Published in final edited form as:

Fungal Genet Biol. 2010 November ; 47(11): 922–929. doi:10.1016/j.fgb.2010.07.005.

Neurospora illuminates fungal photoreception

Chen-Hui Chen¹, Jay C. Dunlap¹, and Jennifer J. Loros^{2,3}

¹Department of Genetics, Dartmouth Medical School, Hanover, NH 03755

²Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755

Light not only is indispensable as an energy source for life on earth but also serves as an essential environmental cue conveying the information of daily and seasonal time to organisms across different kingdoms. Although the molecular mechanisms underlying light responses are actively explored in various light-sensitive organisms, these studies are either hindered by the complexity of the systems or an incomplete familiarity with the light signaling components involved in the scheme. Therefore, study of a simple and well-characterized model system is desirable to expand our knowledge of basic properties underlying the regulation of biological light responses. This review will briefly introduce the basic light sensing machinery in *Neurospora crassa*, a filamentous fungus, and then focus on the most recent advances in employing *Neurospora* as a model to study light signaling cascades, photoadaptation, and circadian clock-modulated effects in eukaryotic cells. Also, we will summarize the functions of a number of putative photoreceptors in *Neurospora*, and discuss the implications of the study of *Neurospora* to the field of fungal photobiology and some challenges for future studies.

Photobiology and the basic light sensing machinery in *Neurospora*

Light responses in the fungal kingdom, a family comprising an estimated 1.5 million species (Casadevall et al., 2008), have been extensively studied for several decades in the long-established model system *Neurospora crassa*. Light affects a variety of physiological processes in *Neurospora*, including entrainment and resetting of the circadian clock, biosynthesis of the photo-protective pigments, induction of asexual conidiospores, development of sexual structures, and the direction of ascospore dispersal (Bahn et al., 2007; Corrochano, 2007; Dunlap and Loros, 2005; Herrera-Estrella and Horwitz, 2007; Idnurm and Heitman, 2005a). Underlying these biological phenomena is the regulation of many *Neurospora* genes by light. Many studies have focused on understanding light-induced gene regulation of individual or specific groups of genes (Arpaia et al., 1995a; Bell-Pedersen et al., 1996; Corrochano et al., 1995; Kaldenhoff and Russo, 1993; Lauter et al., 1992). Recently, of the 5,600 detected genes on a whole-genome microarray, approximately 5.6% or 314, responded to a light stimulus by a relatively rapid increase in transcript amount (Chen et al., 2009). This ratio is higher but close to a prior estimate (3%) from spotted cDNA microarrays covering 1343 genes (Lewis et al., 2002). Based on the timing of induction, most of these light-responsive genes can be classified as either early or late light responders, with average peaks of mRNA expression at approximately 15 to 30 and 60 to 90

© 2010 Elsevier Inc. All rights reserved.

³To whom correspondence should be addressed. jennifer.loros@dartmouth.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

minutes, respectively. Reflecting the temporal regulation of various cellular events, genes related to distinct functional categories are enriched in different stages of light responses.

To perceive light, organisms must be equipped with proteins capable of reacting to light, mostly through a light-dependent, reversible covalent binding to a chromophore through which the energy of light is absorbed and transferred. The proteins competent in responding to light with a reversible conformational change (i.e. photocycle) are referred to as photoreceptors. After decades of studies, all known light responses in *Neurospora* are restricted to near UV/blue-light, suggesting the presence of a blue-light photoreceptor(s) (Chen and Loros, 2009; Linden et al., 1997; Sargent and Briggs, 1967). Extensive genetic screenings and molecular characterization have led to the identification of only two blind mutants, *wc-1* and *wc-2* (Ballario et al., 1996; Collett et al., 2002; Lee et al., 2003; Linden et al., 1997; Linden and Macino, 1997). WC-1 is both a flavin adenine dinucleotide (FAD) binding photoreceptor and a GATA zinc finger transcription factor (Ballario et al., 1996; Froehlich et al., 2002; He et al., 2002). Through their PAS (PER-ARNT-SIM) domains (Ballario et al., 1998; Cheng et al., 2002; Cheng et al., 2003; Crosthwaite et al., 1997), WC-1 and WC-2, another GATA zinc finger transcription factor, form the White Collar Complex (WCC) to transfer light signals to their target genes. WC-1 senses light through a flavin chromophore binding LOV domain (light, oxygen or voltage) (Froehlich et al., 2002; He et al., 2002). LOV domains are a PAS domain variant initially found in the phototropins, a family of blue-light sensitive plant photoreceptors first identified in *Arabidopsis* (Christie et al., 1999), and also found in the *Neurospora* VVD protein discussed below. Upon illumination, the chromophore becomes covalently bound to a cysteine in the LOV domain and undergoes a photochemical cycle, or photocycle, reflecting structural changes in the protein and these changes can be monitored by absorbance or fluorescence spectroscopy (Briggs, 2007). After light activation, WC-1 polymerizes and the WCC transiently binds to the promoter of early light-responsive genes at defined elements within 15 minutes or less (Chen et al., 2009; Cheng et al., 2003; Froehlich et al., 2002; He and Liu, 2005; Olmedo et al., 2010a) to activate transcription, presumably through chromatin remodeling events. WCC-dependent histone modifications have been associated with the light activation of *al-3* (Grimaldi et al., 2006) and *frq* (Belden et al., 2007) genes. Notably, over-expression of WC-1 in the dark is not sufficient to activate gene expression (Lewis et al., 2002). Through a transient light-induced phosphorylation, hyperphosphorylated WC-1 is degraded and removed from the light-responsive elements (He and Liu, 2005; Schafmeier et al., 2005; Talora et al., 1999). Although WC-1 and WC-2 control most light responses in *Neurospora* (Chen et al., 2009; Collett et al., 2002; Lee et al., 2003), some transient, low amplitude light responses have been found in the absence of *wc-1* (Chen et al., 2009). In addition to its ability to initiate light responses, the WCC has function in the dark as a central circadian clock component in *Neurospora*, responsible for activating expression of the *frequency* (*frq*) gene. Studies exploring this function have been reviewed in detail elsewhere (Dunlap et al., 2007; Heintzen and Liu, 2007; Liu and Bell-Pedersen, 2006).

Light signaling cascades in *Neurospora*: light-responsive transcription factors pave the way

To differentially activate light responses in a temporal fashion, the WCC might initiate a hierarchical activation of regulatory factors to induce waves of light responses with distinct timing, presumably through a sequential activation of transcription factors. As predicted, by following light responses in a number of light-inducible transcription factor knockout strains, a GATA family transcription factor called *sub-1* (*submerged protoperithecia-1* whose knockout strain is defective in protoperithelial formation (Colot et al., 2006)), was identified as both light-responsive and essential for regulating most of the late light responsive genes (Chen et al., 2009). Consistent with *sub-1*'s induction as an early light

responsive gene, the WCC can directly bind to the early light-responsive elements (ELREs) on the *sub-1* promoter within 15 minutes after lights on. Interestingly, several genes affected in the $\Delta sub-1$ strain remain transcriptionally responsive to light stimuli but with a lower amplitude, indicating that SUB-1 is not the sole regulator for activating these genes, and may modulate the action of other regulator(s) accordingly. In contrast to the well-established binding kinetics between the WCC and the ELREs, the molecular connection between SUB-1 and late light-responsive elements (LLREs) remains to be determined (Chen et al., 2009). Several transcription factors in addition to WC-1 and SUB-1 are identified to be light-responsive, but the respective knockout strains do not show changes in gene induction within the two hours of light stimulation given to vegetative hyphae growing in liquid culture, regardless of their various defects in light-regulated development (Chen and Loros, 2009; Chen et al., 2009). This suggests that their roles in propagating light signals might become evident at specific, yet to be identified, developmental stages or metabolic conditions.

Notably, despite differences in the timing of induction and the signaling components involved, the microarray analysis also indicates that the late light responses still share many regulatory features with the early light responses (e.g. complete dependence upon functional WCC to respond to light; the induction amplitude modulated by both FRQ and VVD, see discussion below).

Photoadaptation in *Neurospora*: a *vivid* solution

VVD has been the most intensely studied photoreceptor in *Neurospora* after the WCC, but whose molecular actions have been less understood. Mutants in *vvd* were identified due to the unique bright orange appearance of the mycelia when grown in constant light (Heintzen et al., 2001; Schwerdtfeger and Linden, 2001; Shrode et al., 2001), presumably due to the persistent activation of carotenoid biosynthesis (Chen et al., 2009; Olmedo et al., 2010b). Studies have shown that VVD acts as a universal repressor to WCC activity for most light responses (Chen et al., 2009; Heintzen et al., 2001; Schwerdtfeger and Linden, 2001; Schwerdtfeger and Linden, 2003; Shrode et al., 2001). In *vvd* mutants, once gene transcription is turned on by light-activated WCC, the level of transcription will remain up-regulated for many hours in constant light, referred to as “photoadaptation defects”. In contrast, in a wild-type strain, elevated levels of light-induced gene transcription is transient, usually returning to pre-induction baseline within two to four hours (Arpaia et al., 1999; Chen et al., 2009; Lauter and Yanofsky, 1993; Navarro-Sampedro et al., 2008; Schwerdtfeger and Linden, 2001; Schwerdtfeger and Linden, 2003). Some genes display features of a slower or imperfect adaptation and may stay elevated after 4 hours of constant light, like *frq* or *vvd* while others, although repressed, do not return to dark baseline levels within this time frame, and others, such as *al-3*, *sub-1* and *wc-1*, that display a fast return to dark, baseline levels. Interestingly, in the presence of *vvd*, once photoadaptation repression takes place, light responses can be transcriptionally re-activated by a new light stimulus if the culture was returned to the dark (Arpaia et al., 1999; Schwerdtfeger and Linden, 2001) or by an increase in light intensity if the culture has remained in the light (Arpaia et al., 1999; Chen et al., 2010; Schwerdtfeger and Linden, 2003).

Molecularly, VVD is a small, 21 kDa flavin-binding blue-light photoreceptor consisting a LOV domain and an N-terminal cap (Zoltowski et al., 2007). Upon light activation, the formation of a protein-flavin covalent bond within the LOV domain induces a reversible conformational change with a very slow photocycle (Zoltowski et al., 2009). Point mutations in either cysteine 108 that forms a cysteinyl-flavin adduct in response to blue light, or cysteine 71, at the hinge predicted to be essential for allowing the N-terminal cap to swing in response to light, result in a loss-of-function VVD (Schwerdtfeger and Linden, 2003;

Zoltowski et al., 2007). In solution, VVD forms a rapidly exchanging dimer in light, raising the possibility that the VVD LOV domain might interact in vivo with PAS domains from other proteins like those in WC-1 or WC-2 (Lamb et al., 2009; Zoltowski and Crane, 2008). In addition to repressing the WCC activity in constant light, VVD also regulates various WCC-mediated circadian clock properties in darkness, including gating of light input to the clock, maintenance of a daytime oscillator promoting dusk resetting, and temperature compensation of the clock phase (Elvin et al., 2005; Heintzen et al., 2001; Hunt et al., 2007). Until recently, the subcellular localization of VVD and the WCC appeared to conflict with the strong genetic data supporting a robust connection between the two proteins. Fractionation studies found VVD to localize exclusively in the cytosol while the majority of the WCC resides in the nucleus (Denault et al., 2001; Hong et al., 2008; Schafmeier et al., 2008; Schwerdtfeger and Linden, 2003). Small proteins might leak out of the nucleus during the process of nuclei purification and recently two groups have independently demonstrated nuclear, as well as cytoplasmic, localization of GFP-VVD as well as biochemical interaction between VVD and the WCC (Chen et al., 2010; Hunt et al., 2010). Using live cell imaging, a fully-functional version of GFP-conjugated VVD (48 kDa) accumulates in the nucleus upon light induction, and this accumulation is shown to be independent of light activation. Additionally, VVD can directly interact with the WCC and the level of interaction between VVD and the WCC is correlated with the levels of WCC repression in constant light. Raising the amount of VVD protein in the cell before exposure to light results in effective repression of the light responses within 15 minutes, indicating that VVD alone is sufficient to alter the dynamics of photoadaptation, thus suggesting a molecular explanation of the photoadaptation process. Similar work is underway in the Brunner lab (Malzahn et al., 2010).

Light responses and the clock in *Neurospora*: *frequency* determines the amplitude

In natural environments with light-dark cycles, the circadian clock is essential for organisms to anticipate and be prepared for upcoming changes in the environment (Dodd et al., 2005; Michael et al., 2003; Woelfle et al., 2004). In *Neurospora* as in many other light-sensitive organisms, light resets the phase and entrains the period of the biological clock (Crosthwaite et al., 1995), while the clock also regulates the amplitude of light inputs (Chen et al., 2009; Heintzen et al., 2001; Merrow et al., 2001) and photoperiodism (Tan et al., 2004). Molecularly, the WCC not only is the primary photoreceptor complex in the *Neurospora* cell but also is the positive factor at the core of the circadian oscillator (Crosthwaite et al., 1997; Dunlap and Loros, 2006; Liu and Bell-Pedersen, 2006). A well-described transcription-translation-based auto-regulatory negative feedback loop (primarily involving both WC proteins, FRQ and an associated helicase are integral to the *Neurospora* clock system. On a daily basis and in the dark the WCC complex is responsible for the rhythmic activation of *frq* transcription, providing the “positive element” to the feedback loop. FRQ protein, as the “negative element” in the negative-feedback loop of the clock, blocks its own transcription by mediating repression of WCC transcriptional activity by physically binding to the WCC (Dunlap, 1998; Froehlich et al., 2003; Heintzen and Liu, 2007; Schafmeier et al., 2005). There are numerous points of regulation and delay built into the basic feedback loop, including at the highly complex frequency transcription unit, in the surprisingly large number of phosphorylations events on the FRQ and WC-1 proteins by several kinases resulting in a temporally regulated cascade of phospho-isoforms and finally, the creation of additional interlocked feedback loops when FRQ positively regulates WC-1 protein synthesis (Lee et al., 2000) and increases the abundance of *wc-2* mRNA (Cheng et al., 2001). Depending on time-of-day, the activation of the WCC by light is affected by the amount of FRQ in the system (Heintzen et al., 2001; Merrow et al., 2001), which oscillates

dramatically in a circadian manner. By following light responses in both *frq*⁺ and the long-period *frq*⁷ strains, microarray analysis confirms and expands this clock-modulated effects to both early and late light responses (Chen et al., 2009). In the absence of the clock (i.e. in a *frq*-null strain), the expression of WC-1 is low (Lee et al., 2000). Some light responses may be severely affected (Morrow et al., 2001; Tan et al., 2004), while some genes remain robustly light-induced (Arpaia et al., 1993; Arpaia et al., 1995b), presumably due to gene-specific requirements for the amount of the WC-1 in the system (Lee et al., 2003).

In addition to affecting the amplitude of light responses, the circadian clock is also involved in sensing seasonal changes of day length for proper responses or development (i.e. photoperiodism) as demonstrated in plants, insects, and animals (Hazlerigg and Wagner, 2006; Imaizumi, 2010; Nelson et al., 2009). In *Neurospora*, variations in photoperiod have been shown to affect various developmental processes, including asexual conidia production, sexual protoperithecia formation, and the accumulation of carotenoid pigments. As expected, a functional clock is required for these effects (Tan et al., 2004). However, the detailed molecular mechanisms remain elusive. Given the complicated and intertwined relationship between light responses and the clock, *Neurospora* may be an attractive simple system for studies in the field.

Other light signaling components in *Neurospora*

In addition to forward genetic screens, the *Neurospora* Genome Project (Galagan et al., 2003) identified three additional putative photoreceptors: two phytochrome orthologs (*phy-1* and *phy-2*) and one cryptochrome orthologue (*cry*) based on a bioinformatics approach. Although phytochromes in *Arabidopsis thaliana* are *bona fide* red-light/far red-light photoreceptors and mediate various developmental processes (Franklin and Quail, 2010), the biological function of phytochromes in *Neurospora* remains to be determined due to lack of either phenotypic or molecular abnormality in the knockout strains (Chen et al., 2009; Froehlich et al., 2005). However, *Neurospora* PHY-2 is capable of binding either biliverdin or phycocyanobilin with a photocycle *in vitro* (Froehlich et al., 2005), suggesting full photoreceptor capability involved in a yet to be discovered function. Cryptochromes on the other hand are either blue-light photoreceptors or circadian clock components in plants and animals (Guo et al., 1998; Lin and Todo, 2005; Ma et al., 2001; Somers et al., 1998; Yang et al., 2000). Genetic and biochemical analysis (Froehlich et al., 2010) indicate that *Neurospora* CRY is a DASH-type cryptochrome but displays no photolyase activity *in vivo*. The purified CRY protein is capable of binding chromophores (i.e. flavin-adenine dinucleotide and methenyltetrahydrofolate) and single/double stranded ribonucleotides. Both the transcript and the protein of *cry* are strongly induced by light in a *wc-1* dependent manner, and the transcript is circadianly regulated. However, besides a small delay of clock phase under conditions of circadian entrainment, genome-wide microarray analysis and assays for light-regulated phenotypes failed to identify any significant difference between the wild-type and the Δ *cry* strain (Froehlich et al., 2010). Another recent study using different methodology and strains reports small but statistically validated changes in expression of a few genes (Olmedo et al., 2010b), see below. Given regulatory similarities of the circadian clock machinery among eukaryotic model systems, it is somewhat surprising that the cryptochrome in *Neurospora* is neither an essential clock component, nor an indispensable photoreceptor involved in clock resetting.

Based on sequence homology with archaeal opsins, *nop-1* (New eukaryotic opsin-1) was first identified as a putative green-light photoreceptor (Bieszke et al., 1999a). Biochemical analysis indicates that NOP-1 binds to retinal and is capable of undergoing a slow photocycle (Bieszke et al., 1999b). Although extensive examination of Δ *nop-1* strains failed to detect any defects in light-regulated processes, several conidiation-specific genes appear

to be affected in the $\Delta nop-1$ strain at certain developmental stages during asexual development (Bieszke et al., 2007). However, under vegetative growth conditions, microarray analysis indicates that both early and late light responses are not affected in the absence of *nop-1* (Chen et al., 2009).

Interestingly, although most light-responses appear to be unaffected in the absence of *phy-2*, *cry* and *nop-1* (Chen et al., 2009; Froehlich et al., 2010; Froehlich et al., 2005), a recent study by Olmedo *et al* (Olmedo et al., 2010b) provides evidence that under conditions of high-intensity white light, these photoreceptors may modify light responses in a gene-specific manner, possibly through modification of WCC activity. For example, the light induction of *con-10* is slightly enhanced in the absence of *phy-2*, while the induction of the *con-6*, *al-1*, and *vvd* genes in the same strain are not affected. In addition to alternations seen in the $\Delta phy-2$ strain, similar effects were also observed in $\Delta cry-1$ and $\Delta nop-1$ strains. Although the influence of these photoreceptors appears subtle, target-specific, developmental age and stage-specific, and evident only under specific light conditions, these results suggest that the light signaling cascades in *Neurospora* are complicated and communication among various photoreceptors may be essential for organisms living in their native habitats within a range of light conditions.

In contrast to the well-defined blue-light phenotypes in *Neurospora crassa*, red-light-regulated phenotypes are more evident in *Aspergillus nidulans*. Through a phytochrome (*fphA*) and its direct downstream target *veA*, red-light promotes conidiation and represses the formation of fruiting bodies in *Aspergillus* (Blumenstein et al., 2005; Purschwitz et al., 2008). A mutation in the *veA* gene causes impaired red-light signaling, excessive asexual conidiation, and repressed sexual development (Mooney and Yager, 1990). Although neither red-light-regulated responses nor red-light-mediated phenotypes have been documented in *Neurospora crassa* (Chen et al., 2009; Froehlich et al., 2005), a *veA* orthologue, *Neurospora ve-1*, is sufficient to rescue the phenotypes of a *veA* null mutant in *Aspergillus* (Bayram et al., 2008). Except for some light-independent developmental defects (i.e. shortened aerial hyphae and increased asexual conidiation) (Bayram et al., 2008), the *Neurospora* $\Delta ve-1$ strain was recently found to have a three-fold lower accumulation of carotenoids in contrast to a wild-type strain after exposing to white light for 24 hours (Olmedo et al., 2010b), supporting *ve-1*'s potential role in the *Neurospora* light signaling response. It seems likely that additional roles for *Neurospora* PHY-1 and PHY-2 will emerge as additional stages of the life cycle are examined more closely. Nevertheless, it is somewhat surprising to find that *Neurospora* and *Aspergillus*, while both belonging to the Ascomycota, contain such a similar set of light-responding receptor proteins yet appear to use these photoreceptors in very different ways to control physiological responses to light, for example the differential response to red light to direct developmental responses. This may in part be a reflection of the quite large evolutionary distance between the two genera, very roughly estimated to be in the neighborhood of 300 million years, about the same distance between divergence of birds and mammals (Berbee and Taylor, 2010), and the quite different developmental outputs, preferred substrates and overall lifestyles of these fungi.

In addition to photoreceptors, transcription factors, and a number of signaling components, several enzymes, including PKC (protein kinase C) (Arpaia et al., 1999; Franchi et al., 2005; Loros, 2005), NGF-1 (a yeast homologue of *gcn-5* histone acetyl-transferase) (Grimaldi et al., 2006), and NDK-1 (nucleoside diphosphate kinase-1) (Ogura et al., 2001), have been implicated in affecting light responses in *Neurospora*. Alternations in PKC activity lead to changes in the level of the WC-1 protein. PKC appears to interact with WC-1 in a light-dependent manner when it is ectopically over-expressed (Franchi et al., 2005). In contrast, a *ngf-1* mutant was found to be defective in both the acetylation of histone H3 K14 and light responses (Grimaldi et al., 2006), suggesting a connection between chromatin remodeling

events and transcriptional activation by light. In the *ndk-1* mutants, several light-regulated developmental processes are severely affected (Lee et al., 2006; Ogura et al., 2001). However, unlike other light signaling components in *Neurospora*, the knockout or knockdown strains of *pkc*, *ngf-1* and *ndk-1* are either non-viable or show severe growth defects (Loros and Dunlap labs unpublished data). Clearly, it may be difficult to separate the specific activities of these kinases in regulating light responses from general pleiotropic effects arising from their roles in other cellular functions. Similarly, the enzymes involved in the detoxification of ROS (reactive oxygen species), such as CAT-1 (catalase-1) and SOD-1 (superoxide dismutase-1), are reported to affect light-regulated phenotypes in *Neurospora* (Iigusa et al., 2005; Wang et al., 2007; Yoshida and Hasunuma, 2004; Yoshida et al., 2008). Given a wide-range of biological processes in which ROS might participate, the crosstalk between ROS and the WCC activity is likely to be indirect.

Comparing *Neurospora*'s light sensing machinery to other fungi

The successful molecular analysis of the photobiology machinery in *Neurospora crassa* has fostered several subsequent studies in other fungi, demonstrating the presence of conserved light sensing components and regulatory pathways, found across phyla from the Ascomycota, Basidiomycota, and Zygomycota. Many of these efforts have been reviewed in detail elsewhere (Corrochano, 2007; Dunlap and Loros, 2006; Herrera-Estrella and Horwitz, 2007; Idnurm and Heitman, 2005a; Purschwitz et al., 2006). Our discussion here focuses on two recent studies in *Phycomyces* and *Trichoderma*, serving as examples highlighting *Neurospora*'s utility and future implications for the field of fungal photobiology.

The Zygomycetes sit at the base of the fungal evolutionary tree and are the causative agents of several serious mycoses. *Phycomyces*, as an organism, while still very poorly understood, is yet the best understood of all the Zygomycetes. Max Delbrück and co-workers began working on the Zygomycete fungus *Phycomyces blakesleeanus* as a model organism for signaling by examining phototropism (Varju et al., 1961), the ability to grow or bend towards light, leaving a legacy of mutants aberrant in their response to light called the *mad* genes (Bergman et al., 1973). Although these phototropism mutants (*madA* through *madJ*) were isolated 40 years ago, the mutants with the most severe phenotypes (i.e. *madA* and *madB*), remained unidentified until recently. By cloning and analysis of genes with homologous sequences to the *Neurospora* white-collar proteins, *madA* and *madB* were found to be *wc-1* and *wc-2* orthologs (Idnurm et al., 2006; Sanz et al., 2009), allowing researchers to begin getting a handle on these not well understood but incredibly important organisms. As with the WC-1 and the WC-2 in *Neurospora*, MADA and MADB form a transcription factor complex able to drive light responses at the level of gene expression (Sanz et al., 2009).

Trichoderma reesei is an ascomycete fungus with an important industrial application to produce cellulolytic and hemicellulolytic enzymes to degrade biomass (Martinez et al., 2008). Light not only affects the sporulation and growth of *Trichoderma*, but also regulates its capability to express cellobiohydrolase 1 (*cbh1*, a cellulase gene) (Schmoll et al., 2005). The *blr1* and *blr2* genes, orthologs of *Neurospora wc-1* and *wc-2*, are essential to activate most light responses in *Trichoderma* (Casas-Flores et al., 2004; Rosales-Saavedra et al., 2006). A *vvd* homologue has been recently identified in *Trichoderma* called ENVOY (*env1*) and research shows that the *env1* transcript is strongly induced by light (Schmoll et al., 2005) with kinetics similar to the *vvd* transcript. Moreover, the *env1* mutants also display photoadaptation defects in constant light, similar to the Δvvd strain, allowing mutant strains to grow with continuous exposure to light (Castellanos et al., 2010). It seems reasonable to predict that the ENVOY might repress the activity of BLR1 and BLR2 through a direct

physical interaction as VVD and the WCC do in *Neurospora* (Chen et al., 2010; Hunt et al., 2010).

Future challenges for studying fungal photobiology

Recent studies with *Aspergillus* and *Neurospora* have brought to light some challenges in studying fungal photobiology. In addition to red-light, *Aspergillus nidulans* was recently shown to be sensitive to blue-light under certain light conditions, mostly through its WC-1 and WC-2 orthologs, LreA and LreB (Purschwitz et al., 2008). By following light-regulated formation of asexual and sexual structures, blue-light was found to enhance the effects of red-light to promote conidiation while partially restoring the amount of cleistothecia repressed by red-light. Furthermore, by performing co-immunoprecipitation assays, Purschwitz *et al* are able to show a direct interaction between the red light-sensing phytochrome photoreceptor FphA (Blumenstein et al., 2005; Idnurm and Heitman, 2005b) and LreA/LreB, suggesting that red-light and blue-light photoreceptors might act as a complex to differentially regulate various light responses. In *Neurospora*, although red-light-regulated phenotypes remain to be discovered, several genes whose light induction depends on the WCC are affected in the absence of *phy-2* under high-light conditions (Olmedo et al., 2010b), suggesting the existence of some coordinated actions between blue-light and red-light photoreceptors in *Neurospora*. In addition to communication between photoreceptors, there may be other variables, either developmental or environmental, that determine how fungi perceive and respond to light stimuli, many of them currently unexamined. For instance, light-responsive genes involved in the development of sexual structures might be repressed in the process of asexual sporulation and vice versa. The environmental variables include light intensity, the composition of the light spectrum, the duration of light exposure (i.e. repression from photoadaptation), developmental stage, growth conditions, and the time-of-day effects (i.e. influences from the circadian clock). As demonstrated in *Neurospora*, the role of these variables could become more profound when the research focus is gradually shifted from the understanding of the primary photoreceptors to the secondary players in the system.

Summary and conclusion

Light signaling components and light responses in *Neurospora crassa* are summarized in Table 1 and Figure 1. Examination of photoperception and light responses in *Neurospora* has been instructive in elucidating some of the basic properties underlying light responsiveness in eukaryotic cells, including light signaling cascades, photoadaptation, and circadian clock-modulated effects. Furthermore, light responses have been shown to affect various aspects of fungal physiology, including growth, development, metabolism, and virulence (Chen et al., 2009; Idnurm and Crosson, 2009; Idnurm and Heitman, 2005a; Tisch and Schmoll, 2010). Many light-sensitive fungi are known animal and/or plant pathogens or have industrial applications. In addition to understanding the basic biology of the fungi, light application might represent a direct and powerful way to manipulate fungal activities. We expect that the future studies on the mechanisms of light responsiveness in *Neurospora crassa*, one of the best characterized members of the fungal kingdom, will continue to be illuminating and of great interest.

Acknowledgments

This work was supported by grants from the National Institutes of Health to J.J.L. (RO1 GM08336), to J.C.D (PO1GM68087), and by the core grant to the Norris Cotton Cancer Center at Dartmouth. We thank Judith Hertog for critical reading of the manuscript and the Fungal Genetics Stock Center, University of Missouri, Kansas City, for their invaluable service to the fungal community.

References

- Arpaia G, et al. Light and development regulate the expression of the albino-3 gene in *Neurospora crassa*. *Dev Biol*. 1995a; 170:626–635. [PubMed: 7649389]
- Arpaia G, et al. Involvement of protein kinase C in the response of *Neurospora crassa* to blue light. *Mol Gen Genet*. 1999; 262:314–322. [PubMed: 10517328]
- Arpaia G, et al. The interplay of light and the circadian clock. Independent dual regulation of clock-controlled gene *cgc-2(eas)*. *Plant Physiol*. 1993; 102:1299–1305. [PubMed: 8278550]
- Arpaia G, et al. Light induction of the clock-controlled gene *cgc-1* is not transduced through the circadian clock in *Neurospora crassa*. *Mol Gen Genet*. 1995b; 247:157–163. [PubMed: 7753024]
- Bahn YS, et al. Sensing the environment: lessons from fungi. *Nat Rev Microbiol*. 2007; 5:57–69. [PubMed: 17170747]
- Ballario P, et al. Roles in dimerization and blue light photoresponse of the PAS and LOV domains of *Neurospora crassa* white collar proteins. *Mol Microbiol*. 1998; 29:719–729. [PubMed: 9723912]
- Ballario P, et al. White collar-1, a central regulator of blue light responses in *Neurospora*, is a zinc finger protein. *EMBO J*. 1996; 15:1650–1657. [PubMed: 8612589]
- Bayram O, et al. *Neurospora crassa ve-1* affects asexual conidiation. *Fungal Genet Biol*. 2008; 45:127–138. [PubMed: 17631397]
- Belden WJ, et al. Execution of the circadian negative feedback loop in *Neurospora* requires the ATP-dependent chromatin-remodeling enzyme CLOCKSWITCH. *Mol Cell*. 2007; 25:587–600. [PubMed: 17317630]
- Bell-Pedersen D, et al. Distinct cis-acting elements mediate clock, light, and developmental regulation of the *Neurospora crassa eas (cgc-2)* gene. *Mol Cell Biol*. 1996; 16:513–521. [PubMed: 8552078]
- Berbee ML, Taylor JW. Dating the molecular clock in fungi – how close are we? *Fungal Biology Reviews*. 2010 (in press).
- Bergman K, et al. Mutants of *Phycomyces* with abnormal phototropism. *Mol Gen Genet*. 1973; 123:1–16. [PubMed: 4726375]
- Bieszke JA, et al. The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. *Proc Natl Acad Sci U S A*. 1999a; 96:8034–8039. [PubMed: 10393943]
- Bieszke JA, et al. The fungal opsin gene *nop-1* is negatively-regulated by a component of the blue light sensing pathway and influences conidiation-specific gene expression in *Neurospora crassa*. *Curr Genet*. 2007; 52:149–157. [PubMed: 17676324]
- Bieszke JA, et al. A eukaryotic protein, NOP-1, binds retinal to form an archaeal rhodopsin-like photochemically reactive pigment. *Biochemistry*. 1999b; 38:14138–14145. [PubMed: 10571987]
- Blumenstein A, et al. The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. *Curr Biol*. 2005; 15:1833–1838. [PubMed: 16243030]
- Briggs WR. The LOV domain: a chromophore module servicing multiple photoreceptors. *J Biomed Sci*. 2007; 14:499–504. [PubMed: 17380429]
- Casadevall A, et al. The Fungal Kingdom: Diverse and Essential Roles in Earth's Ecosystem. American Academy of Microbiology. 2008
- Casas-Flores S, et al. BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. *Microbiology*. 2004; 150:3561–3569. [PubMed: 15528646]
- Castellanos F, et al. Crucial factors of the light perception machinery and their impact on growth and cellulase gene transcription in *Trichoderma reesei*. *Fungal Genetics and Biology*. 2010 In Press, Corrected Proof.
- Chen CH, et al. Direct physical interaction between VVD and WCC regulates photoadaptation in *Neurospora crassa*. 2010 (manuscript submitted).
- Chen CH, Loros JJ. *Neurospora* sees the light: Light signaling components in a model system. *Commun Integr Biol*. 2009; 2:448–451. [PubMed: 19907715]
- Chen CH, et al. Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. *EMBO J*. 2009; 28:1029–1042. [PubMed: 19262566]

- Cheng P, et al. PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. *Mol Cell Biol*. 2002; 22:517–524. [PubMed: 11756547]
- Cheng P, et al. Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proc Natl Acad Sci U S A*. 2001; 98:7408–7413. [PubMed: 11416214]
- Cheng P, et al. WHITE COLLAR-1, a multifunctional *Neurospora* protein involved in the circadian feedback loops, light sensing, and transcription repression of *wc-2*. *J Biol Chem*. 2003; 278:3801–3808. [PubMed: 12454012]
- Christie JM, et al. LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (*nph1*): binding sites for the chromophore flavin mononucleotide. *Proc Natl Acad Sci U S A*. 1999; 96:8779–8783. [PubMed: 10411952]
- Collett MA, et al. Light and clock expression of the *Neurospora* clock gene *frequency* is differentially driven by but dependent on WHITE COLLAR-2. *Genetics*. 2002; 160:149–158. [PubMed: 11805052]
- Colot HV, et al. A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci U S A*. 2006; 103:10352–10357. [PubMed: 16801547]
- Corrochano LM. Fungal photoreceptors: sensory molecules for fungal development and behaviour. *Photochem Photobiol Sci*. 2007; 6:725–736. [PubMed: 17609765]
- Corrochano LM, et al. Light and developmental regulation of the gene *con-10* of *Neurospora crassa*. *Dev Biol*. 1995; 167:190–200. [PubMed: 7851642]
- Crosthwaite SK, et al. *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. *Science*. 1997; 276:763–769. [PubMed: 9115195]
- Crosthwaite SK, et al. Light-induced resetting of a circadian clock is mediated by a rapid increase in frequency transcript. *Cell*. 1995; 81:1003–1012. [PubMed: 7600569]
- Denault DL, et al. WC-2 mediates WC-1-FRQ interaction within the PAS protein-linked circadian feedback loop of *Neurospora*. *EMBO J*. 2001; 20:109–117. [PubMed: 11226161]
- Dodd AN, et al. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science*. 2005; 309:630–633. [PubMed: 16040710]
- Dunlap JC. Common threads in eukaryotic circadian systems. *Curr Opin Genet Dev*. 1998; 8:400–406. [PubMed: 9729714]
- Dunlap, JC.; Loros, JJ. *Neurospora Photoreceptors*. Briggs, WR.; Spudich, J., editors. Berlin: Wiley-VCH; 2005.
- Dunlap JC, Loros JJ. How fungi keep time: circadian system in *Neurospora* and other fungi. *Curr Opin Microbiol*. 2006; 9:579–587. [PubMed: 17064954]
- Dunlap JC, et al. A circadian clock in *Neurospora*: how genes and proteins cooperate to produce a sustained, entrainable, and compensated biological oscillator with a period of about a day. *Cold Spring Harb Symp Quant Biol*. 2007; 72:57–68. [PubMed: 18522516]
- Elvin M, et al. The PAS/LOV protein VIVID supports a rapidly dampened daytime oscillator that facilitates entrainment of the *Neurospora* circadian clock. *Genes Dev*. 2005; 19:2593–2605. [PubMed: 16264193]
- Franchi L, et al. Protein kinase C modulates light responses in *Neurospora* by regulating the blue light photoreceptor WC-1. *Mol Microbiol*. 2005; 56:334–345. [PubMed: 15813728]
- Franklin KA, Quail PH. Phytochrome functions in *Arabidopsis* development. *J Exp Bot*. 2010; 61:11–24. [PubMed: 19815685]
- Froehlich AC, et al. Genetic and molecular characterization of a cryptochrome from the filamentous fungus *Neurospora crassa*. *Eukaryot Cell*. 2010; 5:738–750. [PubMed: 20305004]
- Froehlich AC, et al. White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. *Science*. 2002; 297:815–819. [PubMed: 12098706]
- Froehlich AC, et al. Rhythmic binding of a WHITE COLLAR-containing complex to the frequency promoter is inhibited by FREQUENCY. *Proc Natl Acad Sci U S A*. 2003; 100:5914–5919. [PubMed: 12714686]

- Froehlich AC, et al. Genetic and molecular analysis of phytochromes from the filamentous fungus *Neurospora crassa*. *Eukaryot Cell*. 2005; 4:2140–2152. [PubMed: 16339731]
- Galagan JE, et al. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*. 2003; 422:859–868. [PubMed: 12712197]
- Grimaldi B, et al. The *Neurospora crassa* White Collar-1 dependent blue light response requires acetylation of histone H3 lysine 14 by NGF-1. *Mol Biol Cell*. 2006; 17:4576–4583. [PubMed: 16914525]
- Guo H, et al. Regulation of flowering time by Arabidopsis photoreceptors. *Science*. 1998; 279:1360–1363. [PubMed: 9478898]
- Hazlerigg DG, Wagner GC. Seasonal photoperiodism in vertebrates: from coincidence to amplitude. *Trends Endocrinol Metab*. 2006; 17:83–91. [PubMed: 16513363]
- He Q, et al. White collar-1, a DNA binding transcription factor and a light sensor. *Science*. 2002; 297:840–843. [PubMed: 12098705]
- He Q, Liu Y. Molecular mechanism of light responses in *Neurospora*: from light-induced transcription to photoadaptation. *Genes Dev*. 2005; 19:2888–2899. [PubMed: 16287715]
- Heintzen C, Liu Y. The *Neurospora crassa* circadian clock. *Adv Genet*. 2007; 58:25–66. [PubMed: 17452245]
- Heintzen C, et al. The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. *Cell*. 2001; 104:453–464. [PubMed: 11239402]
- Herrera-Estrella A, Horwitz BA. Looking through the eyes of fungi: molecular genetics of photoreception. *Mol Microbiol*. 2007; 64:5–15. [PubMed: 17376067]
- Hong CI, et al. Closing the circadian negative feedback loop: FRQ-dependent clearance of WC-1 from the nucleus. *Genes Dev*. 2008; 22:3196–3204. [PubMed: 18997062]
- Hunt SM, et al. The PAS/LOV protein VIVID controls temperature compensation of circadian clock phase and development in *Neurospora crassa*. *Genes Dev*. 2007; 21:1964–1974. [PubMed: 17671094]
- Hunt SM, et al. The role of VIVID in light and temperature responses of the *Neurospora* circadian system. *Fungal Genetics Reports*. 2010; 57:13.
- Idnurm A, Crosson S. The photobiology of microbial pathogenesis. *PLoS Pathog*. 2009; 5:e1000470. [PubMed: 19956669]
- Idnurm A, Heitman J. Light controls growth and development via a conserved pathway in the fungal kingdom. *PLoS Biol*. 2005a; 3:e95. [PubMed: 15760278]
- Idnurm A, Heitman J. Photosensing fungi: phytochrome in the spotlight. *Curr Biol*. 2005b; 15:R829–R832. [PubMed: 16243020]
- Idnurm A, et al. The *Phycomyces madA* gene encodes a blue-light photoreceptor for phototropism and other light responses. *Proc Natl Acad Sci U S A*. 2006; 103:4546–4551. [PubMed: 16537433]
- Iigusa H, et al. Oxygen and hydrogen peroxide enhance light-induced carotenoid synthesis in *Neurospora crassa*. *FEBS Lett*. 2005; 579:4012–4016. [PubMed: 16004990]
- Imaizumi T. Arabidopsis circadian clock and photoperiodism: time to think about location. *Curr Opin Plant Biol*. 2010; 13:83–89. [PubMed: 19836294]
- Kaldenhoff R, Russo VE. Promoter analysis of the *bli-7/eas* gene. *Curr Genet*. 1993; 24:394–399. [PubMed: 8299154]
- Lamb JS, et al. Illuminating solution responses of a LOV domain protein with photocoupled small-angle X-ray scattering. *J Mol Biol*. 2009; 393:909–919. [PubMed: 19712683]
- Lambregts R, et al. A high-density single nucleotide polymorphism map for *Neurospora crassa*. *Genetics*. 2009; 181:767–781. [PubMed: 19015548]
- Lauter FR, et al. Developmental and light regulation of *eas*, the structural gene for the rodlet protein of *Neurospora*. *Genes Dev*. 1992; 6:2373–2381. [PubMed: 1459459]
- Lauter FR, Yanofsky C. Day/night and circadian rhythm control of *con* gene expression in *Neurospora*. *Proc Natl Acad Sci U S A*. 1993; 90:8249–8253. [PubMed: 8367490]

- Lee B, et al. Photomorphogenetic characteristics are severely affected in nucleoside diphosphate kinase-1 (*ndk-1*)-disrupted mutants in *Neurospora crassa*. *Mol Genet Genomics*. 2006; 275:9–17. [PubMed: 16307287]
- Lee K, et al. Roles for WHITE COLLAR-1 in circadian and general photoperception in *Neurospora crassa*. *Genetics*. 2003; 163:103–114. [PubMed: 12586700]
- Lee K, et al. Interconnected feedback loops in the *Neurospora* circadian system. *Science*. 2000; 289:107–110. [PubMed: 10884222]
- Lewis ZA, et al. Overexpression of White Collar-1 (WC-1) activates circadian clock-associated genes, but is not sufficient to induce most light-regulated gene expression in *Neurospora crassa*. *Mol Microbiol*. 2002; 45:917–931. [PubMed: 12180913]
- Lin C, Todo T. The cryptochromes. *Genome Biol*. 2005; 6:220. [PubMed: 15892880]
- Linden H, et al. Blue light regulation in *Neurospora crassa*. *Fungal Genet Biol*. 1997; 22:141–150. [PubMed: 9454641]
- Linden H, Macino G. White collar 2, a partner in blue-light signal transduction, controlling expression of light-regulated genes in *Neurospora crassa*. *EMBO J*. 1997; 16:98–109. [PubMed: 9009271]
- Liu Y, Bell-Pedersen D. Circadian rhythms in *Neurospora crassa* and other filamentous fungi. *Eukaryot Cell*. 2006; 5:1184–1193. [PubMed: 16896204]
- Loros J. A kinase for light and time. *Mol Microbiol*. 2005; 56:299–302. [PubMed: 15813724]
- Ma L, et al. Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. *Plant Cell*. 2001; 13:2589–2607. [PubMed: 11752374]
- Malzahn E, et al. Molecular mechanism of photo-Adaptation in *Neurospora crassa*. *Fungal Genetics Reports*. 2010; 57(suppl):12.
- Martinez D, et al. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nat Biotechnol*. 2008; 26:553–560. [PubMed: 18454138]
- Morrow M, et al. Circadian regulation of the light input pathway in *Neurospora crassa*. *EMBO J*. 2001; 20:307–315. [PubMed: 11157738]
- Michael TP, et al. Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science*. 2003; 302:1049–1053. [PubMed: 14605371]
- Mooney JL, Yager LN. Light is required for conidiation in *Aspergillus nidulans*. *Genes Dev*. 1990; 4:1473–1482. [PubMed: 2253875]
- Navarro-Sampedro L, et al. A genetic selection for *Neurospora crassa* mutants altered in their light regulation of transcription. *Genetics*. 2008; 178:171–183. [PubMed: 18202366]
- Nelson, RJ., et al. *Photoperiodism: The Biological Calendar*. Oxford University Press; 2009.
- Ogura Y, et al. A point mutation in nucleoside diphosphate kinase results in a deficient light response for perithecial polarity in *Neurospora crassa*. *J Biol Chem*. 2001; 276:21228–21234. [PubMed: 11287415]
- Olmedo M, et al. Regulation by blue light of the fluffy gene encoding a major regulator of conidiation in *Neurospora crassa*. *Genetics*. 2010a; 184:651–658. [PubMed: 20026679]
- Olmedo M, et al. A complex photoreceptor system mediates the regulation by light of the conidiation genes *con-10* and *con-6* in *Neurospora crassa*. *Fungal Genet Biol*. 2010b; 47:352–363. [PubMed: 19932184]
- Purschwitz J, et al. Seeing the rainbow: light sensing in fungi. *Curr Opin Microbiol*. 2006; 9:566–571. [PubMed: 17067849]
- Purschwitz J, et al. Functional and Physical Interaction of Blue- and Red-Light Sensors in *Aspergillus nidulans*. *Curr Biol*. 2008; 18:255–259. [PubMed: 18291652]
- Rosales-Saavedra T, et al. Novel light-regulated genes in *Trichoderma atroviride*: a dissection by cDNA microarrays. *Microbiology*. 2006; 152:3305–3317. [PubMed: 17074901]
- Sanz C, et al. Phycomyces MADB interacts with MADA to form the primary photoreceptor complex for fungal phototropism. *Proc Natl Acad Sci U S A*. 2009; 106:7095–7100. [PubMed: 19380729]
- Sargent ML, Briggs WR. The Effects of Light on a Circadian Rhythm of Conidiation in *Neurospora*. *Plant Physiol*. 1967; 42:1504–1510. [PubMed: 16656687]

- Schafmeier T, et al. Circadian activity and abundance rhythms of the *Neurospora* clock transcription factor WCC associated with rapid nucleo-cytoplasmic shuttling. *Genes Dev.* 2008; 22:3397–3402. [PubMed: 19141472]
- Schafmeier T, et al. Transcriptional feedback of *Neurospora* circadian clock gene by phosphorylation-dependent inactivation of its transcription factor. *Cell.* 2005; 122:235–246. [PubMed: 16051148]
- Schmoll M, et al. Envoy, a PAS/LOV domain protein of *Hypocrea jecorina* (Anamorph *Trichoderma reesei*), modulates cellulase gene transcription in response to light. *Eukaryot Cell.* 2005; 4:1998–2007. [PubMed: 16339718]
- Schwerdtfeger C, Linden H. Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. *Mol Microbiol.* 2001; 39:1080–1087. [PubMed: 11251826]
- Schwerdtfeger C, Linden H. VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. *EMBO J.* 2003; 22:4846–4855. [PubMed: 12970196]
- Shrode LB, et al. *vvd* is required for light adaptation of conidiation-specific genes of *Neurospora crassa*, but not circadian conidiation. *Fungal Genet Biol.* 2001; 32:169–181. [PubMed: 11343403]
- Smith KM, et al. Transcription factors in light and circadian clock signaling networks revealed by genome-wide mapping of direct targets for *Neurospora* WHITE COLLAR COMPLEX. *Eukaryot Cell.* 2010 In press.
- Somers DE, et al. Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. *Science.* 1998; 282:1488–1490. [PubMed: 9822379]
- Talora C, et al. Role of a white collar-1-white collar-2 complex in blue-light signal transduction. *EMBO J.* 1999; 18:4961–4968. [PubMed: 10487748]
- Tan Y, et al. Photoperiodism in *Neurospora crassa*. *J Biol Rhythms.* 2004; 19:135–143. [PubMed: 15038853]
- Tisch D, Schmoll M. Light regulation of metabolic pathways in fungi. *Appl Microbiol Biotechnol.* 2010; 85:1259–1277. [PubMed: 19915832]
- Varju D, et al. Interplay between the reactions to light and to gravity in *Phycomyces*. *J Gen Physiol.* 1961; 45:47–58. [PubMed: 13780281]
- Wang N, et al. Loss of Catalase-1 (*Cat-1*) results in decreased conidial viability enhanced by exposure to light in *Neurospora crassa*. *Mol Genet Genomics.* 2007; 277:13–22. [PubMed: 17077971]
- Woelfle MA, et al. The adaptive value of circadian clocks: an experimental assessment in cyanobacteria. *Curr Biol.* 2004; 14:1481–1486. [PubMed: 15324665]
- Yang HQ, et al. The C termini of *Arabidopsis* cryptochromes mediate a constitutive light response. *Cell.* 2000; 103:815–827. [PubMed: 11114337]
- Yoshida Y, Hasunuma K. Reactive oxygen species affect photomorphogenesis in *Neurospora crassa*. *J Biol Chem.* 2004; 279:6986–6993. [PubMed: 14625272]
- Yoshida Y, et al. Conidiation rhythm and light entrainment in superoxide dismutase mutant in *Neurospora crassa*. *Mol Genet Genomics.* 2008; 279:193–202. [PubMed: 18084778]
- Zoltowski BD, Crane BR. Light activation of the LOV protein vivid generates a rapidly exchanging dimer. *Biochemistry.* 2008; 47:7012–7019. [PubMed: 18553928]
- Zoltowski BD, et al. Conformational switching in the fungal light sensor Vivid. *Science.* 2007; 316:1054–1057. [PubMed: 17510367]
- Zoltowski BD, et al. Mechanism-based tuning of a LOV domain photoreceptor. *Nat Chem Biol.* 2009; 5:827–834. [PubMed: 19718042]

Figure 1. Light responses in *Neurospora crassa*

In the presence of light, the White-collar complex (WCC), made up of WC-1 and WC-2, transiently binds to the early light-responsive elements (ELREs) in the promoters of early light-responsive genes to activate the first wave of light induced transcription (i.e. early light responses), including the expression of *frq*, encoding the clock protein FRQ, the photoreceptor VVD, the transcription factor SUB-1, and others. Depending on the specific time-of-day at which light is imposed, FRQ modulates the WCC activity in a circadian manner to determine the amplitude of the light responses. In response to light, VVD protein is highly expressed and accumulates in the nucleus to repress transcriptional activity through a physical interaction with the WCC. Meanwhile, the induction of SUB-1, essential for most late light responses, results in a second wave of light-induced gene expression, in many cases regulated through identified late light response elements (LLRE's) on their promoters. Several secondary photoreceptors and other proteins might affect light responses in a gene-specific or developmental stage-specific manner, including CRY, PHY-2, NOP-1, and VE-1. Finally, the roles of light-inducible transcription factors, such as CSP-1, SAH-1 and VAD-3, are yet to be determined. Components capable of sensing light directly via a chromophore are shown by the wavelength color they absorbed and marked with dashed lines. Flavin moieties are shown by yellow hexagons.

Table 1

Light signaling components in *Neurospora crassa*

NCU # ¹	Gene symbol	Structural features	Light-induced?	Light responses/phenotypes in the knockout strain	References
Components affecting all light responses					
NCU02356.4	<i>wc-1</i>	photoreceptor & transcription factor	Yes	Blind to all known light-regulated responses; k/o shows repressed carotenogenesis	Ballario et al., 1996; Froehlich et al., 2002; He et al., 2002; Cheng et al., 2003; Lee et al., 2003; He and Liu, 2005; Liu and Bell-Pedersen, 2006; Chen et al., 2009
NCU00902.4	<i>wc-2</i>	transcription factor	Yes	Blind to all known light-regulated responses; k/o shows repressed carotenogenesis	Ballario et al., 1996; Froehlich et al., 2002; He et al., 2002; Collett et al., 2002; Chen et al., 2009
NCU03967.4	<i>vvd</i>	photoreceptor	Yes	Regulates photoadaptation upon prolonged light exposure; k/o shows enhanced carotenogenesis	Heintzen et al., 2001; Shrode et al., 2001; Schwerdtfeger and Linden, 2001; Schwerdtfeger and Linden, 2003; Chen et al., 2009; Olmedo et al., 2010b
NCU02265.4	<i>frq</i>	transcriptional regulator	Yes	Regulates light input in a circadian manner; influences expression of WC-1; k/o shows repressed conidiation	Heintzen et al., 2001; Lee et al., 2000; Denault et al., 2001; Heintzen and Liu, 2007; Chen et al., 2009
Components affecting a subset of light responses					
NCU01154.4	<i>sub-1</i>	transcription factor	Yes	Some early and most late light responses are severely impaired; k/o shows submerged protoperithecia in the sexual cycle	Colot et al., 2006; Chen et al., 2009
NCU00582.4	<i>cry</i>	photoreceptor	Yes	Affects amplitude of light responses for a few genes; k/o has a slight phase delay under conditions of circadian entrainment.	Chen et al., 2009; Olmedo et al., 2010b; Froehlich et al., 2010
NCU05790.4	<i>phy-2</i>	photoreceptor	No	Affects amplitude of light responses for a few genes	Froehlich et al., 2005; Chen et al., 2009; Olmedo et al., 2010b
NCU10055.4	<i>nop-1</i>	photoreceptor	No	Affects amplitude of light responses for a few genes	Bieszke et al., 2007; Chen et al., 2009; Olmedo et al., 2010b
NCU01731.4	<i>ve-1</i>	undetermined	No	k/o shows shortened aerial hyphae, increased conidiation and reduced carotenogenesis	Bayram et al., 2008; Olmedo et al., 2010b
Components with undetermined effects on light responses					
NCU02713.4	<i>csp-1</i>	transcription factor	Yes	k/o defective in conidiospore maturation	Chen et al., 2009; Lambreghts et al., 2009
NCU04179.4	<i>sah-1</i>	transcription factor	Yes	k/o shows shortened aerial hyphae	Colot et al., 2006; Chen et al., 2009
NCU06407.4	<i>vad-3</i>	transcription factor	Yes	k/o slows basal and aerial hyphal extension	Colot et al., 2006; Chen et al., 2009
NCU03643.4	none	transcription factor	Yes	k/o shows no phenotype	Chen et al., 2009
NCU04834.4	<i>phy-1</i>	putative photoreceptor	No	k/o shows no phenotype	Froehlich et al., 2005; Chen et al., 2009; Olmedo et al., 2010b

¹NCU numbers are from Neurospora annotation (<http://www.broad.mit.edu/annotation/genome/neurospora/Home.html>).