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The *Aspergillus nidulans* Velvet-interacting protein, VipA, is involved in light-stimulated heme biosynthesis

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Summary

Filamentous fungi are able to differentiate morphologically and adapt the metabolism to internal and external cues. One major regulator is the so-called velvet protein, VeA, best studied in *Aspergillus nidulans*. The protein interacts with several other proteins to regulate light sensing, the balance between asexual and sexual development, penicillin biosynthesis or mycotoxin production. Here, we characterized a novel VeA-interacting protein, VipA. The 334 amino acid long protein comprises a FAR1-like DNA-binding domain, known from plant transcription factors like FHY3 (Far-red elongated hypocotyl 3). VipA interacted not only with VeA, but also with the WC orthologue LreA in the nuclei and with the phytochrome FphA in the cytoplasm. Conidia and cleistothecia formation was similarly affected in a *vipA*-deletion strain as in an *fphA* mutant. However, the effect was less pronounced, suggesting a modulating and not an essential role in light sensing. In addition, VipA modulated heme biosynthesis in response to light through association with the *hemB* promoter, the gene encoding 5-aminolevulinic acid dehydratase. After illumination of *A. nidulans* mycelia with white light the intracellular heme concentration increased by 30% in comparison to a *vipA*-deletion mutant. Hence, VipA couples heme biosynthesis to the illumination conditions.

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Introduction

Besides its essential role as energy source for phototrophic organisms, light may serve as important signal for the orientation in the environment or the adaptation to environmental changes. For instance, if fungi are growing within their substrate or within soil, the conditions may differ dramatically in comparison to the fungus growing at the air–substrate interphase. In the latter case, light is a reliable physical parameter to alert the fungus before stresses like high temperature or osmotic stress become established (Bayram *et al*., 2010; Rodriguez-Romero *et al*., 2010; Dasgupta *et al*., 2015; Fuller *et al*., 2015, 2016). Likewise, in *A. nidulans* light regulates many different processes, ranging from spore germination to development and metabolism. In darkness, the fungus develops mainly sexually by producing ascospore-containing cleistothecia (Pontecorvo *et al*., 1953). Light switches development toward the formation of conidiophores and asexual conidiospores (Mooney and Yager, 1990). In addition, the production of secondary metabolites like the aflatoxin precursor sterigmatocystin or the antibiotic penicillin is affected by illumination (Kato *et al*., 2003). Recently, it was shown that light exposure, especially red light, results in a delay of asco- and conidiospore germination (Fuller *et al*., 2013; Röhrig *et al*., 2013). This may help to emerge the rather sensitive germtubes preferably at dusk or night (higher humidity, no UV light) rather than during the day.

To sense light, *A. nidulans* uses at least three different photoreceptors, two for blue and one for red light. The two blue-light sensing systems are comprised of the White Collar homologues LreA and LreB and the cryptochrome CryA, which furthermore has photolyase activity (Bayram *et al*., 2008; Purschwitz *et al*., 2008). In *Neurospora crassa* the White Collar system and, therefore, the blue-light response is one of the best-studied light-perception systems in fungi (Dasgupta *et al*., 2015). WC-1 and WC-2 are involved in several processes like the regulation of sexual development or sporulation,

carotenoid biosynthesis and entrainment of the circadian clock. WC-1 is a phototropin-like blue-light sensor containing a flavin as chromophore (Froehlich *et al*., 2002; He *et al*., 2002). WC-1 interacts with WC-2, both of which harbor a C-terminal zinc-finger domain required for DNA binding, and they are known to bind to the promoters of light-regulated genes (Chen *et al*., 2009). Transcriptional activation takes place at least partly through WC-dependent chromatin modification (Grimaldi *et al*., 2006). The White-Collar system is well conserved throughout the fungal kingdom and homologues are found for example in *Trichoderma atroviride*, *Magnaporthe oryzae* or *Cryptococcus neoformans* (Casas-Flores *et al*., 2004; Idnurm and Heitman, 2005; Lee *et al*., 2006). *Phycomyces blakesleeanus* even harbors three WC-1 and four WC-2 homologues (Idnurm *et al*., 2006; Sanz *et al*., 2009). In *A. nidulans* LreA and LreB have activating function for sexual development and secondary metabolite production (Purschwitz *et al*., 2008).

Besides blue-light perception, *A. nidulans* is able to sense red and far-red light (Mooney and Yager, 1990). It contains a functional phytochrome, FphA, which is responsible for the delay of asco- and conidiospore germination and in addition represses sexual development (Blumenstein *et al*., 2005; Röhrig *et al*., 2013). Phytochromes are inter-convertible proteins containing a linear tetrapyrrol chromophore covalently bound to the N-terminal photosensory domain (Quail, 2002). Moreover, phytochromes harbor a C-terminal signal output domain. In FphA this domain resembles a bacterial two-component signaling system consisting of a histidine kinase and a response regulator (Blumenstein *et al*., 2005). Red light illumination leads to photoconversion of the tetrapyrrole chromophore and results in structural changes of the protein, converting it into the P_{fr} form. Dark red-light illumination reconverts the protein to the P_r form (Bae and Choi, 2008; Brandt *et al*., 2008).

In *A. nidulans*, the blue- and red-light sensors were shown to interact with each other. This complex interplay includes another protein, namely VeA. VeA has no light-sensory function, but represents the main regulator bridging light sensing to the developmental or metabolic output (Kim *et al*., 2002; Purschwitz *et al*., 2009; Bayram and Braus, 2012; Calvo and Cary, 2015; Rauscher *et al*., 2015). Besides VeA, *A. nidulans* harbors at least three other velvet family members, VosA, VelB and VelC. They all share the *velvet domain*, a 150 amino acid long motif necessary for DNA binding and likely also for protein-protein interactions (Ni and Yu, 2007; Park *et al*., 2012; Ahmed *et al*., 2013; Park *et al*., 2014; Rauscher *et al*., 2015). The three proteins together with the methyltransferase LaeA, comprise different protein complexes that are coordinating sexual and asexual development, production of secondary metabolites as well as spore viability (Bok and Keller, 2004; Bayram and Braus, 2012; Sarikaya-Bayram *et al*., 2015). Interestingly, LaeA is not the only methyltransferase affecting the velvet-mediated output. The LaeA-like methyltransferase LlmF influences secondary metabolism and development through controlling the VeA nuclear to cytoplasmic ratio. LlmF directly interacts with VeA (Palmer *et al*., 2013). Two other velvet-interacting methyltransferases VipC and VapB form a heterotrimeric complex with the FYVE zinc-finger protein VapA, which localizes the complex to the plasma membrane. Through environmental stimuli VipC and VapB are set free and can influence the localization of VeA counteracting LlmF (Sarikaya-Bayram *et al*., 2014). Furthermore VipC and VapB are thought to control gene transcription via chromatin modification (Sarikaya-Bayram *et al*., 2015). The different protein-protein interactions and thereby the different roles of VeA in such different processes are partly regulated through a phosphorylation code (Rauscher *et al*., 2015). Four amino acids were identified, which can be phosphorylated and then change the specificity or activity of VeA. Likely even more amino acids are reversibly modified since in the recent study by Rauscher *et al*. (2015) only part of VeA was analyzed.

In general, VeA is well conserved throughout the fungal kingdom and exhibits in most cases a role in regulating metabolism and development (Calvo, 2008). The exact function, however, differs among different fungi. In *A. parasiticus* VeA acts as an activator of conidiospore formation (Calvo *et al*., 2004). *A. flavus* exhibits a similar regulatory network than *A. nidulans*, although it produces sclerotia instead of cleistothecia (Calvo and Cary, 2015). In *A. niger* the length of the conidiospore chains as well as the formation of the conidiophore stalk and hyphal morphology are strongly influenced by VeA (Wang *et al*., 2015). In *N. crassa ve1* deletion leads – among other phenotypes – to differences in hyphal morphology (Bayram *et al*., 2007). In *Fusarium verticilloides* colony hydrophobicity is influenced because FvVE1 regulates hydrophobin expression. In addition, *FvVE1* deletion leads to problems in polar growth and strains produce less aerial hyphae (Li *et al*., 2006). In *Botrytis cinerea*, the velvet regulatory network controls development and secondary metabolism and is required for full virulence (Schumacher *et al*., 2015).

In this study, we describe a new *A. nidulans* VeA-interaction partner, VipA, as part of the dynamic light-regulator complex by interacting with LreA and phytochrome. We postulate a second function for VipA in the regulation of the heme biosynthesis pathway and, therefore, potentially fine-tuning the phytochrome-chromophore level.

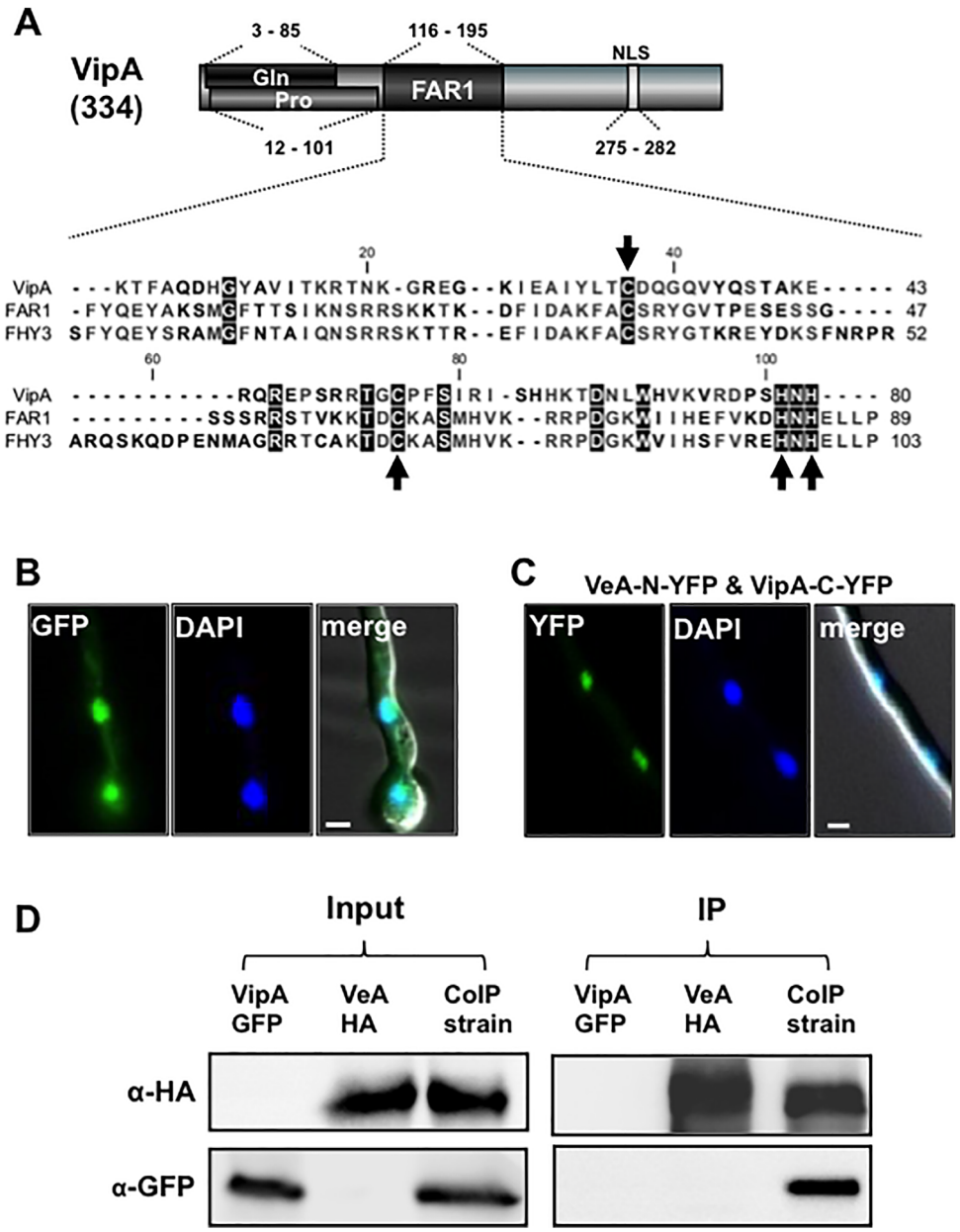


Fig. 1. Characterization of VipA.
A. Scheme of the VipA protein and alignment of the FAR1 domain of VipA to the FAR1 domains of *A. thaliana* FAR1 and FHY3. Conserved amino acids are shaded in black. The arrows indicate the conserved Zn^{2+}-interacting amino acids necessary for the formation of a zinc-finger domain in *A. thaliana* FHY3.
B. Localization of VipA-GFP in the nuclei (strain SRJ1).
C. Interaction of VipA and VeA using bimolecular fluorescence complementation (strain SRJ2). Strains were inoculated onto cover slips, incubated for 10 h at 37°C and then observed in the fluorescence microscope. The size bar equals 2 μm.
D. Interaction between VipA and VeA confirmed by Co-immunoprecipitation analysis (CoIP). CoIP strains SSM12, SRJ1 and SZY70 were cultured in liquid minimal medium containing 2% threonine and 0.2% glucose for 24 h at 37°C. CoIP performed with anti HA agarose beads was followed by SDS PAGE and Western blot analysis.

Results

VipA as a new interaction partner of the velvet protein VeA

Several years ago new VeA-interaction partners were identified in a yeast two-hybrid screening. Whereas the roles of the methyltransferases VipB and VipC have been studied, the function of VipA (AN0859) remained elusive and is subject of this article (Calvo, 2008; Sarikaya-Bayram *et al.*, 2014). The 334 amino acid long VipA protein harbors a FAR1 WRKY-like DNA-binding domain (aa 116–195), which is known from plant transcription factors like *Arabidopsis thaliana* FHY3 or LOM1 from rice (Lin *et al.*, 2007). The cysteine and histidine residues C146, C169 H193 and H195, necessary for DNA binding, are conserved (Fig. 1A and Supporting Information Fig. S1). The protein appears to be conserved among filamentous fungi like *Penicillium roqueforti* (GI: 584408902; 37% identity) or *Talaromyces stipitatus* (GI: 242825902; 37% identity). VipA harbors a potential nuclear localization signal (NLS) and indeed a GFP-tagged version of VipA localized within nuclei, when *vipA* was expressed from the *alcA*-promoter (Fig. 1B). The *alcA* promoter was not fully induced with ethanol or threonine, but only de-repressed with glycerol. This allows low-level expression (Waring *et al.*, 1989).

Expression from the own promoter did not allow us to detect any signal, indicating that the expression level under natural conditions is very low (data not shown). Nuclear localization of VipA-GFP shows that the NLS is functional and VipA is probably involved in regulatory pathways. Bimolecular fluorescence complementation analysis (BIFC or Split YFP) confirmed the interaction of VipA with VeA within nuclei (Fig. 1C). The interaction was further confirmed by Co-immunoprecipitation (CoIP) with HA tagged VeA and GFP tagged VipA (Fig. 1D). In this case, the promoter was moderately induced through growth on 2% threonine and 0.2% glucose.

VipA interacts with phytochrome and the blue-light sensing system

Because VeA is a major regulator of light-dependent processes and itself interacts with phytochrome (Purschwitz *et al.*, 2008), we tested for protein-protein interaction between VipA and other proteins involved in light-sensing, such as phytochrome FphA, LreA and LreB. Bimolecular fluorescence complementation (BIFC or Split YFP) suggested that VipA interacts with all three proteins. But whereas the interaction with the blue-light sensing system, consisting of LreA and LreB, mainly took place within nuclei, the interaction with FphA occurred mostly in the cytoplasm (Fig. 2A). Recently, it was shown that FphA signals into the SakA (stress-activated kinase) pathway through interaction with YpdA in the cytoplasm, which clearly indicated a cytoplasmic function (Yu *et al.*, 2016). For comparison, the interaction between VeA and FphA occurred mainly in the nuclei, suggesting a nuclear function as well (Purschwitz *et al.*, 2008). With Co-immunoprecipitation (CoIP) with HA and GFP tagged versions of the different proteins only the interactions between VipA and FphA and VipA and LreA could be confirmed (Fig. 2B).

VipA influences development and the production of secondary metabolites

To investigate the role of *vipA*, a corresponding deletion strain was constructed (Supporting Information Fig. S2). Compared to the wild type strain SJR7 and the re-complemented strain SJR22 (*vipA*-open reading frame with the natural promoter ectopically integrated in the *vipA*-deletion strain) the *vipA*-deletion strain produced less conidiospores and more cleistothecia under illumination (Fig. 3A–C). In darkness all strains showed a similar amount of conidiospores and cleistothecia. Next, sterigmatocystin production was analyzed by thin-layer chromatography. All three strains produced comparable amounts in darkness and illumination led to

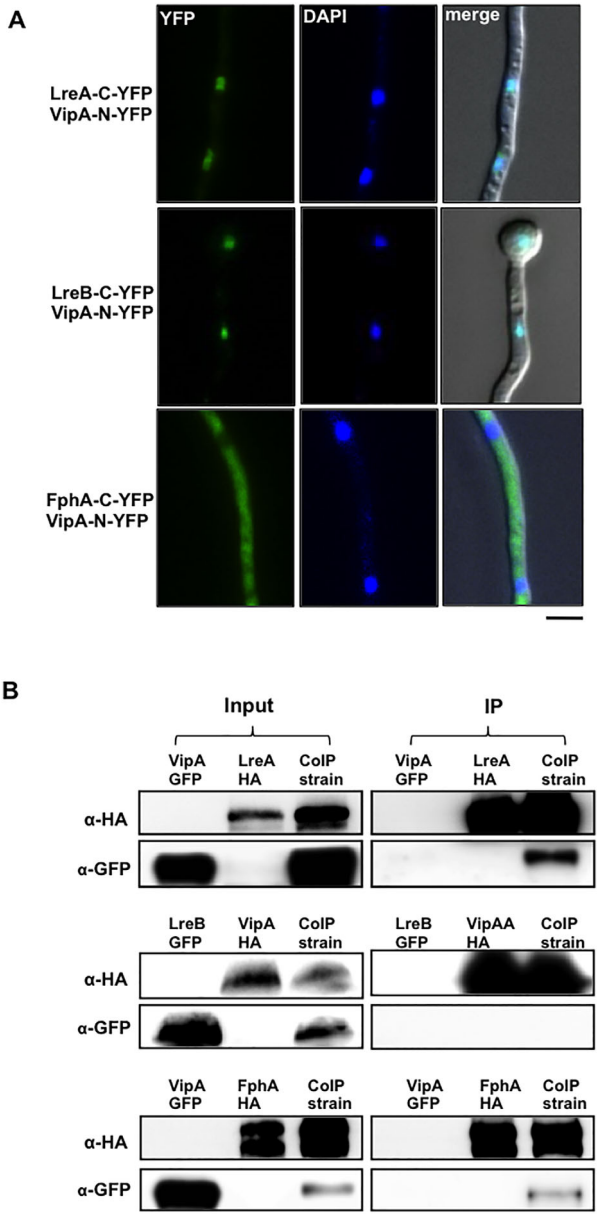


Fig. 2. Interaction of VipA with LreA and FphA.
A. Bimolecular fluorescence complementation analysis shows interactions of VipA with LreA and LreB in the nuclei as well as with FphA in the cytoplasm. Strains SRJ14, SRJ15 and SRJ16 were inoculated onto cover slips, grown for 10 h at 37°C and then observed under the microscope. The size bar equals 5 μm.
B. Co-immunoprecipitation analysis (CoIP) confirmed the interaction between GFP tagged VipA and LreA and FphA, both tagged with HA. HA tagged VipA and GFP tagged LreB did not show an interaction. CoIP strains SRJ11, SRJ 12 and SRJ13 as well as control strains SRJ1, SRJ9, SSM39, SSM45 and SSM46 were cultured in liquid minimal medium containing 2% threonine and 0.2% glucose for 24 h at 37°C. CoIP was performed with anti HA agarose beads was followed by SDS PAGE and Western blot analysis.

elevated sterigmatocystin levels in wild type and the re-complemented strain, but is less pronounced in the *vipA*-deletion strain, however, the difference was not

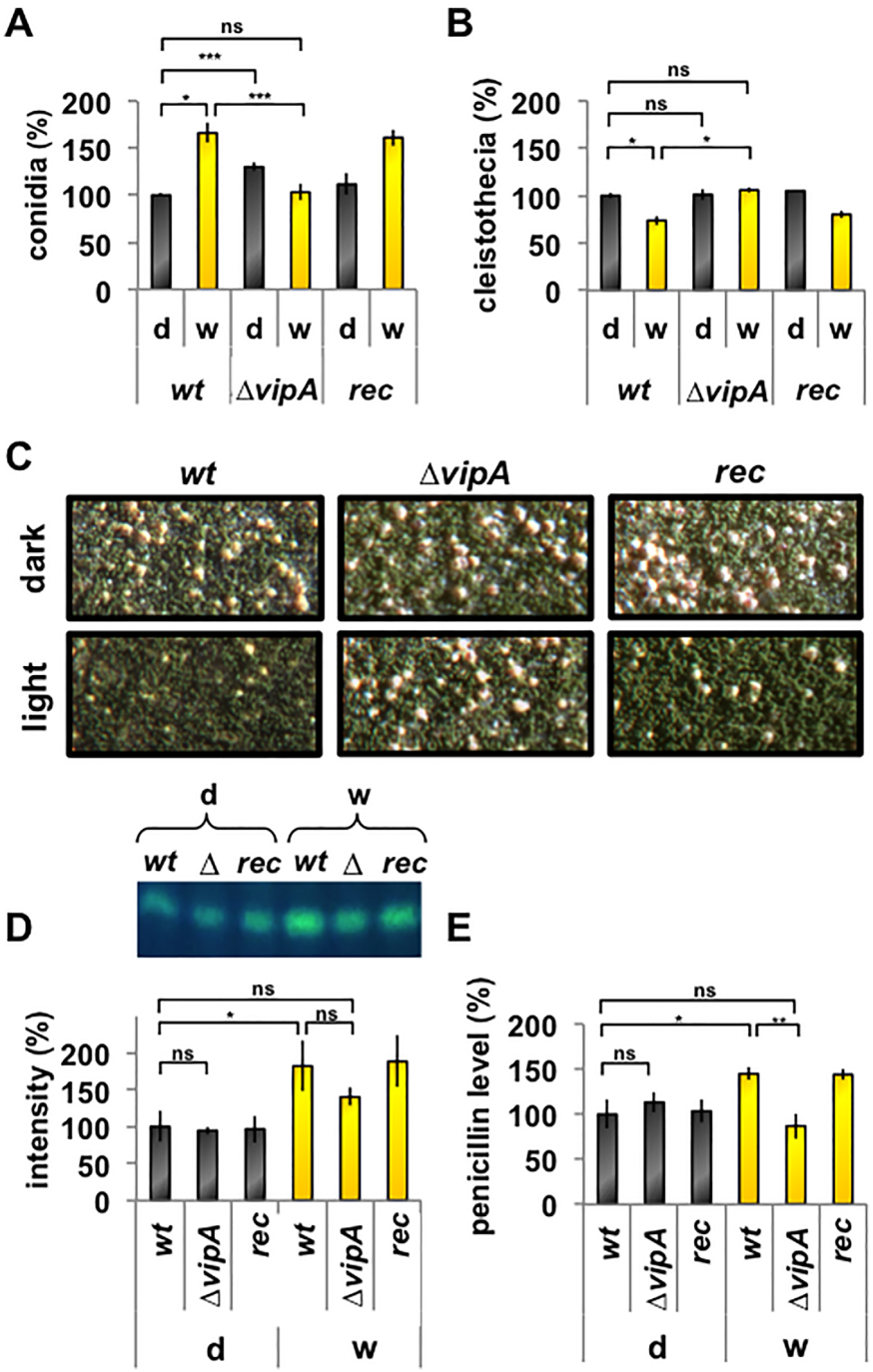


Fig. 3. Role of VipA in asexual and sexual spore formation and the production of sterigmatocystin and penicillin.
A. Conidiospore amount of the wild type strain SRJ7 (wt), the *vipA*-deletion strain SRJ4 (Δ*vipA*) and the *vipA*-complemented strain SRJ22 (rec) after 24 h of dark incubation followed by either 24 h of incubation in darkness or in white light at 37°C.
B. Quantification of young cleistothecia.
C. Colony surface images of strains SRJ7 (wt), SRJ4 (Δ*vipA*) and SRJ22 (rec) after 24 h of dark incubation followed by either 48 h of incubation in darkness or in white light at 37°C.
D. Quantification of sterigmatocystin by thin layer chromatography of extracts derived from strains SRJ7 (wt), SRJ4 (Δ*vipA*) and SRJ22 (rec) grown for 24 h in darkness followed by either 24 h of incubation in darkness or in white light at 37°C. Values derived from band intensity measurements by ImageJ (http://imagej.nih.gov/ij/).
E. Quantification of penicillin in strains SRJ7 (wt), SRJ4 (Δ*vipA*) and SRJ22 (rec). Penicillin amounts were quantified from the inhibition zones with Aspergillus culture supernatants on *Geobacillus stearothermophilus* containing agar plates. *A. nidulans* shaking cultures with corn steep medium were incubated at 26°C for 24 h in darkness followed by either 24 h in darkness or in white light. Error bars represent the standard deviation. All experiments were performed at least in triplicate. Significant differences were calculated using the two-sample *t*-test, two-sided with no equal variance, *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, ns, not significant.

significant (Fig. 3D). In a previous paper, it was shown that the glucose concentration in the medium is important for sterigmatocystin formation. Whereas with 1% glucose sterigmatocystin production was stimulated by light, with 2% glucose the amount of the toxin was higher in the dark (Atoui *et al.*, 2010). We also compared penicillin production using a bioassay. In this case, the lack of VipA caused a similar effect as for sterigmatocystin production leading to reduced penicillin production in light (Fig. 3E).

Light-induction of vipA *depends on VeA and phytochrome*

To analyze if the decreased light response in the *vipA*-deletion strain resulted from reduced abundance of FphA or VeA, the corresponding RNA levels were quantified. No significant differences in darkness compared to illumination were detected (Fig. 4A). However, the expression level of *vipA* was elevated around 2.4-fold

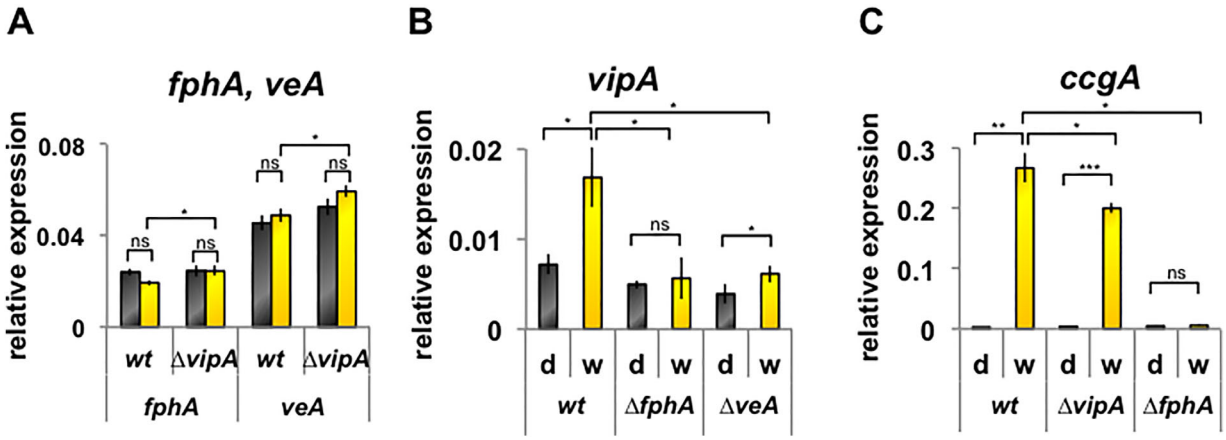


Fig. 4. Expression analysis of *fphA, veA, vipA* and *ccgA*. Spore suspensions were grown on top of liquid medium for 18 h at 37°C in darkness followed by 30 min of illumination (right bar) or in darkness (left bar). Expression levels are normalized to histone H2B expression.
A. Investigation of *fphA*- and *veA*-expression levels showed no differences in the *vipA*-deletion strain (SRJ3) compared to wild type (SKV103).
B. *vipA* expression is dependent on light in a wild type strain (SKV103). In the phytochrome (SJP1) and the *veA*-deletion strain (DVAR1) light upregulation was not observed. Expression data were normalized to the expression in wild type incubated in darkness.
C. Analysis of *ccgA* expression in SKV103 (wt), SRJ3 (Δ*vipA*) and SJP1 (Δ*fphA*). White light led to elevated *ccgA* expression in wild type. In SRJ3 *ccgA* expression after illumination was lower than in wild type, and in strain SJP1 no induction was observed. Error bars represent the standard deviation deriving at least from three biological and two technical replicates. Significant differences were calculated using the two-sample *t*-test, two-sided with no equal variance, *P*-values: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, ns, not significant.

after white-light incubation compared to the expression in darkness. This elevation required FphA and VeA (Fig. 4B).

To test whether VipA is involved in FphA-dependent gene regulation, *ccgA* expression was analyzed. *ccgA* was identified in a genome-wide analysis of light-regulated genes (Ruger-Herreros *et al*., 2011) and used to elucidate the mechanism of light regulation (Hedtke *et al*., 2015). Whereas in the absence of FphA expression of *ccgA* was completely abolished, the absence of VipA caused only a 25% reduction compared to wild type (Fig. 4C). Hence, VipA plays a modulating rather than an essential function with regards to light induction of *ccgA*.

VipA modulates the adaptation of heme biosynthesis to light

In *A. thaliana*, the FAR1 domain protein FHY3 regulates the heme biosynthesis pathway resulting in fine-tuning chlorophyll production in dependence on illumination (Tang *et al*., 2012). Since VipA seems not only to share the FAR1-DNA binding domain with FHY3 but both genes are light regulated, we analyzed a potential role of VipA in heme biosynthesis. The heme biosynthesis pathway is evolutionarily well conserved (Franken *et al*., 2013). We were able to detect a potential FAR1-binding motif in the promoter of *A. nidulans hemB* (AN1403), a homologue of *A. thaliana* HEMB. *hemB* encodes 5-aminolevulinic acid dehydratase (ALAD), which catalyzes the second step in heme biosynthesis. Quantitative real time RT-PCR analysis revealed that *hemB* is upregulated in light. This upregulation was completely lost without VipA whereas overexpression of *vipA* by the H2B promoter led to higher *hemB* expression in both darkness and light (Fig. 5A), suggesting a regulatory role of VipA in this process. With regards to asexual and sexual spore formation the overexpression strain was similar to wild type (data not shown). To show that VipA binds to the promoter of *hemB*, we performed chromatin immunoprecipitation by expressing a heme agglutinin (HA) tagged version of VipA under the control of the inducible *alcA* promoter. The precipitated *hemB* promoter-specific DNA was quantified by real time RT-PCR. VipA indeed bound to the promoter of *hemB* at the potential FAR1-binding motif (700 bp upstream of the start codon) (Fig. 5B). The distance of the putative binding site from the ATG is quite large for fungal promoters. Interestingly, in *A. thaliana* it was shown that a transcription factor belonging to the WRKY zinc finger family binds at a similar distance to the motif in two genes encoding the transcription factors, FAR-RED ELONGATED HYPOCOTYL 3 and FAR-RED IMPARIRED RESPONSE 1 (Tang *et al*., 2013).

As a result of the light- and VipA-dependent change of the expression level of *hemB*, a change in the porphobilinogen (PBG) level was analyzed (Fig. 5C). PBG is the product of the turnover from amino levulinic acid (ALA) catalyzed by ALAD. Whereas the PBG level increased on illumination in wild type, the increase was very much reduced in the *vipA*-deletion strain.

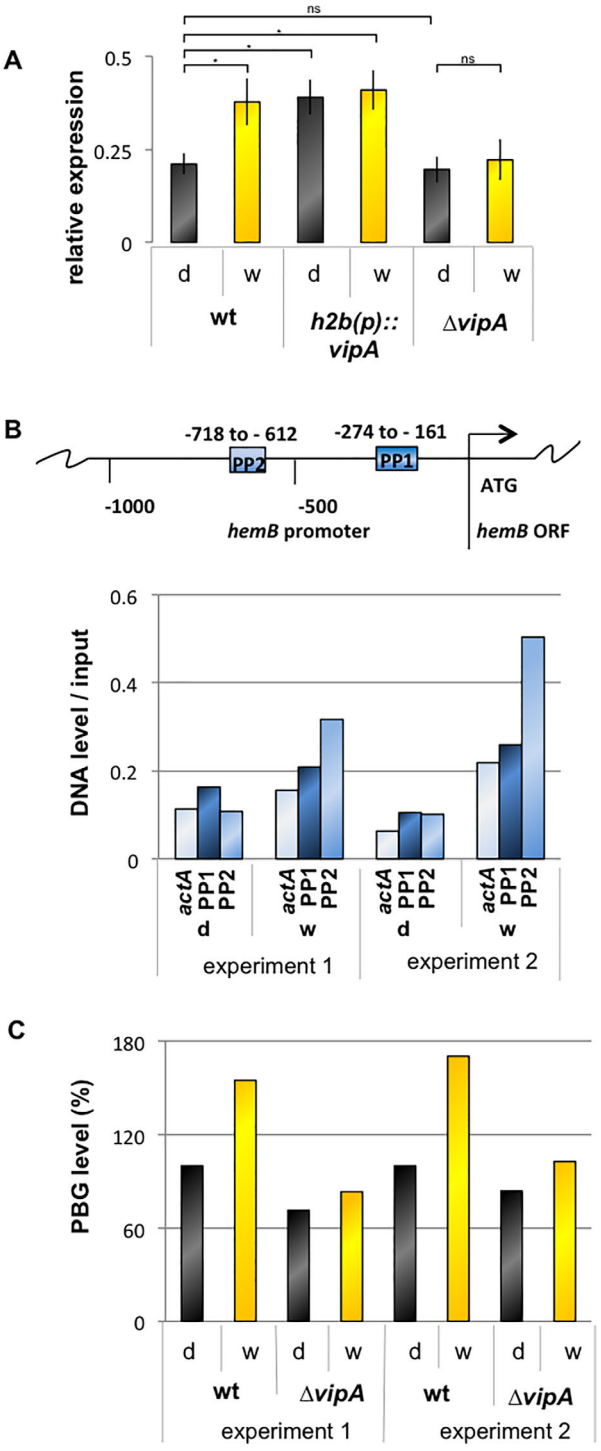


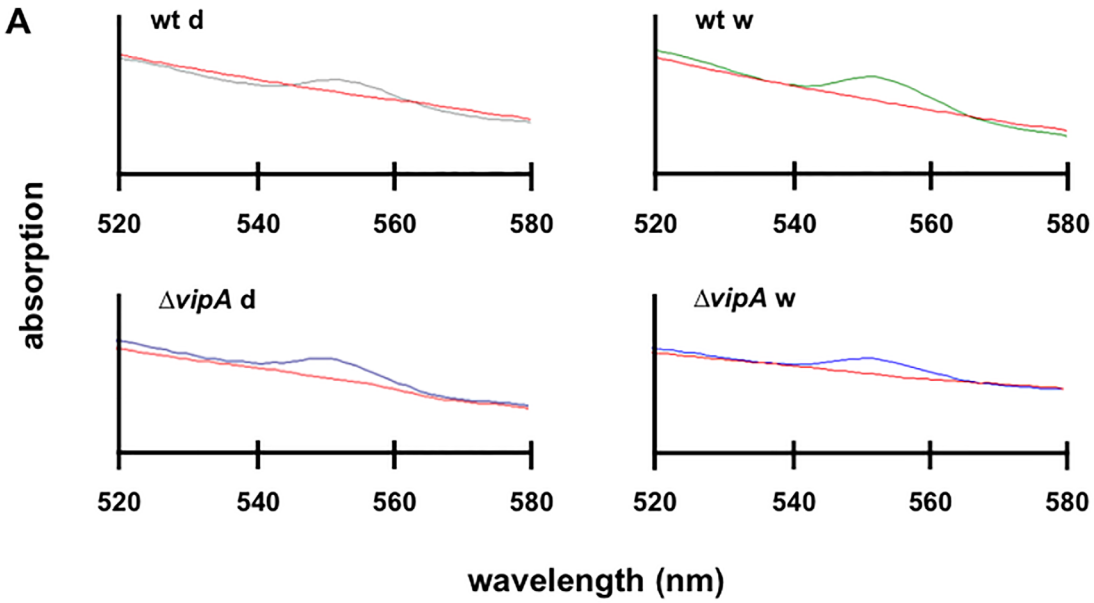
Fig. 5. The role of VipA in light-dependent *hemB* expression stimulation.
A. Relative expression of *hemB* in dependence of light in strains SRJ7 (wt), SZY71 (vipA OE) and SRJ4 (Δ*vipA*) normalized to *benA* expression. Quantitative real time PCR analysis was performed with three biological replicates. Error bars represent the standard deviation. Significant differences were calculated using the two-sample *t*-test, two-sided with no equal variance, *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, ns, not significant.
B. Chromatin immunoprecipitation (ChIP) with HA tagged VipA and quantification of precipitated DNA by quantitative RT-PCR normalized to an input DNA control with primer pair 1 (PP1) spanning a part of the *hemB* promoter near the open reading frame and primer pair 2 (PP2) spanning the potential FAR1 binding motif 700 bp upstream of the ORF. Actin primer pairs (*actA*) were used as a control. Strain SRJ9 (VipA-HA) was incubated for 48 h at 37°C followed by 15 min of dark incubation or white light illumination. The results for two independent experiments (two technical replicates for each) are shown.
C. Determination of the PBG level in SRJ7 (wt) and SRJ4 (Δ*vipA*) in dependence on light. Spores were shaken for 12 h in darkness at 30°C followed by 24 h incubation in white light or darkness respectively. PBG amounts from whole cell extracts were measured with the ClinRep Kit (Recipe, Freiburg). The results for two independent experiments (two technical replicates) are shown.

To directly show an influence of *vipA* on heme production the amount of C type cytochromes was compared in wild type and the *vipA*-deletion strain, each incubated in darkness or under white light respectively (Fig. 6A–C). Whereas in wild type illumination led to an increase in C type cytochrome bound heme from 50.7 pmol/mg (dark) to 72.3 pm/mg (light), in the *vipA*-deletion strain light induction of heme biosynthesis was not observed (48.8 pmol/mg in the dark and 49.9 pmol/mg in light).

Discussion

A. nidulans responds to light in many ways. Besides the decision between asexual and sexual development, the production of secondary metabolites like sterigmatocystin and penicillin is regulated by light (Purschwitz *et al.*, 2008; Atoui *et al.*, 2010). Here we described a novel effect of light, the fine-tuning of heme biosynthesis, as well as VipA as a further interaction partner of VeA.

We characterized VipA as a novel protein involved in light-dependent regulation of the heme biosynthesis pathway via transcriptional control of *hemB* in *A. nidulans* (Fig. 7). There are three lines of evidence that VipA is involved in light-dependent upregulation of heme biosynthesis. (i) Association of VipA with the *hemB* promoter. (ii) Light-stimulation of *hemB* gene expression. (iii) Increase of the heme content on illumination. HemB encodes the ALAD, essential for converting ALA to porphobilinogen (Franken *et al.*, 2013). The induction of heme biosynthesis in *A. nidulans* in response to illumination is probably advantageous, because light may be used as an indicator for stressful growth conditions in nature (Rodriguez-Romero *et al.*, 2010). If *A. nidulans* grows in soil or within an organic substrate, it has to adapt to most likely very different growth conditions once it reaches the surface and is exposed to changing temperatures, desiccation, high-osmolarity and light



B

heme level	wt dark	wt light	ΔvipA dark	ΔvipA light
pmol/mg ± SD	50.7 ± 4.6	72.3 ± 4.1	48.8 ± 3.9	49.9 ± 4.3
%	100	142	96	98

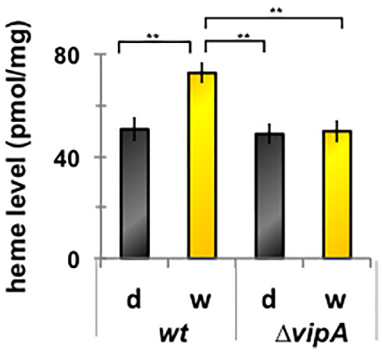


Fig. 6. VipA regulates heme biosynthesis. Absorption spectrum of whole cell extracts from strains SRJ7 (wt) and SRJ4 (Δ*vipA*) first oxidized by NaOH and $K_3FE(CN)_6$ and afterwards completely reduced by adding sodium dithionite to the samples.
A. Absorption spectra.
B. Calculated values following (Berry and Trumpower, 1987) and **(C)** graphical illustration. Error bars represent standard deviations derived from three biological replicates. Significant differences were calculated using the two-sample *t*-test, two-sided with no equal variance, *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, ns, not significant.

exposure. Intracellularly, the concentration of reactive oxygen species probably rises, and *A. nidulans* has to detoxify those molecules with the help of catalases and superoxide dismutase. Increased heme levels may be advantageous to cope with this situation, because, e.g., catalase and superoxide dismutase are heme enzymes (Martinez *et al.*, 2016). In agreement with this hypothesis is the recent finding that the light-signaling cascade is connected to the stress-signaling SakA/HogA pathway. It was shown that mutants in several components of the SakA pathway are unable to transmit the light signal to the nuclei (Yu *et al.*, 2016). Conversely, higher heme levels may also be required for higher biliverdin concentrations, the linear tetrapyrrole and likely the co-factor of phytochrome (Blumenstein *et al.*, 2005) (Fig. 7). Although there is no increased expression of *fphA* in light compared to dark, it could be that the amount of active phytochrome increases in light. If this were the case, this could explain the observed minor effect of *vipA* deletion on other light-responsive processes, such as conidiophore or cleistothecia production or at the molecular level on the expression of *ccgA*. The involvement of VipA in the modulation of heme biosynthesis was initially inferred from its similarity to plant Far1-domain containing proteins such as Far-Red Elongated Hypocotyl3 (FHY3) and Far-Red-Impaired Response1

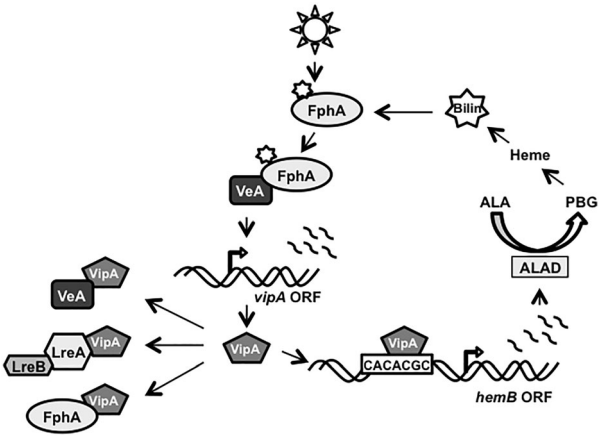


Fig. 7. Proposed model for VipA-dependent heme regulation influencing bilin chromophore synthesis and light signaling through various protein interactions. Light leads to an upregulation of *vipA* expression dependent on phytochrome and VeA. VipA interacts with VeA, phytochrome and LreA and binds in a light-dependent manner to the promoter of *hemB* in the region of a potential FAR1 binding site (CACACGC), stimulating *hemB* expression. This yields elevated levels of the *hemB* product 5-ALAD, converting ALA to PBG. Increased PBG amounts result in higher heme levels, which may cause higher bilin amounts and thereby directly influencing phytochrome-dependent light perception.

(FAR1). These two proteins are positive regulators in phytochrome A signaling and together modulate phyA nuclear accumulation (Hudson *et al*., 1999; Wang and Deng, 2002; Hiltbrunner *et al*., 2005). In a more recent study, Tang *et al*. searched for components involved in tetrapyrrole biosynthesis (Tang *et al*., 2012). Because it was known that most genes involved in chlorophyll biosynthesis are regulated by light, they aimed at identifying new components triggering that response. They tested a number of positive regulators and found decreased protochlorophyllid amounts in *fhy3* and *far1* mutants. In addition to chlorophyll biosynthesis, heme biosynthesis was affected. Finally, they demonstrated that the *HEMB1* gene, encoding 5-ALAD, is a direct target of the control mechanism.

Besides the evidence that VipA adapts the heme content of the cell to the light conditions, it is also conceivable that VipA plays a direct role in light regulation (Fig. 7). It interacts with two important photoreceptors in the cell, with FphA in the cytoplasm and with LreA in nuclei. In addition, it interacts with VeA. These interactions – in addition to the fact that *vipA* deletion partly phenocopies *fphA* deletion – suggest a modulating function of VipA on the activities of the corresponding regulators. However, we have no evidence that VipA may modulate nuclear accumulation of any of the regulators as it was shown for PhyA in Arabidopsis (Hiltbrunner *et al*., 2005).

In this work, we characterized VipA as a novel VeA-binding protein and thus the number of VeA-interaction partners increased to at least nine proteins (LaeA, LlmF, VapA, VipA, VipC, VelB, MpkB, FphA, LreB). Hence, VeA appears to be a hub for the control of different signaling pathways as diverse as asexual and sexual development, light-sensing and production of secondary metabolites (Sarikaya-Bayram *et al*., 2015). Given that VeA probably is a transcription factor with a NF-κ-B like DNA-binding domain (Ahmed *et al*., 2013), it is likely that the different interaction partners only transiently interact with VeA and change their specificity for certain promoters at certain times. Thus VeA not only harbors a putative NF-κ-B like DNA binding domain, but its molecular function has many parallels to the functions of the NF-κ-B transcription factor. The activity of both proteins is elegantly regulated at several layers, such as their expression, their subcellular localization and their interaction with a large number of proteins. In part those interactions are regulated by different phosphorylation state of VeA (Bayram *et al*., 2012; Rauscher *et al*., 2015). Like NF-κ-B, VeA is involved in the control of many cellular functions and this great diversity of genetic targets is most likely achieved through the various interaction partners (Feldman *et al*., 2007). Human NF-κ-B is a member of a family of five related transcription factors, harboring a Rel homology domain in their N-terminus, necessary for DNA binding and homo- and heterodimerization. Various homo- and heterodimers control a large number of different target genes and cellular processes (Hayden and Ghosh, 2008). The next challenge in *A. nidulans* thus will be the study of the interactions at the molecular and structural level and to determine their exact contribution to differential gene expression and fungal differentiation.

Experimental procedures

In silico *analyses*

For *in silico* analysis DNA and protein sequences were obtained from www.aspergillusgenome.org. Similarity searches were performed via NCBI BLASTp and BLAST search. Alignments were done with Clustal Omega (Sievers and Higgins, 2014) and displayed with the CLC sequence viewer (CLC bio, Quiagen). VipA domain analysis was performed via Pfam and Wolf PSORTII. For protein structure analysis RaptorX and USCF Chimera was used (Pettersen *et al*., 2004; Källberg *et al*., 2014).

Strains, plasmids and culture conditions

Supplemented minimal (MM) and complete media (CM) for *A. nidulans* were prepared as described, and standard strain construction procedures were used (Käfer, 1977). Expression of tagged genes under the control of the

Table 1. *A. nidulans* and *E. coli* strains used in this study.

Strain	Genotype	Reference
A. nidulans		
SKV103	*pyrG89; pyroA4; veA+*	(Vienken *et al.*, 2005)
SJR2	*pyrG89; pyroA4, nkuA::bar; veA+*	Julio Rodriguez, Karlsruhe
DVAR1	*pabaA1; yA2; ΔargB::trpC; trpC801; ΔveA::argB*	(Kim *et al.*, 2002)
SJP1	*pyrG89; ΔargB::trpCΔB; pyroA4; ΔfphA::argB; veA+*	(Purschwitz *et al.*, 2008)
SSM12	GR5 transformed with pSM17 (*alcA::HA::veA; pyr4*)	(Purschwitz *et al.*, 2008)
SSM39	SKV103 transformed with pAB14 (*alcA::fphA*::3xHA*; argB*) and pNZ11(*pyroA gene*)	Sylvia Müller, Karlsruhe
SSM45	SKV103 transformed with pSM15 (*alcA*::3xHA*::lreA; pyr4*)	Sylvia Müller, Karlsruhe
SSM46	SKV103 transformed with pSM41 (*alcA*::GFP*::lreB; pyroA*)	Sylvia Müller, Karlsruhe
SRJ1	pCK33 (*alcA*::GFP*::vipA; pyr4*) in SKV103*; pyrG89; pyroA4; veA+*	this work
SRJ2	SKV103 transformed with pRJ1(*alcA(p)::YFP_NT::vipA; pyroA*) and pCK1 *(alcA(p)*::YFP_CT*::veA*)	this work
SRJ3	SJR2 transformed with *vipA* KO cassette; *pyrG89; pyroA4, nkuA::bar; ΔvipA::AfpyrG, veA+*	this work
SRJ4	SRJ3 X SRJ5; *pyroA4; ΔvipA::AfpyrG, veA+*	this work
SRJ5	SKV103 transformed with pNZ11 (*pyroA* gene)	this work
SRJ7	SKV103 transformed with pAB4–1(*pyrG* gene)	this work
SRJ9	SKV103 transformed with pRJ10 (*alcA(p)*::3xHA::*vipA; pyr4*)	this work
SRJ11	SSM39 transformed with pCK33 (*alcA*::GFP*::vipA; pyr4*)	this work
SRJ12	SSM45 transformed with pRJ4 (*alcA*::GFP*::vipA; pyroA*)	this work
SRJ13	SJR9 transformed with pCK5 (*alcA*::GFP::lreB, *pyr4*) and pNZ11(*pyroA* gene)	
SRJ14	SKV103 transformed with pRJ1 (*alcA(p)::YFP_NT::vipA; pyroA*) and pCK7 (*alcA(p)*::YFP_CT*::lreA; pyr4*)	this work
SRJ15	SKV103 transformed with pRJ1 (*alcA(p)::YFP_NT::vipA; pyroA*) und pCK3 (*alcA(p)*:: YFP_CT*::lreB; pyr4*)	this work
SRJ16	SKV103 transformed with pRJ1 (*alcA(p)::YFP_NT::vipA; pyroA*) und pJP5 (*alcA(p)*:: YFP_CT*::fphA*; *pyr4*)	this work
SRJ22	SRJ4 transformed with pRJ5 (*vipA* gene, *pyroA*)	this work
SRJ13	SJR9 transformed with pCK5 and pNZ11	this work
SZY70	SSM12 transformed with pRJ4 (*alcA::GFP::vipA; pyroA*)	this work
SZY71	SRJ4 transformed with pZY36 (*H2B(p)::vipA; pyroA*)	this work
E. coli		
Top10	F- *mcrA* D(*mrr-hsd*RMS-*mcr*BC), Æ80*lacZDM15* D*lacX74, recA1, araD139* D*(ara-leu)7679, galU, galK, rpsL* (StrR) *endA1, nupG*	Invitrogen, Leek, NL

alcA-promoter was regulated by the carbon source; repression on glucose, derepression on glycerol and induction on threonine or ethanol (Waring *et al.*, 1989). A list of *A. nidulans* strains used in this study is given in Table 1. Standard laboratory *Escherichia coli* strains (Top 10 F') were used. Plasmids are listed in Table 2.

Table 2. Plasmids used in this study.

Plasmid	Description	Reference
pMCB17apx	*alcA(p)::GFP; pyr4* from *N. crassa;* for N-terminal Fusion of GFP to the protein of interest	V. Efimov, Piscataway, USA
pNZ11	Co-transformation vector *AfpyroA* 1700bp PCR fragment with *Not*I in TOPO pCR2.1 (Invitrogen)	Nadine Zekert, Karlsruhe
pAB4–1	Containing *A. niger pyrG* gene	(Hartingsveldt *et al.*, 1987)
pJP5	*alcA(p)*:: YFP_CT*::fphA*; *pyr4*	(Vienken *et al.*, 2005)
pCK1	*alcA(p)*:: YFP_CT*::veA;* ORF with *Asc*I and *Pac*I in pJP5; *pyr4*	(Purschwitz *et al.*, 2008)
pCK3	*alcA(p)*:: YFP_CT*::lreB*, in pJP5; *pyr4*	(Purschwitz *et al.*, 2008)
pCK5	*alcA::GFP::lreB, pyr4*	(Purschwitz *et al.*, 2008)
pCK7	*alcA(p)*:: YFP_CT*::lreA*, in pJP5; *pyr4*	(Purschwitz *et al.*, 2008)
pCK33	*vipA*_ORF with *Asc*I and *Pac*I in pMCB17apx; *pyr4*	Christian Kastner, Karlsruhe
pJP5	*alcA(p)*::YFP_CT::*LreB*, (backbone of pMCB17apx); *pyr4*	(Purschwitz *et al.*, 2008)
pDV7	GFP changed with YFP_N-term and *pyr4* changed with *pyroA4* in pMCB17apx	Daniel Veith, Karlsruhe
pSM14	pMCB17apx with 3xHA-Tag in *Kpn*I *& Asc*I, *pyr4 (GFP changed with HA-Tag)*	Sylvia Müller, Karlsruhe
pRJ1	*alcA(p)*::YFP_NT::*vipA* ORF with *Asc*I and *Pac*I in pDV7*; pyroA*	this work
pRJ4	*alcA::GFP::vipA*, ORFwith *Asc*I and *Pac*I in pNZ56 (pMCB17apx with *pyroA* instead of *pyr4*)	this work
pRJ5	*vipA* ORF +1000bp up/down with *Avr*II and *Pac*I in pNZ56 (pMCB17apx with *pyroA* instead of *pyr4*)	this work
pRJ6	*cryA* ORF 1000bp up/down with *Avr*II and *Pac*I in pRM12 (backbone: pMCB17apx with *pyroA*)	this work
pRJ10	*alcA(p)*::3xHA::*vipA*, ORF with *Asc*I and *Pac*I in pSM14; *pyr4*	this work
pZY36	*H2B(p)::vipA, H2B* promoter cloned into pJR4 with *Avr*II and AscI; *pyroA*	this work

Table 3. Oligonucleotides used in this study.

Name	Sequence (from 5' to 3')	Description
P1 vipA KO	GGC TAC TAG TCA AAC CCA CTC T	Fusion PCR for amplification of the *vipA* ORF knockout cassette
P2 vipA KO	AGC TGT CAC CTA TAT TCG CCA TAG	
P3 vipA KO pyrGlink	AGG GTG AAG AGC ATT GTT TGA GGC GCA TCG GGC TTG TAC CTG ACA G	
P4 vipA KO	CGCATCAGTGCCTCCTCTCAGACAG GAT TCT AGT ATA GGC GTT TTT G	
VipA P5	GTT GTT CTT GGT TCG CCG ACT G	
VipA P6	CGA CAT CGA GAG AAA GAA GT	
pyrG_cas_F	CGCCTCAAACAATGCTCTTCACC	Primer for *AfpyrG* knockout cassette
pyrG_cas_R	CTGTCTGAGAGGAGGCACTGAT	Primer for *AfpyrG* knockout cassette
vipA_ORF forward with AscI	CAGTTAGGCGCGCCTATGGAGCAGGCTCAGCAGTTC	*vipA* Open Reading Frame for insertion in pMCB17apx
vipA_ORF reverse with PacI	CAGTTATTAATTAACTCAGAACAGCGTGTCGTACAG	
Vip_RekFor_AvrII	CTTG CCTAGG CGG TTC ACT ATC TAC CAG GGA	*vipA* complementation in pMCB17apx
VipA_Rek R_PacI	GCAGGTTAATTAACCGAAGAGGTATAGAGGAAGG	
h2b(p)_for	GGACCTAGGAACCCTTTACAGCTCCTGGCTG	Primers for *vipA* overexpression
h2b(p)_rev	GGGGCGCGCCTTTAAAAGTTGATAAATTCGAAAGTTGAATTCGG	
vipA_ORF_for RTQ	GATGCCACATATCGCACCGA	*vipA* for qRT PCR
vipA_ORF_rev RTQ	CACGACCTTTGTTGGTTCGCT	
veA qPCR for	CTCACAGCCGAAACCAATCC	*veA* für qRT PCR
veA qPCR rev	AAAGTCGTGTGTGCGAACCC	
PJR4	GGCGTCGAGGCCATGTT	*benA* for qRT PCR as reference
PJR3	CAAGTGTGGTAACCAGGTTGGT	
H2B_fw_low_RTQ	TGCCGAGAAGAAGCCTAGCA	*H2B* for qRT PCR as reference
H2B_rev_low_RTQ	GAGTAGGTCTCCTTCCTGGT	
fphA_RTQ_fw	ATGGAGGCGCTGTTGGATACAA	*fphA* for qRT PCR
fphA_RTQ_rev	CAGATCTATGCTCATCGTCGGA	
hemB fw RTQ	CTCAGCAGGTCATGGCAAGC	*hemB* for qRT PCR
hemB rev RTQ	GTTGATTTGGGAGTGACGGAATAG	
ChIP_RTQ 1403f1	CACATGGTCGAAGTCTTAACGG	*hemB* for ChIP qRT PCR PP1
ChIP_RTQ 1403r1	CTGATTACTGCTGTGGTCGAAAC	
ChIP_RTQ 1403f2	GAACTGGAGGCGTGTGTGC	*hemB* for ChIP qRT PCR PP2 spanning binding motif
ChIP_RTQ 1403r2	CTGACCAAAGGAGGGCCAC	
Real-actA fw	CTTCTCAACATCCAACTCCC	actin negative control (*actA(p)*) for ChIP qRT PCR
Real-actA rv	GGTGGATTAGAATCGAACTAC	

Molecular techniques

Standard DNA transformation procedures were used for *A. nidulans* (Yelton *et al.*, 1984) and *E. coli* (Sambrook and Russel, 1999). For PCR experiments, standard PCR protocols were used with Taq or Phusion polymerase. Denaturation was achieved at 95°C (Taq) or 98°C (Phusion), annealing temperatures were chosen according to the corresponding DNA oligonucleotides, and the polymerization temperature varied from 68°C to 72°C. RNA extraction and realtime PCR analysis were performed as described (Röhrig *et al.*, 2013; Hedtke *et al.*, 2015). For expression analysis the $2^{-\Delta Ct}$ value was calculated, where $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{housekeeping gene}}$. DNA oligo-nucleotides used in this study are listed in Table 3. ChIP and coupled quantitative RT-PCR as well as Co-immunoprecipitation and bimolecular fluorescence complementation assays were also performed as described (Hedtke *et al.*, 2015).

VipA deletion

The *vipA*-deletion strain SJR3 was created by protoplast transformation and homologous integration of a fusion PCR derived knock-out cassette constructed with Primers P1 to P6 and *pyrG* cassette oligonucleotides according to (Nayak *et al.*, 2006; Szewczyk *et al.*, 2006). Deletion was confirmed by PCR and Southern blot (Supporting Information Fig. S2).

Quantification of conidiospores and primordia/cleistothecia

5×10^5 conidiospores were spread on 15 ml solid MM (with the required supplements) containing petri dishes using glass beads. Plates were incubated for 24 h in darkness on 37°C followed by either 24 h (conidiospore formation) or 48 h (primordia formation) incubation in darkness or under white-light illumination (LED panels). Conidiospores of one plate were then scraped off and suspended in 2% Tween 20 solution and the volume adjusted to 50 ml. After vortexing the conidiospore number was determined in a Neubauer hemocytometer. Primordia/cleistothecia were counted using a binocular microscope.

Fluorescence microscopy

Fluorescence and DIC microscopy were performed with the AxioImager Z1 (Zeiss), with the software AxioVision V4.5

and Zen with the AxioCam MR. A 63× or a 100× Plan-ApoChromat Objective was used. Light source: Osram HBO100.

Determination of the porphobilinogen and heme concentrations

10^6 conidiospores were cultivated in 75 ml MM (supplemented with corresponding vitamins) in 100 ml Erlenmeyer flasks for 12 h on 30°C, 200 rpm followed by another 24 h incubation step (37°C, 200 rpm) under white light illumination (halogen lamp) or in darkness. Protein extraction was as described (Hedtke *et al*., 2015) and the protein amount adjusted to 2 mg/ml (for PBG determination) or 1.5 mg/ml (for heme determination).

For PBG determination 300 µl of the protein extract were mixed with buffer 1 (183 µl H_2O, 21 µl 1M TrisCl pH8, 96 µl $MgCl_2$ 50 mM and 200 µg 5-ALA) and incubated for 2.5 h on 37°C. PBG was then determined with the ClinRep Kit (Recipe, Freiburg). For heme determination 200 µl per protein sample were mixed with 40% pyridine in 200 mM NaOH and with 1.2 µl 0.1 M $K_3FE(CN)_6$ solution to oxidize all heme groups. After recording an absorption spectrum, heme groups were completely reduced by sodium dithionite followed by a second absorption spectrum. Heme concentration was determined as described (Berry and Trumpower, 1987).

Penicillin quantification

10^7 conidiospores were incubated in 50 ml corn steep medium supplemented with required vitamins in 100 ml Erlenmeyer flasks for 24 h on 26°C, 200 rpm in darkness followed by another 24 h of incubation in darkness or under white light illumination (halogen lamp). After filtering the mycelium through Miracloth and a centrifugation step (5 min, 5000 rpm), the supernatant was used in the penicillin assay with the indicator bacterium *Geobacillus stearothermophilus* (Herr and Fischer, 2014).

Analysis of sterigmatocystin

5×10^5 conidiospores were spread on 15 ml solid MM (containing the appropriate supplements and 2% glucose) containing petri dishes with glass beads followed by two days of incubation at 37°C under white light illumination (LED panels) or in darkness respectively. Sterigmatocystin extraction and thin layer chromatography were performed as described (Atoui *et al*., 2010)

Light sources

For the analysis of development, expression analyses by real time PCR, sterigmatocystin production and chromatin immunoprecipitation white light generated by LED panels with 14 LEDs (Roithner Lasertech) with an intensity of 0.5 W/m^2 were used. For the analysis of penicillin, PBG and heme a halogen lamp (100 W) was used as white light source in front of a shaking incubator (Inforce Minitron).

Statistical analysis

Significant differences were calculated using the two-sample *t*-test, two-sided with no equal variance, *P*-values: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, ns = not significant.

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References

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

Atoui, A., Kastner, C., Larey, C.M., Thokala, R., Etxebeste, O., Espeso, E.A., *et al*. (2010) Cross-talk between light and glucose regulation controls toxin production and morphogenesis in Aspergillus nidulans. *Fungal Genet Biol* **47**: 962–972.

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

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

Bayram, Ö., Krappmann, S., Seiler, S., Vogt, N., and Braus, G.H. (2007) *Neurospora crassa ve-1* affects asexual conidiation. *Fungal Genet Biol* **45**: 127–138.

Bayram, Ö., Biesemann, C., Krappmann, S., Galland, P., and Braus, G.H. (2008) More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. *Mol Biol Cell* **19**: 3254–3262.

Bayram, Ö., Braus, G.H., Fischer, R., and Rodriguez-Romero, J. (2010) Spotlight on *Aspergillus nidulans* photosensory systems. *Fungal Genet Biol* **47**: 900–908.

Bayram, Ö., Bayram, Ö.S., Ahmed, Y.L., Maruyama, J., Valerius, O., Rizzoli, S.O., *et al*. (2012) The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. *PLoS Genet* **8**: e1002816.

Berry, E.A., and Trumpower, B.L. (1987) Simultaneous determination of hemes a, b, and c from pyridine hemochrome spectra. *Anal Biochem* **161**: 1–15.

Blumenstein, A., Vienken, K., Tasler, R., Purschwitz, J., Veith, D., Frankenberg-Dinkel, N., *et al*. (2005) The

Aspergillus nidulans phytochrome FphA represses sexual development in red light. *Curr Biol* **15**: 1833–1838.
Bok, J.W., and Keller, N.P. (2004) LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryot Cell* **3**: 527–535.
Brandt, S., von Stetten, D., Günther, M., Hildebrandt, P., and Frankenberg-Dinkel, N. (2008) The fungal phytochrome FphA from *Aspergillus nidulans*. *J Biol Chem* **283**: 34605–34614.
Calvo, A.M. (2008) The VeA regulatory system and its role in morphological and chemical development in fungi. *Fungal Genet Biol* **45**: 1053–1061.
Calvo, A.M., and Cary, J.W. (2015) Association of fungal secondary metabolism and sclerotial biology. *Front Microbiol* **6**: 62.
Calvo, A.M., Bok, J.W., Brooks, W., and Keller, N.P. (2004) *veA* is required for toxin and sclerotial production in *Aspergillus parasiticus*. *Appl Environ Microbiol* **70**: 4733–4939.
Casas-Flores, S., Rios-Momberg, M., Bibbins, M., Ponce-Noyola, P., and Herrera-Estrella, A. (2004) BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. *Microbiology* **150**: 3561–3569.
Chen, C.H., Ringelberg, C.S., Gross, R.H., Dunlap, J.C., and Loros, J.J. (2009) Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in Neurospora. *EMBO J* **28**: 1029–1042.
Dasgupta, A., Fuller, K.K., Dunlap, J.C., and Loros, J.J. (2015) Seeing the world differently: Variability in the photosensory mechanisms of two model fungi. *Environ Microbiol* **18**: 5–20.
Feldman, I., Feldman, G.M., Mobarak, C., Dunkelberg, J.C., and Leslie, K.K. (2007) Identification of proteins within the nuclear factor-kappa B transcriptional complex including estrogen receptor-alpha. *Am J Obstet Gynecol* **196**: 394.e1-11.
Franken, A.C., Werner, E.R., Haas, H., Lokman, B.C., van den Hondel, C.A., Ram, A.F., *et al*. (2013) The role of coproporphyrinogen III oxidase and ferrochelatase genes in heme biosynthesis and regulation in *Aspergillus niger*. *Appl Microbiol Biotechnol* **97**: 9773–9785.
Froehlich, A.C., Liu, Y., Loros, J.J., and Dunlap, J.C. (2002) White collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. *Science* **297**: 815–819.
Fuller, K.K., Ringelberg, C.S., Loros, J.J., and Dunlap, J.C. (2013) The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. *MBio* **4**: e00142–e00113.
Fuller, K.K., Loros, J.J., and Dunlap, J.C. (2015) Fungal photobiology: Visible light as a signal for stress, space and time. *Curr Genet* **61**: 275–288.
Fuller, K.K., Dunlap, J.C., and Loros, J.J. (2016) Fungal light sensing at the bench and beyond. *Adv Genet* **96**: 1–51.
Grimaldi, B., Coiro, P., Filetici, P., Berge, E., Dobosy, J.R., Freitag, M., *et al*. (2006) The *Neurospora crassa* White Collar-1 dependent built light response requires acetylation of histone H3 lysine 14 by NGF-1. *Mol Biol Cell* **17**: 4576–4583.
Hartingsveldt, W.V., Mattern, I.E., van Zeijl, C.M.J., and Pouwels, P.H. (1987) Development of a homologous transformation system for *Aspergillus niger* based on the *pyrG* gene. *Mol Genet Genomics* **206**: 71–75.
Hayden, M.S., and Ghosh, S. (2008) Shared principles in NF-kappaB signaling. *Cell* **132**: 344–362.
He, Q., Cheng, P., Yang, Y., Wang, L., Gardner, K.H., and Liu, Y. (2002) White collar-1, a DNA binding transcription factor and a light sensor. *Science* **297**: 840–843.
Hedtke, M., Rauscher, S., Röhrig, J., Rodriguez-Romvero, J., Yu, Z., and Fischer, R. (2015) Light-dependent gene activation in *Aspergillus nidulans* is strictly dependent on phytochrome and involves the interplay of phytochrome and white collar-regulated histone H3 acetylation. *Mol Microbiol* **97**: 733–745.
Herr, A., and Fischer, R. (2014) Improvement of Aspergillus nidulans penicillin production by targeting AcvA to peroxisomes. *Metab Eng* **25**: 131–139.
Hiltbrunner, A., Viczian, A., Bury, E., Tscheuschler, A., Kircher, S., Toth, R., *et al*. (2005) Nuclear accumulation of the phytochrome A photoreceptor requires FHY1. *Curr Biol* **15**: 2125–2130.
Hudson, M., Ringli, C., Boylan, M.T., and Quail, P.H. (1999) The FAR1 locus encodes a novel nuclear protein specific to phytochrome A signaling. *Genes Dev* **13**: 2017–2027.
Idnurm, A., and Heitman, J. (2005) Light controls growth and development via a conserved pathway in the fungal kingdom. *PLoS Biol* **3**: 615–626.
Idnurm, A., Rodriguez-Romero, J., Corrochano, L.M., Sanz, C., Iturriaga, E.A., Eslava, A.P., *et al*. (2006) The Phycomyces *madA* gene encodes a blue-light photoreceptor for phototropism and other light responses. *Proc Natl Acad Sci USA* **103**: 4546–4551.
Käfer, E. (1977) Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. *Adv Genet* **19**: 33–131.
Källberg, M., Margaryan, G., Wang, S., Ma, J., and Xu, J. (2014) RaptorX server: A resource for template-based protein structure modeling. *Methods Mol Biol* **1137**: 17–27.
Kato, N., Brooks, W., and Calvo, A.M. (2003) The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by *veA*, a gene required for sexual development. *Eukaryot Cell* **2**: 1178–1186.
Kim, H.-S., Han, K.-Y., Kim, K.-J., Han, D.-M., Jahng, K.-Y., and Chae, K.-S. (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. *Fungal Genet Biol* **37**: 72–80.
Lee, K., Singh, P., Chung, W.C., Ash, J., Kim, T.S., Hang, L., *et al*. (2006) Light regulation of asexual development in the rice blast fungus, *Magnaporthe oryzae*. *Fungal Genet Biol* **43**: 694–706.
Li, S., Myung, K., Guse, D., Donkin, B., Proctor, R.H., Grayburn, W.S., *et al*. (2006) FvVE1 regulates filamentous growth, the ratio of microconidia to macroconidia and cell wall formation in *Fusarium verticillioides*. *Mol Microbiol* **62**: 1418–1432.
Lin, R., Ding, L., Casola, C., Ripoll, D.R., Feschotte, C., and Wang, H. (2007) Transposase-derived transcription factors regulate light signaling in Arabidopsis. *Science* **318**: 1302–1305.
Martinez, J.L., Petranovic, D., and Nielsen, J. (2016) Heme metabolism in stress regulation and protein production:

From Cinderella to a key player. *Bioengineered* **7**: 112–115.
Mooney, J.L., and Yager, L.N. (1990) Light is required for conidiation in *Aspergillus nidulans*. *Genes Dev* **4**: 1473–1482.
Nayak, T., Szewczyk, E., Oakley, C.E., Osmani, A., Ukil, L., Murray, S.L., *et al*. (2006) A versatile and efficient gene targeting system for *Aspergillus nidulans*. *Genetics* **172**: 1557–1566.
Ni, M., and Yu, J.H. (2007) A novel regulator couples sporogenesis and trehalose biogenesis in *Aspergillus nidulans*. *PLoS One* **2**: e970.
Palmer, J.M., Theisen, J.M., Duran, R.M., Grayburn, W.S., Calvo, A.M., and Keller, N.P. (2013) Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. *PLoS Genet* **9**: e1003193.
Park, H.S., Ni, M., Jeong, K.C., Kim, Y.H., and Yu, J.H. (2012) The role, interaction and regulation of the velvet regulator VelB in *Aspergillus nidulans*. *PLoS One* **7**: e45935.
Park, H.S., Nam, T.Y., Han, K.H., Kim, S.C., and Yu, J.H. (2014) VelC positively controls sexual development in *Aspergillus nidulans*. *PLoS One* **9**: e89883.
Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., *et al*. (2004) UCSF Chimera–a visualization system for exploratory research and analysis. *J Comput Chem* **25**: 1605–1612.
Pontecorvo, G., Roper, J.A., Hemmons, L.M., MacDonald, K.D., and Bufton, A.W.J. (1953) The genetics of *Aspergillus nidulans*. *Adv Genet* **5**: 141–238.
Purschwitz, J., Müller, S., Kastner, C., Schöser, M., Haas, H., Espeso, E.A., *et al*. (2008) Functional and physical interaction of blue and red-light sensors in *Aspergillus nidulans*. *Curr Biol* **18**: 255–259.
Purschwitz, J., Müller, S., and Fischer, R. (2009) Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the white collar protein LreB. *Mol Genet Genomics* **281**: 35–42.
Quail, P.H. (2002) Phytochrome photosensory signalling networks. *Nat Rev* **3**: 85–93.
Rauscher, S., Pacher, S., Hedtke, M., Kniemeyer, O., and Fischer, R. (2016) A phosphorylation code of the *Aspergillus nidulans* global regulator VelvetA (VeA) determines specific functions. *Mol Microbiol* **99**: 909–924.
Rodriguez-Romero, J., Hedtke, M., Kastner, C., Müller, S., and Fischer, R. (2010) Fungi, hidden in soil or up in the air: Light makes a difference. *Annu Rev Microbiol* **64**: 585–510.
Röhrig, J., Kastner, C., and Fischer, R. (2013) Light inhibits spore germination through phytochrome in *Aspergillus nidulans*. *Curr Genet* **59**: 55–62.
Ruger-Herreros, C., Rodriguez-Romero, J., Fernandez-Barranco, R., Olmedo, M., Fischer, R., Corrochano, L.M., *et al*. (2011) Regulation of conidiation by light in *Aspergillus nidulans*. *Genetics* **188**: 809–822.
Sambrook, J., and Russel, D.W. (1999) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Sanz, C., Rodrigues-Romero, J., Idnurm, A., Christie, J.M., Heitman, J., Corrochano, L.M., *et al*. (2009) Phycomyces MADB interacts with MADA to form the primary photoreceptor complex for fungal phototropism. *Proc Natl Acad Sci USA* **106**: 7095–7100.
Sarikaya-Bayram, O., Bayram, Ö., Feussner, K., Kim, J.-H., Kim, H.-S., Kaever, A., *et al*. (2014) Membrane-bound methyltransferase complex VapA-VipC-VapB guides epigenetic control of fungal development. *Dev Cell* **29**: 406–420.
Sarikaya-Bayram, O., Palmer, J.M., Keller, N., Braus, G.H., and Bayram, Ö. (2015) One Juliet and four Romeos: VeA and its methyltransferases. *Front Microbiol* **6**: 1.
Schumacher, J., Simon, A., Cohrs, K.C., Traeger, S., Porquier, A., Dalmais, B., *et al*. (2015) The VELVET complex in the gray mold fungus *Botrytis cinerea*: Impact of BcLAE1 on differentiation, secondary metabolism and virulence. *Mol Plant-Mic Int* **28**: 659–674.
Sievers, F., and Higgins, D.G. (2014) Clustal omega. *Curr Protoc Bioinf* **48**: 3.13.1–3.13.16.
Szewczyk, E., Nayak, T., Oakley, C.E., Edgerton, H., Xiong, Y., Taheri-Talesh, N., *et al*. (2006) Fusion PCR and gene targeting in *Aspergillus nidulans*. *Nature Prot* **1**: 3111–3120.
Tang, W., Wang, W., Chen, D., Ji, Q., Jing, Y., Wang, H., *et al*. (2012) Transposase-derived proteins FHY3/FAR1 interact with PHYTOCHROME-INTERACTING FACTOR1 to regulate chlorophyll biosynthesis by modulating HEMB1 during deetiolation in Arabidopsis. *Plant Cell* **24**: 1984–2000.
Tang, W., Ji, Q., Huang, Y., Jiang, Z., Bao, M., Wang, H., and Lin, R. (2013) FAR-RED ELONGATED HYPOCOTYL3 and FAR-RED IMPAIRED RESPONSE1 transcription factors integrate light and abscisic acid signaling in Arabidopsis. *Plant Physiol* **163**: 857–866.
Vienken, K., Scherer, M., and Fischer, R. (2005) The $Zn(II)_2Cys_6$ transcription factor RosA (repressor of sexual development) triggers early developmental decisions in the filamentous fungus *Aspergillus nidulans*. *Genetics* **169**: 619–630.
Wang, F., Djiksterhuis, J., Wyatt, T., Wösten, H.A.B., and Bleichrodt, R.-J. (2015) VeA of *Aspergillus niger* increases spore dispersing capacity by impacting conidiophore architecture. *Ant Leeuwenh* **107**: 187–199.
Wang, H., and Deng, X.W. (2002) Arabidopsis FHY3 defines a key phytochrome A signaling component directly interacting with its homologous partner FAR1. *EMBO J* **21**: 1339–1349.
Waring, R.B., May, G.S., and Morris, N.R. (1989) Characterization of an inducible expression system in *Aspergillus nidulans* using *alcA* and tubulin coding genes. *Gene* **79**: 119–130.
Yelton, M.M., Hamer, J.E., and Timberlake, W.E. (1984) Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. *Proc Natl Acad Sci USA* **81**: 1470–1474.
Yu, Z., Armant, O., and Fischer, R. (2016) Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling. *Nat Microbiol* **1**: 16019.

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