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Review

Trichoderma in the light of day – Physiology and development

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ABSTRACT

In recent years, considerable progress has been made in the elucidation of photoresponses and the mechanisms responsible for their induction in species of the genus *Trichoderma*. Although an influence of light on these fungi had already been reported five decades ago, their response is not limited to photoconidiation. While early studies on the molecular level concentrated on signaling via the secondary messenger cAMP, a more comprehensive scheme is available today. The photoreceptor-orthologs BLR1 and BLR2 are known to mediate almost all known light responses in these fungi and another light-regulatory protein, ENVOY, is suggested to establish the connection between light response and nutrient signaling. As a central regulatory mechanism, this light signaling machinery impacts diverse downstream pathways including vegetative growth, reproduction, carbon and sulfur metabolism, response to oxidative stress and biosynthesis of peptaibols. These responses involve several signaling cascades, for example the heterotrimeric G-protein and MAP-kinase cascades, resulting in an integrated response to environmental conditions.

Contents

1. Introduction

During evolution nearly all forms of life have been exposed to the electromagnetic radiation emitted by the sun, which for our purpose we will call light. Given the optic properties of light, it may be considered that it is non-randomly structured in time

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and space (low entropy), and such properties have to important consequences for living organisms: it can be used to produce thermodynamic work and carries information. In order to survive and compete in their natural habitat all forms of life are continuously obtaining and decoding information from their environment (including that contained in light), which they use for their own benefit.

Our sun emits light in a wide wavelength range, of which the radiation of longer wavelength is called infrared, and is mostly transformed in molecular movement (heat). Radiation of shorter wavelength, corresponds to the ultraviolet (UV), and can initiate photochemical reactions. Among the molecules that can be affected by UV, DNA must be highlighted, since the result of one of such reactions can be transmitted as a mutation to the next generation. UV radiation can also damage molecules through its capacity to initiate uncontrolled free radical reactions, in most cases involving reactive oxygen species (ROS). Additionally, visible light can indirectly act in photosensitive reactions in which ROS may be produced through energy transfer from a molecule that can be activated by light such as flavin or porphyrin. In this way, blue light is potentially harmful (Lledias and Hansberg, 2000). In this context, it is understandable that sunlight is a significant element for life, and that besides the utilization of its energy and information, during evolution many mechanisms to resist its negative effects have been selected for. Thus, light has contrasting roles in relation to life, on one side all organisms depend on its energy and information, and on the other it is potentially harmful, and even deadly. For fungi life in light requires significant adjustments in numerous regulatory processes, a fact reflected in the widespread effects on their behavior (Herrera-Estrella and Horwitz, 2007; Tisch and Schmoll, 2009).

2. The discovery of light responses and the initial experiments

Even though *Phycomyces* was probably the first fungus in which the effect of light was analyzed, the study of the "informational" use of light by plants started much earlier. Darwin became interested in plant movements such as orientation towards the sun or the escape from the excess of light provoked or influenced by blue-light, and dedicated a complete volume to them, in which he described the use of a yellow-orange solution of potassium dichromate as a filter to eliminate phototropism (Darwin, 1880). This phenomenon was considered as key to solve the identity of the blue-light photoreceptor. The similarity of the action spectra for various biological responses to blue light in organisms as diverse as plants, bacteria, ferns and fungi, was intriguing. This led to the proposal that all such responses should be controlled by the same type of photoreceptor of ancestral origin (Bergman et al., 1969), which was named simply the "near UV/blue" receptor or "Blue Light-Receptor" (BLR). Other authors adopted the nickname "cryptochrome"; a term coined by Jonathan Gressel, while studying in detail the effects of blue light in *Trichoderma* to highlight its hidden absorption (cryptic), and its preponderance in lower plants (cryptogams) and fungi (Gressel, 1979).

In several species of the genus *Trichoderma* a brief pulse of light triggers conidiation. In contrast to the organisms mentioned above this was the only obvious response of *Trichoderma* to light and hence the reason, which led to the use of this fungus as a simple photomorphogenic model.

Two action spectra of photoconidiation, which depict the relative effectiveness of different wavelengths of light in eliciting the physiological response, were determined (Gressel and Galun, 1967; Kumagai and Oda, 1969). Both action spectra show the characteristic shape attributed to the "cryptochrome", including a sharp peak in the near UV 350–380 nm, and a wider peak in the blue with a maximum at 440–450 nm. Accordingly, experiments with the riboflavin structural analog roseoflavin indicated the participation of a flavin as the photoreceptive pigment (Horwitz et al., 1984a).

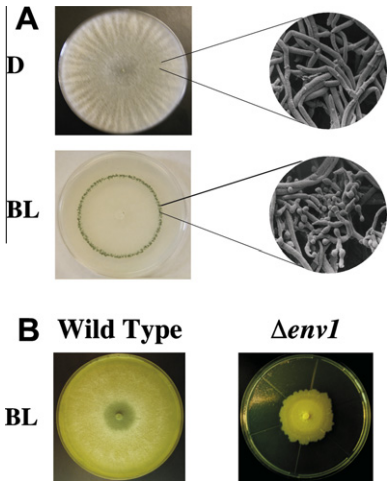

Fig. 1. Effect of blue light on *Trichoderma*. (A) The pictures show the effect of a short (5 min) pulse of blue light (BL) on *T. atroviride*. The upper photographs show a colony of *Trichoderma* growing in the dark (D). The lower photograph shows a colony of *Trichoderma* 36 h after exposure to light, with the characteristic ring of green conidia at the what was the colony perimeter at the time of exposure. Photographs at the right correspond to scanning electron micrographs of the indicated area (fine lines), hyphae (top), and hyphae and conidiophores (bottom). (B) The pictures show the dramatic effect of light on colony growth in the Denv1 mutant of *T. reesei* (left), as compared to the wild type strain (right). *T. reesei* was grown under continuous exposure to blue light (BL) for 72 h.

3. The early studies of *Trichoderma* photoresponses

The first description of the effect of light on conidiation of *Trichoderma* was made in 1957 (Gressel and Galun, 1967; Gutter, 1957). In the dark *Trichoderma* grows indefinitely as mycelium, and a brief pulse of light applied to the actively growing zone of the mycelium leads to the formation of dark green mature conidia, forming a ring at what was the edge of the colony when light was applied (Fig. 1A). The first event induced by light is a fast, first-order, photochemical reaction that does not require the presence of molecular oxygen and is independent of temperature. The fungus appears to be responsive to light (competent) only after 16 h of growth (Gressel and Galun, 1967). Three to seven hours after the induction abundant branching of aerial hyphae with an increased number of septa can be observed, as well as the formation of new aerial hyphae, leading to conidiophore development (Galun, 1971).

This developmental program can be suppressed using RNA synthesis inhibitors, such as 5-fluorouracil, once it was triggered by light, but only in a time-window of approximately 7 h after illumination (Galun and Gressel, 1966; Gressel and Galun, 1967). *T. atroviride* photoconidiation obeys the Bunsen–Roscoe law of reciprocity for pulses of blue light lasting from nanoseconds to minutes. Based on these data, it would appear that in *Trichoderma* photoconidiation is triggered by a single receptor system that is neither recycled to the photoreceptive form nor counted by enzymatic processes during or immediately following irradiation (Horwitz et al., 1990). This conclusion is supported by the observation that photoinduction is "remembered" while the culture is maintained in conditions that do not allow cellular growth (cold or absence of oxygen), as soon as growth is resumed, under optimal conditions, the colony conidiates (Gressel et al., 1975; Horwitz et al., 1990).

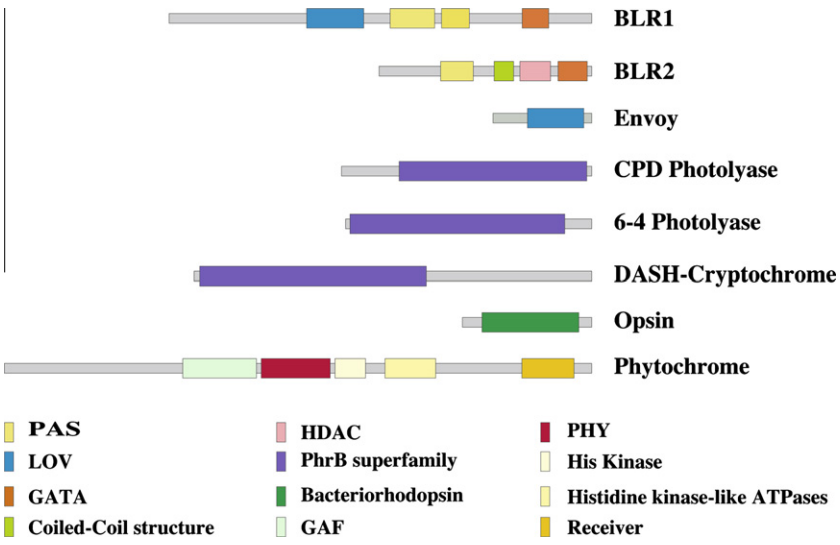

Fig. 2. Schematic representation of the eight potential photoreceptors found in *Trichoderma* species and their domain structure. Conserved domains in all putative photoreceptors were determined using Pfam (http://pfam.sanger.ac.uk/search) and SMART (http://smart.embl-heidelberg.de/). Nomenclature: HDAC histone deacetylase interaction domain; LOV, Light, Oxygen, and Voltage; PAS, domain first found in the Per – period circadian protein, Arnt – Ah receptor nuclear translocator protein, and Sim – single-minded protein; GATA, GATA-type Zinc finger; PHY, characteristic phytochrome domain; GAF, cGMP phosphodiesterase/Adenylate cyclase/FhlA; HIS kinase, Histidine kinase domain.

Recently, sexual development has been discovered in *T. reesei* (teleomorph *Hypocrea jecorina*), but so far in no other *Trichoderma* species (Kuhls et al., 1996; Seidl et al., 2009). Both, asexual sporulation and sexual development, in *T. reesei* require light (Castellanos et al., 2010; Eveleigh, 1985 Seidl et al., 2009).

4. The search for photoreceptors

Using a mutagenesis approach, Horwitz and coworkers (Horwitz et al., 1985) first attempted to isolate the photoreceptor involved in photoconidiation. They screened for *T. atroviride* mutants that did not conidiate in response to light but did in response to starvation. Despite thorough screenings they could not obtain the desired type of mutants, which in their view suggested that the photoreceptor could be essential. Interestingly, they isolated mutants that required more light than the wild type strain to get an equivalent response and overproduced a yellow pigment (*dim*Y, for *dimsighted* & *Yellow*). It was then suggested that the mutants were affected in the photoreceptor (Horwitz et al., 1985, 1986). However, the molecular nature of the *dim*Y mutants still remains to be determined.

A second strategy used to clone a blue-light photoreceptor from *T. atroviride* in the 1990s was based on the early proposal made by Benjamin Horwitz on the likelihood that such a photoreceptor could have evolved from a common ancestor for the receptor and DNA photolyases (Sancar, 1996). The approach led to the cloning and characterization of *phr1*, a gene with photolyase activity that regulates its own expression (Berrocal-Tito et al., 1999, 2007), and the closest homolog of which is the *A. nidulans* cryptochrome (*cryA*), recently shown to control sexual development (Bayram et al., 2008).

Finally, a few years ago two genes (*blr-1* and *blr-2*) from *T. atroviride* were identified (Casas-Flores et al., 2004). The genes encode transcription factors homologous to the *N. crassa* White-Collar (WC) proteins (Ballario et al., 1996; Linden and Macino, 1997; Liu et al., 2003). BLR1 has a DNA binding domain and three PAS domains (Fig. 2). The first PAS domain is considered a LOV (Light–Oxygen–Voltage) sensory domain that has all amino acids necessary to interact with the chromophore FAD, including the cysteine that forms a photoaduct with the flavin. Both BLR1 and BLR2 are essential for photoconidiation and gene expression regulated by blue light (Casas-Flores et al., 2004; Rosales-Saavedra et al., 2006). Consequently, it has been postulated that in *T. atroviride*, BLR-1 acts as the photoreceptor, in association with BLR-2. Recently, mutants in the *T. reesei* homologs of the *blr* genes have been obtained and shown to behave similarly to the *T. atroviride* corresponding mutants (Castellanos et al., 2010). Interestingly, overexpression of BLR2 resulted in enhanced sensitivity to light (Esquivel-Naranjo and Herrera-Estrella, 2007). Although BLR1 and BLR2 form a blue/UV-A receptor, these proteins also have light-independent functions. The BLR proteins are required for conidiation induced by a sudden carbon deprivation and by addition of cAMP in darkness (Casas-Flores et al., 2006). However, although sexual development requires light, it is not known yet, whether BLR1 and BLR2 are essential for this process.

In contrast to the *blr*-genes, identification of the *Trichoderma* ortholog of the second blue-light photoreceptor described in *N. crassa*, VIVID (Heintzen et al., 2001; Schwerdtfeger and Linden, 2001, 2003) was not the result of a study targeted at elucidation of photoreceptors. It was initially found as a signaling factor putatively involved in cellulase gene expression in *T. reesei* (Schmoll et al., 2004). Although not yet proven to act as a photoreceptor in *Trichoderma* species, the PAS/LOV domain protein ENVOY (encoded by *env1*) plays an important role in light responses. In fact, Δenv1 mutants express light-induced genes for a long time under constant illumination, indicating that ENVOY is a negative regulator of the light input, switching off the expression of genes regulated by BLR1 and BLR2 (Castellanos et al., 2010). Thus, ENVOY regulates photoadaptation similarly to the function performed by VIVID in *N. crassa*. Despite considerable sequence similarity, and comparable induction and regulatory characteristics, ENVOY cannot complement a VIVID non-functional mutant, suggesting that the mechanism used by ENVOY to transmit the light signal to the BLR complex may differ from that used by VIVID. The *vvd* non-functional mutant accumulates high amounts of carotenoids, and is not affected in growth. In contrast growth of the *env1* mutant is severely affected by light with reduced hyphal extension rate and loss of polar growth (Fig. 1B), indicating a function of *env1* in light tolerance (Castellanos et al., 2010; Schmoll et al., 2005). On

the other hand, mutants in *blr1* and *blr2* that do not express *env1* are not severely affected in growth (Casas-Flores et al., 2004; Castellanos et al., 2010).

In darkness, *env1* is transcribed at a very low basal level. Upon illumination, strong induction of transcription within minutes results in 50- and 500-fold increase in transcript abundance in both *T. atroviride* and *T. reesei*, respectively, which is mediated by the photoreceptors BLR1 and BLR2 (Castellanos et al., 2010; A. Herrera-Estrella et al., unpublished results). Interestingly, it was found that a light-dependent complex binds to the promoter of *env1* (Schuster and Schmoll, 2009). This observation might reflect that in addition to its transcriptional control by the BLR proteins, the recently described ENVOY upstream motif 1 (Schmoll et al., 2005) may play a relevant role in its regulation. Despite its function as a putative photoreceptor, ENVOY was shown to also have a regulatory function in darkness. Moreover, the signaling function of ENVOY must involve additional, light dependent auxiliary components or it could be post-translationally regulated by light, since overexpression of ENVOY in darkness did not eliminate the requirement of light (Schuster et al., 2007).

5. Genome based discovery

Nowadays three *Trichoderma* genome sequences are available, that of *T. reesei* (Martinez et al., 2008; http://genome.jgi-psf.org/Trire2/Trire2.home.html), *T. atroviride* (http://genome.jgi-psf.org/Triat1/Triat1.home.html) and *T. virens* (http://genome.jgi-psf.org/Trive1/Trive1.home.html). In all three fungi homologs of the photoreceptors *blr1* and *blr2*, and *env1* are present (Fig. 2). It is noteworthy that basidiomycetes and zygomycetes have no homologous of *env1/vvd*, indicating that another mechanism for photoadaptation is operating in them. It has been observed that there are light responses even in Δblr mutants (see Sections 6 & 11.1), which suggests that *Trichoderma* species should have additional genes encoding functional photoreceptors. Indeed, there is a CPD photolyase (PHR1), a DASH-cryptochrome (Ta-12806, Tv-28006 and Tr-59726) and a cryptochrome/6-4 photolyase (Ta-86846, Tv-37166 and Tr-77473) encoded in its genome (Fig. 2). In contrast, in the *N. crassa* genome there is one CPD photolyase and a DASH cryptochrome, and *A. nidulans* has only one CPD photolyase but with cryptochrome like roles. The cryptochromes/6-4 photolyases of *Trichoderma* form part of the animal cryptochromes and 6-4 photolyases subfamily and have a COOH-terminal extension of 79 aa with a similar size to animal cryptochromes and the recently described 6-4 photolyase of *Cercospora zeae-maydis* (Bluhm and Dunkle, 2008). Another notable difference is that the *Trichoderma* DASH cryptochromes have longer COOH-termini (Fig. 2) than those described in animals, plants, bacteria and other fungi. The *T. atroviride* Cry-DASH has a relatively short extension (391 amino acids) compared to those of *T. virens* and *T. reesei*, 710 and 661 amino acids long, respectively. This region is highly variable among all three *Trichoderma species* and it showed no homology to any other known protein in the databases, suggesting that the *Trichoderma* cryptochromes might form a subdivision with novel features within DASH-cryptochromes.

In *Trichoderma*, red light provokes a reduction in mycelial growth and has also an impact on the transcriptional regulation of some genes, suggesting the participation of a phytochrome in these responses (Casas-Flores et al., 2004; Rosales-Saavedra et al., 2006). As found in many fungal genomes, *Trichoderma* species, have a phytochrome containing putative GAF, phytochrome, histidine kinase, and receiver domains, characteristic of these type of photoreceptors (Fig. 2).

The impact of light on the cAMP-signaling pathway suggests that a GPCR (G-Protein Coupled Receptor) photoreceptor could be involved in this signaling pathway. Surprisingly, among *Trichoderma* species only the *T. atroviride* genome contains a gene encoding a putative sensory opsin, containing a bacteriorhodopsin superfamily domain (Ta-83833; Fig. 2), with homology to opsins ORP-1 (NCU01735.3; opsin related protein, 46% identity) and NOP-1 (NCU10055; 27% identity) from *N. crassa* (Borkovich et al., 2004), among others. However, the known light responses of *T. atroviride* and *T. reesei* are very similar, suggesting that opsin plays only a minor role, if any, in the photobiology of these species.

Homologs of other major components of the light response machinery in *N. crassa* and *D. melanogaster* (FRQ1, FWD1, BLI1, SHAGGY, TIMELESS) appear to be present in *T. reesei*, *T. virens* and *T. atroviride*. Even though homologs of FREQUENCY (*frq*) are present in the *Trichoderma* species genomes, and the *T. atroviride* gene is expressed, no obvious sign of circadian rhythms (Brunner and Kaldi, 2008) or their regulation by BLR1/BLR2 or ENVOY has been reported in these fungi.

6. Effects on gene expression

The use of cDNA microarrays representing 1438 *T. atroviride* genes allowed the discovery of 40 light-regulated genes, 30 of which were up-regulated and ten down-regulated. Surprisingly, not all light responsive genes are regulated by the BLR proteins, but all those regulated through them contain in their promoter regions GATA elements, similar to the LRE (light response element) consensus sequence described in light-regulated genes in *N. crassa* (He and Liu, 2005; Rosales-Saavedra et al., 2006). Although the participation of LREs in *Trichoderma* light responses has not been demonstrated, it would seem that those cis-elements might be functionally conserved among fungi. In fact, the *env1* gene driven by its own promoter was regulated by light in *N. crassa*, in a similar manner as in *Trichoderma* (Schmoll et al., 2005). The fact that the promoter region of the blue light down-regulated genes of *T. atroviride* also contains LRE-like elements, suggest a more complex mechanism for the control of transcriptional activity by the BLR proteins in this fungus, where combined arrangements of *cis* acting elements and additional trans-factors (i.e., co-activators or co-repressors) can be decisive for the regulatory output (Rosales-Saavedra et al., 2006).

In a recent genome wide analysis of gene expression using pyrosequencing, 331 early light-regulated genes were identified, 70 of which appear to be *blr*-independent, supporting the existence of additional, functional, light receptors. This set includes genes encoding transcription factors, DNA repair enzymes, and metabolic enzymes. Interestingly, 39 out of the 178 light-induced genes are related to stress responses. Significantly, seventeen of these stress-induced genes are related to oxidative stress. This set of genes includes key elements such as components of the MAPK (p38/Hog1) cascade. In *N. crassa*, the corresponding gene (*os-2*) is also induced by light and its phosphorylation shows circadian oscillations, regulating rhythmic expression of output genes (de Paula et al., 2008). These data suggest that light is perceived as a stress signal impacting a MAPK cascade, perhaps anticipating harmful effects of light, to provide protection against them.

In *T. reesei*, ENVOY influences gene expression not only in the light but also in darkness (Schuster et al., 2007). Nevertheless, ENVOY is dependent on the presence of additional – presumably light responsive – factors to perform its regulatory function. The variety of genes found to be regulated by light and partially by ENVOY in *T. reesei* ranges from those involved in transcription, translation and signal transduction to genes involved in metabolism and transport (Schuster et al., 2007).

Hydrophobins are also induced by light in *Trichoderma* species. In *T. reesei* it was shown that transcription of *hfb2* is influenced by

light (Nakari-Setala et al., 1997) and that the G-protein alpha subunit GNA1 is involved in this regulation (Seibel et al., 2009). Moreover, in *T. atroviride* the photoreceptors BLR1 and BLR2 contribute to adjustment of hydrophobin levels to environmental conditions (Mikus et al., 2009). The mechanism triggering hydrophobin gene expression in *Trichoderma* is likely to be highly sophisticated and involves light dependent splicing of pre-mRNA (Vargovic et al., 2006).

7. Impact of light on cellulase production

The first report identifying the signal transduction pathways that are involved in regulating cellulase gene expression in *T. reesei* was published only in 2005. Surprisingly, the first signaling component involved in regulation of cellulase gene expression was the light-regulatory protein ENVOY (Schmoll et al., 2005, 2004). It was demonstrated that transcription of cellulase genes increased during growth on cellulose in constant light compared to constant darkness. Subsequently, ENVOY was shown to be involved in the regulation of cellulase formation as well as in the light signal transduction process (Schmoll et al., 2005). Later, it was found that the *T. reesei* photoreceptor complex BLR1/BLR2 regulates cellulase gene expression and hence corroborates the link between cellulase gene expression and light response (Castellanos et al., 2010).

8. Utilization of different carbon sources

If fungi are grown under suboptimal conditions, differences in growth in light and darkness can be observed (Carlisle, 1965). For *T. atroviride* this phenomenon is confirmed by experiments analyzing growth of this fungus on numerous carbon sources in light and darkness (Friedl et al., 2008a,b). Hence the presence or absence of light appears to be relevant for utilization and/or uptake of a specific carbon source (Tisch and Schmoll, 2009) also in *Trichoderma* species.

The initial evidence of the direct influence of light on carbon metabolism came from the fact that *T. atroviride* mutants in either *blr1* or *blr2* were unable to produce conidia in response to a sudden deprivation of carbon source (Casas-Flores et al., 2004). Analysis of conidiation of *T. atroviride* on various carbon sources revealed that this process is strongly carbon source dependent both in light and darkness and that light only plays a catalytic role enhancing the extent of conidiation. Interestingly, conidiation was not completely repressed in the largely blind photoreceptor mutants Δblr1 and Δblr2, but still occurred on several carbon sources (Friedl et al., 2008a). These different conidiation patterns are suggested to be due to different redox potentials upon catabolism of the various carbon sources used. This suggestion is in agreement with the hypothesis that BLR1 and BLR2 could act as redox and oxygen sensors (Casas-Flores et al., 2006).

However, not only conidiation, but also vegetative growth on different carbon sources is influenced by light in *T. atroviride* and here mutants in *blr1* and *blr2* show a different residual response to light. From the growth patterns on these carbon sources it can be concluded that BLR1 is responsible for carbon source selectivity, but that the intensity of the response requires both BLR proteins (Friedl et al., 2008b). Consistent with these data, a comparable study in *T. reesei* also revealed an enhanced growth rate as well as indications of an oxidative stress response in light on several carbon sources. However, in contrast to the photoreceptor mutants studied in *T. atroviride*, which still showed a residual light response with respect to growth and no apparent growth defect in light, similar experiments with the *T. reesei env1* deletion strain showed a severe growth phenotype on all but one carbon source (Schuster et al., 2007).

9. Sulfur metabolism

Many sulfur compounds; especially cysteine, methionine and S-adenosylmethionine are essential for the viability of most cells. Thus, many organisms have developed a complex regulatory circuit that governs the expression of enzymes involved in sulfur assimilation and metabolism (reviewed by Marzluf (1997)). Nevertheless, a relevance of light for this process has only been investigated in *T. reesei* so far. Intriguingly, it was shown that the uptake of sulfate is dependent on the light status, and crucial for growth on cellulose in light and cannot be compensated by addition of the organic sulfur source methionine.

Transcription of the sulfur control gene *lim1*, which encodes an E3 ubiquitin ligase, is responsive to light as well as cellulase inducing conditions and regulated by ENVOY. In correlation with this result, the organic sulfur source methionine impacts cellulase gene transcription positively in darkness, but negatively in light. Therefore the presence and nature of the sulfur source must have a light dependent significance beyond just signaling the availability of a nutrient (Gremel et al., 2008).

10. Biosynthesis of secondary metabolites

Trichoderma species produce peptaibols, which are important for its antagonistic activity and represent a potential alternative to conventional antibiotics (Duclohier, 2007; Kubicek et al., 2007; Szekeres et al., 2005). The biosynthesis of peptaibols is associated with initiation of sporulation and influenced by light. Biosynthesis of secondary metabolites by fungi is dependent on the light status in many cases (Tisch and Schmoll, 2009). Therefore it is not surprising that no peptaibols have been detected in *T. atroviride* mutants of *blr1* or *blr2*, hence indicating that the photoreceptors of *Trichoderma* play an important role in regulation of peptaibol production. However, this response is also observed upon starvation and in this case independent of light or the BLR proteins. Consequently, they are not essential for this process (Komon-Zelazowska et al., 2007).

11. Links to other signaling pathways

11.1. Cyclic AMP

The effects of light in the physiology of *Trichoderma* include the hyperpolarization of the plasma membrane (Gresik et al., 1991; Horwitz et al., 1984b), increase in intracellular ATP levels, increase in the activity of adenylate cyclase, and a transient biphasic oscillation in intracellular cAMP levels, and protein phosphorylation. Such changes are associated with the activation of a signaling pathway modulated by cAMP (Gresik et al., 1988; Kolarova et al., 1992). The rapid changes in membrane potential and increase of both metabolites suggested the participation of a transmembrane receptor (Gresik et al., 1988), perhaps associated with heterotrimeric G proteins.

The addition of an analog of cAMP (dibutyryl-cAMP) to a colony growing in the dark, triggers conidiation, while that of an adenylate cyclase inhibitor (atropin) blocks light induced conidiation (Berrocal-Tito et al., 2000). Neither of these inhibitors alters the transcriptional activation of *phr-1*, suggesting the existence of two at least partially independent signaling pathways that are activated by blue light. In this sense, Casas-Flores and coworkers (Casas-Flores et al., 2006) observed an increase in cAMP dependent protein kinase (PKA) activity after a pulse of blue light. This activation occurred even in the Δ*blr*-1 and Δ*blr*-2 mutant strains, confirming the existence of an alternative system for light perception linked to cAMP. They also showed that transformants

expressing an antisense version of *pkr*-1, a gene encoding the regulatory subunit of PKA, which have increased levels of PKA activity, did not produce conidia when a pulse of blue light was applied. In contrast, decreased levels of PKA activity achieved by overexpression of the *pkr*-1 gene, result in the production of conidia even in the dark. This evidence suggests complex mechanisms involved in the cAMP-signaling pathway that regulate asexual reproduction in *T. atroviride*. However, the light receptor responsible for the activation of the cAMP-pathway has not been identified.

In *T. atroviride* PKA activity has a direct impact not only on photoconidiation, but also on the expression of genes regulated by blue light through the BLR proteins (Casas-Flores et al., 2006). Transformants with low levels of PKA activity are blocked in the activation of gene expression regulated by blue light. These results are clear evidence that PKA is involved in light perception, and it has been postulated that the BLR proteins could be a substrate for PKA, or that this kinase could regulate a factor necessary for the transcriptional activity of the BLR complex (Casas-Flores et al., 2006).

11.2. Heterotrimeric G protein signaling

In all *Trichoderma* species investigated so far, three G-protein α-subunits and one β-subunit as well as one G-protein γ subunit have been detected (Schmoll, 2008); Schmoll, unpublished data). In the course of studies on light dependent cellulase regulation, a clear impact of light on signaling via the heterotrimeric G-protein pathway became obvious. The most promising candidate for a function not only in cellulase gene expression, but also light response was GNA3, because of its putative interrelationship with cAMP signaling, which plays a role in both processes in *Trichoderma* (Sestak and Farkas, 1993; Tisch and Schmoll, 2009).

Transcription of *gna3* was indeed found to be significantly stimulated by light. Analysis of a recombinant strain expressing a constitutively active version of GNA3 resulted in strong light dependent up-regulation of the major cellulase *cbh1* (cellobiohydrolase 1) transcript. Nevertheless, this G-alpha subunit is not solely responsible for cellulase induction, since the presence of an inducer is still required (Schmoll et al., 2009). In addition to the light dependent function of GNA3 in cellulase gene expression in *T. reesei* also a function in peptaibol biosynthesis was observed in *T. atroviride*. Deletion of *gna3* abolished peptaibol formation, despite illumination or addition of cAMP and irrespective of conidiation (Komon-Zelazowska et al., 2007). Hence the signal transmitted by GNA3 is essential for peptaibol production in contrast to the light signal.

The second G-protein alpha subunit investigated in *T. reesei* (GNA1), is also involved in the light dependent regulation of cellulase gene transcription albeit in a different way than GNA3. In a *T. reesei* mutant strain lacking the *gna1* gene no transcription of *cbh1* in light on cellulose could be observed, whereas a strong increase in transcript abundance was detected under the same conditions in darkness (Seibel et al., 2009). Nevertheless, also in the case of this G-alpha subunit, expression of a constitutively active version of GNA1 did not lead to inducer independent expression of the cellulase genes on the repressing carbon sources glucose and glycerol or cause relieve from carbon catabolite repression. Hence, GNA1 is involved in light dependent regulation of cellulase gene expression, but not essential for induction of this process. In *T. atroviride*, the ortholog of *gna1* (*tga1*) was shown to play a role as negative regulator of conidiation. Transformants silenced in this gene formed conidia constitutively. In contrast, transformants overexpressing the gene or expressing a constitutively active allele were unable to produce conidia in response to light (Rocha-Ramirez et al., 2002). Intriguingly, these effects on conidiation were not observed in *T. reesei* (Seibel et al., 2009).

12. Perspectives

Although considerable progress has been made in elucidating the mechanisms of light response in *Trichoderma* and other fungi, we still lack a comprehensive understanding on how this machinery works and how its function is modulated under different environmental conditions. Our first insights into the light signaling process and its implications in *Trichoderma* species as integrated from the data reviewed above are summarized in Fig. 3. It will be

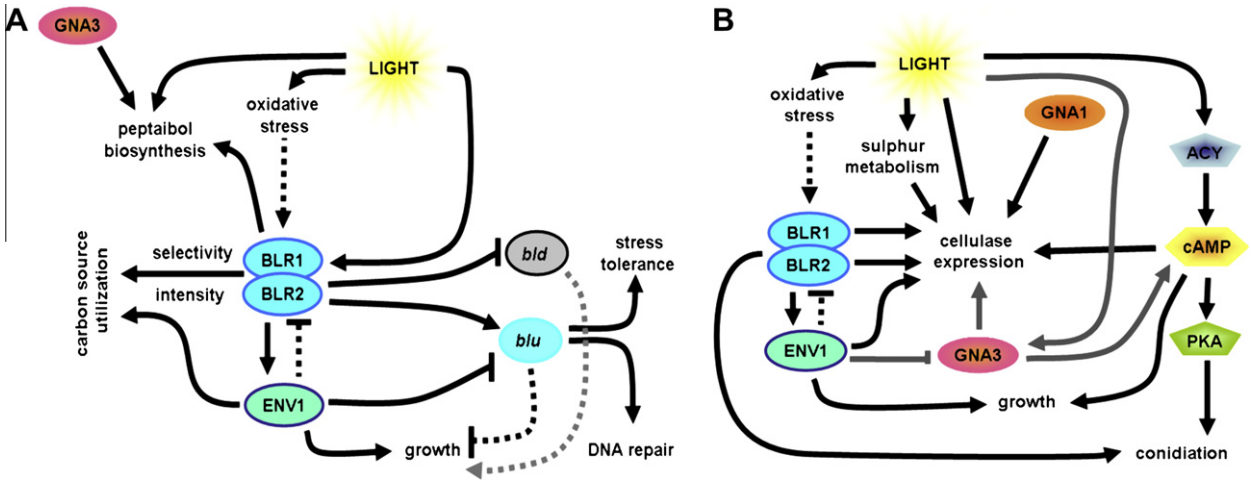

Fig. 3. Diagram of the signaling network involved in regulation of light responses in *Trichoderma* species. The diagram has been divided in two sections for simplicity. (A) The photoreceptor complex BLR1/BLR2 controls expression of blue light up-regulated (blu) and down-regulated (bld) genes. The BLR proteins control transcription of the *env1* gene. ENV1 in turn establishes a feedback regulatory loop with the BLR proteins, and is involved in photoadaptation. Blu genes impact stress tolerance and DNA repair. Both blu and bld genes regulate growth. The photoreceptor BLR1/BLR2 is also crucial for light dependent carbon source utilization. In addition, light causes oxidative stress and influences peptaibol biosynthesis, which is also regulated by the G-protein alpha subunit GNA3. (B) The BLR1/BLR2 complex regulates photoconidiation, which is also influenced by a second light input involving the cAMP-pathway, constituted by adenylate cyclase (ACY), cAMP, and protein kinase A (PKA). Light enhanced cellulase gene expression involves the function of the photoreceptors BLR1/BLR2 and ENV1, the sulfur signaling pathway, the heterotrimeric G-protein alpha subunits GNA1 and GNA3 and the cAMP-pathway. Arrows indicate positive regulation and lines with a bar at the end indicate negative regulation. Solid lines are used when there is direct experimental evidence and dotted lines when the role is still hypothetical. Overlapping lines appear in gray for clarity.

important to delineate the pathways that directly or indirectly interact with components of the light signaling machinery in order to establish how a light signal is transmitted and integrated into other physiological pathways. Genome wide analyses of gene expression suggest that light may be a cue for the fungus to protect itself from the harmful effects of light, the fungus would in turn activate a variety of mechanisms to ensure its preservation. However, the link of the light signal to other stress responses requires further investigation. Similarly, it will be important to determine whether light-induced ROS have detrimental consequences or function as signaling molecules in light responses (Aguirre et al., 2005). Knowledge on the underlying processes will not only enable us to exploit these properties for the countless applications of *Trichoderma* species, but we will also gain a deeper insight into how fungi manage to survive and succeed in their natural habitat.

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