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Review

Photoreception in *Neurospora*: a tale of two White Collar proteins

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Abstract. *Neurospora crassa* is the best-understood fungal organism in terms of the mechanism of light responses. All known *Neurospora* photoresponses are mediated by blue light. Two Per-Arnt-Simdomain containing transcription factors, WHITE COLLAR-1 (WC-1) and WC-2, are essential components for almost all light responses. Recently, WC-1 was shown to be a blue-light photoreceptor. How light affects the DNA binding of the WC proteins to the promoter of the circadian clock gene *frequency* was also demonstrated. These studies established a mechanism that explains the light responses mediated by activation of transcription. The purpose of this review is to summarize the findings of recent studies on the molecular mechanism of photoreception in *Neurospora*.

Key words. White collar-1; circadian clock; LOV domain; frequency; light responses; blue light photoreceptor.

Introduction

Light regulates a wide variety of physiological processes in many fungal organisms. Physiological, genetic, molecular and biochemical studies in the ascomycete *Neurospora crassa* have made it the best-understood fungal system in terms of the molecular mechanism of light responses. In *Neurospora*, all known light responses are sensitive only to ultraviolet (UV)/blue-light. These blue-light responses include induction of carotenoid synthesis, induction of protoperithecia, phototropism of perithecial peaks, induction of hyphae growth and asexual spore formation, changes of membrane potential, induction of gene expression and protein modifications, and entrainment of the circadian clock (reviewed in [1–4]).

Identification of the two WHITE COLLAR (WC) proteins, WC-1 and WC-2, as the key components of the light responses in *Neurospora* is the first major advance in our understanding of the light-signaling mechanism in a fungal organism [5, 6]. Recently, WC-1 was identified as the photoreceptor for circadian clock and other light responses in *Neurospora* [7, 8]. In addition, the light input pathway for the circadian clock gene *frequency (frq*) was demonstrated. The purpose of this review is to summarize recent advances in our understanding of the molecular mechanisms of light responses in *Neurospora*.

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Light-induced transcription in *Neurospora*: immediate early genes vs. late-response genes

Many blue-light responses in *Neurospora* are the results of light-induced transcription of genes involved in different physiological processes. For example, light-induced carotenoid biosynthesis is due to light induction of the transcription of the *albino* genes (*al-1*, *al-2* and *al-3*) that are required for the biosynthesis of carotenoid [9–11]. Light stimulation of conidiation can be explained by light induction of genes involved in the conidiation process [12–14]. One of the best-studied light-inducible genes in

Neurospora is *frq*. In constant darkness, levels of *frq* and FRQ protein oscillate daily [15, 16], and *frq* transcription is rapidly induced by light [17, 18]. This light-induced transcription of *frq* is the molecular basis for light resetting of the circadian clock [3, 19].

Traditional methods and complementary DNA (cDNA) microarray analysis revealed that the transcription of more than 30 genes in *Neurospora* is induced by light [1, 20]. These genes are involved in a variety of known and unknown processes. The results of the cDNA microarray experiments suggest that ~3% of *Neurospora* genes are stimulated by light. This number is most likely an underestimate, because cDNA microarray analysis tends to favor highly expressed genes. To date, there is no known confirmed example of a gene that is repressed by light.

Judging from the kinetics of light induction of transcription, the light-induced genes in *Neurospora* can be grouped into two classes: immediate early genes and late-response genes [1, 20]. For the immediate early light-inducible genes, induction of transcription can be observed 5 min after a light pulse and reaches its peak after 15–30 min. Known examples of the immediate early genes include *frq*, the *al* genes, *con-6* and *con-10*, the *bli* genes and several *clock-controlled genes (ccgs)* (reviewed in [1]). The immediate light induction of these genes suggests that they are the direct targets of the *Neurospora* light input pathway and may share similar cis-acting light-responsive elements in their promoters. Indeed, as described below, similar cis-acting light-responsive elements have been found in the *frq* and *al-3* genes, and direct binding of the blue-light photoreceptor to these promoter elements has been shown [5–8, 10]. For the late response genes, such as *ccg-1* and *ccg-2*, light induction of transcription usually is not observed in the first 15 min after light exposure and does not reach its peak until 1–2 h later [20–22]. Such delayed light responses suggest that these genes are not immediately downstream of the light input pathway. Therefore, light induction of the late response genes is likely a result of the induction of the immediate early genes.

Although many *Neurospora* light responses can be explained by light-induced transcription, several light responses suggest that they do not require changes in transcription. Blue light has been shown to induce post-translational phosphorylation of several proteins, including WC-1 and nucleoside diphosphate kinase (NDK)-1 [7, 23–25]. These light-induced phosphorylation events are rapid (~15 min after light) with kinetics similar to those of the immediate early genes. In addition, light-induced changes of membrane input resistance and ion transport can occur within a few minutes of light treatment, and changes in membrane conductance can be observed within a few seconds after light on [26, 27]. Therefore, these non-transcription-based light responses must have different light input signaling mechanisms from the light-induced transcriptional events.

Photoadaptation

When *Neurospora* cultures are transferred from dark to constant light (LL), the kinetics of light induction of most of the genes indicate that the light induction is transient and that a light desensitization mechanism is working to mediate light responses. For immediate early genes such as *al-1*, *al-2*, *al-3,* and the *con* genes, after the initial strong light induction peaks at ~15–30 min in LL, their RNA levels quickly decrease and become barely detectable after 2 h in LL [28–31]. A similar photoadaptation phenomenon was observed for the light-induced hyperphosphorylation of WC-1, a process that may lead to its degradation [23, 24]. However, the light induction of *frq* in LL is different from that of most immediately early genes. Although the *frq* level decreases after initial induction, light appears to constantly elevate the expression of *frq* so that its level in LL is always significantly higher than that in the dark [17]. Such constant activation of *frq* transcription in LL leads to constant high levels of FRQ, which explains the fact that the circadian clock is suppressed by LL [32].

Photoadaptation of light responses is also demonstrated by the results of two-pulse experiments [28, 29]. After a first light pulse, ~2 h dark incubation time is needed before a second light pulse can efficiently trigger another light response. Despite the adaptation of the light responses in LL at a given light intensity, *Neurospora* also has the ability to sense changes of light intensities. When *Neurospora* cultures are shifted from a low light intensity to a high light intensity condition, a second light induction can be observed [28, 29]. Currently, the mechanism of photoadaptation is unclear, although *vivid* (*vvd*) has been shown to be an important component in this process (see below). Pharmacological experiments also suggest the involvement of de novo protein synthesis and protein kinase C in photoadaptation [28, 29].

WC-1 and WC-2, two PAS-domain-containing transcription factors, are essential for light responses in *Neurospora*

Mutants of *wc-1* or *wc-2* were initially isolated as mutants with deficiencies in light-induced carotenoid synthesis, and the genes were proposed as components of the *Neurospora* blue-light input pathway [33, 34]. In true *wc-1* or *wc-2* null mutants, all light responses examined are abolished, including the light induction of *frq* and the entrainment of the circadian clock [1, 2, 7, 18, 35], indicating that these genes are essential for almost all light responses in

Neurospora. Extensive genetic screens for mutants that are completely blind for light responses yielded close to 30 mutants; however, all of these mutations belong to different alleles of *wc-1* or *wc-2* [36, 37]. The absence of additional *white collar* loci suggests that *wc-1* and *wc-2* are the only two essential genes for blue light responses in *Neurospora*. It is possible, however, that there are unknown *wc*-independent light responses, given the existence of several putative photoreceptors in *Neurospora*. A recent study suggested that the conidiation process could be driven by light/dark cycles at high light intensity under certain conditions in a *wc-2* mutant strain [38].

In addition to their essential role in light responses, both WC-1 and WC-2 proteins are also the positive elements of the circadian feedback loop in *Neurospora*. In constant darkness, they form a heterodimeric WC complex and bind to the light response elements (LREs) in the *frq* promoter, activating transcription of *frq* [8, 19, 23, 39]. In turn, FRQ proteins act as the negative elements in the loop, repressing the transcription of *frq*, probably by interacting directly with the WC proteins [15, 40–42]. Thus, this negative feedback loop generates rhythmic expression of *frq*. In either *wc-1* or *wc-2* null mutants, *frq* messenger RNA (mRNA) and FRQ protein levels are low, and the circadian clock is defective under normal conditions [7, 18, 43]. Like all circadian oscillators, the *Neurospora* circadian clock can be entrained by light, and the rapid induction of *frq* transcription by light is the molecular basis for the light entrainment of the clock [17]. In true *wc-1* and *wc-2* null mutants, light induction of *frq* is completely abolished [7, 18, 35]. Therefore, the WC proteins have at least two distinct functions, activating gene expression in the dark and after light exposure.

The *Neurospora wc-1* and *wc-2* genes were cloned by Macino and his colleagues by genetic approaches [5, 6]. Sequence analysis of the WC-1 and WC-2 proteins revealed that they are both Per-Arnt-Sim (PAS) domain-containing transcription factors with GATA type Zn-finger DNA binding domains (fig. 1). WC-1 protein has three PAS domains, the most N-terminal of which belongs to a specialized class of PAS domains known as LOV (light, oxygen or voltage) domains. WC-2 has only one PAS domain. Consistent with their roles as transcription factors, most of the WC proteins were found in the nucleus [23, 24]. In vivo, WC-1 and WC-2 form complexes, and the PASC (third) domain of WC-1 and the WC-2 PAS domain are essential for the formation of these complexes [23, 44, 45]. In mutant strains in which the WC-1/WC-2 interaction was disrupted, the light and circadian functions of the WC proteins were eliminated, indicating that formation of the WC complex is essential for their functions [44, 45]. In addition, such complex formation is important for maintaining the steady state level of WC-1 [44], the limiting factor in the WC complex [39, 41]. Thus, WC-2 is a positive regulator of WC-1. On the other hand, WC-1 negatively regulates the transcription of *wc-2* independent of light [45]. Therefore, *wc-1* and *wc-2* form another feedback loop. This feedback loop may be important for maintaining a proper level of the WC complexes in the cell to achieve their functions in circadian clock and light sensing. Unlike *wc-2*, the transcription of *wc*-1 is light induced, and its light induction requires functional *wc* genes [5, 6]. But the biological significance of the light induction of *wc-1* is unclear.

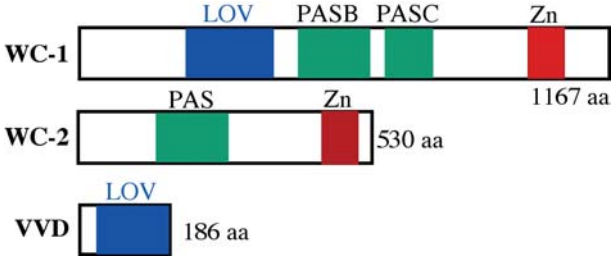

Figure 1. Schematic depictions of the domain structures of WC-1, WC-2 and VVD proteins. Zn: zinc-finger DNA binding domain.

Sensitivity of light responses in *Neurospora* is gated by the circadian clock: light response is most sensitive to light around dawn [31, 42]. The circadian gating of light responses appears to be due to the circadian expression of WC-1 and VIVID (see below). In addition to being to negative element of the circadian feedback loop, FRQ positively regulates expression of both WC-1 and WC-2, forming positive feedback loops that interlocked with the negative feedback loop [39, 42, 44, 46]. Such positive regulation of WC-1 by FRQ leads to the rhythmic expression of WC-1, peaking at around subjective dawn, the time when light responses are most sensitive to light. In contrast to the rhythmic expression of WC-1, the level of WC-2 is not rhythmic [39–41].

Light-dependent binding of a WC complex to promoters, a mechanism that triggers light-induced transcription

How does light trigger light responses? Since the WC proteins are DNA-binding transcription factors that are required for activation of gene transcription after light exposure, it is reasonable to predict that they will bind to the promoters of the light-inducible genes and activate their expression. Promoter analysis of the *al-3* gene has identified the first cis-acting element that is required for light-induced transcription [10]. After cloning *wc* genes, it was shown that, in vitro, the recombinant WC proteins can indeed bind to this promoter element [5, 6, 10]. It is unclear, however, whether light mediates the DNA-binding ability of the WC proteins. Studies of *ccg-2* also identified a promoter fragment essential for its light induction [47]. Since *ccg-2* is one of late light-regulated genes, it is possible that

WC proteins do not activate *ccg-2* light induction directly. In the process of determining how light induces rapid transcription of *frq*, a process that is critical for circadian clock resetting, Froehlich and colleagues have functionally mapped two cis-acting LREs in the promoter of *frq* [8]. Although neither element is essential, elimination of both elements abolished light induction of *frq*. In addition, both elements are sufficient on their own to drive light-induced transcription of a reporter construct. Using nuclear extracts in an in vitro DNA binding assay, two complexes with different sizes were found to bind both LREs. Not surprisingly, both of the complexes contained WC-1 and WC-2. The smaller WC complex (its size is similar to a WC-1/WC-2 heterodimer) binds to the LREs in the dark, but its binding was reduced after light exposure. In contrast, the larger WC complex does not bind to the LREs in the dark, but its binding to LREs is induced by light. Thus, after light exposure, the larger WC complex replaces the smaller complex and binds to the LREs (fig. 2). Furthermore, this light-induced LRE/WC binding in vitro using cell-free nuclear extracts has a fluence threshold and wavelength response similar to those of light-induced clock resetting in vivo [17, 48, 49]. Therefore, the small WC complex appears to be the complex that activates *frq* expression in the dark, while the large WC complex is responsible for light-induced activation of *frq* transcription. The existence of at least two types of WC-1/WC-2 complexes in vivo was also confirmed by immunoprecipiation assays [45]). In addition to the WC-1/WC-2 heterodimeric complex, ~20% of WC-1 was found to self-associate to form a large complex. On the other hand, no WC-2 was found to self-associate in vivo.

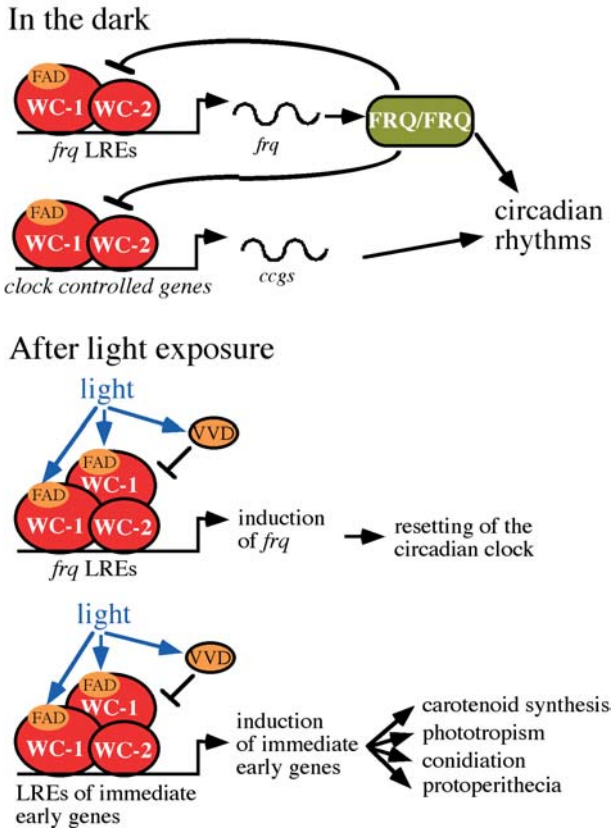

Figure 2. How WC complexes function in the dark and after light exposure in *Neurospora*. In the dark, the heterodimeric WC complex bind to the LREs of *frq* and to the promoters of clock-controlled genes that are direct targets of the WCs to activate their transcription. Homodimeric FRQ complex physically interacts with this WC complex to inhibit its activity. This negative feedback loop generates the circadian rhythms in the cell. After light exposure, a large WC complex, probably containing more than one WC-1 molecule, replaces the heterodimeric WC complex and binds to the LREs of *frq* to trigger rapid light induction of *frq*. The same complex or a similar complex binds to the LREs of other immediate early genes to activate their transcription. These rapid light-induced transcriptional events result in various light responses in *Neurospora*. After light irradiation, VVD protein is expressed, and it acts directly or indirectly to inhibit the activity of the large WC complex.

These results demonstrate how light induces *frq* transcription and resets the circadian clock, and it provides a mechanism that can explain the light induction of transcription of other *Neurospora* immediate early genes (fig. 2). Because of the delayed light responses of the late-response genes, their promoters are probably not the direct targets of a light WC complex. Instead, they may be under regulation of the immediate early genes. On the other hand, for light responses such as light-induced protein modifications and membrane channel activities [23–25, 27, 50] that do not require transcription, the mechanism of light signaling should be different. Some of these light responses have been shown to require the *wc* genes; thus, WC proteins must have novel modes of action to mediate these processes.

WC-1, a blue-light photoreceptor

What is the blue-light photoreceptor(s) that mediates light responses in *Neurospora*? Previous evidence has suggested that a flavin-containing blue-light photoreceptor(s) is responsible. First, the action spectra for several activities, including entrainment of circadian clock and photoinduction of carotenoid biosynthesis, are similar to typical flavin absorption spectra, with peak activity at ~465 nm and no response above ~520 nm [48, 49, 51]. In addition, two *Neurospora* flavin deficiency mutant strains were found to have reduced photosensitivity for clock resetting and induction of carotenogenesis [52, 53]. The fact that *wc* mutants are the only mutants that have been repeatedly isolated in genetic screens that show loss of almost all light responses [1, 2, 36] raises the possibility that one or both of the WC proteins are photoreceptors responsible for these light responses.

If the WC proteins are photoreceptors, they should bind a chromophore. The possibility was strengthened after the protein sequence of the LOV/PAS domain of WC-1 was

found to be similar to the chromophore-binding LOV domain of the phototropins, the plant blue-light photoreceptors for phototropism [54–56]. Biochemical analyses of several LOV domains from the phototropins have shown that each LOV domain noncovalently binds a flavin mononucleotide (FMN) molecule via 11 conserved protein residues and is able to undergo fully reversible photocycles. All of the flavin-contacting residues are also conserved in the WC-1 LOV domain, suggesting that WC-1 may also bind flavin and function as a blue-light photoreceptor. Consistent with this, several *wc-1* mutants were found to have mutations in the LOV domain [57]. Results of a study using a *wc-2* mutant also suggested that WC-2 is not the photoreceptor and that it acts downstream of WC-1 [24].

To demonstrate the essential role of the WC-1 LOV domain in light responses, a *Neurospora* mutant without the entire WC-1 LOV domain was made [7]. As expected, all light responses examined were eliminated in this mutant, including light induction of the transcription of *frq* and *al-3*, and light-induced hyperphosphorylation of WC-1. In addition, the circadian conidiation rhythm in this mutant could not be entrained by light/dark cycles. However, the expression of *frq* in the dark was near normal in this mutant, and a functional circadian clock could be observed after temperature treatments. Therefore, the WC-1 LOV domain appears to be essential only for the light function of the protein and not for its transcription activation role in the dark.

To show that WC-1 protein is associated with a chromophore in vivo, an epitope-tagged *wc-2* strain was created so that the endogenous WC complex could be biochemically purified directly from *Neurospora* [7]. As expected, the purified WC complex was found to be associated with a flavin species. Surprisingly, the flavin species it contains is not FMN, but flavin adenine dinucleotide (FAD). Importantly, FAD binds WC-1 stoichiometrically, with a molar ratio of about 1 : 1. The fact that WC-1 is associated with FAD and not FMN probably reflects the differences between the LOV domains of phototropins and WC-1, especially in one of the linker regions between two critical helices in these LOV domains [7].

Although these results strongly suggest that WC-1 is the blue-light photoreceptor for the circadian clock and other light responses, they did not directly show the light-sensing role of WC-1. This role of WC-1 was demonstrated by Froehlich and colleagues using the in vitro DNA binding assay [8]. Using the in vitro translated WC-1 and WC-2, gel retardation results showed that two different WC-1/WC-2 complexes (similar to the two complexes found using the nuclear extracts) bind to the LREs. As observed with the nuclear extracts, light caused a significant increase in the binding of the larger WC complex to LREs and a decrease in the binding of the small WC complex. However, such light effects on DNA binding could be observed only in the presence of FAD, not FMN. Importantly, when the in vitro translated WC-1 and FAD that had been exposed to light were mixed with the in vitro translated WC-2 made in the dark, light-induced DNA binding could be observed. Thus, WC-1, not WC-2, can directly sense light.

Together, these findings demonstrate that WC-1, using FAD as the chromophore, is the blue-light receptor responsible for light resetting of the clock and other light responses in *Neurospora*.

Although WC-2 is not responsible for direct sensing of light signal, it is an essential component in the light input pathway. First, the presence of WC-2 and the formation of the WC-1/WC-2 complex are important for maintaining the steady-state level of WC-1 [44]. Second, WC-2 and its DNA binding domain are essential for DNA binding of the WC complexes and transcriptional activation, and WC-1 cannot bind to DNA in the absence of WC-2 [6, 8, 18, 44]. In the dark, WC-1 and WC-2 bind LREs of *frq* as a heterodimer. As for the phototropin LOV domains [55], formation of an adduct between FAD and the highly conserved cysteine in the WC-1 LOV domain after light exposure should trigger conformational changes of WC-1 that result in binding of the larger WC complex to *frq* LREs. Because the larger WC complex was also found when the in vitro translated WC proteins were used, it suggests that the large complex is the result of the multimerization of the small complex. The existence of a large WC complex containing more than one WC-1 molecule was confirmed in vivo, but self-association of WC-2 was not detected [45]. Thus, the large WC complex may consist of one WC-2 molecule with more than one WC-1 molecule (fig. 2). It is unclear, however, whether the large WC complex exists in the dark or can form only after light exposure.

Differential regulation of light induction of *frq* and other light-inducible genes by WC proteins

Although light-induced binding of the WC complexes to the gene promoters may be a general theme in the mechanisms of light-induced transcription in *Neurospora*, several lines of evidence indicate that light induction of *frq* and of other genes differs significantly in the requirements of the WC proteins. First, fully functional WC proteins are not required for the light induction of *frq*. In mutants that contain a point mutation in the DNA binding domain of WC-2 [18, 43, 44] or in the LOV domain of WC-1 [58], near normal light induction of *frq* could still be observed, while light induction of other genes was mostly abolished. Second, light-induction of *frq* needs only very low levels of the WC proteins [39]. In strains in which expression of the WC proteins is under the control of an inducible promoter, the light-induced expression of FRQ is near normal

even in the absence of induction of the WC proteins. The extremely low threshold levels of the WC proteins for *frq* light induction is highlighted by one of the *wc-1* mutants [35, 45]. In this mutant, a mutation in the N-terminal of WC-1 eliminates the expression of the full-length protein from the normal AUG start codon, but a truncated WC-1 protein, which results from alternative protein initiation and cannot be detected by regular immunoblotting, is expressed at a level that is less than 1% that of the wild-type strain. Even though light induction of other genes is either completely or mostly abolished in this strain, light induction of *frq* is near normal. When the WC-1 alternative protein initiation was not taken into consideration for one of the *wc-1* mutants, it was once concluded that WC-1 was not required for light induction of *frq* [38]. Taken together, these data indicate that although all known light-induced transcription requires both WC proteins, they differentially regulate light induction of *frq* and of other genes.

On the basis of requirements of WC proteins for light induction, the immediate early light-inducible genes in *Neurospora* can be grouped into at least three classes [18, 35, 44, 45]. In contrast to the unique sensitivity of *frq* expression to WC complex levels, genes such as *al-3* and *al-1* belong to a group that is most sensitive to changes in WC levels and activities, while light induction of *vvd* shows intermediate dependence on the WCs. Such differential regulation of light-inducible genes by the WCs suggests that there are different WC complexes controlling different genes or that various light-regulated promoter elements have different affinities for the same WC complexes.

Both WC proteins contain GATA-type Zn-finger DNA binding domains. Indeed, the determined LREs of *frq* do contain two imperfect repeats with GATN sequence [8]. This is similar but not identical to the LRE previously identified for *al-3* [10]. Understanding whether these two known LREs bind to different WC complexes or have different affinities for the same WC complex will provide answers to how WC proteins differentially regulate different light-inducible genes. Interestingly, the Zn-finger DNA binding domain of WC-1 is not required for light induction of genes, although it is essential for expression of *frq* in the dark [45]. Similarly, a mutation of the WC-2 DNA binding domain also abolished the dark expression of *frq* and the light induction of *al* genes, while the light induction of *frq* was maintained [6, 18, 43, 44]. For *frq* light induction, therefore, only one DNA binding domain from either of the WC proteins is needed, but both DNA binding domains are required for the dark function of the WC proteins. For *al* genes, however, the DNA binding domain of the WC-2 protein appears to be critical.

WC complexes bind the same LREs in the *frq* promoter in the dark and after light exposure, mediating *frq* transcription under both conditions [8]. Are other light-inducible genes also activated by the WCs in the dark? A recent screening of *Neurospora* clock-controlled genes and light-induced genes using c-DNA microarray analysis suggested that many clock-controlled genes are controlled directly at the transcriptional level by the WC proteins in constant darkness [20]. However, the vast majority of the genes that are controlled by the WC proteins in the dark were not light-inducible. On the other hand, among the 22 light-inducible genes identified, only 4 were found to be activated by WC-1 in the dark, suggesting that in the dark, most of the light-inducible genes in *Neurospora* are not directly activated by WC proteins. Therefore, the mode of regulation of *frq* transcription by WC proteins is not common among clock- and light-regulated genes in *Neurospora*.

VIVID, a modulator of light responses, is a *wc*-dependent blue-light photoreceptor

vivid (*vvd*) mutants were originally isolated as mutants with bright orange coloration [59]. Recent evidence has shown that VVD is it is an important regulator of all light responses examined in *Neurospora* [29–31]. First, in *vvd* mutants, all light-induced gene expression examined was elevated, including that of *frq* and other light-inducible genes. Second, the photoadaptation of gene expression is partially lost in *vvd* mutants. The increased light induction of the genes involved in carotenoid biosynthesis and the deficiency in photoadaptation result in the bright orange color of the *vvd* mutants. Third, because of the increased sensitivity of *frq* transcription to light in *vvd* mutants, phase of the circadian clock and light-induced phase shifting are affected. Finally, the circadian gating of light responses is partially lost in *vvd* mutants. Therefore, VVD acts as an important negative regulator of light responses to control photoadaptation and light input of the circadian clock in *Neurospora*.

Cloning of the *vvd* gene revealed that its protein product is a small protein that is composed essentially of a single LOV domain (fig. 1) [31]. The expression of *vvd* is strongly induced by light, and its light induction requires both WC proteins [31, 38, 45]. In addition, *vvd* expression is controlled by the circadian clock. The circadian oscillation of *vvd* expression in the first day in constant darkness partially explains the circadian gating of light responses. After long incubation in constant darkness (>2 days), however, the amount of *vvd* decreases to an undetectable level. Thus, VVD is dependent on the presence of WC proteins and exposure to light to be expressed and to function. The LOV domain of VVD is very similar to that of WC-1; thus, VVD may also bind FAD and function as a blue photoreceptor in *Neurospora* [7]. In a *Neurospora* strain in which the LOV domain of WC-1 was swapped with that of VVD, we showed that the LOV domain of VVD could partially replace the light function of

the WC-1 LOV domain [58]. Schwerdtfeger and colleagues recently showed that VVD protein expressed in *Escherichia coli* is noncovalently associated with a flavin chromophore, and it undergoes a photocycle in vitro [60]. Together, these results strongly suggest that the functions and structures of the VVD and WC-1 LOV domains are similar, and that VVD is a *wc*-dependent blue light photoreceptor that regulates photoadaptation and light responses. Although it is not known how VVD functions, it is likely that after light exposure, it directly or indirectly influences the activity of the WC proteins (fig. 2).

Conclusion

Since the cloning of the *wc* genes, significant progress has been made toward understanding the molecular mechanism of light responses in *Neurospor*a. With the demonstration that WC-1 is a photoreceptor and how light affects the DNA binding of the two WC proteins with *frq* promoter, a molecular model has been proposed to explain light responses mediated by transcriptional events, such as resetting of the circadian clock and induction of carotenoid biosynthesis. However, such a model could not explain some of the light responses that do not require changes in transcription, such as light-induced protein phosphorylation, and membrane potential and channel activities [23–25, 27, 50]. Just like light-induced transcription of the immediate early genes, these light responses are rapid, and some of them have been shown to require the *wc* genes. Thus, the WC proteins must have other novel modes of action to regulate these light responses. The significantly different WC requirements for the light induction of *frq* and of other genes, and the unknown mechanism of function of VVD suggest that other regulators of light responses exist. Consistent with this notion, there are several light-insensitive mutants with reduced light sensitivity [61]. Finally, in the finished *Neurospora* genome, there are several potential photoreceptors with one putative cryptochrome gene, two phytochrome-like genes and two genes similar to the archaeal rhodopsins [62]. The functions of these genes in light responses are currently unknown; it is possible that these genes mediate some light responses that do not require the *wc* genes.

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