Seeing the rainbow: light sensing in fungi
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Light is essential for photosynthetic organisms, but also serves as an important environmental cue for non-photosynthetic species; thus, light sensing is evolutionarily conserved throughout the kingdoms, from archaea and fungi to humans. Light sensors are chromoproteins, the low-molecular weight compound of which absorbs specific wavelengths and induces a reaction from the protein. In fungi, three light-sensing systems have been described at the molecular level. Blue-light sensing is achieved by a flavin-based photoreceptor, which itself acts as a transcription factor, and red-light sensing is achieved by a phytochrome, a molecule until recently thought to be confined to plants. A retinal-based opsin-system was discovered recently, although a biological function remains to be determined. The challenge for future research will be the identification of further components of signalling cascades, the identification of light-regulated genes and the unravelling of possible functional interplays between the different light control systems.

Introduction
As fungi are saprophytic organisms, one of the intriguing questions is, why do they ‘see’ light? The most obvious answer is provided for asexual spore production in filamentous fungi. Because the fungi are sessile and spores serve as their distribution units in the environment, the spores should only be produced when the fungus grows at a water–air interface, for example on the soil surface, and light is a reliable signal to indicate if this is the case. Given that light influences many different physiological responses such as asexual conidiation, the circadian clock, secondary metabolism, pigmentation and sexual development, it is not surprising that fungi are capable of sensing light over a broad spectrum range, from ultraviolet to far-red light. The range of perceptible light intensities covers more than ten orders of magnitude, from gloomy starlight to full sunshine. Hence, a variety of photoreceptors is conserved in fungi, some of which have been analyzed in the past few years (Figure 1).

The blue-light response
The first blue-light response was reported in 1881 by Darwin when he described a blue light induced phototropic response in plants [1], and have been identified in all three domains (eukaryotes, bacteria and archaea) since. Blue-light receptors can be divided into two general classes: the phototropins and the cryptochromes. With phototropins, the photosensory N–terminal part consists of several characteristic domains, one of which is a LOV domain (light, oxygen or voltage). The C–terminus harbours a kinase domain and, in some cases, additional motifs. Cryptochromes show high similarity to photolyases — which are thought to be very ancient molecules — because they share an important role as a photo-defence system. Through gene duplication and functional changes, cryptochromes might have evolved from ancestral photolyases. The cryptochrome Cry1 from Neurospora crassa shows strong sequence homologies to cryptochromes from other organisms, but its role in photobiology has not yet been elucidated [2].

The best-described blue-light receptor in the fungal kingdom is the phototropin-like protein White Collar 1 (WC–1) from the ascomycete N. crassa. In this fungus, all known light responses, such as carotenoid biosynthesis, induction of protoperithecia and their phototropism, induction of hyphal growth, asexual spore formation and the entrainment of the circadian clock, are sensitive to UV or blue light (Figure 2) [3]. The WC–1 protein was discovered through the analysis of a wc–1 mutant. The name ‘white collar’ derives from the observation that carotenoid biosynthesis in the mycelium of this mutant is impaired, whereas conidia are still pigmented: this leads to the appearance of colonies with a non-pigmented border or (white collar). A second mutant with the same phenotype led to the discovery of White Collar 2 (WC–2). Sequence analyses revealed that WC–1 and WC–2 are GATA-type zinc-finger domains containing transcription factors [4,5]. Both proteins share another motif, the Per-Amt-Sim (PAS) domain [6]. WC–1 has three PAS domains, of which the first is a LOV domain. It shares high similarity to the LOV domain containing proteins from plants, such as FKF1 (flavin-binding, Kelch repeat, F–Box 1) from Arabidopsis [7]. In this domain of WC–1, 11 conserved residues were identified that are necessary for chromophore-binding in plants. Using the third PAS
**Figure 1**

Light sensing in the fungal kingdom. Different examples of light-regulated processes and the photosensory systems characterized are displayed. Descriptions are from top to bottom. *A. nidulans*, asexual conidiophores with thousands of conidiospores (3 μm in diameter) are produced under light conditions. In the dark and under far-red-light conditions, meiotic spores are favoured. *N. crassa*: circadian rhythm of sporulation. Inoculation on one side of a Petri dish and incubation for several days in darkness. The half rings indicate rhythmic spore formation. *C. cinereus*: the heterothallic basidiomycete produces fruiting bodies with a height of 5–10 cm. Several steps of fruiting-body formation are light-dependent. *Cryptococcus neoformans*: The yeast-like pathogen *C. neoformans* forms an outer capsule of carbohydrates that is essential for infection and cause of cryptococcal meningitis. *P. blakesleeanus*: phototropic bending of sporangiophores toward white light (illumination from the left; image reproduced from [25]). *P. polycephalum*: plasmodium of the true slime mould *P. polycephalum*. The plasmodium is a large multinuclear cell (syncytium), which is the main vegetative phase of the life cycle. It can grow to an area of up to 1 m². Image acknowledgements: *N. crassa*, picture kindly provided by J Dunlap (Hanover, NH, USA); *C. cinereus*, picture kindly provided by M Navarro-Gonzalez and U Kuees (Göttingen, Germany); *C. neoformans*, picture kindly provided by A Idnurm (Durham, NC, USA); *P. blakesleeanus*, reprinted from [25], with permission; *P. polycephalum*, picture kindly provided by M Etzrodt and M von der Helm (Germering, Germany). ¹J Purschwitz, unpublished data. ²G Braus, personal communication.
domain, WC–1 forms a complex with the PAS domain of WC–2 [8,9]. This complex acts as transcriptional activator of light-regulated genes (Figure 2). Direct involvement of WC–1 in light perception has been shown by two different approaches. Dunlap and colleagues [10] characterized light-response elements (LRE) in the promoter of frq. The frq transcript level is very low. Upon light exposure the frq mRNA level increases immediately and wc–1 expression is also induced. By contrast, the WC–2 level is high under all conditions. PKC dissociates from WCC, leaving a phosphorylated WCC behind at the LRE site. Transcription of frq reaches its peak and FRQ is synthesized. One hour after light exposure, WCC experiences hypophosphorylation and is subsequently degraded. FRQ binds newly synthesized WCC and prevents its own transcriptional activation. As a result frq expression is again reduced to a basal level and the photo-cycle can start again. It has not yet been analyzed in such a detail if all other light-regulated processes, indicated on the right, have a similar regulation mechanism. (c) The phytochrome system in A. nidulans. (Top) Domain organization of phytochromes in plants, bacteria and fungi. (Middle) Phenotype of a phytochrome deletion strain. (Bottom) Localization of GFP-tagged phytochrome. Nuclei were stained with DsRed (assembled from [35**]).

Molecular basis for blue-light and red-light sensing. (a,b) The white collar system in N. crassa (adapted from [40]). In darkness, the WCC associates with PKC and binds to LREs in the promoter of frq. The frq transcript level is very low. Upon light exposure the frq mRNA level increases immediately and wc–1 expression is also induced. By contrast, the WC–2 level is high under all conditions. PKC dissociates from WCC, leaving a phosphorylated WCC behind at the LRE site. Transcription of frq reaches its peak and FRQ is synthesized. One hour after light exposure, WCC experiences hypophosphorylation and is subsequently degraded. FRQ binds newly synthesized WCC and prevents its own transcriptional activation. As a result frq expression is again reduced to a basal level and the photo-cycle can start again. It has not yet been analyzed in such a detail if all other light-regulated processes, indicated on the right, have a similar regulation mechanism. (c) The phytochrome system in A. nidulans. (Top) Domain organization of phytochromes in plants, bacteria and fungi. (Middle) Phenotype of a phytochrome deletion strain. (Bottom) Localization of GFP-tagged phytochrome. Nuclei were stained with DsRed (assembled from [35**]).

WCl and WC–2 are both nuclear-localized, but a fraction of WC–2 is also detected in the cytoplasm. Both proteins undergo light-dependent phosphorylation, but neither light nor phosphorylation has an effect on their localisation pattern [12]. One of the best-known examples
of blue light regulated gene expression in N. crassa is the transcriptional activation of the gene encoding the FRQ protein, the central component of the circadian clock (Figure 2). It was shown that the WC–1 protein concentration is regulated by protein kinase C (PKC) [13] and that hyperphosphorylation of WCC changes its binding activity to the target promoters, and thus is important for photoadaptation [14]. Not only is modification of the WCC at the protein level important for regulation of its activity but is also important for regulation of the complex at the transcriptional level. Kaldi et al. [15] recently showed that three promoters drive the expression of wc–1. In Trichoderma atroviride, a link to protein kinase A (PKA) was shown [16]. Abolishment of functional PKA resulted in a non-sporulating phenotype, whereas overexpression of PKA also induced conidiation in darkness. In N. crassa, apparently at least one additional circadian oscillator exists that also depends on WC–1 and WC–2 for activity and is temperature-entrainable [17,18].

A second blue-light receptor, named VIVID, was discovered recently in N. crassa. It consists of just one LOV domain and binds co- or non-covalently to flavin (FAD or FMN [flavin mononucleotide]). It is hypothesized that VIVID senses changes in light intensity [19], and it is also involved in the modulation of the circadian clock [20]. Once WC–1 undergoes the dark–light transition, WC–1, in combination with WC–2, stimulates the expression of VIVID. In vcd mutants, the circadian gating of light responses is partially lost, which leads to a circadian shift.

Meanwhile, WC–like blue-light receptors have also been described in the ascomycetes T. atroviride (see above) [21], the rice blast fungus Magnaporthe oryzae [22], and Aspergillus nidulans (H Haas, personal communication; Fishet et al. unpublished results), as well as basidiomycetes and zygomycetes [23*,24,25**,26]. The zygomycete Phycomyces blakesleeanus produces up to 10 cm high sporangiophores that bend towards near-UV light and away from far-UV light. In the 1960s, the Nobel Laureate Max Delbrück had already isolated diverse Phycomyces strains with defective phototropism, of which the corresponding madA gene was recently discovered to encode a WC–1-like photoreceptor [25**].

The red-light response

Phytochromes were discovered in plants on the basis of the observation that seed germination of Lactuca sativa is inhibited by far-red light, and this effect is reversed by subsequent illumination with red light [27]. Phytochromes are a family of red/far-red-responsive photoreceptors using a linear tetrapyrrole (named bilin) as the chromophore for light sensing [28]. The attachment of the chromophore is an autocatalytic process resulting from an intrinsic bilin lyase activity of the phytochrome protein. Phytochromes switch between two stable conformations: a red-absorbing (Pr) form and a far-red-absorbing (Pfr) form. This so-called photoconversion involves a Z → E isomerisation in one double bond of the bilin, resulting in a conformational change in the phytochrome protein. All phytochromes share a common or general structure consisting of an N–terminal input module and a C–terminal regulatory module. The photosensory input domain comprises PAS, GAF (cGMP-specific phosphodiesterases; cyanobacterial adenylate cyclases; formate hydrogen lyase) and PHY (phytochrome) subdomains and harbours the chromophore attachment site, which is, depending on the organism, localized in the GAF or in the PAS domain. In the case of fungi and eubacteria, the output module is comprised of a histidine kinase domain (HKD) and a response regulator domain (RRD), whereas plant phytochromes possess only the HKD separated by two PAS domains from the photosensory module (Figure 2). Recently, a breakthrough was achieved with the crystal structure of the chromophore-binding domain from the Deinococcus radiodurans phytochrome with a 2.5 Å resolution [29*] and the structures of two phytochrome-related response regulators (with a resolution below 2 Å) from the cyanobacterium Calothrix PCC7601 [30].

In several fungi, red-light responses — reminiscent of the plant phytochrome response — have been reported, for example in A. nidulans. This fungus reproduces asexually with conidiospores and sexually with ascospores. Whereas asexual reproduction occurs at wavelengths of 680 nm, sexual spore formation is favoured at wavelengths of 740 nm, or in the dark [31]. Surprisingly, the peaks in the difference spectrum obtained with purified FphA expressed in Escherichia coli (at 705 nm and 758 nm) are slightly distinct from the peaks in the action spectrum. Whether this is because of different chromophores in E. coli and in A. nidulans has yet to be analyzed.

Another example of a phytochrome response related to the fungal phytochrome response is found in the true slime-mould Physarum polycephalum, where fragmentation of the plasmodium and sporulation can be induced by far-red light and the induction of sporulation can be suppressed by a red light pulse [32,33]. Thus, the Physarum phytochrome appears to act in reverse when compared to classical phytochrome effects, and is reminiscent of the high-irradiance response in Arabidopsis.

In filamentous fungi, phytochromes were identified in the genomes of several species, but were only analyzed in some detail in N. crassa and A. nidulans. The presence of a response regulator domain in the fungal proteins points to a bacterial origin of phytochromes from an ancient two-component system. In N. crassa two phytochrome-coding genes were identified, phy-1 and phy-2. The expression of both phy genes is not regulated by light, but the transcription of phy-1 appears to be under the control of the circadian clock. The function of the phytochromes in
N. crassa remained unclear, because the phy-deletion strains displayed no abnormalities in any known photoresponses [34]. By contrast, deletion of the single phytochrome gene, fhpA, in A. nidulans caused a developmental phenotype [35**]. Corresponding deletion strains produce more ascospores under light conditions than did the wild type, indicating that the repression of sexual development is overcome. However, the de-repression is not complete, showing that A. nidulans is still able to sense and react to red light. This result points to additional photoreceptors, which remain to be discovered. Meanwhile, we have preliminary results suggesting cross-talk occurs between the phytochrome and the blue-light sensing white collar system (Purschwitz et al., unpublished results).

In A. nidulans, phytochrome appears not to be the only component used for red-light sensing; A. nidulans laboratory strains harbour another mutation, which makes them light-insensitive. The gene was named ve/cer. This mutation is very useful, because corresponding strains conditare well in the dark, and thus light is not required during growth and spore production. It was only recently that the gene was cloned and analyzed [36]; it doesn’t display any significant sequence homology or domain conservation, but is conserved among filamentous fungi. Very recently it was shown that Velvet (VeA) shuttles in a light-dependent manner between the cytoplasm and the nucleus (A Calvo, personal communication). However, there is no evidence that it could also act as a light-sensor, and thus it is conceivable that it interacts genetically or even physically with the phytochrome. The first hints for such an interaction were obtained in our laboratory (Kastner et al., unpublished results). VeA not only regulates the balance between asexual and sexual development but also the production of secondary metabolites [37].

A role for opsins in fungi?

Opsins are retinal-binding proteins, with seven transmembrane helices (7TM), capable of absorbing light, either for signalling (e.g. visual rhodopsins of animal eyes) or for energy-conservation purposes (e.g. archaenal rhodopsins). Although fungi do not have eyes and are not able to use light for energy conservation, they do contain proteins with similarities to opsins. In the N. crassa opsin NOP-1 the chromophore is buried in a pocket within the 7TM structure, and bound by a protonated Schiff base to a lysine. The absorption of green light (λmax 534 nm) leads to an all-trans → 13-cis isomerisation of retinal, followed by the deprotonation of the Schiff base, resulting in a near-UV-absorbing intermediate. Archaeal rhodopsins employ this mechanism in order to pump protons over the plasma membrane and act predominantly as light-driven ion transporters. By contrast, the reaction cycle of NOP-1 is far too long (up to seconds) to operate as an effective ion pump, suggesting rather that it has signalling functions. However, deletion of nop-1 does not cause any discernible phenotype [38,39].

Conclusions

Although it has been known for a long time that fungi sense and react to light, it is only recently that the molecular machinery has been studied in some cases. So far, only very few components have been characterized and the availability of several fungal genome sequences, in combination with the steadily increasing toolbox for the manipulation of fungi, promises a fruitful future for the investigation of light sensing in fungi. Because light not only controls developmental decisions but also the production of secondary metabolites, the results might have implications for biotechnological metabolites, in which fungi play increasing roles in the production of low-molecular weight, as well as high molecular weight compounds.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


