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ORIGINAL PAPER

Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the White Collar protein LreB

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Abstract *Aspergillus nidulans* senses red and blue-light and employs a phytochrome and a *Neurospora crassa* White Collar (WC) homologous system for light perception and transmits this information into developmental decisions. Under light conditions it undergoes asexual development and in the dark it develops sexually. The phytochrome FphA consists of a light sensory domain and a signal output domain, consisting of a histidine kinase and a response regulator domain. Previously it was shown that the phytochrome FphA directly interacts with the WC-2 homologue, LreB and another regulator, VeA. In this paper we mapped the interaction of FphA with LreB to the histidine kinase and the response regulator domain at the C-terminus in vivo using the bimolecular fluorescence complementation assay and in vitro by co-immunoprecipitation. In comparison, VeA interacted with FphA only at the histidine kinase domain. We present evidence that VeA occurs as a phosphorylated and a non-phosphorylated form in the cell. The phosphorylation status of the protein was independent of the light receptors FphA, LreB and the WC-1 homologue LreA.

Keywords *Aspergillus nidulans* · Phytochrome · Red-light response · Blue-light response

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Introduction

Light is an ubiquitous signal in the environment, which may vary concerning wavelength and intensity in different locations. Therefore, several wavelength-specific photoreceptors have evolved in plants, bacteria and fungi (Purschwitz et al. 2006; van der Horst et al. 2007; Bae and Choi 2008). Photoreceptor systems enable organisms to adjust their physiological and developmental processes in a light-quality specific manner. One very common photoreceptor is the red-light receptor phytochrome (van der Horst et al. 2007). Although it was thought for a long time that phytochromes are plant-specific light receptors, in the last decade it was discovered that they are also present in chemotrophic bacteria and fungi and that the fungal phytochromes resemble the bacterial ones (Lamparter et al. 1997; Bhoo et al. 2001; Blumenstein et al. 2005; Froehlich et al. 2005).

Whereas in most bacteria deletion of the corresponding genes does not produce easy visible phenotypes, the filamentous fungus *Aspergillus nidulans* is an excellent model system to study the light response and the role and interplay of different photoreceptors. This fungus develops in light asexual conidiophores with haploid spores containing a single nucleus (Fischer and Kües 2006). Under dark conditions, *A. nidulans* undergoes the sexual cycle (Busch and Braus 2007).

Early on it was described that the most effective light quality for the induction of asexual development was red light, and the effect was reversible with far-red light. This reaction was reminiscent of a plant phytochrome response (Mooney and Yager 1990). The molecular analysis of the light response started with the analysis of the *veA1* mutant, which does not respond to light as strong as the wild type does (Mooney and Yager 1990). The corresponding gene was cloned and subsequently further studied also in other

fungi. It appears that the VeA protein is found exclusively in filamentous fungi and is a regulator of different developmental and metabolic pathways (Calvo 2008). The protein contains a nuclear localization signal (NLS), a nuclear export signal (NES), and a PEST sequence, suggesting fast protein turnover, but there is no evidence that it is a light receptor or a transcription factor itself (Kim et al. 2002; Kato et al. 2003; Li et al. 2006; Bayram et al. 2007; Stinnett et al. 2007). Recently, a light receptor in Aspergilli was identified indeed as a phytochrome (Bhoo et al. 2001; Blumenstein et al. 2005). In addition to a phytochrome, homologues of the *N. crassa* blue-light receptor system (WC-1 = LreA and WC-2 = LreB) were identified in the genome of *A. nidulans* and a blue-light effect described, which had been noticed before in certain mutant backgrounds (Yager et al. 1998; Purschwitz et al. 2008). In addition, the functioning of a cryptochrome was also shown (Bayram et al. 2008a). In *A. nidulans* a multi-component light receptor complex has been proposed, suggesting cross-talk between different light quality signaling pathways. The light-regulator complex triggers development and secondary metabolism (Calvo et al. 2002; Bayram et al. 2008b; Fischer 2008; Purschwitz et al. 2008). The protein complex consists of the phytochrome FphA, the VeA protein, LreA, and LreB and perhaps other proteins like LaeA. However, if the complex composition is always the same or changes according to different physiological conditions, is yet unknown. Direct protein–protein interaction was shown between FphA and VeA, FphA and LreB, and LreA and LreB. Whereas VeA does not contain motifs, which allow a conclusion about the molecular function, LreB harbors a N-terminal PAS domain (*P*ER, *A*RNT, *S*IM, involved in protein-protein interaction), a C-terminal nuclear localization sequence and a Zn finger motif, which suggests that it acts as a transcription factor like in *N. crassa* (Linden and Macino 1997).

To better understand the architecture of the light-regulator complex in *A. nidulans*, we investigated whether VeA and LreB compete for the same FphA interaction site or bind to distinct regions of the large chromoprotein.

Methods

Strains, growth conditions and plasmids

Strains used in this study are listed in Table 1. Supplemented minimal media for *A. nidulans* was prepared as previously described (Pontecorvo et al. 1953; Käfer 1977). Standard DNA transformation procedures were used for *A. nidulans* (Yelton et al. 1984) and *E. coli* (Sambrook and Russel 1999). For cloning the truncated versions of FphA standard protocols were applied and the following primers were used PHY (5′-AscI-FphA GAF PHY for 5′-ggcgcgcc tatgtccgagctcccc-3′, 5′-PacI-FphA GAF PHY rev 5′-gttaatt aagagctctccaaagcggcttc-3′), PHYHK (5′-AscI-FphA PHY HK for 5′-ggcgcgcctatgtccgagctcccctctc-3′, 5′-PacI-FphA PHY HK800 rev 5′-cttaattctaagtctgagcttcgctttcacct-3′), HK (5′-AscI-FphA HK RR for 5′-ggcgcgcctatggagagctccagcctt acc-3′, PacI PHY HK rev 5′-ttaattaactttgatgaggctctatccc-3′), HKRR (5′-AscI-FphA HK RR for 5′-ggcgcgcctatggagagct ccagccttacc-3′, PacI FphA rev 5′-gttaattaaggcgactcccgaga tggcgc-3′), RR (5′-AscI-FphA RR for 5′-ggcgcgcctatggaaa cggcacgttcac-3′, PacI FphA rev 5′-gttaattaaggcgactcccgag atggcgc-3′). For tagging with GFP at the N-terminus fragments were inserted into pMCB17apx (Veith et al. 2005) and for generation of HA fusion proteins in pSM14 (*alcA::HA*, *pyr4*). All plasmids used in this study are listed in Table 2.

BiFC system and epifluorescence microscopy

The proteins of interest were fused with the C- or the N-terminus of YFP. The corresponding plasmids were derived from pMCB17apx by exchanging the GFP against one half of YFP (Blumenstein et al. 2005). These plasmids were transformed into GR5 or SJP1. Germlings were incubated at RT for 24 h in MM containing glycerol as carbon source to derepress the *alcA* promoter, which drives the expression of the fusion proteins. For epifluorescence microscopy a Zeiss Axio Imager Z.1 (Jena, Germany) with a 63× Plan-ApoChromat objective using YFP filtersets. Pictures were taken with the Zeiss AxioCamMR.

Protein extracts, immunoprecipitation and western blotting

For preparing protein extracts *A. nidulans* strains were incubated in liquid MM for 24 h at 37°C. To induce the *alcA* promoter this medium was supplemented with 0.2% glucose and 2% threonine. The mycelium was harvested by filtration through Miracloth (Calbiochem, Heidelberg, Germany), dried between some paper towels and immediately ground in liquid nitrogen. Afterwards the mycelial powder was resuspended in protein extraction buffer (20 mM Tris–HCl, pH 8, 0.05% Triton-X-100, 150 mM NaCl) containing protease inhibitors (1 mM PMSF). Cell debris was pelleted by centrifugation (Eppendorf Centrifuge 5403; Eppendorf, Hamburg, Germany) at 15,000 rpm at 4°C for 10 min. A volume of 1 ml protein extract was adjusted to 300 mM NaCl and incubated with monoclonal antibody HA.11 (dilution 1:200; clone 16B12; Hiss Diagnostics, Freiburg, Germany), GFP-AP11 antibody from Alpha Diagnostics International (San Antonio, CA, USA) or Anti-GFP, N-terminal antibody (Sigma, St Louis, USA) with a dilution of 1:800 each. After 1 h 50 µl Protein-G-Agarose (Roche, Mannheim, Germany) were added and incubated for

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

Strain	Genotype	Source
GR5	pyrG89; wA3; pyroA4; veA1	(Waring et al. 1989)
SSM12	GR5 transformed with pSM17 (alcA::HA::veA)	(Purschwitz et al. 2008)
SSM33	SJP21.3 transformed with pSM17	This study
SJP1	*pyrG89; ΔargB::trpCΔB; pyroA4; ΔfphA::argB; veA+*	(Purschwitz et al. 2008)
SJP21.3	*pyrG89*; Δ*lreB::argB*; Δ*argB:trpCΔB*; *pyroA4*; Δ*lreA::argB*, Δ*fphA::argB*; *veA+*	(Purschwitz et al. 2008)
SJP54	GR5 transformed with pJP29 (*alcA::YFP-NT::PHY*) and pCK3 (*alcA::YFP-CT::lreB*)	This study
SJP55	GR5 transformed with pJP11 (*alcA::YFP-NT::PHYHK*) and pCK3 (*alcA::YFP-CT::lreB*)	This study
SJP56	GR5 transformed with pJP15 (*alcA::YFP-NT::HKRR*) and pCK3 (*alcA::YFP-CT::lreB*)	This study
SJP57	GR5 transformed with pJP13 (*alcA::YFP-NT::RR*) and pCK3 *alcA::YFP-CT::lreB*)	This study
SJP60	GR5 transformed with pJP29 (*alcA::YFP-NT::PHY*) and pCK1 (*alcA::YFP-CT::veA*)	This study
SJP61	GR5 transformed with pJP11 (*alcA::YFP-NT::PHYHK*) and pCK1 (*alcA::YFP-CT::veA*)	This study
SJP62	GR5 transformed with pJP15 (*alcA::YFP-NT::HKRR*) and pCK1 (*alcA::YFP-CT::veA*)	This study
SJP63	GR5 transformed with pJP13 (*alcA::YFP-NT::RR*) and pCK1(*alcA::YFP-CT::veA*)	This study
SJP65	GR5 transformed with pJP8 (*alcA::GFP::HKRR*)	This study
SJP66	GR5 transformed with pJP10 (*alcA::GFP::RR*)	This study
SJP78	SJP65 transformed with pSM16 (*alcA::HA::lreB*)	This study
SJP81	SJP66 transformed with pSM16 (*alcA::HA::lreB*)	This study
SJP96	GR5 transformed with pSM16 *(alcA::HA::lreB)*	This study
SJP102	GR5 transformed with pJP76 (*alcA::GFP::PHYHK*)	This study
SJP105	SJP102 transformed with pSM16 (*alcA::HA::leB)*	This study
SJP106	SJP102 transformed with pSM17 (*alcA::HA:veA*)	This study
SJP108	SJP1 transformed with pSM16 (*alcA::HA::leB*)	This study
SJP109	SJP1 transformed with pJP8 (*alcA::GFP::HKRR*)	This study
SJP110	SJP109 transformed with pSM16 (*alcA::HA::leB*)	This study
SJP113	SJP1 transformed with pJP76 (*alcA::GFP::PHYHK*)	This study
SJP114	SJP113 transformed with pSM16 (*alcA::HA::lreB*)	This study

additional 3 h. Agarose beads were pelleted by centrifugation in an Eppendorf Centrifuge at 15,000 rpm at 4°C for 30 s and washed two times with 1 ml extraction buffer. After denaturation of samples protein extracts and CoIP pellets were loaded on a 7.5% sodium dodecyl sulfate polyacrylamide gel. For western blotting a monoclonal antibody raised against the hemeagglutinine (HA) epitope (dilution 1:1000), or against GFP (Product G 1544; Sigma–Aldrich, München, Germany; dilution 1:4000) was used. For blotting nitrocellulose membranes from Schleicher and Schuell (Dassel, Germany) were used.

Protein phosphatase treatment

Preparation of crude cell extracts of *A. nidulans* was performed as described above. For protein extraction 50 mM Tris–HCl pH 7.5, 100 mM NaCl, 0.1 mM EGTA, 0.05% TritonX 100 either with or without addition of protease inhibitors (1.5 mM PMSF, 1 µM Pepstatin A) was used. Lambda protein phosphatase (New England Biolabs) treatments were performed at 30°C for 1 h. Equal amounts of protein (200 µg per lane) were applied to sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Results

Interaction of FphA with LreB is mediated via the C-terminal signal output domains of FphA

We tested five different truncated versions of FphA for interaction with LreB in a bimolecular fluorescence complementation (BiFC) approach in vivo: PHY (FphA 1-757), PHYHK (1-1089), HK (756-825), HKRR (756-1280), RR (1107-1280). Constructs containing the HK domain either in combination with PHY or with the RR domain at the very C-terminus of FphA resulted in interaction with LreB (Fig. 1). The fluorescent signals were essentially confined to the nuclei and comparable to the localization and signal intensity of full-length FphA and LreB (Purschwitz et al. 2008). About 30 independent original colonies were analyzed for the presence of the two plasmids (which had both

Table 2 Plasmids used in this study

Plasmid	Genotype	Source
pMCBapx	*alcA::GFP*, *pyr-4*	(Veith et al. 2005)
pSM14	*alcA::HA*, *pyr-4*	This study
pSM16	*alcA::HA::lreB*, *pyr-4*	(Purschwitz et al. 2008)
pSM17	*alcA::HA::veA*, *pyr-4*	(Purschwitz et al. 2008)
pCK1	*alcA::YFP-CT::veA*, *pyr-4*	(Purschwitz et al. 2008)
pCK3	*alcA::YFP-CT::lreB*, *pyr-4*	(Purschwitz et al. 2008)
pJP8	*alcA::GFP::HKRR*, *pyr-4*	This study
pJP10	*alcA::GFP::RR*, *pyr-4*	This study
pJP11	*alcA::YFP-NT::PHYHK*, *pyr-4*	This study
pJP13	*alcA::YFP-NT::RR*, *pyr-4*	This study
pJP15	*alcA::YFP-NT::HKRR*, *pyr-4*	This study
pJP29	*alcA::YFP-NT::PHY*, *pyr-4*	This study
pJP76	*alcA::GFP::PHYHK*, *pyr-4*	This study

the same nutritional marker) from which about 50% fulfilled this criterium and showed fluorescence. The N-terminus of FphA (PHY) did not reveal an affinity for LreB. Surprisingly, the HK domain alone also did not interact with LreB in this assay, although the results for the PHYHK and HKRR constructs indicate that this kinase domain should play a major role in mediating physical contact to the WC2 homologous protein. This interaction could also not be shown using a two-hybrid assay (results not shown).

The positive interaction pattern of PHYHK, HKRR and RR was verified by co-immunoprecipitation. For these experiments the FphA constructs were N-terminally tagged with GFP and expressed under the control of the *alcA* promoter whereas in the case of LreB an HA-fusion protein was generated (Fig. 2). These tests were done in the strain GR5, which harbors a wild type copy of *fphA*. Because FphA is able to self-interact (Blumenstein et al. 2005), it was possible that full-length FphA mediated the observed interactions between the different FphA domains with LreB. To exclude this possibility we repeated the co-immunoprecipitation experiments for PHYHK and HKRR in a *ΔfphA* background (Fig. 2). Also in the *ΔfphA* background interaction of the FphA domains with LreB was demonstrated.

FphA interacts with VeA via the HK domain

We first tested the different truncated versions of FphA described above for interaction with the global regulator VeA in a BiFC experiment (Fig. 1). Almost all constructs showed negative results except the PHYHK fragment. Like in the case of LreB, this fragment displayed the same pattern as the full-length FphA tested for interaction with

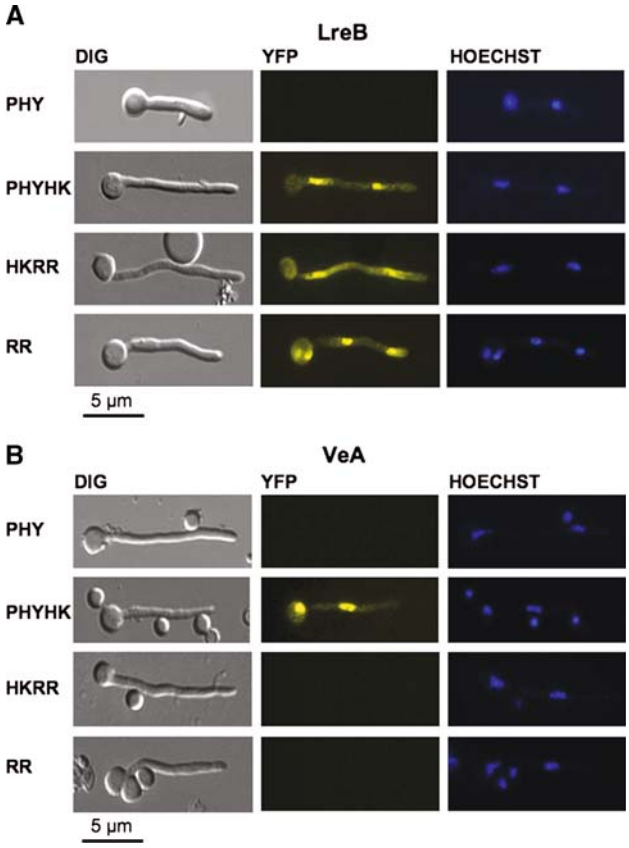

Fig. 1 Mapping of the interaction domains between FphA and LreB (**a**) and VeA (**b**) using bimolecular fluorescence complementation in *A. nidulans*. Strain GR5 was transformed with the split YFP plasmid pCK3 encoding LreB (**a**) or pCK1 encoding VeA (**b**) in combination with different split YFP plasmids encoding different domains of FphA: the sensory domain PHY (FphA1-757) (pl. pJP29), the histidine kinase domain PHYHK (FphA1-1089) (pl. pJP11), the histidine kinase domain plus the response regulator HKRR (FphA756-1280) (pl. pJP15), and the response regulator RR (FphA1107-1280) (pl. pJP13). Strains harboring pCK3 or pCK1 and a corresponding FphA domain encoding plasmid were grown in minimal medium with 2% glycerol for moderate induction of the *alcA* promoter

VeA. In all cases the fluorescence signals were mainly restricted to the nuclei (Fig. 1). Neither in combination with the RR domain nor alone the HK domain displayed an interaction signal. The N-terminus of FphA, the PHY domain didn't show physical contact, either. In conclusion, the combination of PHY and the HK domain appears to form a special structure enabling the physical contact to VeA. Like for LreB, we analyzed the putative interaction of the single HK domain and VeA in a yeast-two-hybrid approach. Also in this assay no interaction could be observed.

We also asked if light influences the affinity of the phytochrome FphA to its binding partners. We tested the photosensory module of FphA for binding of LreB and VeA under constant dark or constant illumination with white light. No differences were observed.

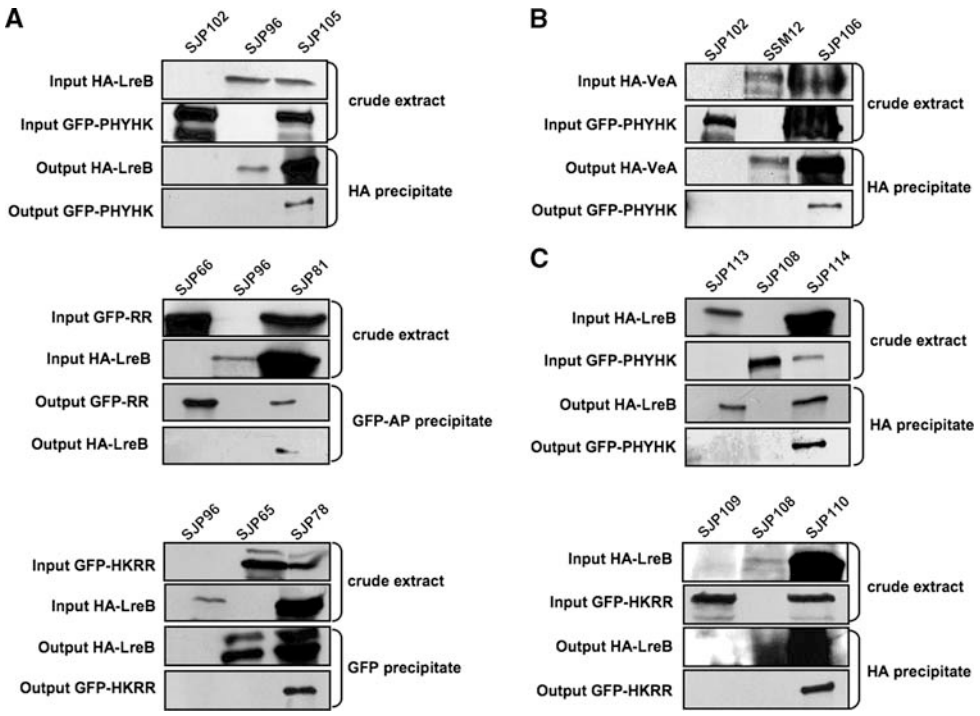

Fig. 2 Mapping of the interaction domains between FphA and LreB (**a**) and VeA (**b**) using co-immunoprecipitation in wild type (**a**, **b**) and Δ*fphA* background (**c**). **a** Strain GR5 was transformed with LreB N-terminally fused to the hemeagglutinine epitope (pSM16) to yield strain SJP96 or transformed with pSM16 and a second plasmid containing different FphA domains fused to GFP resulting in SJP105 (PHYHK), SJP81 (RR) and SJP78 (HKRR). Transformation of GR5 with pJP76, pJP8 or pJP10 resulted in corresponding GFP control strains. **b** Strain GR5 was transformed with plasmid pSM17, coding for the HA::VeA fusion protein, to produce SSM12. Strain SJP106 resulted from transformation of GR5 with pSM17 and pJP76, whereas strain SJP102 harbors only pJP76. **c** The Δ*fphA*-deletion strain SJP1 was transformed with LreB N-terminally fused to the heme agglutinine epitope (pSM16) and plasmids containing fragments of FphA fused to GFP. For the study of an interaction between LreB and PHYHK, plasmids pSM16 and pJP76 were transformed alone or in combination resulting in strains SJP108 (pSM16), SJP113 (pJP76) and SJP114 (pSM16 and pJP76). To verify the interaction of LreB with HKRR strain SJP1 was transformed with plasmids pSM16 (strain SJP108) or pJP8 (strain SJP109) as well as with both plasmids in case of strain SJP110. For co-immunoprecipitation, antibodies directed against the hemeagglutinine epitope (Klon 16B12 derived from mouse, Hiss Diagnostics, Freiburg, Germany) or the GFP epitope (Anti-GFP N-terminal, derived from rabbit, Sigma–Aldrich, St Louis, USA or GFP11-AP derived from goat, Alpha Diagnostics International, San Antonio, USA) were used as indicated in **a**–**c**. Precipitation was performed in 1 ml crude extract of approximately 10 mg/ml total protein with the indicated antibodies and 50 µl Protein G-Agarose (Roche, Mannheim, Germany). For more details see the "Methods"

Phosphorylation of VeA is independent of FphA, LreA and LreB

In order to analyze the function of the interaction between FphA and VeA, phosphorylation of VeA was tested (Fig. 3). We treated crude extract of strain SSM12 (containing a HA–VeA fusion protein) with Lambda protein phosphatase (λ-PPase) that removes phosphates bound to serine, threonine and tyrosine. We observed a shift towards a faster migrating VeA form after treatment of the extracts with λ-PPase. This band probably represents none-phosphorylated VeA. Without λ-PPase treatment a more diffuse VeA signal was observed, which possibly represents a mixture of phosphorylated and none-phosphorylated forms. To test whether the different signals were due to protein degradation rather than to the phosphorylation status, we made a control assay with and without protease inhibitors. No shift was observed in this case. The same experiment was repeated with a strain in which three components of the light signaling, FphA, LreA, and LreB, were absent (strain SSM33). Also in this strain VeA occurred in the phosphorylated form (Fig. 3). We also tested phosphorylation of LreB, but could not detect any evidence for that under the employed experimental conditions (Fig. 3).

Discussion

Phytochromes are light sensing chromoproteins with serine/threonine kinase activity (Rockwell and Lagarias 2006). As phytochromes do not posses DNA-binding motives and have no other biochemical than kinase activity, downstream procession must be mediated by interaction with other proteins and thereby modulation of their

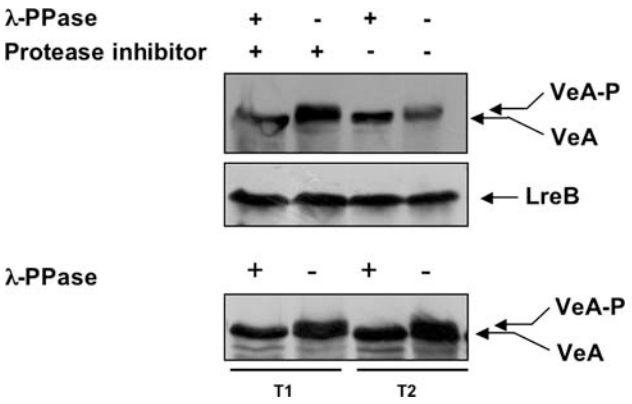

Fig. 3 Detection of the phosphorylation of VeA. Mobility shift analysis of VeA in a 7.5% SDS polyacrylamide gel in the presence or absence of Lambda protein phosphatase (Lambda-PPase). Strain SSM12 (*upper panel*), SJP96 (*middle panel*), and SSM33 (*lower panel*) were grown in MM containing 0.2% glucose and 2% threonine as carbon sources for at least 28 h at 37°C, 180 rpm. Protein extraction and Westernblot analysis were performed as described in "Methods". In the case of SSM33 two independent transformants (T1 and T2) were analyzed

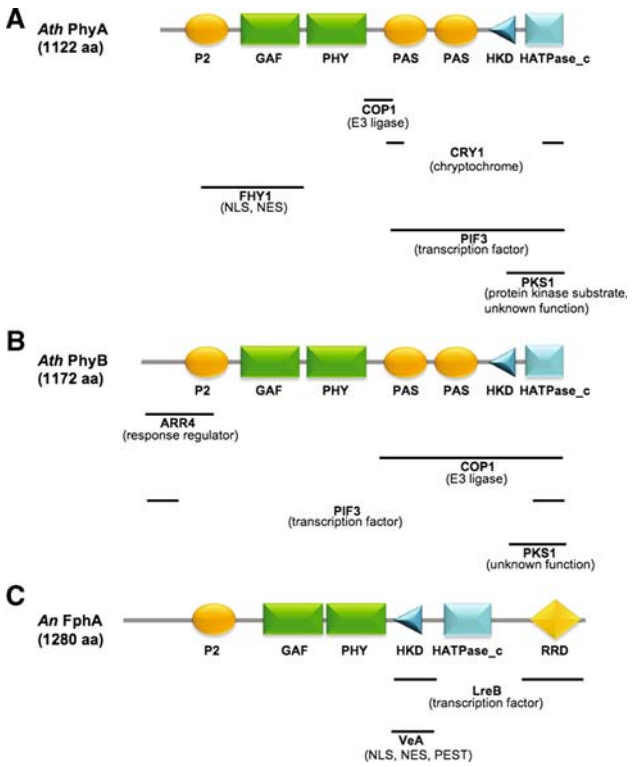

Fig. 4 Summary of the mapping of interactions of *A. thaliana* PhyA and PhyB and *A. nidulans* FphA. **a** The cryptochrome Cry1, the transcription factor PIF3 and PKS1 were shown to interact with the signal output module of PhyA. The putative E3 ligase COP1 was shown to bind to a small part of PhyA, which resides between the PHY and the following PAS domain corresponding to the hinge region between the photosensory and the signal output module. The only protein shown to interact with PhyA via the photosensory module is FHY1 and its homologue FHL. **b** PhyB interacts with COP1 at the C-terminus. PIF3 interaction with phytochrome differs between PhyA and PhyB. This might indicate that signal transduction mechanisms differ between different phytochromes. The response regulator ARR4 was also shown to bind to the N-terminus of PhyB. This region has been discussed to include the kinase activity. **c** Summary of *A. nidulans* phytochrome interactions

activities. Vice versa, interacting proteins could modulate phytochrome activity. Signal transduction can be mediated by phosphorylation or by changing the protein-protein interactions. Hence, for the understanding of light signaling, the identification of phytochrome interaction partners is crucial. Several screens for interaction partners have been performed with plants in the past and more than 20 different proteins have been identified so far (Bae and Choi 2008). These proteins can be divided into three subgroups. Members of the first group are involved in the regulation of nuclear localization of phytochromes. Plant phytochrome resides in the cytoplasm but upon illumination is translocated into the nucleus. Accessory proteins, such as FHY1 or FHL, for nuclear import are crucial for phytochromes without an own NLS, as in the case of *Arabidopsis thaliana* PhyA (Hiltbrunner et al. 2006). A second group can be involved in the modulation of the output activity of phytochrome, such as ARR4, which binds to PhyB and stabilizes the Pfr form (Sweere et al. 2001). The third group comprises proteins whose own activities are modulated by the red light receptor (Ni et al. 1998; Ni et al. 1999). In *A. thaliana* PhyA and PhyB some regions were identified, which mediate the interactions. In PhyA the C-terminus appears to be important for several interactions, for PhyB C- but also N-terminal interaction regions were characterized (Fig. 4).

In this paper we studied the interaction of *A. nidulans* phytochrome FphA with two interaction partners, VeA and LreB. As a difference to other studies, we studied the interaction in vitro by co-immunoprecipitation but also in vivo using the bimolecular fluorescence complementation assay. VeA interacted with the histidine kinase domain of FphA. Although direct interaction of the isolated histidine kinase domain could not be shown, the summary of the other interaction results, suggests that the interaction occurs through this domain. The failure of the experiments to show the direct interaction could be due to the instability of the isolated domain expressed in *A. nidulans* or the lack of a NLS. However, both arguments should have been invaluable in the yeast-two-hybrid assay, where we could also not detect the interaction between VeA and the FphA histidine kinase domain. This failure could be due to additional sequences in the neighborhood of the histidine kinase required for the interaction. However, with this approach also an interaction between the full-length FphA and VeA could not be detected, even in the presence of exogenous biliverdin. It could therefore be that the interaction between VeA and FphA depends on other *A. nidulans* proteins not present in *S. cerevisiae*. In any case, we conclude from our results that FphA and VeA interact through the histidine kinase domain

and envisaged two possibilities for the role of this interaction. (1) VeA could regulate the activity of the histidine kinase or (2) VeA could be a substrate for the phosphorylation reaction and thereby change itself its properties. As we could demonstrate that the phosphorylation status of VeA is independent of the presence of the phytochrome, we hypothesize that VeA regulates the histidine kinase and thereby phytochrome activity. Given that VeA phosphorylation was independent of the light receptors or the light conditions, the protein could link the light response to other developmental and physiological responses of *A. nidulans*. The fact that it has been shown recently, that the VeA concentration in the cell is lower under light than under dark conditions (Bayram et al. 2008b), suggests a possible feedback regulation between FphA and VeA. It has been shown recently that the Pfr form—in contrast to plant phytochrome—has higher kinase activity than the Pr form (Frankenberg-Dinkel, personal communication). If and how this increase of the kinase activity is transmitted to the VeA protein, is certainly an important question for future studies. Another very interesting open question is, which are the factors regulating the phosphorylation status of VeA.

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