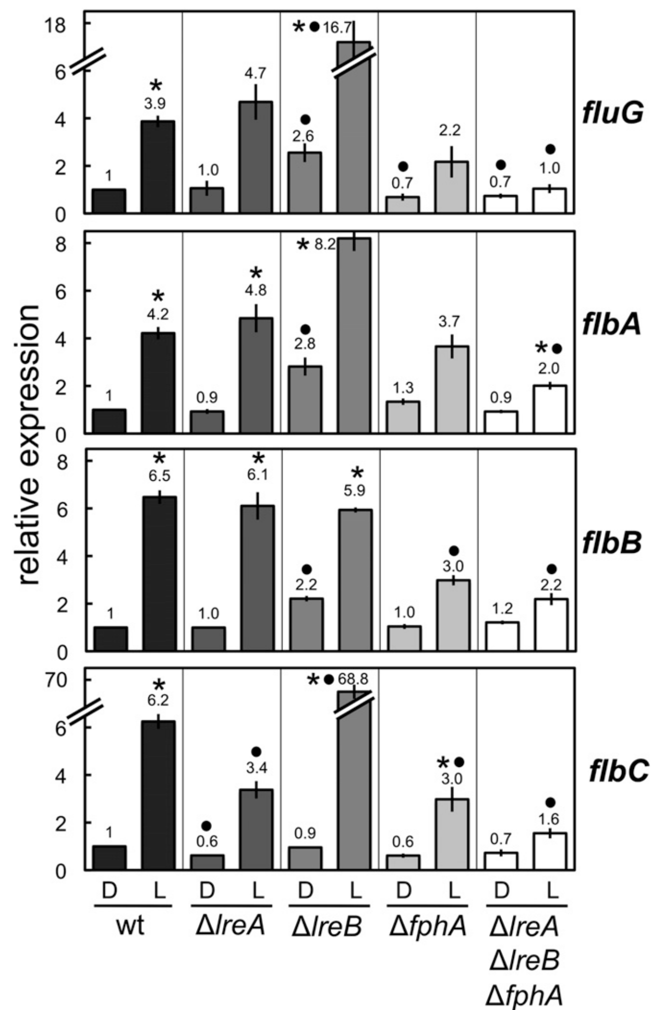

Figure 5 The activation of the upstream regulators of *brlA* by light requires the photoreceptor complex. Mycelia of the wild-type, Δ*lreA*, Δ*lreB*, Δ*fphA*, or Δ*lreA*Δ*lreB*Δ*fphA* strains were exposed to white light for 60 min or kept in the dark (D) prior to RNA extraction. The amount of *fluG*, *flbA*, *flbB*, or *flbC* mRNAs were assayed by quantitative RT–PCR. Results are shown as relative to control samples of the wild type kept in the dark. The plot shows the average and standard error of the mean of the relative photoactivation values in at least three independent experiments. Differences between dark and light conditions for the same strain, indicated by * and between different strains under the same conditions by •, are statistically significant according to the Wilcoxon–Mann–Whitney test and a *P* value <0.05.

fluG is performed upstream of *flbA–C* (Yu *et al.* 2006), we considered the possibility that FlbA–C were actually responsible for the light-dependent induction of *fluG*. Thus, the expression of *fluG* was assayed by quantitative RT–PCR in the wild type, Δ*flbA*, Δ*flbB*, or Δ*flbC* strains after 60 min of light exposure and compared to the accumulation of *fluG* mRNA in mycelia kept in the dark. The *fluG* gene was induced fourfold in the wild-type strain (Figure 7). In the Δ*flbA* strain, the expression of *fluG* was sevenfold higher than in the wild-type strain, regardless of light or dark conditions, showing that *fluG* was derepressed in the absence of *flbA* and unable to respond to light. A reduction of the light-

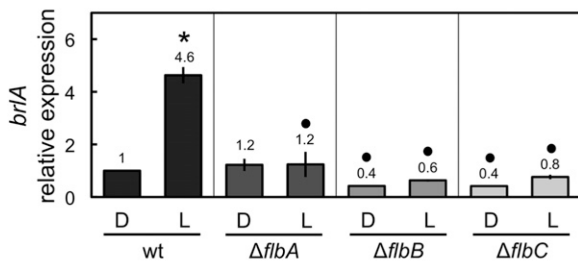

Figure 6 Light-dependent induction of the *brlA* gene is mediated by FlbA, FlbB, and FlbC. Mycelia of the wild-type and deletion strains were exposed to white light for 60 min and the amount of *brlA* mRNA was assayed by quantitative RT–PCR. Results are shown as relative to the wild-type samples kept in the dark. The plot shows the mean value and standard error of the mean of at least three independent experiments. Differences between dark and light conditions for the same strain, indicated by * and between different strains under the same conditions by •, are statistically significant according to the Wilcoxon–Mann–Whitney test and a *P* value <0.05.

dependent mRNA accumulation was observed in the Δ*flbB* strain, suggesting that FlbB was needed for full light-dependent induction of *fluG*. In the Δ*flbC* strain, no light-dependent induction of *fluG* was detected compared to the dark controls. However, the sample grown in the dark showed an expression level higher (twofold) than in the wild-type strain. The difference between the amount of *fluG* mRNA in light-exposed mycelia in Δ*flbA* or Δ*flbC* mutants compared to the amount observed in light-exposed mycelia of the wild type was not statistically significant. However, under dark conditions the difference between the amount of *fluG* mRNA in these two mutants to that observed in the wild type was statistically significant (Figure 7). These results suggest that FlbA–C were needed for the correct regulation of *fluG* expression, including its capacity to respond to the induction by light, and that FlbA and FlbC act as repressors of *fluG*.

To find out whether just one of the three *fluffy* genes or all (*flbA*, *flbB*, and/or *flbC*) were required for the reception of the signal from the photoreceptors, we also assayed the light-dependent mRNA accumulation for *flbA*, *flbB*, and *flbC* in each single deletion mutant (Figure 7). The expression of *flbA* was not affected in the Δ*flbB* or Δ*flbC* strains. However, the expression of *flbB* and *flbC* was reduced in the deletion mutants. In particular, *flbB* did not respond to the light stimulus in the Δ*flbA* and Δ*flbC* strains. The expression of *flbC* was reduced but it still retained some response to light in the Δ*flbB* strain. These results suggested that *flbA* was required for the full light-dependent response of *fluG* and *flbB* and only partially required for *flbC* (still showing ~30% of induction compared to the wild-type strain). *flbB* was not required for the expression of the other *fluffy* genes, and *flbC* was required for the light-dependent induction of *fluG*, *flbB*, and *flbA*. In addition, deletion of *flbC* resulted in a slight derepression of *fluG* and *flbA*. The results show that FlbA and FlbC play a major role in the regulation of the *fluffy* genes by light.

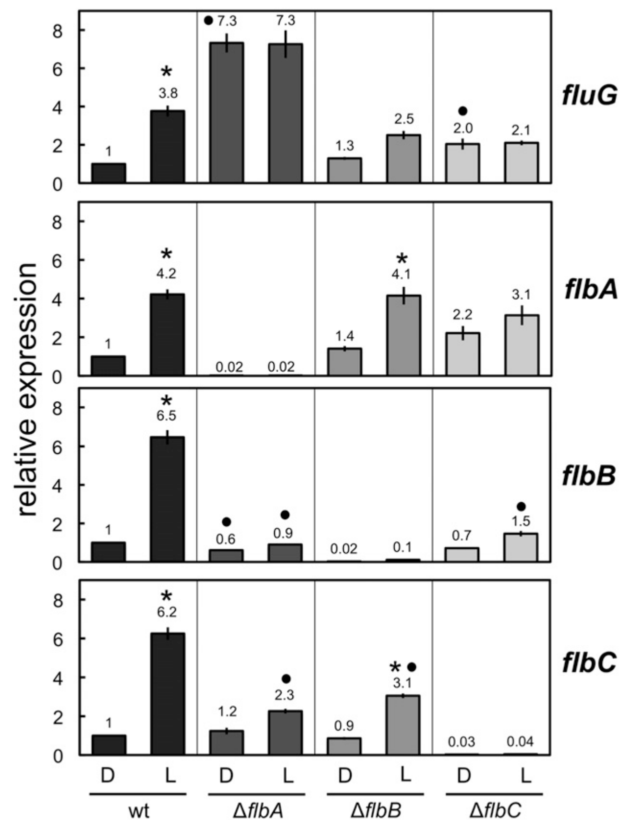

Figure 7 Light-dependent cross-regulation of the *fluffy* genes. Mycelia of the wild-type and deletion strains were exposed to white light for 60 min and the amount of the *fluG*, *flbA*, *flbB*, or *flbC* mRNAs were assayed by quantitative RT–PCR. Results are shown as relative to the wild-type control samples kept in the dark. The plot represents the mean value and standard error of the mean of at least three independent experiments. Differences between dark and light conditions for the same strain indicated by * and between each strain and the wild type under the same conditions by •, are statistically significant according to the Wilcoxon–Mann–Whitney test and a *P* value <0.05.

Discussion

The response to light involves complex molecular mechanisms that regulate many different aspects of the biology of organisms. These responses and the corresponding molecular mechanisms have been extensively studied in plants and many fungi, in particular in *N. crassa* (Chen *et al.* 2004, 2010b). One of the most fascinating biological processes controlled by light is development. *A. nidulans* has been a model for the study of fungal development for more than three decades. However, unlike *N. crassa*, the study of the light-regulated processes has not been approached in detail in the Aspergilli but light sensing, including the regulation by red light of conidiation, was described in *A. nidulans* and other fungi many years ago (Tan 1974; Mooney and Yager 1990; Bayram *et al.* 2010; Rodriguez-Romero *et al.* 2010). Complete expression analysis has been performed by microarray hybridization experiments with different fungi (Rosales-Saavedra *et al.* 2006; Chen *et al.* 2009; Greenwald *et al.* 2010; Idnurm and Heitman 2010). In *N. crassa*

changes in transcriptional levels of many different families of genes drive physiological and developmental changes (Dong *et al.* 2008; Greenwald *et al.* 2010). These changes in transcription were observed over a period of time broader than in our microarray experiment. Most of the changes in transcription levels during development and circadian cycles are not due to direct action of light but rather to a cascade of signals leading the fungus to change its physiological and/or developmental state. The number of blue-light–regulated genes was estimated to be 6% in *N. crassa* (Chen *et al.* 2009). In *T. atroviride*, 40 genes regulated by white light have been identified using cDNA microarrays, which represents 2.8% of the genes printed in the array. Thirty *T. atroviride* genes were upregulated (2%) and 10 were downregulated (0.8%) (Rosales-Saavedra *et al.* 2006). Here we report for the first time in *A. nidulans* the global effect of light exposure on transcription. In *A. nidulans* 5% of the genes were differentially regulated under our experimental conditions, which is similar to the response found in *N. crassa*. We used a short time of broad-spectrum light to discern a fast and initial transcriptional response, and to try to avoid secondary mycelial changes and adaptation to light. The transcriptomics results showed that several aspects of the biology of *A. nidulans* are affected by light. The response to light in *A. nidulans* included many upregulated genes that could be required to avoid stressing environmental changes like reactive oxygen species, osmotic stress, heat shock, etc., that may occur after light exposure or under natural conditions. In addition, we found the upregulation of genes that participate in the carbohydrate metabolism and the downregulation of genes that participate in nitrogen metabolism, suggesting that both carbohydrate and nitrogen metabolisms are regulated by light in *A. nidulans*.

A minimum of 15–30 min of illumination is necessary to elicit conidiation in a *veA*$^{+}$ genetic background (Mooney and Yager 1990). This is consistent with our results showing that *brlA* expression reaches a plateau at ~30 min of illumination. However, *brlA* is transiently activated by light as maximum light-dependent *brlA* mRNA accumulation was observed after 30–60 min of light, but longer exposures to light (up to 12 hr) did not increase the amount of *brlA* mRNA over the values obtained in the dark.

Photoadaptation has been described for light-regulated genes in *N. crassa* (Lauter and Yanofsky 1993; Arpaia *et al.* 1999; Schwerdtfeger and Linden 2003) and *P. blakesleeanus* (Rodriguez-Romero and Corrochano 2006). Photoadaptation in *N. crassa* requires the product of the *vvd* gene (Heintzen *et al.* 2001; Schwerdtfeger and Linden 2003; Zoltowski *et al.* 2007), such that the activity of the white-collar complex (WCC) is controlled by the VVD protein through the direct interaction of VVD with the components of the WCC (Chen *et al.* 2010a; Hunt *et al.* 2010; Malzahn *et al.* 2010). A homolog of *vvd* is not found in the genome *A. nidulans*. Photoadaptation of several genes also occurs in the zygomycete *P. blakesleeanus* despite the absence of a *vvd* homolog in its genome (Rodriguez-Romero and Corrochano 2006; Sanz *et al.* 2009). It is possible that a novel mechanism is responsible for the observed photoadaptation of *brlA* in the absence of a *vvd* homolog in *A. nidulans*.

The expression of the *brlA* gene is activated by several regulators. Recently, it has been reported that FlbB together with FlbD bind to the promoter of *brlA* to activate transcription (Garzia *et al*. 2010). FlbC is another transcriptional regulator that binds to the promoter of *brlA* and is involved in its activation (Kwon *et al.* 2010). We have found that *flbB* and *flbC* are induced by light and that deletion of *flbB* or *flbC* disrupts the activation of *brlA* by light. These results suggest that these two proteins are not only required for the activation of *brlA* but also for its regulation by light. One possibility is that the photoreceptor complex is signaling some other regulators, including FlbB and FlbC, to bind to the promoter of *brlA* or that the photoreceptor complex binds itself to the promoter to activate *brlA* transcription in a mechanism that requires FlbB and FlbC. LreA and LreB are homologs of the white-collar proteins, which are known DNA-binding proteins (Froehlich *et al.* 2002; He *et al.* 2002; Belden *et al.* 2007b; Olmedo *et al.* 2010a) and may directly regulate gene expression in *A. nidulans* in a similar way. It is interesting that the induction levels of all the genes that we have characterized are similar, possibly reflecting that an increase in the expression of the *fluffy* genes triggers an equivalent increase in *brlA* expression. The absence of light-dependent *brlA* mRNA accumulation in strains with deletions in *flbA*, *flbB*, or *flbC* suggests that the light-dependent activation of *brlA* occurs through the induction of these genes. However, deletion of *fphA* did not appear to have a dramatic effect in the expression of the *fluffy* genes, which leaves open the possibility that the photoreceptor complex is also binding to the promoter of *brlA* and is required for the full light-dependent activation of *brlA*. Thus, what remains unknown is how the photoreceptors induce the expression of the regulators of conidiation and whether it is a direct or indirect event through other components in the signaling pathway, yet to be discovered.

FluG deserves special attention. The expression of the gene is induced by illumination (Figure 3). FluG is responsible for the synthesis of an unknown diffusible factor that triggers conidiation (Lee and Adams 1994) by acting upstream of and derepressing all the *fluffy* genes (*flbA–E*). Surprisingly, our results in Figure 7 revealed that *fluG* is controlled by *flbA*, *flbB*, and *flbC*. Lee and Adams (1995) reported that *fluG* and *flbA* work interdependently to activate the transcription of *brlA*, *i.e.*, each of them requires the presence of the other to promote conidiation (Lee and Adams 1995). We have found that FlbA is responsible for the correct regulation of *fluG* expression. In the absence of FlbA, *fluG* is deregulated: the gene is derepressed and also failed to be induced under illumination. FlbB and FlbC also seem to be involved in the correct light-dependent regulation of *fluG* expression. This shows that there is a feedback loop of regulation in the conidiation pathway. Yager *et al.* (1998) isolated a *fluG701* mutant that failed to respond to

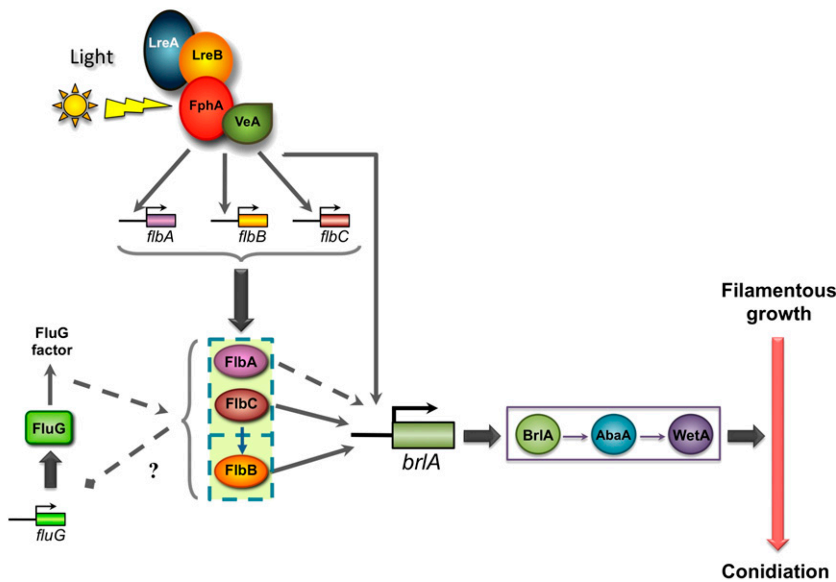

Figure 8 A model for light-dependent induction of conidiation in *A. nidulans*. Light is detected by the photoreceptor complex, which in turn activates the expression of the *fluffy* genes *flbA* and *flbC*. They are both involved in the light-dependent expression of *flbB*. In addition, *flbA* and flbC regulate *fluG* expression, which creates a feedback loop. The *fluffy* genes *flbA–C* are essential for the light-dependent induction of *brlA* expression. The photoreceptor complex may also interact directly with *brlA*. The resulting activation of *brlA* by light activates a regulatory cascade that results in the activation of the developmental program of conidiation.

red light, which suggests that FlbA–C could activate the transcription of another regulator that acts on FluG (Yager *et al.* 1998). The possibility that this feedback loop allows the amplification of the signal is possible but unlikely, since once the developmental pathways are triggered, there is a balance between them, rather than a yes/no response in *A. nidulans*, which would originate from signal amplification. Another possibility is that the FluG-dependent factor is sensitive to light or oxidation, and this feedback loop increases the synthesis of the unknown compound to satisfy new demands and maintain the derepressed state for conidiation.

The conidiation results obtained by Purschwitz *et al.* (2008) showed an interesting pattern that we could also observe with our expression data (Purschwitz *et al.* 2008). They observed a nonstatistically significant increase of conidial number in the Δ*lreA* mutant strain (both in dark and light conditions), which is consistent with the induction of some conidiation genes in the Δ*lreA* strain in comparison to the wild-type strain (Figure 5). Although in both cases the differences are statistically not significant, they show a clear trend that would be consistent with a dual role of LreA in induction and repression of genes. Our data on the expression of the *fluffy* genes in the Δ*lreB* mutant suggest that the role of the WC complex in *A. nidulans* and *N. crassa* differ. Deletion of *lreB* resulted in a strong increase of the expression of some genes compared to the wild type. However, the Δ*lreB* strain was blind for *brlA* expression, despite the fact that LreB does not have any motif for photodetection. Purschwitz *et al.* (2008) demonstrated the existence of a photoreceptor complex in which LreB is bridging the two photoreceptor proteins FphA and LreA (Purschwitz *et al.* 2008). One possibility is that LreB is required for DNA binding of the photoreceptor complex and depending on the light conditions, it will act as inducer (recruiting FphA to the promoter) or repressor through binding to LreA. WC-2 binds to hundreds of genomic regions in *N. crassa*. However, not all of the genes that are direct targets of the WCC are light inducible (Smith *et al.* 2010). This may not be surprising for genes with a complex regulation. Our data suggest that the role of WCC differs in *A. nidulans* and in *N. crassa*. LreA/LreB/FphA is a heteromeric protein with a complex role in the interplay between blue and red lights that displays a repression *vs.* activation activity depending on the conditions to trigger conidiation (or other biological function). In addition, the dual induction/repression role of the LreA/B could be achieved through one of the *fluffy* genes, acting as derepressor, while the other(s) would be inducers of the expression of *brlA*. This system would require the activity of all the *fluffy* genes. In a recent review, Etxebeste *et al.* (2010) discussed the possibility of a hierarchical *vs.* a cooperative mode of control of development in *A. nidulans*. Our results together with those from other laboratories (Adams *et al.* 1998; Yu *et al.* 2006; Etxebeste *et al.* 2010) suggest a complex combination of both modes (hierarchical plus cooperative) operating upstream of the master regulator BrlA, where some of the *fluffy* genes were necessary for the correct regulation of other *fluffy* genes, and subsequently, altogether would be required for the expression of *brlA*.

A model for the light-dependent induction of conidiation starts with light detected by the photoreceptor complex (Figure 8). Then, this complex would activate the expression of the *fluffy* genes *flbA* and *flbC*, which are also required for the light-dependent expression of *flbB*. There is cross-regulation between *fluG* and *flbA* and *flbC*. The *fluffy* genes *flbA–C* are essential for the light-dependent induction of *brlA* expression, and the activitation of these genes by light will provide the regulatory proteins for the correct activation of *blrA*. In addition, we propose that the photoreceptor complex interacts directly with the promoter of *brlA*, as deletion of *lreB* resulted in a complete loss of *brlA* expression but

not *fluffy* genes (Figures 4 and 5). The resulting activation of *brlA* by light will activate a regulatory cascade that will result in the activation of the developmental program for conidiation. *A. nidulans* asexual development is a fascinating example of how different environmental signals are integrated and transduced into complex morphogenetic pathways. Our results provide a framework for future experimental validation that will help to understand how light acts as a signal to regulate asexual development.

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Regulation of Conidiation by Light in *Aspergillus nidulans*

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Table S1 Primers used in this study

Primer name	Sequence
brlA-F	TACCGCGACGGGTTTCAG
brlA-R	GAGGTCTGTCGTCGGAGCAT
brlAα-F	CACCCCACCGGCCTTAG
brlAα-R	GATTGAATGAGATAGAGAGACAGAAGATG
brlAß-F	AACCCCTCGACCTCGTTCTT
brlAß-R	GAGGCCTGTGTCTCTGGATAAAA
fluG-F	CTCGAAGAAATCGCCGAAAC
fluG-R	CTCGGCATGGAATTGTTGAA
flbA-F	CTGGCTGATGGACTGTTCGA
flbA-R	CAAAAAGTTCCGCGATCAGAA
flbB-F	CGCTTACGGCGCATACTTACA
flbB-R	TCGGGCTCATTCCTGATGA
flbC-F	GAGAAGCGTCATTCGCTTGTG
flbC-R	CGGAGGTTAGAGACAACGGAAA
benA-F	CCAGTGTGGTAACCAGGTTGGT
benA-R	GGCGTCGAGGCCATGTT

Table S2 Full list of light regulated genes.

Table S3 Gene ontology of light regulated genes.
Classification of molecular process, biological function and cellular component of the up-regulated genes (A) or down-regulated genes (B). The table includes the frequency of each category over the total number of regulated genes. Genes with unknown ontology or not yet annotated classification were omitted.

Tables S2-S3 are available for download as Excel files at http://www.genetics.org/content/suppl/2011/05/30/genetics.111.130096.DC1.

Table S4 List of conidiation genes, which show differential expression in the microarray hybridization experiment

Locus	Gene	Log2 ratio light vs dark	Fold change	SD
AN2421	*flbC*	1.77	3.41	0.616
AN0973	*brlA*α	0.84	1.79	0.721
AN7542	*flbB*	0.78	1.72	0.653
AN4819	*fluG*	0.26	1.19	0.103