[Communicative & Integrative Biology 2:5, 448-451; September/October 2009]; ©2009 Landes Bioscience

Mini-Review

Neurospora sees the light

Light signaling components in a model system

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Key words: light, microarray, Neurospora, photoreceptors, *wc-1*, *wc-2*, *vvd*, *frq*, *sub-1*, *nop-1*, *ve-1*

Light is a key environmental signal for most life on earth. Over 5% of *Neurospora crassa* genes are expressed in response to light stimulation in a temporally regulated cascade that includes several transcription factors. Fungal genomes, including Neurospora's, may encode several different proteins capable of binding chromophores with the ability to harvest light energy as well as proteins that can interact with primary photoreceptors or further propogate the light signal. The best understood photoreceptors are the evolutionarily conserved White Collar proteins, and the related Vivid protein, but fungi may also encode phytochromes, cryptochromes and opsins.

According to a recent report from the American Society of Microbiology, the fungal kingdom comprises an estimated 1.5 million species, many hundreds of which are known animal or plant pathogens.[1] The filamentous fungus *Neurospora crassa* is a leading research model, including studies aimed at understanding light responses in fungal cells.[2-7] Decades of effort from several labs, have established the White Collar complex (WCC) as an essential as well as dominant light signaling component. The heterodimeric transcription factor WCC senses light directly through bound FAD, and binds to the promoters of many light-responsive genes, activating gene expression. We have shown that light regulated expression falls into two distinct temporal classes, both under WCC control. SUB-1, identified as an "early" light-responsive transcription factor, was found to regulate most of the "late" light gene expression. Chromatin-immunoprecipitation (ChIP) and bioinformatics analysis further established the hierarchical relationship between early and late light responses.[8] Here, we present a brief summary of recent studies on the molecular components involved in Neurospora photobiology.

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Submitted: 04/23/09; Accepted: 04/24/09

Previously published online as a *Communicative & Integrative Biology* E-publication:
http://www.landesbioscience.com/journals/cib/article/8835

Light-Regulated Biology in *Neurospora crassa*

Light acts as an essential cue to regulate a variety of physiological processes in Neurospora, including the resetting of the circadian clock, biosynthesis of the photo-protective carotenoid pigments, asexual conidiospore formation, perithecial development in the sexual cycle, and the direction of ascospores release.[2-7] Underlying this biology is the regulation of many Neurospora genes by light. Microarrays representing the approximately 10,000 genes in *Neurospora crassa* were used as probes against light induced cDNA. Of the 5,600 detectable genes, 314 (approximately 5.6%) responded to the light stimulus by increasing transcript levels.[8] Most of the identified genes (92%) were either early (45%), with peak expression between 15 and 45 minutes, or late (55%), with the induced expression peaking between 45 and 90 minutes after lights on. Genes related to the synthesis of photoprotective pigments (7.1%), vitamins, cofactors, and prosthetic groups (4.7%), secondary metabolism (4.7%), DNA processing (6.3%), cellular signaling (5.5%) and environmental sensing and response (1.6%) were found enriched in the early light response. In contrast, genes involved in carbohydrate metabolism (20%), oxidation of fatty acids (1.9%) and oxygen detoxification reaction (2.5%) were found enriched in the late light response. Within the early group were several transcription factors, most of which show mutant phenotypes during development (see Table 1).

All currently known light responses in Neurospora are restricted to near UV/blue light,[5,7] suggesting the presence of a master photoreceptor dedicated to blue light sensing and signal transduction. Extensive genetic screening and analysis has resulted in the isolation of only two fully blind mutants, *wc-1* and *wc-2*,[3,9,10] both GATA family zinc finger transcription factors.[11] The direct connection between light sensing and gene activation has subsequently been demonstrated both in vitro and in vivo.[12-15] The photoreceptor WC-1 forms an obligate complex with WC-2 to bind to specific DNA sequences,[16,17] including the promoter of a light-responsive transcription factor, *sub-1*, the function of which is essential for the late light response.[8] Several additional components are or may be involved in the light signaling mechanism (Fig. 1). As the WCC has been reviewed in some detail elsewhere,[2-7] our discussion below will focus on the additional players.

Table 1 **Real and putative light signaling components in *Neurospora crassa***

NCU #[1]	Gene	Light-sensing chromophore	TF?	Light-related or other phenotypes in mutants	Light-responsive[2]	Refs
NCU02356.2	*wc-1*	FAD	Yes	Blind to most if not all light responses, carotenogenesis repressed in mycelia	+	2–8
NCU00902.2	*wc-2*	None	Yes	Blind to most if not all light responses, carotenogenesis repressed in mycelia	No	2–8
NCU03967.2	*vvd*	FAD	No	Affects photoadaptation, excess accumulation of carotenoids	+ + +	8, 18–21
NCU04834.2	*phy-1*	Undetermined	No	Wild-type light responses, k/o wild-type	No	8, 28
NCU05790.2	*phy-2*	Biliverdin or Phycocyanobilin	No	Wild-type light responses, k/o wild-type	No	8, 28
NCU00582.2	*cry*	FAD	No	Wild-type light responses, k/o wild-type	+ + +	8, 29
NCU10055.2	*nop-1*	Retinal	No	Wild-type light responses, involved in late-stage asexual development	No	8, 31–33
NCU02265.2	*frq*	None	No	Affects amplitude of light induced gene expression	+	8, 34, 35
NCU01154.2	*sub-1*	None	Yes	Affects some early and most late light responses, submerged protoperithecia in the sexual cycle	+	8, 36
NCU02713.2	*csp-1*	None	Yes	Wild-type light responses, defective in conidiospore maturation	+	8, 37
NCU04179.2	*sah-1*	None	Yes	Wild-type light responses, shortened aerial hyphae	+	8, 36
NCU06407.2	*vad-3*	None	Yes	Wild-type light responses, slowed basal and aerial hyphal extension	+	8, 36
NCU03643.2	None	None	Yes	Wild-type light responses, k/o wild-type	+	8
NCU01731.3	*ve-1*	None	No	Wild-type light responses, shortened aerial hyphae, increased conidiation	No	40

[1]NCU numbers are from Neurospora annotation (http://www.broad.mit.edu/annotation/genome/neurospora/Home.html). [2]Fold change of mRNA transcripts in response to a white light stimulus; +, less than 10-fold; + +, 10-~100-fold; + + +, more than 100-fold. The data adapted from ref. 8.

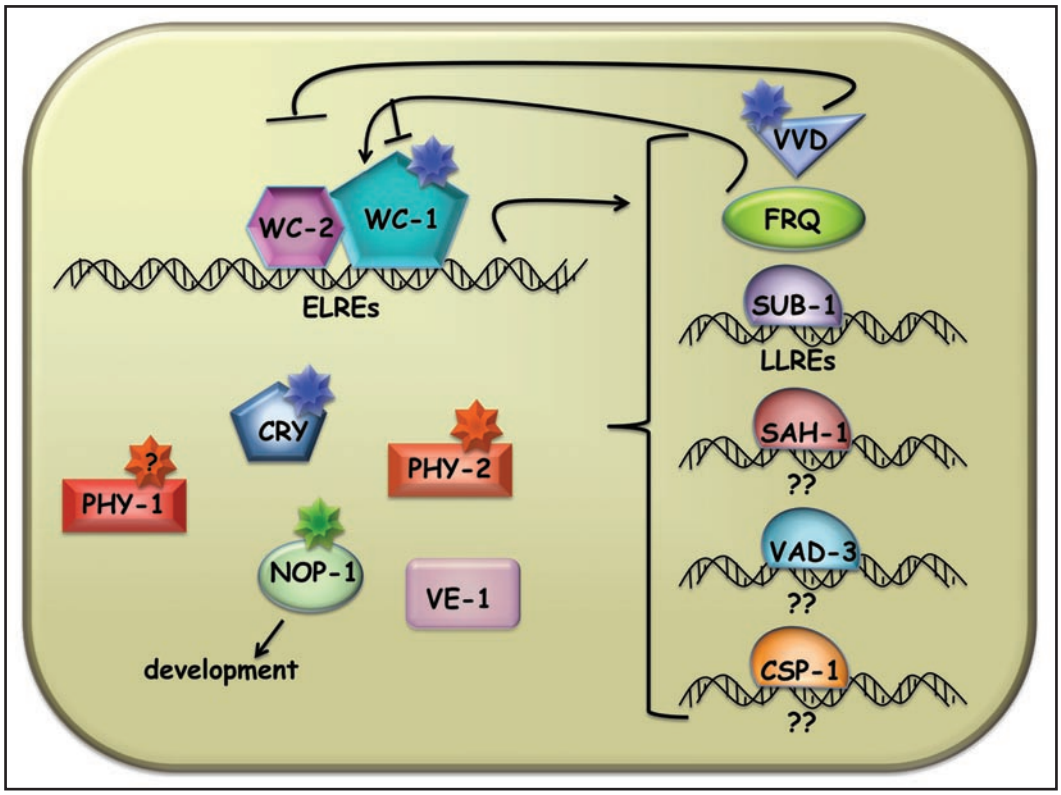

Figure 1. Established and putative molecular components involved in Neurospora light signaling. WC-1 and WC-2 form a heterodimeric transcription factor (WCC) that binds to early light responsive elements (ELREs). In response to a light signal, transcription is rapidly activated, resulting in the expression of several downstream transcription factors, as well as the VVD and FRQ proteins. The SUB-1 transcription factor is required for expression of most late-light responsive genes, many of which have a specific late light responsive element (LLRE), A/GTGAC/TG/ATCA. VVD acts as a potent repressor of WCC activity on light regulated genes. FRQ may block further activity on some genes while at the same time promotes expression of the WCC. Several proteins can bind chromophores (WC-1, PHY-1, NOP-1, VVD, CRY and maybe PHY-2). Chromophores are shown as stars in the color of light they absorb. Several proteins have no described responses to light in Neurospora (CRY, PHY-1, PHY-2, VE-1) although they do in other fungi and other organisms.

Real and Putative Light Signaling Components in *Neurospora crassa*

Real and putative light signaling components in Neurospora are summarized in Table 1. After the WCC, VVD has been the next most intensely studied photoreceptor in the fungi. Our study and others have clearly shown that VVD acts as a universal repressor for most if not all light-induced gene expression controlled by the WCC.[8,18-21] In *vvd* mutants, once gene transcription is turned on by the light-activated WCC, transcript levels will remain upregulated for many hours in constant light, so-called "photoadaptation defects". In contrast, in a wild-type strain, light-induced gene expression is transient, usually returning to pre-induction levels within two to four hours.[8] Molecularly, VVD is a small, 21 kD flavin-binding photoreceptor consisting of a LOV (light, oxygen or voltage) domain and N-terminal cap.[22] Upon light activation, the formation of a protein-flavin bond in the LOV domain induces a conformational change at the N-terminus which appears to be essential for the light function of VVD.[22] Formation of a rapidly exchanging VVD dimer in light has been proposed recently.[23] Interestingly, VVD appears to localize exclusively in the cytosol while the majority of WCC is in the nucleus.[19,24] This raises the question of how VVD communicates with the WCC to repress light responses and regulate various circadian clock properties.[21,25,26] The answer to this question will certainly shed light on the molecular mechanisms of photoadaptation in general.

After completion of the Neurospora genome project,[27] two putative red-light photoreceptors (*N. crassa* phytochrome orthologs *phy-1* and *phy-2*) and one additional blue-light photoreceptor (*N. crassa* cryptochrome orthologue *cry*) were identified. Although there is yet no report of red light-regulated biology in Neurospora, a collaborative effort has shown that PHY-2 can covalently bind either biliverdin or phycocyanobilin and is capable of undergoing a photocycle in vitro.[28] The *cry* gene encodes a member of the cryptochrome-DASH family. We have found it capable of binding FAD and MTHF, with both transcript and protein levels strongly induced by blue light in a *wc-1* dependent manner.[29] However, due to the lack of a detectable phenotype or atypical light responses in the respective knockout strains,[8,28,29] the biological function(s) of PHY-1, PHY-2 and CRY remains to be discovered in Neurospora, although function has been reported for homologs in other fungi.[3,4,6,30] The opsin, NOP-1, is a putative green-light photoreceptor identified via sequence homology with archaeal rhodopsins.[31] NOP-1 has been shown to both bind retinal and undergo a slow photocycle[32] and the expression levels of several genes are known to be affected in a knockout strain during late asexual development.[33] Our microarray data, not carried past two hours after light stimulus in the knock-out strain, suggest that NOP-1 does not play a role in either early or late light regulated gene induction.[8]

Instead of sensing light directly, which requires the ability to interact with chromophores that absorb light energy, the FRQ and SUB-1 proteins are indirectly involved in light signaling. Our array analysis has confirmed and extended the role of FRQ in regulating the light function of the WCC by affecting the amplitude of induction for both types of light responses.[8] Given that FRQ is an oscillating circadian clock component and has been shown to physically interact with the WCC, previous studies[34,35] together with our recent microarray data highlight the clock-modulating effect of the light input pathway. The novel GATA family transcription factor, SUB-1, was identified as essential for regulating a subset of the early and most of the late light responses.[8] A previously described phenotype associated with the *sub-1* (*submerged protoperithecia*-1) knockout strain[36] might easily be the functional consequence of impaired late light responses (i.e., the formation of protoperithecia in Neurospora is a light-regulated developmental process), which might also hold true for developmental defects seen in knockout strains of other light-responsive transcription factors.[36,37]

Finally, the *veA* locus has been shown to be required for both light-regulated development and secondary metabolism in *Aspergillus nidulans*[38,39] and the promoter and coding sequences of the *N. crassa* ortholog, *ve-1*, is sufficient to complement the role of *veA* null mutants in *A. nidulans*.[40] However, unlike its counterpart, VE-1 knockout strains in Neurospora lack light-dependent phenotypes[40] and have largely normal gene expression in response to white light (Chen C-H and Loros J, unpublished data) suggesting that *ve-1* may not have a significant role in regulating light signals in *N. crassa*, at least under the conditions tested.

Fungal Light Signaling Components are Conserved

Sequence and functional orthologs of WC-1, WC-2 and most of the other light signaling components are widespread among the fungal kingdom. Recent studies have demonstrated that WC-1- and WC-2-like molecules in various fungal species play an essential role in mediating light signals from the Ascomycota, Basidiomycota and Zygomycota phyla.[2-4,6,7] Of broader evolutionary interest, WC-1 and the animal circadian-clock-associated bHLH transcription factors, CYC from insects and BMAL1 and NPAS2 from mammals, share a common ancestor. The bHLH transcription factors do not bind chromophores but, like WC-1 in Neurospora, they are critical for light resetting, as well as the maintenance of circadian rhythms in animals, highlighting the close evolutionary relationship between photobiology and circadian rhythmicity.[41,42] Successful work on the WCC in Neurospora has led to fundamental breakthroughs in understanding photobiology in other fungi. We predict that future work on the underlying mechanisms of Neurospora light signaling components will continue to illuminate other light-sensitive eukaryotic cells.

Acknowledgements

This work was supported by grants from the National Institutes of Health to J.J.L. (RO1 GM08336), to Jay C. Dunlap and J.J.L. (PO1GM68087), and by the core grant to the Norris Cotton Cancer Center at Dartmouth. We thank Randy Lambreghts for critical reading of the manuscript and are deeply grateful to the Fungal Genetics Stock Center at the University of Missouri, Kansas City in supporting our work with Neurospora.

References

1. Casadevall A, Heitman J, Buckley M. The Fungal Kingdom: Diverse and Essential Roles in Earth's Ecosystem. American Academy of Microbiology 2008.
2. Herrera-Estrella A, Horwitz BA. Looking through the eyes of fungi: molecular genetics of photoreception. Mol Microbiol 2007; 64:5-15.
3. Corrochano LM. Fungal photoreceptors: sensory molecules for fungal development and behaviour. Photochem Photobiol Sci 2007; 6:725-36.
4. Purschwitz J, Muller S, Kastner C, Fischer R. Seeing the rainbow: light sensing in fungi. Curr Opin Microbiol 2006; 9:566-71.
5. Dunlap JC, Loros JJ. How fungi keep time: circadian system in Neurospora and other fungi. Curr Opin Microbiol 2006; 9:579-87.
6. Idnurm A, Heitman J. Photosensing fungi: phytochrome in the spotlight. Curr Biol. 2005;15:829-32.
7. Dunlap JC, Loros JJ. Neurospora Photoreceptors. Handbook of Photosensory Receptors. Briggs WR, Spudich JL Eds. Weinheim Wiley-VCH 2005; 18:371-388.
8. Chen CH, Ringelberg CS, Gross RH, Dunlap JC, Loros JJ. Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in Neurospora. EMBO J 2009; 8:1029-42.
9. Lee K, Dunlap JC, Loros JJ. Roles for WHITE COLLAR-1 in circadian and general photoperception in *Neurospora crassa*. Genetics 2003; 163:103-14.
10. Collett MA, Garceau N, Dunlap JC, Loros JJ. Light and clock expression of the Neurospora clock gene frequency is differentially driven by but dependent on WHITE COLLAR-2. Genetics 2002; 160:149-58.
11. Linden H, Ballario P, Macino G. Blue light regulation in *Neurospora crassa*. Fungal Genet Biol 1997; 22:141-50.
12. He Q, Cheng P, Yang Y, Wang L, Gardner KH, Liu Y. White collar-1, a DNA binding transcription factor and a light sensor. Science 2002; 297:840-3.
13. Froehlich AC, Liu Y, Loros JJ, Dunlap JC. White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. Science 2002; 297:815-9.
14. Cheng P, Yang Y, Wang L, He Q, Liu Y. 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; 278:3801-8.
15. He Q, Liu Y. Molecular mechanism of light responses in Neurospora: from light-induced transcription to photoadaptation. Genes Dev 2005; 19:2888-99.
16. Belden WJ, Loros JJ, Dunlap JC. Execution of the circadian negative feedback loop in Neurospora requires the ATP-dependent chromatin-remodeling enzyme CLOCKSWITCH. Mol Cell 2007; 25:587-600.
17. Froehlich AC, Loros JJ, Dunlap JC. Rhythmic binding of a WHITE COLLAR-containing complex to the *frequency promoter* is inhibited by FREQUENCY. Proc Natl Acad Sci USA 2003; 100:5914-9.
18. Schwerdtfeger C, Linden H. Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. Mol Microbiol 2001; 39:1080-7.
19. Schwerdtfeger C, Linden H. VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J 2003; 22:4846-55.
20. Shrode LB, Lewis ZA, White LD, Bell-Pedersen D, Ebbole DJ. *vvd* is required for light adaptation of conidiation-specific genes of *Neurospora crassa*, but not circadian conidiation. Fungal Genet Biol 2001; 32:169-81.
21. Heintzen C, Loros JJ, Dunlap JC. The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating and regulates clock resetting. Cell 2001; 104:453-64.
22. Zoltowski BD, Schwerdtfeger C, Widom J, Loros JJ, Bilwes AM, Dunlap JC, et al. Conformational switching in the fungal light sensor Vivid. Science 2007; 316:1054-7.
23. Zoltowski BD, Crane BR. Light activation of the LOV protein vivid generates a rapidly exchanging dimer. Biochemistry 2008; 47:7012-9.
24. Cha J, Chang SS, Huang G, Cheng P, Liu Y. Control of WHITE COLLAR localization by phosphorylation is a critical step in the circadian negative feedback process. EMBO J 2008; 27:3246-55.
25. Hunt SM, Elvin M, Crosthwaite SK, Heintzen C. The PAS/LOV protein VIVID controls temperature compensation of circadian clock phase and development in *Neurospora crassa*. Genes Dev 2007; 21:1964-74.
26. Elvin M, Loros JJ, Dunlap JC, Heintzen C. The PAS/LOV protein VIVID supports a rapidly dampened daytime oscillator that facilitates entrainment of the Neurospora circadian clock. Genes Dev 2005; 19:2593-605.
27. Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, et al. The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 2003; 422:859-68.
28. Froehlich AC, Noh B, Vierstra RD, Loros J, Dunlap JC. Genetic and molecular analysis of phytochromes from the filamentous fungus *Neurospora crassa*. Eukaryot Cell 2005; 4:2140-52.
29. Froehlich AC, Chen C-H, Belden WJ, Loros JJ, Dunlap JC. Genetic and molecular characterization of a cryptochrome from the filamentous fungus *Neurospora crassa*. In preparation 2009.
30. Veluchamy S, Rollins JA. A CRY-DASH-type photolyase/cryptochrome from *Sclerotinia sclerotiorum* mediates minor UV-A-specific effects on development. Fungal Genet Biol 2008; 45:1265-76.
31. Bieszke JA, Braun EL, Bean LE, Kang S, Natvig DO, Borkovich KA. The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Proc Natl Acad Sci USA 1999; 96:8034-9.
32. Bieszke JA, Spudich EN, Scott KL, Borkovich KA, Spudich JL. A eukaryotic protein, NOP-1, binds retinal to form an archaeal rhodopsin-like photochemically reactive pigment. Biochemistry 1999; 38:14138-45.
33. Bieszke JA, Li L, Borkovich KA. 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; 52:149-57.
34. Tan Y, Merrow M, Roenneberg T. Photoperiodism in *Neurospora crassa*. J Biol Rhythms 2004; 19:135-43.
35. Merrow M, Franchi L, Dragovic Z, Gorl M, Johnson J, Brunner M, et al. Circadian regulation of the light input pathway in *Neurospora crassa*. EMBO J 2001; 20:307-15.
36. Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, et al. A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. Proc Natl Acad Sci USA 2006; 103:10352-7.
37. Lambreghts R, Shi M, Belden WJ, Decaprio D, Park D, Henn MR, et al. A high-density single nucleotide polymorphism map for *Neurospora crassa*. Genetics 2009; 181:767-81.
38. Calvo AM. The VeA regulatory system and its role in morphological and chemical development in fungi. Fungal Genet Biol 2008; 45:1053-61.
39. Bayram O, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, et al. VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 2008; 320:1504-6.
40. Bayram O, Krappmann S, Seiler S, Vogt N, Braus GH. *Neurospora crassa ve-1* affects asexual conidiation. Fungal Genet Biol 2008; 45:127-38.
41. Tauber E, Last KS, Olive PJ, Kyriacou CP. Clock gene evolution and functional divergence. J Biol Rhythms 2004; 19:445-58.
42. Lee K, Loros JJ, Dunlap JC. Interconnected feedback loops in the Neurospora circadian system. Science 2000; 289:107-10.