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

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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.

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 (Kurdistan 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 (Luksens, 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
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 *ireA*, *ireB* or *veA* reduced the expression in white light only to about 50% (Fig. 2A). Interestingly, double deletion of *ireA* and *ireB* 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 *ireA/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...
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).
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 \textit{fphA}- and \textit{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 \textit{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 \textit{actA} promoter, which served as control. In an \textit{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 \textit{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 \textit{ccgA} light induction in wild type and \textit{gcnE}- and \textit{hdaA}-deletion strains. GcnE was chosen because the orthologue in \textit{N. crassa} NGF-1 is involved in WC-dependent histone H3 acetylation (Grimaldi \textit{et al.}, 2006; Brenna \textit{et al.}, 2012). In addition, in \textit{A. nidulans}, GcnE is important for acetylation of secondary metabolite gene clusters (Nützmann \textit{et al.}, 2011). It also contributes to the regulation of the asexual developmental pathway in \textit{A. nidulans} (Canovas \textit{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

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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
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 *veA*1 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.
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 acetyltransferase 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 pmD and pmB 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 transcriptional 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 ireA or ireB 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 ireB reduces photoinduction, but only to about 50%. This reduction could even be reverted through deletion of ireA. 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 &
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 minutes at 95°C for the reverse transcription reaction, followed by 5 min at 95°C for its inactivation and 40 PCR cycles (10 s at 95°C, 1 min at 60°C). 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.

**Table 1. Strains used in this study.**

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<tr>
<th>Strain</th>
<th>Genotype</th>
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<td>FGSCA4</td>
<td>Glasgow Wild Type</td>
<td>FGSC Missouri</td>
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<td>SJP1</td>
<td>pyrG89; argB::trpC; pyroA4; fphA::argB; veA+</td>
<td>Purschwitz et al. (2008)</td>
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<td>Reyes-Dominguez et al. (2008)</td>
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<td>A1153sadaB</td>
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<td>SRF200 (argB2; pyrG89; pyroA4) transformed with pJR87 (argB2; alcA::fphA::770A::3xHA)</td>
<td>Janina Purschwitz</td>
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<td>SRF200 (argB2; pyrG89; pyroA4) transformed with pJP88 (argB2; alcA::fphAD1181A::3xHA)</td>
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<td>SJR2</td>
<td>FGSCA1153 X SKV103; pyroA4; pyrG89; nkuA::bar; veA+</td>
<td>This study</td>
</tr>
<tr>
<td>SJR10</td>
<td>pyrG89; argB::trpC; pyroA4; pyrG89; nkuA::bar; fphA::argB; veA+</td>
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<tr>
<td>SMH4</td>
<td>pyrG89; pyroA4; nkuA::bar; fphA::argB; veA+</td>
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<tr>
<td>SMH8</td>
<td>DVAR1 (ΔveA) transformed with pSM15 (alcA::3xHA::lreA; pyr-4)</td>
<td>This study</td>
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<tr>
<td>SMH9</td>
<td>alcA::n-yfp::gcnE; alcA::c-yfp::fphA; veA+</td>
<td>This study</td>
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<tr>
<td>SMH10</td>
<td>pyrG89; argB::trpC; pyroA4; nkuA::bar; fphA::argB; veA+</td>
<td>This study</td>
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<tr>
<td>SMH15</td>
<td>alcA::n-yfp::gcnE; alcA::c-yfp::lreA; veA+</td>
<td>This study</td>
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<tr>
<td>SMH16</td>
<td>SSR66 transformed with pSM17 (alcA::3xHA::veA)</td>
<td>This study</td>
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<tr>
<td>SMH17</td>
<td>alcA::n-yfp::gcnE; alcA::c-yfp::veA; veA+</td>
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<td>SRJ28</td>
<td>alcA::n-yfp::hdaA; alcA::c-yfp::lreA; veA+</td>
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<tr>
<td>SRJ30</td>
<td>alcA::n-yfp::hdaA; alcA::c-yfp::veA; veA+</td>
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<td>SSR6</td>
<td>SSM39  transformed with pSR16 (alcA::gfp::gcnE, pyr-4) and pNZ11 (pyroA4); veA+</td>
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<td>SSR7</td>
<td>SSM45 transformed with pMH11 (alcA::GFP::hdaA) and pNZ11 (pyroA4); veA+</td>
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<tr>
<td>SSR8</td>
<td>SKV103 transformed with pSM16 (alcA::GFP::gcnE; pyr-4); veA+</td>
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<tr>
<td>SSR26</td>
<td>ΔgcnE; alcA::GFP::gcnE (-400 bp terminator); ptrA</td>
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<td>SSR31</td>
<td>yA2; ΔgcnE::AfpyrG; pyroA4; veA+</td>
<td>This study</td>
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<tr>
<td>SSR55</td>
<td>yA2; adaB::argB2; pyroA4; veA+</td>
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<tr>
<td>SSR66</td>
<td>pyroA4; papaA1; nkuA::bar; lreA::ptrA; veA+</td>
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<tr>
<td>SSR89</td>
<td>yA2; pyroA4; lreA::ptrA; pyroA4; lreB::argB; veA+</td>
<td>This study</td>
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<td>SZY6.1</td>
<td>SJR2 co-transformed with pZY8 (hH3-LB (1kb):hH3K14Q::hH3-RC (1kb)) and FNO3 (pyr4); pyroA4; veA+</td>
<td>This study</td>
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<tr>
<td>SZY7</td>
<td>SJR2 co-transformed with pZY9 (hH3-LB (1kb):hH3K9Q::hH3-RC (1kb)) and FNO3 (pyr4); pyroA4; veA+</td>
<td>This study</td>
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<tr>
<td>SZY8</td>
<td>SJR2 co-transformed with pZY10 (hH3-LB (1kb):hH3K9/14Q::hH3-RC (1kb)) and FNO3 (pyr4); pyroA4; veA+</td>
<td>This study</td>
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<tr>
<td>SZY9</td>
<td>SJR2 co-transformed with pZY12 (hH3-LB (1kb):hH3K9R::hH3-RC (1kb)) and FNO3 (pyr4); pyroA4; veA+</td>
<td>This study</td>
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<tr>
<td>SZY16.1</td>
<td>SJR2 co-transformed with pZZY11 (hH3-LB (1kb):hH3K14R::hH3-RC (1kb)) and FNO3 (pyr4); pyroA4; veA+</td>
<td>This study</td>
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</table>

**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 days 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 H2O/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 Scientific, OVERVIEW 1 460 μl batch) was bound to the samples (1 μl of protein). Then, the samples were washed with wash buffer (1% soluble BSA, 0.1% Tween 20) and coupled to the DNA. After PCR, the samples were resolved on a 1.5% agarose gel and the bands were excised with a scalpel.
Table 2. Oligonucleotides used for real-time PCR, ChIP and site-directed mutagenesis.

<table>
<thead>
<tr>
<th>Oligo name</th>
<th>Sequence (5′-3′)</th>
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<tr>
<td>cccA-102F</td>
<td>CGACGCCTCCTCCTACTCTCTC</td>
</tr>
<tr>
<td>cccA-178R</td>
<td>CATCAGGGAGTCTGGTCCCTGGGT</td>
</tr>
<tr>
<td>H2B-fw_low_RTO</td>
<td>TGCCGCCAGGAAGGCGTTGA</td>
</tr>
<tr>
<td>H2B_rev_low_RTO</td>
<td>GAGGTAGTCTCCTCCCTGTT</td>
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<tr>
<td>real-cccA-fw3</td>
<td>GTGTAAGTACAGAGGAAAGGCGG</td>
</tr>
<tr>
<td>real-cccA-rv3</td>
<td>GAGGTGCTATCAGAGGCGG</td>
</tr>
<tr>
<td>real-actA-fw</td>
<td>CTTCCGAAATCCCAACTCC</td>
</tr>
<tr>
<td>real-actA-rv</td>
<td>GGTGTTAGATACGTCACACT</td>
</tr>
<tr>
<td>hH3_F</td>
<td>ATACGCTTGCCCTACGCGCGGCGC</td>
</tr>
<tr>
<td>hH3_R</td>
<td>AGCGAGCTTCAGTTGTCAGTGGGG</td>
</tr>
<tr>
<td>hH3-fw</td>
<td>GGTGGATTAGAATCGAACTAC</td>
</tr>
<tr>
<td>hH3-rv</td>
<td>CTTCTCAACATCCAACTCCC</td>
</tr>
</tbody>
</table>

Scientific) was added to 50 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). The input control was treated with 200 μl TE/1% SDS under the same conditions as the IP samples.

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

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% bromophenol 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 (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.
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 ($5 \times 10^5$) 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$_2$O/2% Tween 20 and counted in a Neubauer chamber. Three biological replicates were analyzed.

**Acknowledgements**

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**References**


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


Supporting information

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