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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running head: light regulation in *A. nidulans*

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Summary

The ability for light sensing is found from bacteria to human but relies only on a small number of evolutionarily conserved photoreceptors. Fungi react in many ways to light, and in many fungi blue light is the effective wavelengh. *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. Induction strictly depends on phytochrome and its histidine-kinase activity. Full light-induction 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 an ubiquitous signal in the environment, which may vary concerning wavelength and intensity in different locations. Therefore, a small number of wavelength-specific photoreceptors has evolved in plants, bacteria and fungi (Bae & Choi, 2008, Corrochano, 2007, Idnurm *et al.*, 2010, Purschwitz *et al.*, 2006, van der Horst *et al.*, 2007). 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.*, 2010b, Rodriguez-Romero *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 & Melles, 1983) the induction of the synthesis of carotenoids (Harding & Turner, 1981), and very importantly, the regulation of the circadian clock (Baker et al., 2012, Sargent & Briggs, 1967). All the different responses require the *wc-1* and *wc-2* genes (Ballario & G., 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, oxygene 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 & Liu, 2005). In a genome-wide analysis, it was shown that 5.6 % of the known transcripts of *N. crassa* respond to light. Bioinformatics revealed two *cis*-elements in the promoters of such genes, one element for early and one for late light responses (Chen *et al.*, 2009). One of the most interesting open questions was the one concerning the photoreceptor molecule. This question was solved simultaneously in two laboratories. Most surprisingly, WC-1, the transcription factor itself, harbors a flavin (FAD) as light-sensing chromophore (Froehlich *et al.*, 2002, He *et al.*, 2002). WC-1 is therefore related to phototropins of plants with which it also

shares other protein domains.

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 the chromatin structure may be altered, among which are histone acetylation and methylation (Kurdistani & 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 & Berger, 2000). It has also been shown in *A. nidulans* that histone modifications are involved in e.g. the activation of silent gene clusters of secondary metabolites (Nützmann *et al.*, 2011, Nützmann *et al.*, 2013).

In addition to the WC proteins, a number of other factors have been described, such as another blue-light receptor protein named *Vivid*. It also harbors a flavin as chromophor, acts as a light-dependent repressor of the WCC and is involved in an adaptation process (Chen *et al.*, 2010a, Hunt *et al.*, 2010, Malzahn *et al.*, 2010, Schafmeier *et al.*, 2008, Schafmeier *et al.*, 2005, Zoltowski *et al.*, 2007). In an approach to identify targets for the WCC, CSP1 has been identified as repressor of metabolic genes (Sancar *et al.*, 2011, Smith *et al.*, 2010). Besides the apparent interplay between the WCC and Vivid, Vivid appears not to be widely spread among fungi (Rodriguez-Romero *et al.*, 2010).

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 & 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 (Bayram & Braus, 2012, Calvo, 2008, Kim *et al.*, 2002). The structure of the velvet domain of a related protein, VosA, was solved recently, showing similarity to NF-kB and further proofs that VeA is likely to directly bind to DNA (Ahmed *et al.*, 2013). Photobiological experiments suggested already 1990 the involvement of a phytochrome in the light response of *A. nidulans* (Mooney & Yager, 1990). However, the dogma at that time was, that phytochromes are plant-specific molecules, and it took until 2005 that first fungal phytochromes were functionally characterized (Blumenstein *et al.*, 2005, Ulijasz & 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 & 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 Δ*lreA* and Δ*lreB* 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 white-collar complex, 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 red light

Developmental decisions in *A. nidulans* are largely controlled by light. Originally it was reported that red light is the most effective wavelength to trigger asexual development (Mooney & Yager, 1990). Later it was found that also blue light could be detected and led to induction of asexual sporulation (Purschwitz *et al*., 2008). Here 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 was set to be most similar to the intensity of the corresponding wavelengths of the spectrum of the white-light source. **(Fig. 1 A).** 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. 1 B). 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 2 fold 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 in comparison to wild type (**Supporting Fig. 1 B**) (Aronson *et al.*, 1994). However, spore viability was decreased in response to heat and oxidative stress **(Supporting Fig. 1 C)**.

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. 1 C)**. 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. 1 B, C)**. 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 to red light alone and did also not reach the level of white-light induction. In addition to light regulation, *ccgA* was upregulated 12 h post induction of asexual development and thus only slightly after the master regulator *brlA* **(Supporting Fig. 2)**.

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 only to about 50% **(Fig. 2 A)**. Double deletion of *lreA* and *lreB* resulted in an expression pattern similar to wild type. During the course of these experiments we observed derepression of *ccgA* in the dark in the absence of VeA. 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 a function of phytochrome in the dark. 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).

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. 2 B)**.

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 (ChIP) 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. All proteins also bound to some extend to this promoter and therefore this unspecific binding could have been used to normalize the obtained values for the *ccgA* promoter. However, we preferred to actually show both, binding to the actin and binding to the *ccgA* promoter.

Whereas FphA did not bind to the *ccgA* promoter, neither in the dark nor upon illumination, VeA was always found at the promoter **(Fig. 3 A)**. Precipitating LreA revealed binding to the *ccgA* promoter in darkness but release from the DNA after short time of illumination. The error bar appears rather large in this experiment. This is due to differences in the kinetics of the release. In some experiments LreA was released already before 15 min, in others only slightly later.

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. 3 B)**. 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. 3 B)**.

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 neither of lysine 9 nor of lysine 14 increased after the light stimulus. This was also the case for the deletion

of *lreA*.

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 and 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 (Brenna *et al.*, 2012, Grimaldi *et al*., 2006). 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 both of FphA and LreA with GcnE and HdaA and also of VeA with HdaA **(Fig. 5 A and Supporting Fig. 3)**. 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. Nevertheless, only the interactions of LreA were, so far, reproducible with co-immunoprecipitation assays **(Fig. 5 B)**. It could be that the other proteins only transiently interact and that those interactions are stabilized and fixed through the split YFP halfs and 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 if 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. 6 A)**. 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 to 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 to VeA+. In order to judge the obtained result, the overall phenotypes of the deletion strains needs to be considered. Whereas deletion of *hdaA* did not affect colony growth, the *gcnE*-deletion strain appeared very sick and produced only small, non-sporulating colonies **(Supporting Fig. 4)** (Canovas *et al.*, 2014, Reyes-Dominguez *et al.*, 2008). 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) **(Supporting Fig. 4).** 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 to 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 non-acetylatable 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 to 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. 6 B)**.

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 (Chen *et al*., 2009, Rosales-Saavedra *et al.*, 2006, 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 the induction with white light. First, it has to be considered that *ccgA* induction was measured already 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. 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.

We found that the molecular mechanism of photoinduction comprises a number of different proteins and the regulation appears to be rather complex. The only essential component for *ccgA* induction is apparently phytochrome, although it does not bind to the promoter of the studied reporter gene. Nevertheless, it controls binding of two other factors, the velvet protein, VeA and the blue-light transcription factor, LreA. FphA together with LreA control 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, 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 to 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). 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. 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 all light responses are regulated by blue light and the WC complex, phytochrome plays a prominent role in *A. nidulans*. The acetylation level of lysine 9 in H3 was indeed dependent on phytochrome, although there was also reduction of acetylation of the *ccgA* promoter in the *lreA*-deletion strain, 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 the light intensity and phytochrome B and histone deacetylase were identified as positive regulators in this process (Jang *et al.*, 2011, Tessadori *et al.*, 2009). However, the data rely on the quantification of the heterochromatin index in nuclei, and the exact mechanisms of gene control remained enigmatic.

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 either blue or red light cause 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. Thus FphA could phosphorylate LreA in the dark and after illumination, the phosphorylation level would decrease (Brandt *et al*., 2008). We propose that different forms of LreA act different 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*.

One facet 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, which turn on gene expression. Likewise, *A. nidulans* phytochrome interacts with the transcription factor LreB and the putative transcription factor VeA. But none 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. 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.

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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 & 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 hours of incubation in constant darkness at 37°C the mycelial mat was illuminated with white light (11 W/m^2), red light (1.45 W/m^2) or blue light (0.72 W/m^2) LED lamps for 5 min or 30 min at 22ºC. Control samples were harvested in complete darkness. Samples were frozen in liquid nitrogen and stored at -80ºC until RNA isolation. RNA was isolated with the RNeasy kit (Quiagen). Cell disruption was made in a homogenizer with glass beads at max. speed for 5 minutes. The isolated RNA was quantified and an aliquot treated with DNase I. RNA samples were diluted to a final concentration of 50 ng/µl in DEPC water.

Real Time PCR

Quantitative PCR experiments were performed to determine relative mRNA abundance using ONE-STEP SYBR Green RT-PCR on an iClycler from BIO-RAD. Each reaction of 25 µl contained

0.5 µl of iScript RT enzyme for One-Step (BioRad), 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 95ºC 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 *benA* to correct for sampling errors. 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 3 to 6 biological replicates. *brlA* expression was quantified as control during asexual development. For Oligos used in this study see **Table 2**.

Chromatin-Immunoprecipitation (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, 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 (13000 rpm). Anti-HA agarose (Thermo 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 (13000 rpm, 1 min) and the beads washed successively with 1 ml of lysis buffer (3x), 3x 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), 3x 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 3x with TE (10 mM Tris pH 7.5; 1 mM EDTA). 125 µl elution buffer (50 mM Tris pH 7.5; 1 mM EDTA; 1 % SDS) were 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 & concentrator kit (Zymo research) and purified DNA was used in quantitative real time 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 was 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).

For the ChIP studying histone H3 acetylation, strains were grown in medium containing only glucose. 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.

At least two biological replicates were used (usually 4) 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 rpm. 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 (13000 rpm, 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 4000 rpm pelleted the

beads. The pellet was then washed four times with extraction buffer. Adding 2x SDS sample buffer (4x 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 1x PBS; 100 mM β-mercaptoethanol; 2 % SDS for 30 min at 50 °C, 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 x 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.

References

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

Ahmed, Y. L., J. Gerke, H. S. Park, O. Bayram, P. Neumann, M. Ni, A. Dickmanns, S. C. Kim, J. H. Yu, G. H. Braus & R. Ficner, (2013) The velvet family of fungal regulators contains a DNA-binding domain structurally similar to NF-kappaB. *PLoS Biol* **11**: e1001750.

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

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

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

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

Ballario, P., P. Vittorioso, A. Margrelli, C. Toalora, A. Cabibo & G. Macino, (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. & G. H. Braus, (2012) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. *FEMS Microbiol. Rev.* **36**: 1-24.

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

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

Brenna, A., B. Grimaldi, P. Filetici & P. Ballario, (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., A. T. Marcos, A. Gacek, M. S. Ramos, G. Gutierrez, Y. Reyes-Dominguez & J. Strauss, (2014) The histone acetyltransferase GcnE (GCN5) plays a central role in the regulation of Aspergillus asexual development. *Genetics* **197**: 1175-1189.

Chen, C. H., B. S. DeMay, A. S. Gladfelter, J. C. Dunlap & J. J. Loros, (2010a) Physical

interraction between VIVID and white collar complex regulates photoadaptation in Neurospora. *Proc. Natl. Acad. Sci. USA* **107**: 16715-16720.

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

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

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

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

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

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

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

Gacek-Matthews, A., L. M. Noble, C. Gruber, H. Berger, M. Sulyok, A. T. Marcos, J. Strauss & A. Andrianopoulos, (2015) KdmA, a histone H3 demethylase with bipartite function, differentially regulates primary and secondary metabolism in Aspergillus nidulans. *Mol Microbiol*.

Grimaldi, B., P. Coiro, P. Filetici, E. Berge, J. R. Dobosy, M. Freitag, E. U. Selker & P. Ballario, (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. & S. Melles, (1983) Genetic analysis of phototropism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. *Plant Physiol.* **1983**.

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

He, Q., P. Cheng, Y. Yang, L. Wang, K. H. Gardner & Y. Liu, (2002) White collar-1, a DNA binding transcription factor and a light sensor. *Science* **297**: 840-843.

He, Q. & Y. Liu, (2005) Molecular mechanism of light responses in Neurospora: from light-induced transcription to photoadaptation. *Genes Dev.* **19**: 2888-2899.

Herdman, M., T. Coursin, R. Rippka, J. Houmard & N. Tandeau de Marsac, (2000) A new

appraisal of the prokaryotic origin of eukaryotic phytochromes. *J. Mol. Evol.* **51**: 205-213.

Hunt, S. M., S. Thompson, M. Elvin & C. Heintzen, (2010) VIVID interacts with the WHITE COLLAR complex and REQUENCY-interacting RNA helicase to alter light and clock responses in Neurospora. *Proc. Natl. Acad. Sci. USA* **107**: 16709-16714.

Idnurm, A., S. Verma & L. M. Corrochano, (2010) A glimpse into the basis of vision in the kingdom Mycota. *Fungal Genet. Biol.* **47**: 881-892.

Jang, I. C., P. J. Chung, H. Hemmes, C. Jung & N. H. Chua, (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., K.-Y. Han, K.-J. Kim, D.-M. Han, K.-Y. Jahng & K.-S. Chae, (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. *Fungal Genet. Biol.* **37**: 72-80.

Kurdistani, S. K. & M. Grunstein, (2003) Histone acetylation and deacetylation in yeast. *Nature* **4**: 276-284.

Lamparter, T., F. Mittmann, W. Gärtner, T. Borner, E. Hartmann & J. Hughes, (1997) Characterization of recombinant phytochrome from the cyanobacterium Synechocystis. *Proc. Natl. Acad. Sci. USA* **94**: 11792-11797.

Law, D. J. & W. E. Timberlake, (1980) Developmental regulation of laccase levels in *Aspergillus nidulans*. *J Bacteriol* **144**: 509-517.

Linden, H., P. Ballario & G. Macino, (1997) Blue light regulation in *Neurospora crassa*. *Fungal Genet. Biol.* **22**: 141-150.

Lukens, R. J., (1965) Reversal by red light of blue light inhibition of sporulation in *Alternaria solani*. *Phytophathology* **55**: 1032.

Malzahn, E., S. Ciprianidis, K. Káldi, T. Schafmeier & M. Brunner, (2010) Photoadaptation in Neurospora by competitive interaction of activating and inhibitory LOV domains. *Cell* **142**: 762-772.

Mooney, J. L. & L. N. Yager, (1990) Light is required for conidiation in *Aspergillus nidulans*. *Genes Dev.* **4**: 1473-1482.

Nützmann, H. W., J. Fischer, K. Scherlach, C. Hertweck & A. A. Brakhage, (2013) Distinct amino acids of histone H3 control secondary metabolism in *Aspergillus nidulans*. *Appl. Environ. Microbiol.* **79**: 6102-6109.

Nützmann, H. W., Y. Reyes-Dominguez, K. Scherlach, V. Schroeckh, F. Horn, A. Gacek, J.

Schürmann, C. Hertweck, J. Strauss & B. A.A., (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.

Purschwitz, J., S. Müller, C. Kastner & R. Fischer, (2006) Seeing the rainbow: light sensing in fungi. *Curr. Opin. Microbiol.* **9**: 566-571.

Purschwitz, J., S. Müller, C. Kastner, M. Schöser, H. Haas, E. A. Espeso, A. Atoui, A. M. Calvo & R. Fischer, (2008) Functional and physical interaction of blue and red-light sensors in *Aspergillus nidulans*. *Curr. Biol.* **18**: 255-259.

Reyes-Dominguez, Y., F. Narendja, H. Berger, A. Gallmetzer, R. Fernandez-Martin, I. Garcia, C. Scazzocchio & J. Strauss, (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. & J. C. Lagarias, (2010) A brief history of phytochromes. *ChemPhysChem* **11**: 1-10.

Rodriguez-Romero, J., M. Hedtke, C. Kastner, S. Müller & R. Fischer, (2010) Fungi, hidden in soil or up in the air: light makes a difference. *Annu. Rev. Microbiol.* **64**: 585-510.

Rosales-Saavedra, T., E. U. Esquivel-Naranjo, S. Casas-Flores, P. Martínez-Hernández, E. Ibarra-Laclette, C. Cortes-Penagos & A. Herrera-Estrella, (2006) Novel light-regulated genes in *Trichoderma atroviride*: a dissection by cDNA microarrays. *Microbiology* **152**: 3305-3317.

Ruger-Herreros, C., J. Rodriguez-Romero, R. Fernandez-Barranco, M. Olmedo, R. Fischer, L. M. Corrochano & D. Canovas, (2011) Regulation of conidiation by light in *Aspergillus nidulans*. *Genetics* **188**: 809-822.

Sancar, G., C. Sancar, B. Brügger, N. Ha, T. Sachsenheimer, E. Gin, S. Wdowik, I. Lohmann, F. Wieland, T. Höfer, A. Diernfellner & M. Brunner, (2011) A global circadian repressor controls antiphasic expression of metabolic genes in Neurospora. *Mol. Cell* **44**: 687-697.

Sánchez-Arrguín, A., A. S. Pérez-Martínez & A. Herrera-Estrella, (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. & W. R. Briggs, (1967) The effects of light on a circadian rhythm of conidiation in Neurospora. *Plant Physiol.* **42**: 1504-1510.

Schafmeier, T., A. Diernfellner, A. Schäfer, O. Dintsis, A. Neiss & M. Brunner, (2008) Circadian activity and abundance rhythms of the Neurospora clock tanscription factor WCC associated with rapid nucleo-cytoplamic shuttling. *Genes Dev.* **22**: 3397-3402.

Schafmeier, T., K. Kaldi, A. Diernfellner, C. Mohr & M. Brunner, (2005) Phosphorylation-dependent maturation of *Neurospora* circadian clock protein from a nuclear repressor toword a cytoplasmic activator. *Genes Dev.* **20**: 297-306.

Smith, K. M., G. Sancar, R. Dekhang, C. Z. Sullivan, S. Li, A. G. Tag, C. Sancar, E. L. Bredeweg, H. D. Priest, R. F. McCormick, T. L. Thomas, J. C. Carrington, J. E. Stajich, D. Bell-Pedersen, M. Brunner & M. Freitag, (2010) Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for Neurospora white collar complex. *Eukaryot. Cell* **9**: 1549-1556.

Sterner, D. E. & S. L. Berger, (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., M. van Zanten, P. Pavlova, R. Clifton, F. Pontvianne, L. B. Snoek, F. F. Meillenaar, R. K. Schulkes, R. van Driel, L. A. Voesenek, C. Spillane, C. S. Pikaard, P. Fransz & A. J. Peeters, (2009) Phytochrome B and histone deacetylase 6 control light-induced chromatin compaction in *Arabidopsis thaliana*. *PLoS Genet.* **5**: e1000638.

Tribus, M., J. Galehr, P. Trojer, G. Brosch, P. Loidl, F. Marx, H. Haas & S. Graessle, (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. & R. D. Vierstra, (2011) Phytochrome structure and phtochemistry: recent advances toward a complete molecular picture. *Curr. Opin. Plant Biol.* **14**: 498-506.

van der Horst, M. A., J. Key & K. J. Hellingwerf, (2007) Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *T Microbiol* **15**: 554-562.

Vienken, K. & R. Fischer, (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., J. S. Brunzelle, K. T. Forest & R. D. Vierstra, (2005) A light-sensing knot revealed by the structure of the chromophore-binding domain of phytochrome. *Nature* **438**: 325-331.

Zoltowski, B. D., C. Schwerdtfeger, J. Widom, J. J. Loros, A. M. Bilwes, J. C. Dunlap & B. R. Crane, (2007) Conformational switching in the fungal light sensor Vivid. *Science* **316**: 1054-1057.

Figure legends

Figure 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 3 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.

Figure 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 white light or kept in the dark. The plot shows the average and standard deviation of the relative photoactivation to dark of the wild type in a minimum of 3 experiments.

Figure 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.

Figure 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 to the Δ*fphA* strain in darkness and after 20 min of illumination with white light. Real time PCR was performed for *ccgA* and the control. The plot shows the average and standard error of the mean

Figure 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, 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.

Figure 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 non-acetylatable 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 3 experiments.

Supporting Figure 1: Deletion of *ccgA*. **A** Southern Blot of the *ccgA*-deletion strain. Digestion with *Bgl*II; expected band after hybridization with the ORF is 1.8 kb, after hybridization with the left border (LB): 1.8 kb for WT and 1.01 kb for the deletion strain, for the right border (RB): 1.8 + 1.3 kb for WT and 1.3 kb for the deletion strain. **B** Deletion of *ccgA* shows no discernible phenotype, colonies grow like wild type. **C** Spore viability of the *ccgA*-deletion strain was tested in response to heat or oxidative stress. Spores were heated to 50 °C for 15 min or treated with 100 mM or 200 mM H_2O_2. Colonies of wild type and the *ccgA*-deletion strain were counted.

Supporting Figure 2: *ccgA* expression during asexual development. Real time PCR for *ccgA* during asexual development under dark conditions. Total RNA was isolated from mycelia growing in liquid medium for 18 h in the dark and then asexual development was activated by filtration and exposing the mycelia on solid medium. All steps were performed in darkness. Samples were collected at different times after asexual development induction. The gene *brlA* was used as a control. The plot shows the average and standard error of the mean of the relative expression in a minimum of 3 experiments.

Supporting Figure 3: BiFC experiments of FphA and VeA with GcnE and HdaA. GcnE and HdaA are fused to the N-terminus of YFP, FphA and VeA are fused to the C-terminus. Spores were inoculated in medium containing 2 % glycerol and grown over night at 28°C.

Supporting Figure 4: Recomplementation of the *gcnE* deletion and phenotypes of the *hdaA*- and *adaB*-deletion strains. The deletion strain of *gcnE* (upper panel) was recomplemented with *alcA::GFP::gcnE* restoring wild type phenotype when growing on inducing medium (containing 2 % glycerol). Deletion of *hdaA* (middle panel) shows growth comparabale to wild type but less conidiation. The *adaB*-deletion strain (lower panel) grows significantly slower than wild type and conidiation is impaired.

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 & 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*	This study

	(alcA::3xHA::lreA; pyr-4)	
SMH9	*alcA::n-yfp::gcnE; alcA::c-yfp::fphA; veA+*	This study
SMH10	*pyrG89; ∆argB::trpCDB; pyroA4, nkuA::bar; ∆fphA::argB; veA+*	This study
SMH15	*alcA::n-yfp::gcnE; alcA::c-yfp::lreA; 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	Δ*gcnE; alcA::GFP::gcnE* (+400bp 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; ∆lreA::ptrA; veA+*	This study
SSR89	*yA2; pyroA4; ∆lreA::ptrA; ∆lreB::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

Table 2: **Oligonucleotides used for Real Time PCR, ChIP and site directed mutagenesis.**

Oligo name	Sequence (5'-3')
benA-30F	CAAGTGTGGTAACCAGGTTGGT
benA-96R	GGCGTCGAGGCCATGTT
brlA-841F	TACCGCGACGGGTTTCAG
brlA-905R	GAGGTCTGTCGTCGGAGCAT
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