Molecular Microbiology (2015) 97(4), 733–745 ■
doi:10.1111/mmi.13062
First published online 26 June 2015

Light-dependent gene activation in *Aspergillus nidulans* is strictly dependent on phytochrome and involves the interplay of phytochrome and white collar-regulated histone H3 acetylation

Maren Hedtke,[1†] Stefan Rauscher,[1†] Julian Röhrig,[1†] Julio Rodríguez-Romero,[2] Zhenzhong Yu[1] and Reinhard Fischer[1*]

[1]*Department of Microbiology, Karlsruhe Institute of Technology, Institute for Applied Biosciences, Hertzstrasse 16, D-76187 Karlsruhe, Germany.*
[2]*Centre for Plant Biotechnology and Genomics (CBGP) U.P.M. – I.N.I.A., Campus de Montegancedo, Autopista M-40 (Km 38), 28223 Pozuelo de Alarcón, Madrid, Spain.*

Summary

The ability for light sensing is found from bacteria to humans but relies only on a small number of evolutionarily conserved photoreceptors. A large number of fungi react to light, mostly to blue light. *Aspergillus nidulans* also responds to red light using a phytochrome light sensor, FphA, for the control of hundreds of light-regulated genes. Here, we show that photoinduction of one light-induced gene, *ccgA*, occurs mainly through red light. Induction strictly depends on phytochrome and its histidine-kinase activity. Full light activation also depends on the Velvet protein, VeA. This putative transcription factor binds to the *ccgA* promoter in an *fphA*-dependent manner but independent of light. In addition, the blue light receptor LreA binds to the *ccgA* promoter in the dark but is released after blue or red light illumination and together with FphA modulates gene expression through histone H3 modification. LreA interacts with the acetyltransferase GcnE and with the histone deacetylase HdaA. *ccgA* induction is correlated to an increase of the acetylation level of lysine 9 in histone H3. Our results suggest regulation of red light-induced genes at the transcriptional level involving transcription factor(s) and epigenetic control through modulation of the acetylation level of histone H3.

Accepted 14 May, 2015. *For correspondence. E-mail reinhard.fischer@kit.edu; Tel. +49 721 6084 4630; Fax +49 721 6084 4509. Homepage: http://www.iab.kit.edu/microbio †Equal contribution.

Introduction

Light is a ubiquitous signal in the environment, which may vary concerning wavelength and intensity in different locations. Therefore, a small number of wavelength-specific photoreceptors have evolved in plants, bacteria and fungi (Purschwitz *et al.*, 2006; Corrochano, 2007; van der Horst *et al.*, 2007; Bae and Choi, 2008; Idnurm *et al.*, 2010). Blue light responses have been observed in many different fungi from zygomycetes and ascomycetes to basidiomycetes, and the response has been studied best in *Neurospora crassa*, which developed into a model system for light regulation in fungi (Chen *et al.*, 2010). Blue light responses in *N. crassa* include the induction of sporulation and protoperithecia development (Degli-Innocenti *et al.*, 1984), positive phototropism of perithecial beaks (Harding and Melles, 1983), the induction of the synthesis of carotenoids (Harding and Turner, 1981) and, very importantly, the regulation of the circadian clock (Sargent and Briggs, 1967; Baker *et al.*, 2012). Some genes regulated by the clock have been named clock-controlled genes, *ccg*, and were used as reporter genes to study the clock and light responses (Loros *et al.*, 1989; Arpaia *et al.*, 1995; Bell-Pedersen *et al.*, 1996). All the different responses require the *wc-1* and *wc-2* genes (Ballario and Macino, 1997; Linden *et al.*, 1997). Both *N. crassa* WC proteins are GATA-type transcription factors and contain a zinc-finger motif for DNA binding, a LOV domain (light, oxygen and voltage) for flavin binding and PAS domains for protein–protein interactions (Ballario *et al.*, 1996; Crosthwaite *et al.*, 1997). WC-1 and WC-2 interact and form the *White-Collar complex* (WCC). This complex, upon light exposure, binds transiently to the promoters of light-inducible genes, presumably to activate their transcription (Froehlich *et al.*, 2002; He *et al.*, 2002; He and Liu, 2005).

Besides transcriptional regulation of gene expression through WC-1 and WC-2, there is good evidence that

locus-specific modification of the chromatin structure also contributes to gene induction (Grimaldi *et al*., 2006). There are several possibilities how chromatin structure may be altered, among which are histone acetylation and methylation (Kurdistani and Grunstein, 2003; Gacek-Matthews *et al*., 2015). Whereas methylation is usually associated with gene silencing and gene repression, acetylation normally leads to activation of expression since this modification results in an opening of the chromatin structure and facilitates binding of transcription factors (Sterner and Berger, 2000). It has also been shown in *Aspergillus nidulans* that histone modifications are involved in the activation of silent gene clusters of secondary metabolites (Nützmann *et al*., 2011; 2013).

Many fungi are also able to sense red light. Already more than 40 years ago, red light responses have been described in *Botrytis cinerea* and *Alternaria solani*. Sporulation is inhibited by blue light, and the effect could be reverted by red light exposure (Lukens, 1965; Tan, 1974). Another well-studied example is *A. nidulans*, where asexual conidiospores are produced in light, and durable ascospores are formed in the dark (Mooney and Yager, 1990). However, the photobiology has been largely neglected for more than 40 years, because early on, a 'blind' mutant, named *veA1*, had been isolated, which conidiated very well in the dark (Käfer, 1965). This was very convenient for the cultivation of the fungus under laboratory conditions and was not likely to interfere in experiments studying metabolism. However, even the molecular analysis of a *bona fide* light-dependent process, asexual sporulation, was mainly performed in a *veA1* mutant background. Using developmental mutants, a cascade of regulators along with many regulated genes necessary for asexual development has been discovered (Adams *et al*., 1998; Etxebeste *et al*., 2010). Recently, it was shown that the master regulator, *brlA*, directly and quickly responds to light (Ruger-Herreros *et al*., 2011). Despite the obvious importance of the *veA* gene, the molecular analysis of this gene was only done 40 years after the isolation of the mutant and revealed that it encodes a regulator but not a light sensor (Kim *et al*., 2002; Calvo, 2008; Bayram and Braus, 2012). The structure of the velvet domain of a related protein, VosA, was solved recently, showing similarity to NF-kB and further proves that VeA is likely to directly bind to DNA (Ahmed *et al*., 2013). Photobiological experiments already suggested by 1990 the involvement of a phytochrome in the light response of *A. nidulans* (Mooney and Yager, 1990). However, the dogma at that time was that phytochromes are plant-specific molecules, and it was not until 2005 that the first fungal phytochromes were functionally characterized (Blumenstein *et al*., 2005; Ulijasz and Vierstra, 2011). Some years before, phytochrome had already been identified in the cyanobacteria *Synechocystis* PCC6803 and *Fremyella diplosiphon* (Lamparter *et al*., 1997) and, even more surprisingly, in the heterotrophic bacteria *Deinococcus radiodurans* and *Pseudomonas aeruginosa* (Wagner *et al*., 2005). These discoveries suggested that phytochrome must have evolved billions of years before the emergence of green plants (Herdman *et al*., 2000; Rockwell and Lagarias, 2010).

Some time ago, it was discovered that *A. nidulans* is able to respond to red but also to blue light (Purschwitz *et al*., 2008). The corresponding WC orthologues were named LreA and LreB for WC-1 and WC-2 respectively. Asexual conidiospore production was slightly increased in *ΔlreA* and *ΔlreB* strains, independent of the presence or absence of light. This suggested a repressing function of LreA and LreB for the asexual cycle. On the other hand, they act as positive factors for the sexual cycle. Most interestingly, LreB interacted not only with LreA to form a WCC, but also with the phytochrome FphA, which further interacted with VeA. Thus, a light regulator complex has been proposed, which may be an efficient way of coordinating the red and the blue light response in *A. nidulans* (Purschwitz *et al*., 2008). However, although this complex was found within nuclei, it remained open whether it is a stable protein complex or reflects rather transient interaction during signal transduction. Given that the WC-proteins are likely to directly interact with their target DNA of light-regulated genes, it could be that phytochrome forms a stable complex, which then would also be bound to DNA or it could be that phytochrome only transiently interacts with the WC complex. In this paper, we present evidence that phytochrome controls gene expression through chromatin remodeling. Furthermore, we show that LreA binds to the promoter in the dark and is released upon illumination, whereas FphA does not bind to DNA.

Results

ccgA *is strongly induced by red light*

We aimed at studying the light response at the gene level. In order not to cause any other stress conditions through light, the light sources were adjusted to very low intensities. In addition, the intensities of the red and blue LEDs were set to be most similar to the intensity of the corresponding wavelengths of the spectrum of the white light source (Fig. 1A). Under these low-intensity light conditions, sporulation was still induced by white, red and blue light. The number of spores obtained under blue light illumination was almost as high as the number obtained in white light. Red light was less effective, and only about 70% of the spores were produced after 48 h (24 h dark, 24 h light) of induction of asexual development (Fig. 1B). In order to study the effect of light induction at the gene level, we referred to the genome-wide analyses in *A. nidulans* where

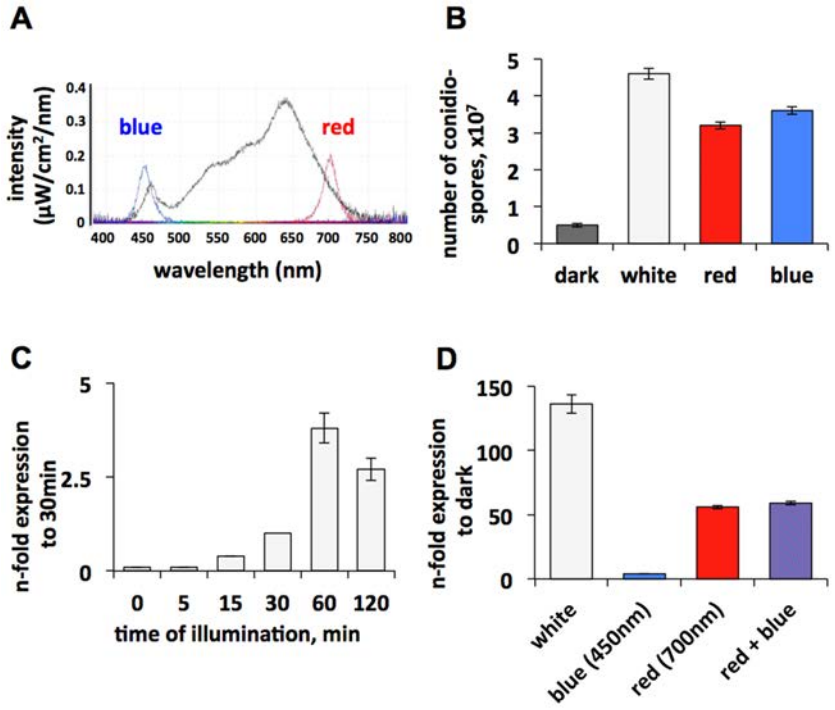

Fig. 1. Light-dependent expression of *ccgA*.
A. Spectrum and intensity of the LEDs for white, red and blue light.
B. Number of conidiospores formed in wild type in the dark and in white, red and blue light. Spores were inoculated, and plates were kept in the dark for 24 h. Then, plates were transferred to white, red or blue light for another 24 h or kept in the dark. Error bars represent standard deviations.
C. Time course of photoactivation of *ccgA* with white light. Total RNA was isolated from mycelia exposed to white light for various periods, or kept in the dark. The signal was normalized to the signal obtained with RNA isolated after 30 min of light. The plot shows the average and standard error of the mean of the relative photoactivation in a minimum of three experiments. RNA was isolated from the wild-type strain FGSC A4.
D. Real-time PCR of *ccgA* from total RNA isolated from mycelia grown for 18 hours in complete darkness and then exposed for 30 min to white, red, or blue light or the combination of red and blue light or kept in the dark. The signal was normalized to the signal obtained with RNA isolated in the dark. Error bars represent standard deviations.

more than 400 genes were induced at least twofold after 30 min of white light induction (Ruger-Herreros *et al.*, 2011). Among the genes with the highest induction was a homologue of a *N. crassa* morning-specific gene called '*clock-controlled gene 1*' (*ccg-1*) (AN9285). The *A. nidulans* gene was named *ccgA*. Deletion of *ccg-1* in *N. crassa* or *ccgA* in *A. nidulans*, respectively, did not lead to any detectable phenotypic differences, with regard to growth rate and conidiation, in comparison with wild type (Aronson *et al.*, 1994) (Fig. S1).

Because *A. nidulans ccgA* apparently responded very well to white light, and because light regulation of the expression has been studied very well in *N. crassa*, *ccgA* was used to analyze the light response of *A. nidulans* and the role of blue and red light photoreceptors in detail. Mycelium was illuminated with white, red or blue light and the combination of both and then processed for real-time RT-PCR analyses to determine the *ccgA* transcript levels. A control was kept in the dark. The experiment was performed with at least three independent biological replicates. In agreement with previous DNA array analyses, *ccgA* was strongly induced under illumination conditions. In a time course ranging from 0 to 120 min of illumination, the expression was steadily increasing until 60 min. The value decreased after 120 min, suggesting adaptation (Fig. 1C). In further experiments, 30 min was taken as the time for illumination in order to detect early induction events and not to interfere with adaptation phenomena. Next, we asked which wavelength would cause *ccgA* induction (Fig. 1D). Both blue and red light were able to induce *ccgA* expression, but whereas blue light reached less than 10% of the value obtained with white light, red light was much more effective and 50% of the expression level with white light was reached. Red and blue light combined were comparable with red light alone and did not reach the level of white light induction.

Red light sensing depends on phytochrome and the velvet protein

Because *ccgA* responded very well to red light, we aimed at understanding the role of known proteins involved in light induction and analyzed the light response to red and to blue light in several light regulator mutants. Because the *veA* gene is crucial for light regulation, all mutant strains (besides the Δ*veA* strain) carried the *veA* wild-type allele.

Deletion of phytochrome had a drastic effect on *ccgA* expression, whereas deletion of *lreA*, *lreB* or *veA* reduced the expression in white light only to about 50% (Fig. 2A). Interestingly, double deletion of *lreA* and *lreB* resulted in an expression pattern similar to wild type under all light conditions tested. During the course of these experiments, we observed derepression of *ccgA* in the dark in the absence of the VeA protein. Deletion of *lreA/B* or *fphA* did not cause such derepression. This suggests VeA acting as a repressor of *ccgA* in the dark. The derepression was still dependent on phytochrome suggesting also

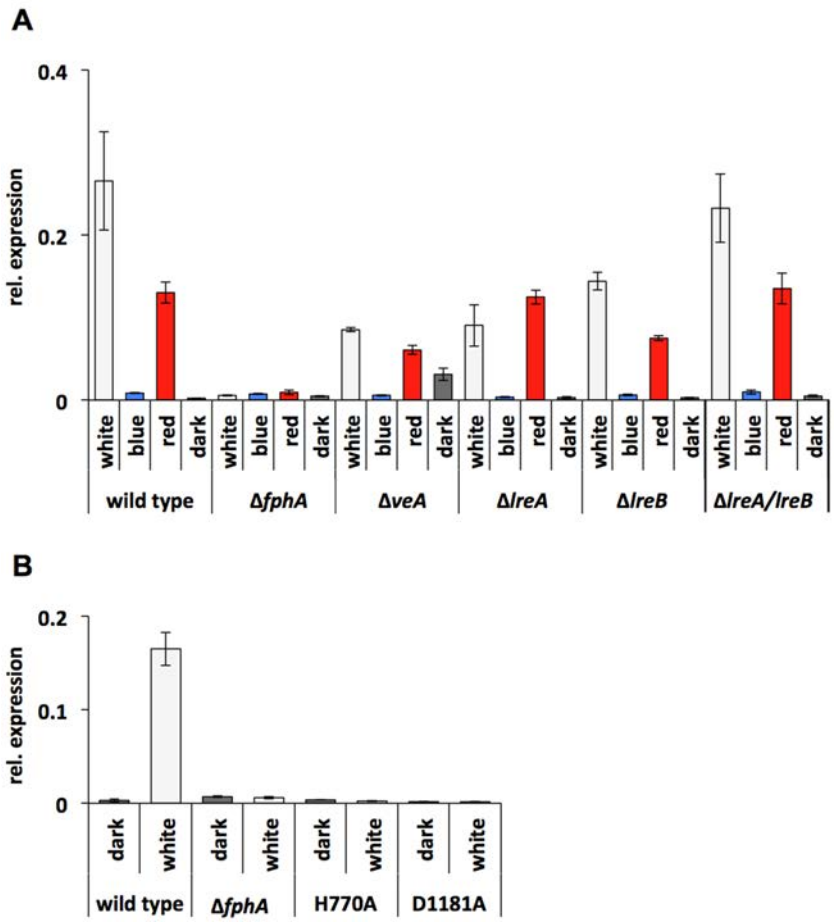

Fig. 2. Analysis light-dependent gene induction in different light-signaling mutants. A. Real-time PCR analyses of *ccgA* in wild type and the mutant strains indicated. B. Analysis of *ccgA* expression in phytochrome kinase mutants. A, B Total RNA was isolated from mycelia grown for 18 hours in complete darkness and then exposed for 30 minutes to indicated light conditions or kept in the dark. The plot shows the average and standard deviation in a minimum of three experiments. The expression of *ccgA* was quantified relative to the expression of the housekeeping gene H2B.

a function of phytochrome in the dark (data of the double mutant not shown). Taken together, these results suggest a repressing function of VeA in the dark and an essential, activating function of phytochrome. This is in agreement with the function of VeA as a repressor of asexual development (Adams *et al.*, 1998; Kim *et al.*, 2002). Red light sensing and *ccgA* expression was not impaired in the *lreA* or the *lreA/B* mutants, although there was a slight reduction in the *lreB*-deletion strain.

Because FphA is an active kinase (Brandt *et al.*, 2008), we hypothesized that *ccgA* induction would require the kinase activity of FphA. Indeed, mutagenesis of the histidine located in the kinase domain (amino acid residue 770) to alanine, and mutagenesis of the conserved aspartate in the response regulator domain (D1181A) caused the loss of the inducing activity of FphA (Fig. 2B).

Velvet and the white collar protein LreA bind to the promoter of ccgA

In order to further elucidate the molecular mechanism for the observed repressing and activating functions of VeA, LreA and FphA, respectively, we hypothesized that the proteins bind to the promoters of light-regulated genes. To test this, chromatin immunoprecipitations (ChIPs) with HA-tagged proteins were performed. Mycelial samples were taken in darkness and after different incubation times in white light. Furthermore, ChIPs of LreA, FphA and VeA were performed in different mutant strains to test whether binding would depend on the presence of any of the other proteins. Bound DNA fragments were quantified by real-time PCR. The actin promoter was used as control.

Whereas FphA did not bind to the *ccgA* promoter, neither in the dark nor upon illumination, VeA was always found at the promoter (Fig. 3A). Precipitating LreA revealed binding to the *ccgA* promoter in darkness but release from the DNA after short time of illumination, between 15 and 30 min.

Next, binding of light regulators in dependence of each other was studied. FphA was required for VeA binding and VeA was required for LreA binding (Fig. 3B). Binding of VeA was not dependent on LreA. Release of LreA from the promoter was not only achieved by illumination with white light but also with blue (high or low intensity) or red light (Fig. 3B).

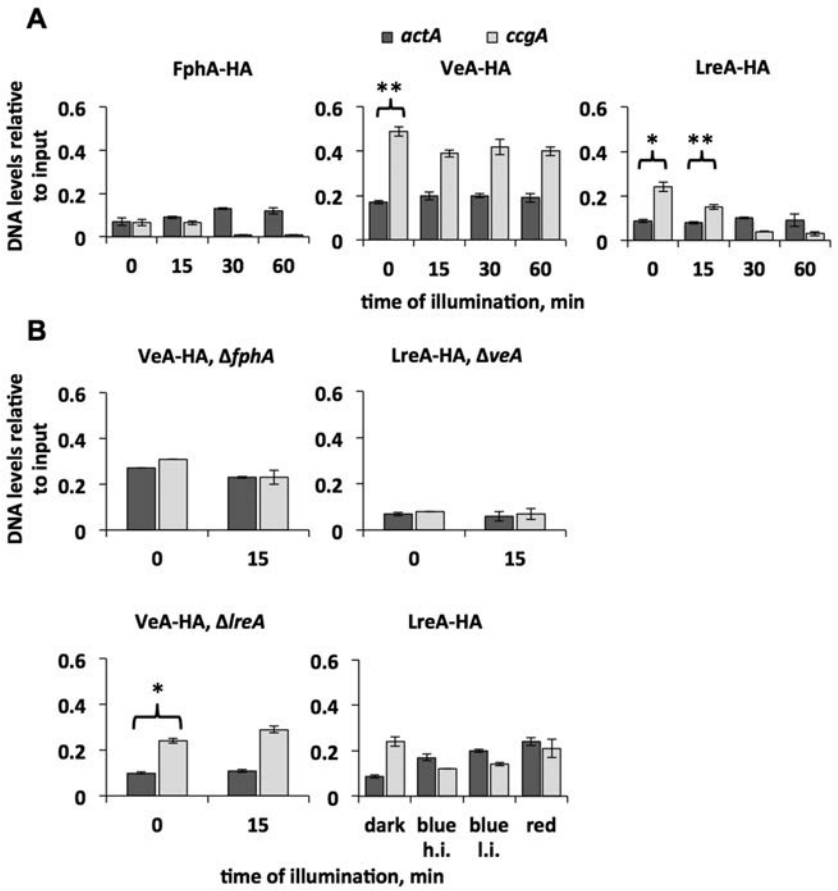

Fig. 3. Chromatin immunoprecipitation (ChIP) with HA-tagged FphA, LreA and VeA in wild type (A) and different mutant strains (B) as indicated.
Spores were inoculated on medium containing glycerol and incubated in darkness at 37°C for 2 days. Samples were taken in the dark and after different times of illumination with white light and crosslinked with formaldehyde. After precipitation with anti-HA agarose, DNA was eluted and purified for use in real-time PCR. Primers derived from the *ccgA* or the actin (*actA*) promoter were used. DNA levels are relative to Input. The plot shows the average and standard error of the mean. Unpaired *t* tests were performed exemplarily for the samples indicated: $P \leq 0.05$ (*), $P \leq 0.01$ (**). l.i. = low intensity, h.i. = high intensity.

Histone H3 acetylation accompanies light-dependent gene expression

Next, we investigated if light regulation involved chromatin remodeling and tested acetylation of histone H3 comparing wild type, the *fphA*- and *lreA*-deletion strain. Acetylation of lysine 9 and 14 of histone H3 was studied in darkness and after 20 min of illumination with white light (Fig. 4). ChIP experiments revealed an increase of acetylation of lysine 9 in the promoter region of *ccgA* after illumination in wild type. This increase was also observed for lysine 14 acetylation but seems not to be specific for light regulation since there was also an increase of acetylation in the *actA* promoter, which served as control. In an *fphA*-deletion strain, the acetylation levels of neither lysine 9 nor of lysine 14 increased after the light stimulus. This was also the case for the deletion of *lreA*. However, in this strain, the acetylation level of lysine 9 was higher in darkness than in wild type and did not increase further upon illumination. This suggests that FphA is important for the increase of the acetylation level in light, whereas LreA is needed to keep the level low in darkness.

In order to test the effect of changes in the acetylation level of histone H3 and if the photoreceptors are directly involved in this mechanism, we performed interaction studies between FphA, LreA and VeA with the histone acetyltransferase GcnE and the deacetylase HdaA. We also investigated *ccgA* light induction in wild type and *gcnE*- and *hdaA*-deletion strains. GcnE was chosen because the orthologue in *N. crassa* NGF-1 is involved in WC-dependent histone H3 acetylation (Grimaldi *et al.*, 2006; Brenna *et al.*, 2012). In addition, in *A. nidulans*, GcnE is important for acetylation of secondary metabolite gene clusters (Nützmann *et al.*, 2011). It also contributes to the regulation of the asexual developmental pathway in *A. nidulans* (Canovas *et al.*, 2014). Indeed, bimolecular fluorescence complementation experiments (BiFC or 'Split-YFP') showed interaction of FphA, VeA and LreA with GcnE and HdaA (Figs 5A and S2). Interaction of LreA with GcnE and HdaA took place in the nucleus. The fluorescent signal of FphA with GcnE was located in the nucleus, and with HdaA, it was observed in the cytoplasm. The signal for VeA and HdaA was also nuclear; the interaction of VeA with GcnE was located in cytoplasmic accumulations. Nevertheless, only the interactions of LreA were, so far, reproducible by co-immunoprecipitation (Fig. 5B). It would be possible that the other proteins only interact transiently and that those interactions are stabilized and fixed through the

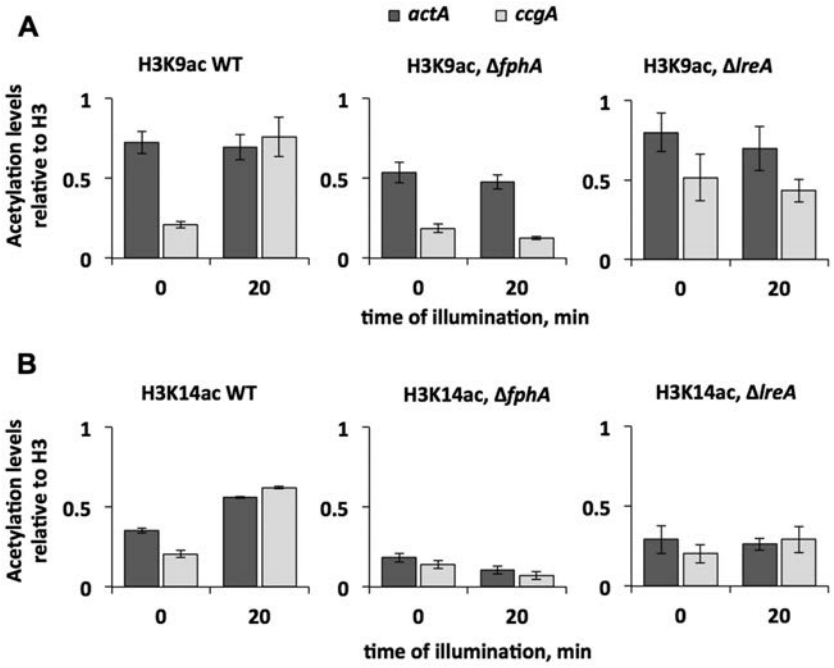

Fig. 4. Light-dependent acetylation of histone H3.
ChIP was performed with antibodies against acetylated lysine 9 (A) or 14 (B) of histone H3. Acetylation levels are shown normalized to histone H3 abundance as determined with the anti H3 antibody. Wild type is compared with the *ΔfphA* and *ΔlreA* strain in darkness and after 20 min of illumination with white light. Real-time PCR was performed for *ccgA* and the control (*actA*). The plot shows the average and standard error of the mean.

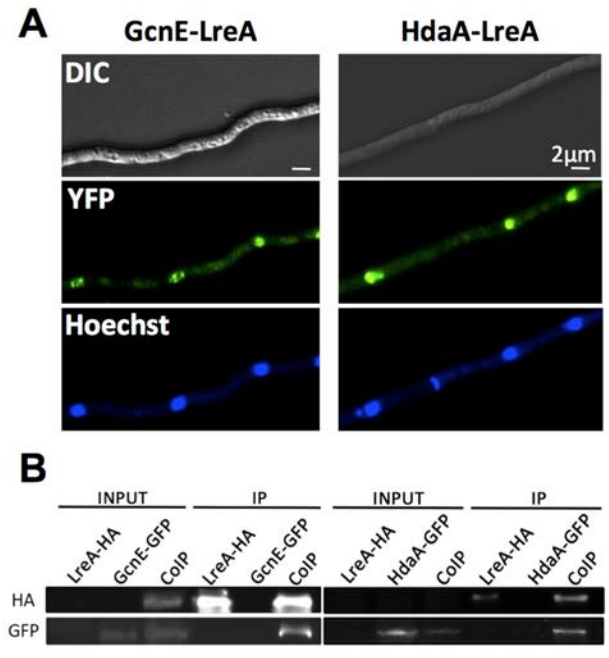

Fig. 5. Analysis of the interaction of LreA with GcnE and HdaA with bimolecular fluorescence complementation (BiFC) and Co-immunoprecipitation.
A. BiFC analysis. GcnE was fused to the N-terminus of YFP, and LreA was fused to the C-terminus. Spores were inoculated in medium containing 2% glycerol and grown over night at 28°C.
B. Co-Immunoprecipitation of GcnE and HdaA with LreA. LreA was HA-tagged, GcnE and HdaA were fused to GFP. Strains were grown for 24–48 h in liquid medium containing 2% threonine. Proteins were isolated, precipitated with anti-HA agarose and SDS PAGE, and Western blot were performed.

split YFP fragments and are therefore not detectable by Co-IPs. The interaction of LreA with histone-modifying enzymes suggested that LreA could trigger the acetylation status of the *ccgA* promoter. We hypothesized that LreA is bound to the promoter in the dark and binds HdaA and GcnE; it could activate HdaA and inactivate GcnE and thereby increase the deacetylated form of histone H3. After illumination, LreA leaves the promoter and GcnE could acetylate histone H3 and contribute to full activation of the promoter. In order to test this, we studied the impact of *gcnE* and *hdaA* deletions on the expression of *ccgA* (Fig. 6A). We anticipated that deletion of *gcnE* would lead to a decrease of the light response, whereas deletion of *hdaA* should lead to further stimulation of light induction. Indeed, deletion of *hdaA* confirmed our hypothesis, and light induction was strongly enhanced. However, deletion of *gcnE* resulted as well in a drastic increase of *ccgA* expression in light compared with wild type. This induction was partly dependent on VeA since truncation of the protein (VeA1) reduced the amount of the *ccgA* transcript in light considerably compared with a strain with full-length VeA. In order to judge the obtained result, the overall phenotypes of the deletion strains need to be considered. Whereas deletion of *hdaA* did not affect colony growth, the *gcnE*-deletion strain appeared very sick and produced only small, nonsporulating colonies (Fig. S3) (Reyes-Dominguez *et al.*, 2008; Canovas *et al.*, 2014). To get independent evidence for the involvement of GcnE or

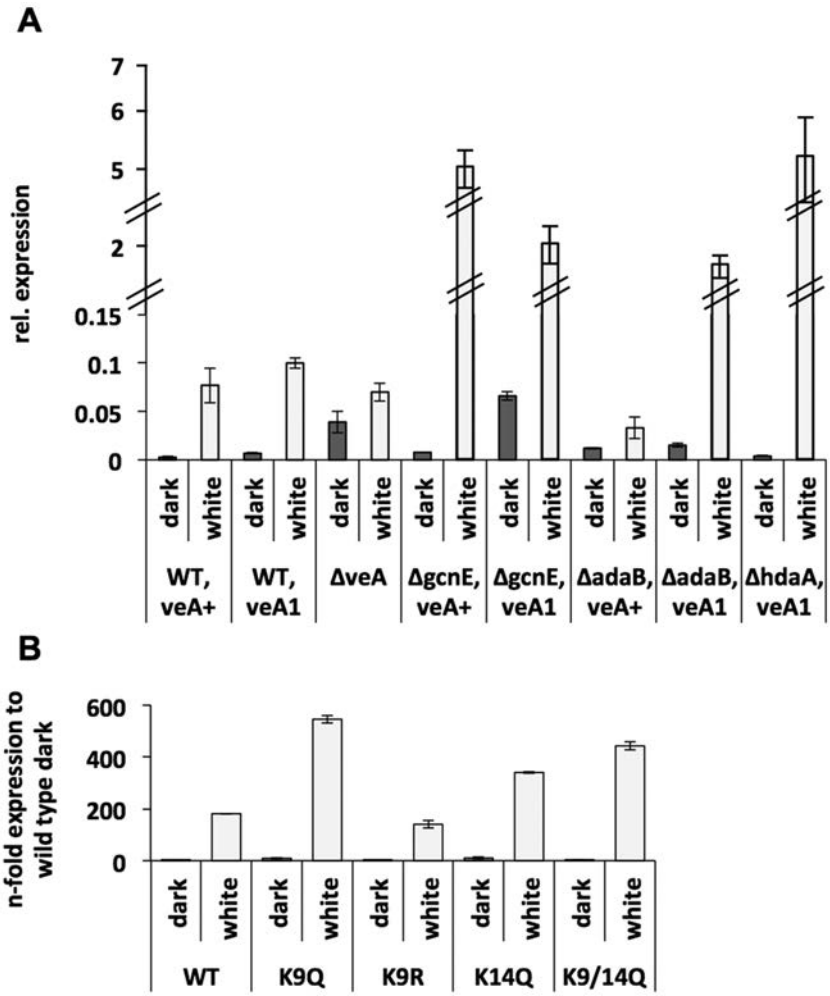

Fig. 6. Influence of GcnE, HdaA, AdaB or mutation of lysine residues of histone H3 on *ccgA* expression.
A. Comparison of *ccgA* expression in deletion mutants of *gcnE*, *adaB* and *hdaA* in dark and after illumination with white light.
B. *ccgA* expression in strains containing mutations of lysine residues 9 and/or 14 in dark and light. Exchange of lysine (K) to glutamine (Q) simulates acetylation, exchange to arginine (R) leads to a nonacetylatable residue. Real-time PCR of *ccgA* using total RNA isolated from mycelia grown for 18 hours in complete darkness. The plot shows the average and standard deviation of the mean of the relative expression in a minimum of three experiments.

the acetylation machinery, *ccgA* expression was studied in a strain where another component of the SAGA/Ada complex, AdaB, was removed. The *adaB*-deletion strain grew also slower than wild type and produced only a small amount of spores (Reyes-Dominguez *et al.*, 2008) (Fig. S3). Expression of *ccgA* in the *adaB* deletion strain was reduced to a very low level. However, this was only the case in a *veA*$^{+}$ background. In a *veA1* strain, expression of *ccgA* was increased in light, comparable with the situation in the *gcnE* deletion strain. This suggests not only involvement of FphA and LreA but also of VeA in chromatin remodeling.

To get direct evidence that the acetylation status of histone H3 in the *ccgA* promoter matters for light induction, lysine 9 and/or 14 of histone H3 was substituted by glutamine (H3K9Q, H3K14Q or H3K9/14Q) or arginine (H3K9R) to simulate an acetylated or nonacetylatable residue respectively. The H3K9Q mutation led to an increase of the expression of *ccgA*, whereas an exchange with arginine reduced gene induction in light. In the K14Q mutant, light induction was slightly lower compared with wild type. The double mutation of K9/14Q showed expression levels slightly higher than in wild type but not as high as in the K9Q mutant (Fig. 6B).

Discussion

Whereas in animals light and vision primarily cause behavioral changes, in plants and lower eukaryotes, light mainly determines major developmental or metabolic decisions and causes massive reprogramming of cells. In *A. nidulans* and other filamentous fungi, light controls the expression of several hundred genes, among which are genes involved in asexual or sexual development but also genes encoding enzymes of the secondary metabolism (Rosales-Saavedra *et al.*, 2006; Chen *et al.*, 2009; Ruger-Herreros *et al.*, 2011; Sánchez-Arrguín *et al.*, 2012). Here, we describe the molecular basis of light sensing in *A. nidulans* using one highly photoinducible gene, *ccgA*. This is the first detailed study on the mechanism of phytochrome functioning in fungi.

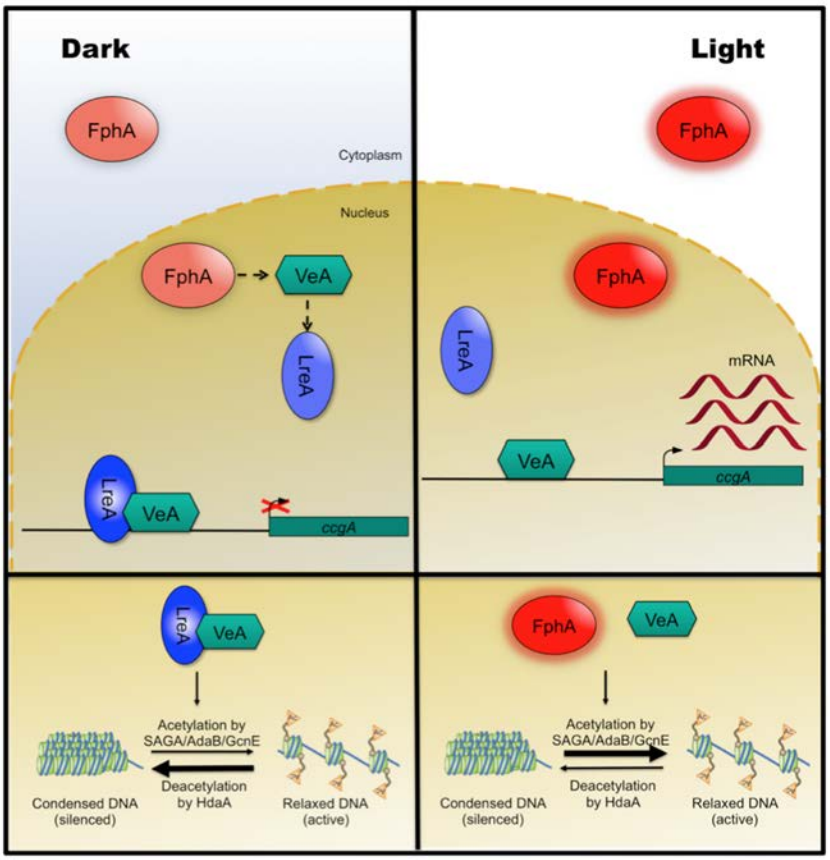

Fig. 7. Proposed model of light regulation of *ccgA*.
In darkness, VeA and LreA are bound to the promoter and interact with HdaA to prevent transcription. Upon illumination, LreA leaves the promoter and VeA and FphA activate acetylation of lysine 9 and 14 by the SAGA/AdaB/GcnE complex to induce expression of the gene.

The first point, which we would like to discuss, is the fact that sporulation was induced by red and by blue light, whereas *ccgA* induction was mainly dependent on red light and the induction did not reach levels induced with white light. First, it has to be considered that *ccgA* induction was measured 30 min after illumination, whereas asexual development requires 24 h. Second, asexual development is the result of the differential expression of hundreds of genes, and their behavior towards different wavelengths could be different. Asexual development is thus an integration of the light responses of many genes, and so far, there is no evidence that *ccgA* plays a role in development (Fig. S1). Therefore, it will be very interesting to study the mechanism(s) of photoinduction or repression for other genes to compare it with the *ccgA* promoter.

The only essential component for *ccgA* induction is apparently phytochrome, although it does not bind to the promoter of the studied reporter gene (Fig. 7). Nevertheless, it controls binding of two other factors: the velvet protein, VeA, and the blue light transcription factor, LreA. FphA together with LreA controls the acetylation level of histone H3. Several lines of evidence suggest epigenetic control of *ccgA* expression. (i) The acetylation level of lysine 9 of histone H3 increases upon illumination, (ii) LreA interacts with the acetyltransferease GcnE and the deacetylase HdaA, (iii) deletion of the SAGA/Ada complex component AdaB causes reduction, whereas deletion of *hdaA* causes induction of the photoinduction of *ccgA*, and (iv) changes of lysine 9 in histone H3 phenocopy the phenotypes of *adaB* or *hdaA* deletion strains. The only result, which is apparently not in agreement with our model, is that the *gcnE* deletion mutant showed an increased photoinduction of *ccgA* compared with wild type although we expected a reduction like in the *adaB* mutant. However, deletion of *gcnE* led to a drastic growth phenotype, and it is likely that many processes in the cell are affected (Canovas *et al*., 2014). This is also true for deletion of *hdaA* and *adaB*, but the phenotypes at the colony level are distinct and suggest that target genes may be affected in different ways. It is indeed likely that *ccgA* expression is controlled by other factors besides light because we found that it is for instance upregulated during asexual development (data not shown). Another explanation for the derepression of *ccgA* in the absence of GcnE could be that the two proteins may have different independent functions in different processes, and more, yet unidentified, acetyltransferases or other modifying enzymes like methylases could be involved in light regulation. Furthermore, modifications of different amino acid residues could depend on each other, and deletion of one

enzyme would then lead to more changes than anticipated. Likewise, deletion of *gcnE* and deletion of *adaB* led to partial derepression of *prnD* and *prnB* although the acetylation level was low (Reyes-Dominguez *et al*., 2008).

In comparison to the situation in *A. nidulans*, in *N. crassa*, acetylation of histone H3 was also shown to be important for photoinduction, but in this case, lysine 14 appears to play the major role. Mutation of lysine 14 resulted in the reduction of light-dependent gene expression of *al-3*, which is important for carotenogenesis (Brenna *et al*., 2012). Of course, one prominent difference between the two fungi is the involvement of phytochrome. Whereas almost all light responses are regulated by blue light and the WC complex, phytochrome plays a uniquely prominent role in *A. nidulans*. The acetylation level of lysine 9 in H3 was indeed dependent on phytochrome, and we propose that LreA recruits and interacts with the histone modification machinery. Recently, it has been shown for *Arabidopsis thaliana* that chromatin compaction is dependent on light intensity, and phytochrome B and histone deacetylase were both identified as positive regulators of this process (Tessadori *et al*., 2009; Jang *et al*., 2011). However, the data rely on the quantification of the heterochromatin index in nuclei, and the exact mechanisms of gene control remained enigmatic. In summary, there is good evidence that histone modification is important for light-dependent gene regulation. Although, one has to keep in mind that genome-wide modifications of histone H3 (e.g. deletion of *gcnE* or mutagenesis of individual lysine residues) usually cause pleiotropic effects that could impact light-dependent regulation of development.

The question now is how the components are linked and what happens at the *ccgA* promoter. We envision the following scenario. In the dark, LreA and VeA are bound to the promoter, and LreA interacts with GcnE and HdaA. It keeps GcnE in an inactive form but stimulates HdaA activity. After illumination, LreA is released from the promoter. It is surprising that blue as well as red light causes this release. In case of blue light, LreA could change the phosphorylation status, which would change the affinity to DNA. In the case of red light, FphA could change the phosphorylation status of LreA. Indeed, FphA has transphosphorylation activity, and this activity is higher in dark than in light (Brandt *et al*., 2008). Thus, FphA could phosphorylate LreA in the dark, and after illumination, the phosphorylation level would decrease. We propose that different forms of LreA act differently on the histone modifying enzymes. Of course, this model and the assumption that LreA is a phosphoprotein lacks experimental evidence yet. Nevertheless, it is highly likely as compared with *N. crassa*. Another interesting aspect was the observation that deletion of *lreA* or *lreB* led to a reduction of the white light-dependent photoinduction of *ccgA*, but double deletion reverted the effect. We cannot explain this phenomenon at the moment, but it points to an even more complex interplay between the different components.

One aspect of the activating function of phytochrome may be the triggering of the activity of LreA and thereby the histone modification machinery. On the other hand, histone modification is probably not sufficient but rather a prerequisite for full *ccgA* induction. Thus, other positive factors are required, which are downstream of FphA. Plant phytochrome indeed interacts with downstream transcription factors that turn on gene expression. Likewise, *A. nidulans* phytochrome interacts with the transcription factor LreB and the putative transcription factor VeA. But neither of those two has an activating function. In contrast, deletion of *veA* causes partial derepression of *ccgA* in the dark. Thus, VeA rather acts as repressor. Deletion of *lreB* reduces photoinduction, but only to about 50%. This reduction could even be reverted through deletion of *lreA*. Hence, other yet unknown factors can be postulated, and the challenge will be to identify those regulators downstream of FphA.

In conclusion, we show that light regulation of *ccgA* is a multifaceted process, which involves a number of different proteins and regulatory principles (Fig. 7). However, the results indicate already that even more components are likely to play a role and probably link light regulation with different metabolic and morphogenetic pathways.

Experimental procedures

Strains and growth conditions

The genotypes of the strains used are given in Table 1. Standard *A. nidulans* molecular and genetic procedures were used (Käfer, 1977). Synchronous development was induced by exposing liquid-grown mycelia to an air interface as described previously (Law and Timberlake, 1980).

RNA isolation

Conidia were inoculated with a loop on the surface of 20–25 ml of complete liquid medium or minimal medium in a Petri dish. After 18 or 25 h of incubation in constant darkness at 37°C, the mycelial mat was illuminated with white light, red light or blue light LED lamps. Control samples were harvested in complete darkness. Samples were frozen in liquid nitrogen and stored at −80°C until RNA isolation. RNA was isolated with the E. Z. N. A. Fungal RNA Mini Kit (VWR). Cell disruption was made in a homogenizer with glass beads at maximum speed for 5 min. The isolated RNA was quantified and an aliquot treated with DNase I. RNA samples were diluted to a final concentration of 50 ng μl^{-1} in DEPC water.

Real-time PCR

Quantitative PCR experiments were performed to determine relative mRNA abundance using SensiFAST SYBR &

Table 1. Strains used in this study.

Strain	Genotype	Source
FGSCA4	Glasgow Wild Type	FGSC Missouri
SJP1	*pyrG89; ΔargB:trpCΔB; pyroA4 ΔfphA::argB veA+*	Purschwitz *et al.* (2008)
SJP70	*pyrG89; ΔargB:trpCΔB; pyroA4 ΔlreB::argB veA+*	Purschwitz *et al.* (2008)
ΔgcnE	*biA1; yA2; ΔgcnE::AfpyrG; veA1*	Reyes-Dominguez *et al.* (2008)
H4	*yA2; hdaA::pyrG; pyrG89; riboB2; chaA1, veA1*	Tribus *et al.* (2005)
SKV103	*pyrG89; pyroA4; veA+*	Vienken and Fischer (2006)
A1153ΔadaB	*adaB::argB2; yA1; pabaA1; pyroA4; nkuA::bar; veA1*	Nützmann *et al.* (2011)
SJP119	SRF200 *(argB2; pyrG89; pyroA4)* transformed with pJP87 *(argB; alcA::fphAH770A::3xHA)*	Janina Purschwitz
SJP120	SRF200 *(argB2; pyrG89; pyroA4)* transformed with pJP88 *(argB; alcA::fphAD1181A::3xHA)*	Janina Purschwitz
SSM12	*alcA::veA::3xHA; pyroA4*	Sylvia Müller
SSM39	*alcA::fphA::3xHA; pyrG89, veA+*	Sylvia Müller
SSM42	*alcA::fphA::3xHA; ΔveA::argB*	Sylvia Müller
SSM45	*alcA::3xHA::lreA; pyroA4; veA+*	Sylvia Müller
SSM50	*alcA::veA::3xHA; pyroA4; ΔfphA::argB*	Sylvia Müller
SJR2	FGSCA1153 X SKV103; *pyroA4; pyrG89; nkuA::bar; veA+*	This study
SJR10	*pyrG89; ΔargB::trpCDB; pyroA4, nkuA::bar; ΔfphA::argB; veA+*	This study
SMH4	*pyrG89; pyroA4, nkuA::bar; ΔccgA::pyroA; veA1*	This study
SMH8	*DVAR1 (ΔveA) transformed with pSM15 (alcA::3xHA::lreA; pyr-4)*	This study
SMH9	*alcA::n-yfp::gcnE; alcA::c-yfp::fphA; veA+*	This study
SMH10	*pyrG89; ΔargB::trpCDB; pyroA4, nkuA::bar; ΔfphA::argB; veA+*	This study
SMH15	*alcA::n-yfp::gcnE; alcA::c-yfp::lreA; veA+*	This study
SMH16	SSR66 transformed with pSM17 *(alcA::3xHA::veA)*	This study
SMH17	*alcA::n-yfp::gcnE; alcA::c-yfp::veA; veA+*	This study
SRJ28	*alcA::n-yfp::hdaA; alcA::c-yfp::lreA; veA+*	This study
SRJ30	*alcA::n-yfp::hdaA; alcA::c-yfp::veA; veA+*	This study
SSR6	SSM39 transformed with pSR16 *(alcA::gfp::gcnE, pyr-4)* and pNZ11 (*pyroA*); *veA+*	This study
SSR7	SSM45 transformed with pMH11 (*alcA::GFP::hdaA*) and pNZ11 (*pyroA*); *veA+*	This study
SSR8	SKV103 transformed with pSR16 *(alcA::GFP::gcnE, pyr-4); veA+*	This study
SSR26	*ΔgcnE; alcA::GFP::gcnE* (+400 bp terminator); *ptrA*	This study
SSR31	*yA2; ΔgcnE::AfpyrG; pyroA4; veA+*	This study
SSR55	*yA2, adaB::argB2; pyroA4; veA+*	This study
SSR66	*yA2; pyroA4; pabaA1; nku::bar; ΔlreA::ptrA; veA+*	This study
SSR89	*yA2; pyroA4; ΔlreA::ptrA; ΔlreB::argB; veA+*	This study
SZY6.1	SJR2 co-transformed with pZY8 (hH3-LB (1kb)::hH3K14Q:: hH3-RB (1 kb)) and FNO3 (*pyr4*); *pyroA4; veA+*	This study
SZY7	SJR2 co-transformed with pZY9 (hH3-LB (1kb)::hH3K9Q::hH3-RB (1 kb)) and FNO3 (*pyr4*); *pyroA4; veA+*	This study
SZY8	SJR2 co-transformed with pZY10 (hH3-LB (1kb)::hH3K9/14Q:: hH3-RB (1 kb)) and FNO3 (*pyr4*); *pyroA4; veA+*	This study
SZY9	SJR2 co-transformed with pZY12 (hH3-LB (1kb)::hH3K9R::hH3-RB (1 kb)) and FNO3 (*pyr4*); *pyroA4; veA+*	This study
SZY16.1	SJR2 co-transformed with pZY11 (hH3-LB (1kb)::hH3K14R::hH3-RB (1 kb)) and FNO3 (*pyr4*); *pyroA4; veA+*	This study

Fluorescein One-Step Kit (Bioline) on an iCycler from Bio-Rad. Each reaction of 25 µl contained 0.2 µl of RT enzyme, 0.2 µM of primers (HPLC grade) and 100 ng of total RNA. The cycle included 10 at 50°C for the reverse transcription reaction, followed by 5 min at 95°C for its inactivation and 40 PCR cycles (10 s at 95°, and 1 min at 60°). After each PCR, we performed melting curve analyses to show the specific amplification of single DNA segments and the absence of nonspecifically amplified DNA.

The results for each gene were normalized to the corresponding results obtained with histone H2B. Then, the results obtained with each sample were normalized to the RNA sample obtained from wild-type mycelia in darkness or exposed to light for 30 minutes and are the average of three to six biological replicates. For Oligonucleotides used in this study, see Table 2.

ChIP and coupled qRT-PCR

To induce the expression of the protein with HA-tag under the control of the *alcA* promoter, plates with minimal medium containing 0.2% glucose and 2% glycerol were inoculated. After 2 d of incubation at 37°C in the dark, one sample was kept in darkness, and others were illuminated for 15, 30 and 60 min. Spores were harvested in H_2O/0.2% Tween 20. By adding formaldehyde (final concentration 1%), DNA–protein interactions were crosslinked for 30 min, and the reaction was stopped with glycine (final concentration 125 mM). Spores were washed and resuspended in 1 ml of lysis buffer (50 mM HEPES KOH pH 7.5; 140 mM NaCl; 1 mM EDTA; 1% Triton X-100; 0.1% Na-Deoxycholate; Protease Inhibitor Cocktail (Sigma)) and sonicated. The samples were centrifuged for 10 min (13,000 r.p.m.). Anti-HA agarose (Thermo

Table 2. Oligonucleotides used for real-time PCR, ChIP and site-directed mutagenesis.

Oligo name	Sequence (5′-3′)
ccgA-102F	CGACGCTTCCCTCACTTCTC
ccgA-178R	CATCATGGGACTTCTCGTCCTT
H2B_fw_low_RTQ	TGCCGAGAAGAAGCCTAGCA
H2B_rev_low_RTQ	GAGTAGGTCTCCTTCCTGGT
real-ccgA-fw3	GTGGTAATGACAGGAAAGGCC
real-ccgA-rv3	GAGTTCGTCATAAGCATGGGCG
real-actA-fw	CTTCTCAACATCCAACTCCC
real-actA-rv	GGTGGATTAGAATCGAACTAC
hH3_F	ATACGCTTGACACCACCACG
hH3_R	AGCGAGCTTCAGTTGTCATAGG
hH3K9Q_R/ hH3K9K14Q_R	CAGTAGACTGGCCTGTTGGGGTAAAGTTAGCATC
hH3K9Q_F	CCAACAGGCCAGTCTACTGGTGGCAAGGCTCC
hH3K9K14Q_F	CCAACAGGCCAGTCTACTGGTGGCCAGGCTCC
hH3K14Q_R	GGGGAGCCTGGCCACCAGTAGACTTGCCTG
hH3K14Q_F	ACTGGTGGCCAGGCTCCCCGTAAGCAGCTC
hH3K14R_R	GGGGAGCACGGCCACCAGTAGACTTGCCTG
hH3K14R_F	ACTGGTGGCCGTGCTCCCCGTAAGCAGCTC
hH3K9R_R	CAGTAGATCTGCCTGTTGGGGTAAAGTTAGCATC
hH3K9R_F	CCAACAGGCAGATCTACTGGTGGCAAGGCTCC
hH3_out_F	ATGTGACGGTCTTGCGCTTG
hH3_out_R	CGCAGTGGATGATGTGAATCTTTAG

Scientific) was added to 500 µl supernatant and the mixture rotated at 4°C over night; 1/10 (50 µl) of the supernatant was used as a control and was not further treated (Input). The sample was centrifuged (13,000 r.p.m., 1 min) and the beads washed successively with 1 ml of lysis buffer (3×), 3× with high salt buffer (50 mM HEPES KOH pH 7.5; 500 mM NaCl; 1 mM EDTA; 1% Triton X-100; 0.1% Na-Deoxycholate), 3× with wash buffer (10 mM Tris pH 7.5; 1 mM EDTA; 0.25 M LiCl; 0.5% Nonidet P-40; 0.5% Na-Deoxycholate) and 3× with TE (10 mM Tris pH 7.5; 1 mM EDTA); 125 µl of elution buffer (50 mM Tris pH 7.5; 1 mM EDTA; 1% SDS) was added to the bead pellet and the sample heated to 65°C for 15 min. After adding 125 µl TE/1% SDS, the beads were incubated at 65°C for 10 min and then boiled for 5 min at 95°C. The input control was treated with 200 µl TE/1% SDS under the same conditions as the IP samples. Proteinase K was added, and after 2 h at 37°C, the samples were purified with the ChIP clean up and concentrator kit (Zymo research), and purified DNA was used in quantitative real-stime PCR reactions with primers derived from the promoters of the gene of interest, *ccgA*. As control, primers were used that amplify part of the promoter of *actA*; 1 µl of precipitated DNA (IP) and 1 µl of 1:10 diluted Input were used for the reaction to compare enrichment of DNA fragments. qRT-PCR was performed using SensiFAST SYBR & Fluorescein Kit (Bioline) in a 25 µl reaction (40 cycles, 95°C for 10 s and 58°C for 30 s). Immunoprecipitated DNA was divided by Input samples to show the relative enrichment of the *ccgA* promoter compared with *actA*.

For the ChIP studying histone H3 acetylation, strains were grown in medium containing only glucose, and samples were split into three. Antibodies against the N-terminus of histone H3, acetylated lysine 9 of histone H3 (H3K9ac) and acetylated lysine 14 of histone H3 (H3K14ac) (all from Millipore, #06–755, #06–942 and #06–911), and Protein G- Agarose (Roche) were used for precipitation. Results obtained for the acetylated lysines were normalized to H3, and the relative amount of acetylated histones is shown in dependence of H3 abundance.

At least two biological replicates were used (usually four), and standard errors of the mean were calculated. For Oligos used in this study, see Table 2.

Co-immunoprecipitation

Spores were inoculated in liquid minimal medium containing 2% threonine and 0.2% glucose and incubated for 24–48 h at 37°C and 180 r.p.m. Mycelium was filtered, ground in liquid nitrogen, mixed with extraction buffer (50 mM Tris-HCl pH 7.8; 150 mM NaCl, 0.05% Nonidet P – 40; protease inhibitor mix) and incubated on ice for 20 min. The samples were centrifuged twice (13,000 r.p.m., 4°C, 15 min). Protein concentration of the supernatant was determined; 5 mg of total protein was used for co-immunoprecipitations. Anti-HA agarose (Thermo Scientific) was added and incubated for at least 3 h at 4°C, rotating. Centrifugation for 30 s at 4,000 r.p.m. pelleted the beads. The pellet was then washed four times with extraction buffer. Adding 2× SDS sample buffer (4× SDS sample buffer: 240 mM Tris-HCl pH 6.8; 400 mM DTT; 8% SDS; 0.04% bromphenol blue; 30% Glycerol) and boiling the samples for 5 min at 95°C released the proteins from the beads. SDS-PAGE and Western Blot were performed. Monoclonal antibodies against GFP (Roche) and the secondary antibody (anti-mouse) were used. After the chemiluminescent reaction, the membrane was stripped using 1× PBS, 100 mM β-mercaptoethanol, 2% SDS for 30 min at 50°C, and washed twice with PBS-T and blocked. Then, anti-HA antibodies (Sigma) and secondary antibodies (anti-mouse) were added.

Bimolecular fluorescence complementation microscopy

Spores were inoculated on cover slips in liquid minimal medium containing 2% glycerol and 0.2% glucose (and necessary markers) and were incubated over night at 28°C. The next day, cover slips were mounted on microscope slides and hyphae were examined under the microscope (Zeiss) using the YFP channel. GcnE and HdaA were fused to the N-terminal part of YFP; LreA, FphA and VeA were fused to the C-terminal part of YFP under the inducible *alcA* promoter.

Site-directed mutagenesis

Standard PCR and cloning procedures were used to construct plasmids for site-directed mutagenesis. Procedure will be explained for H3K14Q as example. The histone H3 gene was amplified with the primers hH3_F/hH3_R using genomic DNA as template and cloned into pJET1.2/blunt vector, yielding plasmid pZY7. It was used as template to amplify the left and right borders for fusion PCR. hH3K14Q-LB and hH3K14Q-RB cassettes used for fusion PCR were generated with primers hH3_F/hH3K14Q_R and hH3K14Q_F/hH3_R respectively. Fusion PCR was performed with the primers hH3_F/hH3_R. The product was cloned into pJET1.2/blunt vector (Fermentas), yielding pZY8. Wild-type SJR2 was co-transformed with pYZ8 and pFNO3. Putative transformants were checked by

PCR for homologous recombination with the primers hH3_out_F/hH3_out_R. The PCR product was sequenced to screen H3K14Q mutant.

Determination of the spore number

Spores (5×10^5) of FGSCA4 were inoculated on solid medium and grown at 37°C for 24 h in the dark and then 24 h in light (white, red or blue). Spores of the whole plate were harvested with H_2O/2% Tween 20 and counted in a Neubauer chamber. Three biological replicates were analyzed.

Acknowledgements

This work was supported by the German Science Foundation (DFG). J.R. was supported with a fellowship of the Spanish Ministry, and Z.Y. was supported with a CSC fellowship from China. We thank J. Strauss (University of Vienna, Austria), H. Haas (University of Innsbruck, Austria), and A. Brakhage (Hans-Knöll Institute, Jena, Germany) for various *A. nidulans* strains, and L. Corrochano (Sevilla, Spain) for helpful discussions.

References

Adams, T.H., Wieser, J.K., and Yu, J.-H. (1998) Asexual sporulation in *Aspergillus nidulans*. *Microbiol Mol Biol Rev* **62:** 35–54.

Ahmed, Y.L., Gerke, J., Park, H.S., Bayram, O., Neumann, P., Ni, M., *et al*. (2013) The velvet family of fungal regulators contains a DNA-binding domain structurally similar to NF-kappaB. *PLoS Biol* **11:** e1001750.

Aronson, B.D., Lindgren, K.M., Dunlap, J.C., and Loros, J.J. (1994) An efficient method for gene disruption in *Neurospora crassa*. *Mol Genet Genomics* **242:** 490–494.

Arpaia, G., Loros, J.J., Dunlap, J.C., Morelli, G., and Macino, G. (1995) Light induction of the clock-controlled gene *ccg-1* is not transduced through the circadian clock in *Neurospora crassa*. *Mol Genet Genomics* **247:** 157–163.

Bae, G., and Choi, G. (2008) Decoding of light signals by plant phytochromes and their interacting proteins. *Annu Rev Plant Biol* **59:** 281–311.

Baker, C.L., Loros, J.J., and Dunlap, J.C. (2012) The circadian clock of *Neurospora crassa*. *FEMS Microbiol Rev* **36:** 95–110.

Ballario, P., and Macino, G. (1997) White collar proteins: PASsing the light signal in *Neurospora crassa*. *Trends Microbiol* **5:** 458–462.

Ballario, P., Vittorioso, P., Margrelli, A., Toalora, C., Cabibo, A., and Macino, G. (1996) White collar-1, a central regulator of blue light responses in *Neurospora crassa*, is a zinc finger protein. *EMBO J* **15:** 1650–1657.

Bayram, Ö., and Braus, G.H. (2012) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. *FEMS Microbiol Rev* **36:** 1–24.

Bell-Pedersen, D., Shinohara, M.L., Loros, J.J., and Dunlap, J.C. (1996) Circadian clock-controlled genes isolated from *Neurospora crassa* are late night- to early morning-specific. *Proc Natl Acad Sci USA* **93:** 13096–13101.

Blumenstein, A., Vienken, K., Tasler, R., Purschwitz, J., Veith, D., Frankenberg-Dinkel, N., and Fischer, R. (2005) The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. *Curr Biol* **15:** 1833–1838.

Brandt, S., von Stetten, D., Günther, M., Hildebrandt, P., and Frankenberg-Dinkel, N. (2008) The fungal phytochrome FphA from *Aspergillus nidulans*. *J Biol Chem* **283:** 34605–34614.

Brenna, A., Grimaldi, B., Filetici, P., and Ballario, P. (2012) Physical association of the WC-1 photoreceptor and the histone acetyltransferase NGF-1 is required for blue light signal transduction in *Neurospora crassa*. *Mol Biol Cell* **23:** 3863–3872.

Calvo, A.M. (2008) The VeA regulatory system and its role in morphological and chemical development in fungi. *Fungal Genet Biol* **45:** 1053–1061.

Canovas, D., Marcos, A.T., Gacek, A., Ramos, M.S., Gutierrez, G., Reyes-Dominguez, Y., and Strauss, J. (2014) The histone acetyltransferase GcnE (GCN5) plays a central role in the regulation of Aspergillus asexual development. *Genetics* **197:** 1175–1189.

Chen, C.H., Ringelberg, C.S., Gross, R.H., Dunlap, J.C., and Loros, J.J. (2009) Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in Neurospora. *EMBO J* **28:** 1029–1042.

Chen, C.H., Dunlap, J.C., and Loros, J.J. (2010) Neurospora illuminates fungal photoreception. *Fungal Genet Biol* **47:** 922–929.

Corrochano, L.M. (2007) Fungal photoreceptors: sensory molecules for fungal development and behaviour. *Photochem Photobiol Sci* **6:** 725–736.

Crosthwaite, S.K., Dunlap, J.C., and Loros, J.J. (1997) *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. *Science* **276:** 763–769.

Degli-Innocenti, F., Chambers, J.A., and Russo, V.E. (1984) Conidia induce the formation of protoperithecia in *Neurospora crassa*: further characterization of white collar mutants. *J Bacteriol* **159:** 808–810.

Etxebeste, O., Garzia, A., Espeso, E.A., and Ugalde, U. (2010) *Aspergillus nidulans* asexual development: making the most of cellular modules. *T Microbiol* **18:** 569–576.

Froehlich, A.C., Liu, Y., Loros, J.J., and Dunlap, J.C. (2002) White collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. *Science* **297:** 815–819.

Gacek-Matthews, A., Noble, L.M., Gruber, C., Berger, H., Sulyok, M., Marcos, A.T., *et al*. (2015) KdmA, a histone H3 demethylase with bipartite function, differentially regulates primary and secondary metabolism in Aspergillus nidulans. *Mol Microbiol* **96:** 839–860.

Grimaldi, B., Coiro, P., Filetici, P., Berge, E., Dobosy, J.R., Freitag, M., *et al*. (2006) The *Neurospora crassa* White Collar-1 dependent bluit light response requires acetylation of histone H3 lysine 14 by NGF-1. *Mol Biol Cell* **17:** 4576–4583.

Harding, R.W., and Melles, S. (1983) Genetic analysis of phototropism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. *Plant Physiol* **72:** 996–1000.

Harding, R.W., and Turner, R.V. (1981) Photoregulation of the carotenoid biosynthetic pathway in albino and white collar mutants of *Neurospora crassa*. *Plant Physiol* **68:** 745–749.

He, Q., and Liu, Y. (2005) Molecular mechanism of light

responses in Neurospora: from light-induced transcription to photoadaptation. *Genes Dev* **19:** 2888–2899.
He, Q., Cheng, P., Yang, Y., Wang, L., Gardner, K.H., and Liu, Y. (2002) White collar-1, a DNA binding transcription factor and a light sensor. *Science* **297:** 840–843.
Herdman, M., Coursin, T., Rippka, R., Houmard, J., and Tandeau de Marsac, N. (2000) A new appraisal of the prokaryotic origin of eukaryotic phytochromes. *J Mol Evol* **51:** 205–213.
van der Horst, M.A., Key, J., and Hellingwerf, K.J. (2007) Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *T Microbiol* **15:** 554–562.
Idnurm, A., Verma, S., and Corrochano, L.M. (2010) A glimpse into the basis of vision in the kingdom Mycota. *Fungal Genet Biol* **47:** 881–892.
Jang, I.C., Chung, P.J., Hemmes, H., Jung, C., and Chua, N.H. (2011) Rapid and reversible light-mediated chromatin modifiactions of Arabidopsis phytochrome A lucus. *Plant Cell* **23:** 459–470.
Käfer, E. (1965) The origin of translocations in *Aspergillus nidulans*. *Genetics* **52:** 217–232.
Käfer, E. (1977) Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. *Adv Genet* **19:** 33–131.
Kim, H.-S., Han, K.-Y., Kim, K.-J., Han, D.-M., Jahng, K.-Y., and Chae, K.-S. (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. *Fungal Genet Biol* **37:** 72–80.
Kurdistani, S.K., and Grunstein, M. (2003) Histone acetylation and deacetylation in yeast. *Nature* **4:** 276–284.
Lamparter, T., Mittmann, F., Gärtner, W., Borner, T., Hartmann, E., and Hughes, J. (1997) Characterization of recombinant phytochrome from the cyanobacterium Synechocystis. *Proc Natl Acad Sci USA* **94:** 11792–11797.
Law, D.J., and Timberlake, W.E. (1980) Developmental regulation of laccase levels in *Aspergillus nidulans*. *J Bacteriol* **144:** 509–517.
Linden, H., Ballario, P., and Macino, G. (1997) Blue light regulation in *Neurospora crassa*. *Fungal Genet Biol* **22:** 141–150.
Loros, J.J., Denome, S.A., and Dunlap, J.C. (1989) Molecular cloning of genes under control of the circadian clock in Neurospora. *Science* **243:** 385–388.
Lukens, R.J. (1965) Reversal by red light of blue light inhibition of sporulation in *Alternaria solani*. *Phytophathology* **55:** 1032.
Mooney, J.L., and Yager, L.N. (1990) Light is required for conidiation in *Aspergillus nidulans*. *Genes Dev* **4:** 1473–1482.
Nützmann, H.W., Reyes-Dominguez, Y., Scherlach, K., Schroeckh, V., Horn, F., Gacek, A., *et al*. (2011) Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation. *Proc Natl Acad Sci USA* **108:** 14282–14287.
Nützmann, H.W., Fischer, J., Scherlach, K., Hertweck, C., and Brakhage, A.A. (2013) Distinct amino acids of histone H3 control secondary metabolism in *Aspergillus nidulans*. *Appl Environ Microbiol* **79:** 6102–6109.
Purschwitz, J., Müller, S., Kastner, C., and Fischer, R. (2006) Seeing the rainbow: light sensing in fungi. *Curr Opin Microbiol* **9:** 566–571.
Purschwitz, J., Müller, S., Kastner, C., Schöser, M., Haas, H., Espeso, E.A., *et al*. (2008) Functional and physical interaction of blue and red-light sensors in *Aspergillus nidulans*. *Curr Biol* **18:** 255–259.
Reyes-Dominguez, Y., Narendja, F., Berger, H., Gallmetzer, A., Fernandez-Martin, R., Garcia, I., *et al*. (2008) Nucleosome positioning and histone H3 acetylation are independent processes in the Aspergillus nidulans prnD-prnB bidirectional promoter. *Eukaryot Cell* **7:** 656–663.
Rockwell, N.C., and Lagarias, J.C. (2010) A brief history of phytochromes. *Chemphyschem* **11:** 1–10.
Rosales-Saavedra, T., Esquivel-Naranjo, E.U., Casas-Flores, S., Martínez-Hernández, P., Ibarra-Laclette, E., Cortes-Penagos, C., and Herrera-Estrella, A. (2006) Novel light-regulated genes in *Trichoderma atroviride*: a dissection by cDNA microarrays. *Microbiology* **152:** 3305–3317.
Ruger-Herreros, C., Rodriguez-Romero, J., Fernandez-Barranco, R., Olmedo, M., Fischer, R., Corrochano, L.M., and Canovas, D. (2011) Regulation of conidiation by light in *Aspergillus nidulans*. *Genetics* **188:** 809–822.
Sánchez-Arrguín, A., Pérez-Martínez, A.S., and Herrera-Estrella, A. (2012) Proteomic analysis of *Trichoderma atroviride* reveals independent roles for transcription factors BLR-1 and BLR-2 in light and darkness. *Eukaryot Cell* **11:** 30–41.
Sargent, M.L., and Briggs, W.R. (1967) The effects of light on a circadian rhythm of conidiation in Neurospora. *Plant Physiol* **42:** 1504–1510.
Sterner, D.E., and Berger, S.L. (2000) Acetylation of histones and trancription-related factors. *Microbiol Mol Biol Rev* **64:** 435–459.
Tan, K.K. (1974) Red-far-red reversible photoreaction in the recovery from blue-light inhibition of sporulation in *Botrytis cinerea*. *J Gen Microbiol* **8a:** 201–202.
Tessadori, F., van Zanten, M., Pavlova, P., Clifton, R., Pontvianne, F., Snoek, L.B., *et al*. (2009) Phytochrome B and histone deacetylase 6 control light-induced chromatin compaction in *Arabidopsis thaliana*. *PLoS Genet* **5:** e1000638.
Tribus, M., Galehr, J., Trojer, P., Brosch, G., Loidl, P., Marx, F., *et al*. (2005) HdaA, a major class 2 histone deacetylase of Aspergillus nidulans, affects growth under conditions of oxidative stress. *Eukaryot Cell* **4:** 1736–1745.
Ulijasz, A.T., and Vierstra, R.D. (2011) Phytochrome structure and photochemistry: recent advances toward a complete molecular picture. *Curr Opin Plant Biol* **14:** 498–506.
Vienken, K., and Fischer, R. (2006) The $Zn(II)_2Cys_6$ putative transcription factor NosA controls fruiting body formation in *Aspergillus nidulans*. *Mol Microbiol* **61:** 544–554.
Wagner, J.R., Brunzelle, J.S., Forest, K.T., and Vierstra, R.D. (2005) A light-sensing knot revealed by the structure of the chromophore-binding domain of phytochrome. *Nature* **438:** 325–331.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web-site.