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MINI-REVIEW

Light-regulated promoters for tunable, temporal, and affordable control of fungal gene expression

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Abstract
Regulatable promoters are important genetic tools, particularly for assigning function to essential and redundant genes. They can also be used to control the expression of enzymes that influence metabolic flux or protein secretion, thereby optimizing product yield in bioindustry. This review will focus on regulatable systems for use in filamentous fungi, an important group of organisms whose members include key research models, devastating pathogens of plants and animals, and exploitable cell factories. Though we will begin by cataloging those promoters that are controlled by nutritional or chemical means, our primary focus will rest on those who can be controlled by a literal flip-of-the-switch: promoters of light-regulated genes. The *vvd* promoter of *Neurospora* will first serve as a paradigm for how light-driven systems can provide tight, robust, tunable, and temporal control of either autologous or heterologous fungal proteins. We will then discuss a theoretical approach to, and practical considerations for, the development of such promoters in other species. To this end, we have compiled genes from six previously published light-regulated transcriptomic studies to guide the search for suitable photoregulatable promoters in your fungus of interest.

Keywords Regulatable promoters · Photobiology · Fungal genetics · Functional genomics

Introduction

Elucidating a gene's function rests heavily on the ability to impact its expression. The simplest form of this involves blocking protein synthesis wholesale—either through mutagenesis of the genome or knockdown of the message—and then observing the loss-of-function phenotype. Essential genes cannot be scrutinized in this way for the obvious reason, and knockout/knockdown mutants often display little or no phenotype due to homeostatic buffering by redundant genes or pathways (Giaever et al. 2002; Hartman et al. 2001). In these cases, informative phenotypes may be achieved by overexpressing the gene-of-interest to mimic a gain-of-function allele, or titrating down expression to confirm essentiality. This approach involves replacing the endogenous promoter with one that provides the desired level of expression and experimental control, and such systems have been useful in inferring gene function in several eukaryotes (Rørth et al. 1998; Zhang 2003; Sopko et al. 2006). Regulatable promoter systems are highly desirable in bioindustry as well. Whereas constitutive and/or robust overexpression of a heterologous protein may be toxic to the cell, for example, a more tightly controllable system may be desired to keep expression levels low or induce sharply once sufficient biomass has accrued.

This review will focus on regulatable promoter systems in filamentous fungi, an important group of organisms whose members include agriculturally and medically important pathogens (Möller and Stukenbrock 2017; Powers-Fletcher et al. 2016), essential genetic models (Roche et al. 2014; Osmani and Mirabito 2004), and prolific cell factories in industrial fermentations (Druzhinina and Kubicek 2017; Meyer et al. 2015). We will begin with a brief overview of promoter systems already in place for fungal research and industry, which will lead into our main topic concerning the exploitation of fungal photobiology to those ends.

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Current regulatable systems: benefits and limitations

The ideal regulatable promoter has the following traits: (1) *tightness*, i.e., not leaky in the "off-state," (2) *robustness*, allowing for many folds expression above or below endogenous levels, (3) *tunability*, such that expression changes linearly with the concentration of the signal (inducer/repressor), (4) *temporal controllability*, i.e., the signal can be applied and removed from the system at any given time, and (5) *cost-effectiveness*, such that the signal is inexpensive on both a small and large scale. Several promoter systems have been developed for use in molds and are generally regulated either by a primary nutrient or some chemical inducer. A brief overview of these strategies, including a mention of their strengths and weaknesses, will be provided.

Most regulatable systems in fungi utilize conditional metabolic promoters, meaning that the expression state (on/off) varies with qualitative changes in the nutrient composition of the media. This includes several that are induced on alternative carbon sources and repressed by glucose (e.g., P*alcA*, P*cbh-1*, P*crg1*, P*xlnA*), as well as orthologs of the nitrite reductase (P*niiA/nit-6*) which is repressed by ammonium and induced by nitrate (Waring et al. 1989; Liu et al. 2008; Bottin et al. 1996; Amaar and Moore 1998; Exley et al. 1993; de Graaff et al. 2005). These promoters are generally *robust* under inducing conditions and *tight* under repressing ones and have indeed been employed to study essential genes and to create over-expression libraries to screen for phenotypes of interest (Hu et al. 2007; Lee et al. 2005; Romero et al. 2003). Their major limitations, however, stem from the constraints they place on the medium that can be used (i.e., they are *metabolically restrictive*). This is problematic from a research perspective if the global metabolic influence of the medium masks the phenotype otherwise associated with overexpression of the target-gene (Ouyang et al. 2015). Furthermore, as many of these promoters are repressed by glucose, they exclude the use of most low-cost and complex (nutrient rich) media for use in biofermenters. These promoters are further limited by the fact that they are essentially a binary switch (on or off) and consequently do not allow for finely *tunable* expression. Nor do they allow for easy *temporal controllability*, as the entire medium would have to be changed to facilitate a timed induction. Thoroughly removing repressive media from a mycelial mat is laborious and undesirable in a large-scale grow-ups, and trace carryover nutrients may impact induction levels and kinetics. Thus, while primary nutritional promoters may be *robust* and *tight*, other desirable features such as *tunability*, *temporal controllability*, and we can now add *metabolic flexibility* to the list of considerations, are best achieved with a signal that can be spiked into the culture (e.g., a chemical) and operate irrespective of the medium.

Several chemically regulated promoter systems that function in standard (i.e., glucose-containing) media exist for fungi. The human estrogen receptor system, for example, has remarkably been adapted for use in *Aspergillus niger*, which requires the heterologous expression of the human estrogen receptor along with the gene-of-interest being placed downstream of a *URA3* promoter modified to contain several estrogen response elements (EREs). The system is exquisitely sensitive to estrogen (picomolar range) and *tunable*; however, depending upon the exact promoter construct utilized, it is either *robust* or *tight*, but not both (Pachlinger et al. 2005). Additional concerns stem from the inhibitory effect of estrogen on the engineered strains at concentrations as low as 10 nM (Pachlinger et al. 2005). Endogenous fungal promoters may also be used, including P*thiaA* from *Aspergillus oryzae*, which is repressed by thiamine and is *tunable*; however, the system does not function at neutral or weakly alkaline conditions (pH = 7), likely because the loss of thiamine's charge (it is a weak base) affects its import (Shoji et al. 2005). The *qa-2* promoter from *Neurospora* is induced by and tunable across four to five log-orders of quinic acid. This promoter may, however, lack sufficient *robustness* for high degrees of over-expression and is moreover *leaky* under low glucose (Giles et al. 1985; Shi et al. 2010; Larrondo et al. 2009). This is a good place to note that promoter leakiness can be exploited in certain experimental contexts. Our group, for example, could assess phenotypes associated with the essential gene *casein kinase-1* (*ck-1*) due to weak expression from *pqa-2* in the absence of quinic acid (Mehra et al. 2009).

Three systems that have emerged recently utilize apparently non-toxic signals that also provide tight and tunable expression. The copper-repressible promoter of *tuc-1* from *Neurospora* is notable because copper can be spiked into the culture for a timed repression or, alternatively, the copper chelator BCS can be added into a copper-containing culture for a timed induction (Lamb et al. 2013; Ouyang et al. 2015). Importantly, Ouyang and colleagues demonstrated that copper has no discernible impact on the metabolite profile of *Neurospora* when analyzed by proton nuclear magnetic resonance spectroscopy (Ouyang et al. 2015). The tetracycline resistance operon (both the tet-on and tet-off variations) was first developed in *Aspergillus fumigatus* (Vogt et al. 2005; Helmschrott et al. 2013) and has since been adapted for use in *A. niger*, where it is tight and responsive within minutes of adding doxycycline to the culture (Meyer et al. 2011; Wanka et al. 2016). Finally, the benzoate para-hydroyxylase (*bphA*) promoter from *A. niger* is tightly repressed in the absence of benzoic acid and induced within 10 min upon its addition to the medium (Antunes et al. 2016). That the fungus can grow on benzoic acid as the sole carbon source indicates that toxicity is a minimal concern in the system.

In summary, chemically induced promoter systems offer a *tunability* and *temporal controllability* that are typically

lacking with nutritional/conditional promoters. General considerations may include the cost of the inducer as well as its potential toxicity to the fungus. In all cases, the ability to remove the chemical inducer once it is applied requires a wash out step, which can be both laborious and inefficient. Thus, a signal that can be applied and removed instantaneously would be ideal, which is where we can now shed light on the story! While recent work and reviews have focused on the development of optogenetics in yeast and filamentous fungi, which involves the expression of heterologous/synthetic light sensing modules (Drepper and Krauss 2011; Wang et al. 2014; Zhang et al. 2016; Salinas et al. 2017), we will instead focus on promoters that function downstream of endogenous photosensory pathways in your fungus of interest.

A primer on light and fungi

Visible light is a ubiquitous environmental signal that can provide important environmental information to the fungal cell. Sporulation is photoinducible in many fungi, for example, because light presumably signals the surface-to-air interface for optimal dispersal. Other species use visible light primarily as a proxy for co-occurring stresses (e.g., genotoxic ultraviolet radiation) and therefore repress growth and differentiation. Regardless of the response, light perception is owed to highly sensitive and wavelength-specific photoreceptor proteins that transduce the signal either through some biochemical activity (e.g., a kinase) or an altered intermolecular interaction (e.g., protein-protein, protein-DNA) (recently reviewed in Fischer et al. 2016; Fuller et al. 2015). Fungal phytochromes, for example, are histidine kinases that bind a tetrapyrrole chromophore that imparts a sensitivity to the near-infrared/red range (600–850 nm wavelength) (Yu et al. 2016); opsins are transmembrane proteins (ion channels or cyclases) that bind retinal and have a peak sensitivity to green light (495–570 nm) (Avelar et al. 2014; García-Martínez et al. 2015); and the cryptochromes and LOV-domain proteins both bind flavin to detect light in the near-ultraviolet/blue range (400–490 nm) (Losi and Gärtner 2011). In this way, the combined effort of multiple photoreceptor types can incur a visible spectrum to fungi that approximates that of mammals, and their activities may be coordinated to achieve an optimal response to changing light qualities across the day.

The mechanisms of fungal phototransduction are best characterized for the white collar-1 (WC-1) family of proteins, which are GATA-type transcription factors that contain both a Zn-finger DNA-binding domain as well as a specialized PAS (*P*er-*A*rnt-*S*im) domain called the LOV domain (for *l*ight, *o*xygen, and *v*oltage) which binds flavin-adenine dinucleotide (FAD) as a chromophore. First shown to be a photoreceptor in *Neurospora* (Froehlich et al. 2002)—a foundational model for fungal photobiology—WC-1 obligately exists as a heterodimer with another Zn finger protein called WC-2 to form the white collar complex (WCC) (Ballario et al. 1998; Cheng et al. 2002). Upon the absorption of blue light (~ 465 nm wavelength) by FAD, a conformational change alters the ability of the heterodimer to drive expression of genes containing WCC binding sites called LREs (for *l*ight-*r*esponse *e*lement) (Froehlich et al. 2002; Smith et al. 2010; Wang et al. 2015). In addition to being the most-well studied photoreceptor in fungi, the WCC is also the most well-conserved, with orthologs distributed across the Ascomycota, Basidiomycota (Kamada et al. 2010; Brych et al. 2016; Idnurm and Heitman 2005b), Mucormycotina (formerly called Zygomycota) (Idnurm et al. 2006; Sanz et al. 2009; Corrochano et al. 2016; Corrochano and Garre 2010; Stajich 2016), and even the anciently diverged chytrids (Idnurm et al. 2010; Dunlap and Loros 2006). Corresponding to the presence of these genes, blue light responses manifest in key research models (*Neurospora crassa*, *Aspergillus nidulans*), plant pathogens (*Botrytis cinerea*, *Magnaporthe oryzae*), human pathogens (*A. fumigatus*, *Cryptococcus neoformans*), and industrial workhorses (*A. niger*, *Trichoderma reesei*) (reviewed broadly in Purschwitz et al. 2006; Rodriguez-Romero et al. 2010; Fuller et al. 2016). As these physiological responses may be fast, robust, and fluency dependent (titratable), the promoters of light-responsive genes may serve as useful regulatable systems in these diverse and important organisms. We will explore this concept, first through a test case in the form of the *Neuropora vvd* promoter, and then speculate upon the utility of the approach in other fungal systems.

The *vvd* promoter in *Neurospora*: a case study for light-regulated expression systems in fungi

In *Neurospora*, the expression of many light-induced genes begins returning to baseline (dark level) after a few hours in constant illumination. This process, termed "photoadaptation," is due to a negative feedback on the WCC and is facilitated by an additional blue light receptor called VVD. Briefly summarized, the WCC drives strong expression of the *vvd* promoter within minutes of culture illumination. The VVD protein, which also binds FAD as a chromophore, becomes light activated, dimerizes with WC-1, and inhibits the transcriptional activity of the WCC (Chen et al. 2010; Hunt et al. 2010; Malzahn et al. 2010) (Fig. 1a). The upshot of this negative feedback is that the fungus does not constitutively synthesize light-output genes during constant illumination at a fixed intensity but instead remains responsive to increasing intensities over time (Heintzen et al. 2001; Schwerdtfeger and Linden 2001, 2003; Dasgupta et al. 2015). This is evident in *vvd* mutants that hyperaccumulate carotenoids in light, thus giving the mycelium a bright

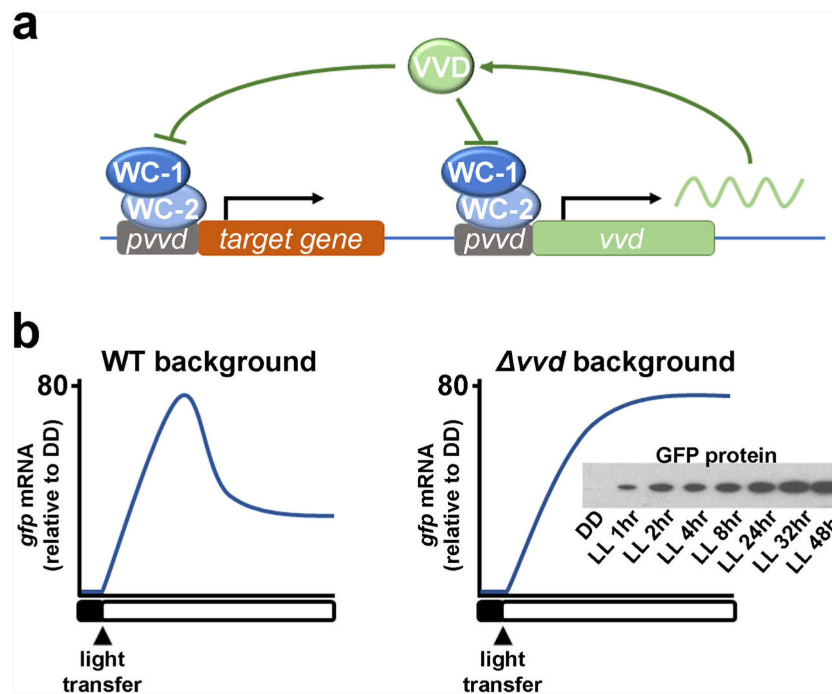

Fig. 1 The use of *pvvd* to drive heterologous gene expression in *Neurospora*. **a** Scheme of *Neurospora* photoadaptation, whereby the WC-1/WC-2 heterodimer (the WCC) drives expression *vvd* in response to light. The VVD protein itself becomes photoactivated and negatively regulates the WCC through a direct interaction, thus attenuating the light response. **b** The left graph depicts the kinetics of heterologous gene expression in a *Neurospora* wild-type background, in which photoadaptation results in reduced expression after an initial induction. The right graft depicts the kinetics of the same gene in a *Δvvd* background, in which photoadaptation is ablated and gene expression remains constitutively high under constant illumination. The protein blot demonstrates a linear accumulation of GFP when driven by *pvvd* in the *Δvvd* mutant (adapted from Hurley et al. 2012)

("vivid") orange color from which the gene derives its name (Heintzen et al. 2001).

In addition to the rapid onset of induction, which can be as high as 300-fold within minutes of light exposure, *vvd* expression is effectively undetectable in constant darkness. Given the tight and robust nature of this response, the *vvd* promoter was investigated by our group as a putative system to drive autologous and heterologous proteins in *Neurospora* (Hurley et al. 2012). As a proof-of-principle, the 3000 bp fragment that immediately precedes the *vvd* translational start (*pvvd*) was placed upstream of the gene encoding green fluorescent protein (*gfp*). Moreover, this construct was expressed ectopically and in a *vvd* knockout background (*Δvvd*) in order to attenuate the photoadaptation response and increase the degree and duration of target-gene overexpression. The *tightness* of the system was evident in that GFP protein remained undetectable by western blot when the fungus was cultured in constant darkness. After 1 h of light exposure, however, GFP was detectable and steady-state protein increased linearly for up to 48 h incubation in constant illumination (Fig. 1b). At the transcript level, *gfp* message was already 50-fold above background (dark) after 1 h of induction, and levels were maintained as high as 80-fold for the duration of the 48 h time course. Notably, similar degrees of tightness and robustness were observed when the same *vvd* promoter fragment drove the *Neurospora* cellulase gene, *gh5-1*, which is used in the production of cellulosic biofuels (Hurley et al. 2012; Sun et al. 2011). Thus, the ability to conditionally and strongly express genes in *Neurospora* to basic research or industrial ends can be achieved with light-regulated systems, and the use of strains deficient in photoadaptation can further prolong overexpression in this organism (Hurley et al. 2012).

To better characterize the kinetics of *vvd* promoter activity, the *pvvd-gfp* strain was exposed to a 1 h light-pulse and then returned to constant darkness for further incubation. In this experiment, a 160-fold increase in *gfp* mRNA was observable directly after the light pulse, but these levels had returned to baseline 7 h after light was removed. This underscores perhaps the greatest advantage to using light as opposed to chemical inducers: It can be applied and removed rapidly (and repeatedly) without manipulation of the culture. This *temporal control* is unmatched compared to nutritionally or chemically driven systems, which require a complete change of the medium (and washing of the mycelium) to achieve multiple cycles of induction/repression. Moreover, *gfp* mRNA and protein displayed an attenuated (but still linear) induction when *Neurospora* was exposed to lower light intensities, thereby demonstrating the *tunability* of the promoter (Hurley et al. 2012).

In summary, work with the *Neurospora vvd* promoter demonstrates generally that the intrinsic photosensing capabilities of fungi can be exploited to control genes-of-interest. In the case of *pvvd* itself, the system was *tight*, *robust*, *tunable*, *temporally controllable*, and presumably, as it was not addressed experimentally, *nutritionally flexible*. We will now set a wider gaze and discuss the use of light-driven expression systems in other fungi, focusing on the identification of putative promoters that might be developed in one's organism of interest.

Light-regulatable promoters in your favorite fungus?

In principle, the *vvd* promoter from *Neurospora* could be used to drive gene expression in heterologous fungal systems so long as (1) the organism contains orthologs to the white collar proteins and (2) those proteins recognize the promoter elements of *pvvd*. The reverse experiment has been tried, in which the apparent *vvd*-ortholog of *T. reesei*, called *env1*, was expressed using its own promoter in a *Neurospora* *Δvvd* mutant. Although the *env1* transgene was (intriguingly) unable to rescue the *Δvvd* photoadaptation phenotype, its expression pattern was conserved: *env1* mRNA was undetectable in the dark but strongly accumulated in *Neurospora* after 10-min light pulses (Schmoll et al. 2005). *Neurospora* and *Trichoderma* are relatively closely relatives, however, as they are both in the Sordariomycetes clade (phylum Ascomycota). Although it is assumed that WCC recognition would be similar across more distantly related fungi (Weirauch et al. 2014), direct experimental evidence—by either sequence analysis of known light-regulated genes, or by WCC chromatin immunoprecipitation (ChIP)—will be greatly informative (Chen et al. 2009; Smith et al. 2010). Even if binding sites are conserved in distant *Neurospora* relatives, the mode of action of the WCC on the promoter may differ. For example, the WC-1 ortholog in *Aspergillus nidulans*, LreA, has been demonstrated to operate in a repressive fashion; that is, it binds DNA in the dark and represses gene expression and then is released from the DNA upon illumination (Hedtke et al. 2015). Moreover, and in contrast to *Neurospora* (Chen et al. 2009), the proper induction of light-regulated genes in *A. nidulans* is not solely dependent on LreA/B (the WCC); rather, proper induction of light-regulated genes depends upon an interplay of LreA/B with the red-light sensing phytochrome FphA (Purschwitz et al. 2008; Purschwitz et al. 2009; Bayram et al. 2010; Hedtke et al. 2015). Thus, the peculiarities of the light sensing mechanisms across divergently related species may impact the tightness or robustness of heterologous light-driven promoters such as *pvvd*.

It instead may be more feasible to turn to the organism-of-interest itself, i.e., identifying light-regulated genes in your fungus and cloning the promoters of those that display the desired photo-kinetics. Sticking with *vvd* as an example, orthologs in both *T. reesei* and *Fusarium fujikuroi* (a model for fungal secondary metabolism and toxin production) are rapidly and robustly induced by light in a manner dependent upon the respective *wc-1* orthologs (Schmoll et al. 2005; Castellanos et al. 2010; Castrillo and Avalos 2014). In a general way, therefore, orthologs of *Neurospora* light-regulated genes may be a good place to start in the search for photoregulatable promoters in a different species. Such an analysis will likely have to extend beyond *vvd* unfortunately, as this family of proteins is restricted to the Sordariomycetes (including *Neurospora*, *Trichoderma*, *Fusarium*, *Botrytis*, *Magnaporthe*), so those working on other fungi, such as any one of the important *Aspergillus* species, will have to look elsewhere. We will let genome-wide studies of light-regulated fungal genes guide the search.

Putative light-inducible promoters

To date, light-regulated transcriptomic studies have been published for only a handful of fungi and include (1) *Neurospora* microarray and RNA-seq, both of which compare dark against multiple timepoints in white light (Chen et al. 2009; Wu et al. 2014); (2) *Botrytis cinerea* microarray comparing dark against 60 min white light illumination (Schumacher et al. 2014); *T. reesei* microarrays comparing 72 h dark against 72 h white light illumination (Tisch et al. 2011, 2014; Tisch and Schmoll 2013); *Trichoderma atroviride* microarray and RNA-seq, both comparing dark against a 4–5 min white light pulse (Rosales-Saavedra et al. 2006; García-Esquivel et al. 2016); *A. nidulans* microarray, comparing dark against 30 min white light illumination (Ruger-Herreros 2011); and *A. fumigatus* microarray, comparing dark against several timepoints in white light (Fuller et al. 2013). Table 1 is a list of the 10 most robustly light-induced genes from each of these organisms; in the case of *Neurospora* and *T. atroviride*, the RNA-seq datasets were preferentially used over the microarray studies. For those interested in developing a light-based expression system in the organisms listed, Table 1 may be useful for selecting genes that provide the greatest robustness. For example, *vvd* appears on the list of all Sordariomycetes in the table (i.e., all those except the two *Aspergilli*); however, even greater induction may be achieved by the *con-6* promoter in *Neurospora* or the hypothetical protein, Ta_131348, promoter in *T. atroviride*. In the case of *con-6*, expression had been previously shown to be undetectable in the dark by northern blot but became abundant within 15 min of white light illumination (Navarro-Sampedro et al. 2008), suggesting that its promoter may be a suitable alternative to *pvvd* when more robust overexpression is required. In general, however, the tightness of each candidate gene in the dark, as well as the overall

Table 1 Top light-induced genes across whole-genome transcriptional datasets

Gene	Description	L/D	Orthologs with a conserved light induction					
			Nc	*Bc*	*Tr*	*Ta*	*An*	*Af*
Neurospora crassa (Wu et al. 2014)								
NCU08769	*con-6*	362	X	–	–	–	An8640	–
NCU00552	Phytoene desaturase, *al-1*	315	X	–	–	–	–	–
NCU08699	*bli-4*	222.9	X	BC1G_15551	–	–	–	–
NCU08770	Hypothetical protein	194	X	–	–	–	–	–
NCU02190	Oxysterol binding	157.6	X	–	–	–	AN2877 AN3807 AN5015	–
NCU07325	*con-10*	137.2	X	–	–	–		–
NCU07267	*bli-3*	111.4	X	–	–	Ta_33514	–	–
NCU03967	*vvd*	97	X	BC1G_04348 BC1G_15404	TR_81609	Ta_150699	–	–
NCU00582	Cryptochrome, *cry*	78.8	X	BC1G_08145 BC1G_13162	TR_107680	–	An0387	AfuA_1g01600
NCU10063	Sugar isomerase	73.5	X	BC1G_09879	TR_21396	Ta_38632	–	–
Botrytis cinerea (Schumacher et al. 2014)								
BC1G_12555	Hypothetical protein	40	–	X	–	–	–	–
BC1G_04348	*BcVVD1*, vivid-like	37.5	NCU03967	X	TR_81609	Ta_150699	–	–
BC1G_15551	Short chain Dehydrogenase/reductase	22.8	NCU08699 NCU01107	X	–	–	–	–
BC1G_08145	*BcCRY2*, cryptochrome 2	17.6	NCU00582 NCU08626	X	TR_107680	Ta_302457	An0387	AFUA_1G01600
BC1G_03556	Alcohol dehydrogenase	16.6	NCU11395	X	–	–	AN2470	–
BC1G_04088	Hypothetical protein	16.1	–	X	–	–	–	–
BC1G_15022	/	15.9	–	X	–	–	–	–
BC1G_16235	Hypothetical protein	15.8	–	X	–	–	–	–
BC1G_02449	Hypothetical protein	15	–	X	–	–	–	–
BC1G_02552	Hypothetical protein	14.7	–	X	–	–	–	–
Trichoderma reesei (Tisch et al. 2011)								
TR_34493	a-type pheromone precursor, *hpp1*	32.8	–	–	X	–	–	–
TR_59364	Sexual differentiation protein, ISP4	13.7	–	–	X	–	–	–
TR_106171	Heteroincompatibility domain protein	13.1	–	–	X	–	–	–
TR_81609	ENVOY, *env1*	10.7	NCU03967	BCG1_04348	X	Ta_150699	–	–
TR_123865	Peptidase S8 and S53, subtilisin, kexin, sedolisin	9.1	–	–	X	–	–	–
TR_72086	ArgE, acetylornithine deacetylase	6.2	–	–	X	–	–	–
TR_124341	Mating type protein MATa1 (MAT1-2-1)	5.7	–	–	X	–	–	–
TR_62693	Putative ABC-transporter	5.3	–	–	X	–	–	–
TR_44278	Rab geranyl transferase escort protein	5.1	–	–	X	–	–	–
TR_23238	Unknown protein	5.0	NCU_04343	–	X	–	AN11095	–
Trichoderma atroviride (García-Esquivel et al. 2016)								
Ta_131348	Hypothetical protein	244.3	NCU06125	–	–	X	–	–
Ta_300570	grg1	105.3	NCU03753	BCG1_11685	TR_64667 TR_73516	X	AN9285 AN5056	AFUA_5G14210
Ta_298329	Aromatic-ring hydroxyla	83	NCU08173	–	–	X	–	–
Ta_131177	Glycoside hydrolase family 15	74.4	–	–	TR_1885	X	–	–
Ta_150699	VIVID	50.5	NCU03967	BCG1_04348	TR_81609	X	–	–
Ta_138208	Putative trans. factor	47.8	–			X	–	–
Ta_255394	Hsp23	44.9	–	BCG1_08326	–	X	–	–

Table 1 (continued)

Gene	Description	L/D	Orthologs with a conserved light induction					
			Nc	*Bc*	*Tr*	*Ta*		
Ta_201457	Hypothetical protein	40.5	–	–	–	X	–	AFUA_1G12620
Ta_151377	Hypothetical protein peroxisomal dehydratase	23.9	–	–	–	X	–	–
Ta_228383	Predicted protein	22.6	–	BCG1_04288	–	X	–	–
Aspergillus nidulans (Ruger-Herreros et al. 2011)								
AN5056	ccgB	238.9	NCU03753	BC1G_11685	TR_64667 TR_73516	Ta_300570	X	AFUA_5G14210
AN9285	ccgA	219.8	NCU03753	BC1G_11685	TR_64667 TR_73516	Ta_300570	X	AFUA_5G14210
AN0045	Aos23	215.3	–	–	–	–	X	–
AN0693	Hypothetical protein	153.3	NCU04266	BC1G_10056	–		X	AFUA_1G13550
AN8339	Hypothetical protein	112.2	–	BC1G_02245	–	–	X	–
AN4299	Clock and temp controlled	93.7	NCU08949	BC1G_04134	–	–	X	AFUA_4G05900
AN7558	Hypothetical protein	82.1	–	–	–	–	X	–
AN8641	Hypothetical protein	77.2	–	–	–	–	X	–
AN8638	cetJ	73.0	NCU05005	–	–	–	X	–
AN8018	Auxin efflux transporter	67.2	–	BCG1G_04005	–	–	X	–
Aspergillus fumigatus (Fuller et al. 2013)								
AFUA_4G01360	MFS transporter	10.2	–	–	–	–	AN9370	X
AFUA_4G01440	Glutathione *S*-transferase family protein	6.2	–	BCG1_09645	–	–	–	X
AFUA_1G01600	Deoxyribodipyrimidine photo-lyase, Phr1	3.6	NCU08626	BCG1_13162 BCG1_08145	TR_107680	Ta_302457	AN0387	X
AFUA_3G01430	Benzodiazepine receptor	3.5	NCU02801	–	–	–	AN8959	X
AFUA_2G00790	Hypothetical protein	2.7	–	–	–	–	–	X
AFUA_6G00510	NADP-dependent alcohol dehydrogenase	2.7	NCU04823 NCU08402	–	–	–	AN8628	X
AFUA_6G14280	Flavin-binding monooxygenase-like protein	2.6	NCU07332	BCG1_03037	–	–	AN5338	X
AFUA_7G05730	Hypothetical protein	2.6	NCU04946	–	–	–	AN10847	X
AFUA_7G00170	Dimethylallyl tryptophan synthase GliD1	2.5	–	–	–	–	–	X
AFUA_3G11550	LEA domain protein	2.5	NCU09057	BCG1_10283	–	–	–	X

A summary of the most light-induced genes in the indicated organism. The predicted protein sequence of each gene in the first column was used in a BLASTP search (NCBI) against the predicted protein database of the other five fungi represented in the table. Putative homologs that appear in the light-regulated dataset of those fungi are provided (columns 5–9). The L/D column represents the ratio of gene expression of light (L) compared to dark (D) samples. For those datasets that include multiple timepoints in the light (*Neurospora*, *Aspergillus fumigatus*), the timepoint with the largest L/D ratio is presented

Nc Neurospora crassa, *Bc Botrytis cinerea*, *Tr Trichoderma reesei*, *Ta Trichoderma atroviride*, *An Aspergillus nidulans*, *Af Aspergillus fumigatus*

photoinduction kinetics, will have to be assessed independently, e.g., by qRT-PCR or western blot.

For those working in species for which transcriptomic data are currently not available, candidate promoters may be selected on the basis of their homology to ones listed in Table 1. For each gene in the table (each row), homologs from the other datasets that display similar light-regulation are noted. Gene AN8640 of *A. nidulans*, for example, is listed in the row corresponding to *Neurospora's con-6* because the two genes are orthologs and they are similarly light-induced in their respective whole-genome experiments; notably, AN8640 was not among the top 10 most induced in *A. nidulans* (Ruger-Herreros et al. 2011). In principle then, those genes with the largest degree of conserved regulation across the species represented in Table 1 are more likely to be light-induced in fungi not in the table. To illustrate, the ortholog of the *T. atroviride grg-1* may be a better candidate in your fungus (as it is upregulated in the other five species in Table 1) than is the ortholog to *T. atroviride* transcription factor Ta_138208 (which only appears in the *T. atroviride* dataset). Indeed, orthologs of the *A. fumigatus* photolyase, *phr1*, are not only upregulated by light in all the species represented in Table 1 but also demonstrate similar patterns of robust light induction in various other ascomycetes (*Trichoderma harzianum*, *Fusarium oxysporum*), the basidiomycete pathogen *Ustilago maydis*, as even the Mucromycotina *Phycomyces blakesleeanus* (Tagua et al. 2015; Brych et al. 2016; Alejandre-Durán et al. 2003; Berrocal-Tito et al. 1999).

Although informative, care must be taken when assessing the transcriptomic studies described above. First, microarray-based studies are relatively insensitive compared to RNA-seq and consequently may have failed to detect subsets of light-induced genes. This is perhaps best exemplified in the basidiomycete yeast *Cryptococcus neoformans*, in which microarrays detected only a single light-induced gene at the 2-fold cutoff, *hem15* (Idnurm and Heitman 2010). For that reason, this study was omitted from Table 1. However, the *hem15* promoter identified may prove to be an excellent candidate in your fungus, as it was demonstrated to be light induced in the ascomycete *Neurospora* and two Mucormycotina species, *Phycomyces blakesleeanus* and *Rhizopus oryzae* (Idnurm and Heitman 2010). A second consideration is that the nutritional source and mode of growth (i.e., liquid versus solid culture) are important variables across the transcriptomic experiments described in Table 1. In one way, this perhaps places greater emphasis on those genes that are conserved across them, i.e., their light induction transcends those variables and makes them more likely to be conservatively regulated in other fungi. However, those experimental variables also represent an important caveat in interpreting the data. For example, the asexual developmental genes induced in the *A. nidulans* array were not found in *A. fumigatus*, but this may reflect the fact that *A. fumigatus* was grown in submerged liquid culture (Fuller et al. 2013), a condition that may suppress sporulation (Lee et al. 2016). Finally, one must consider the length of the light treatment employed in each of the studies. Most involve acute light inductions (minutes to a couple of hours), which likely reveal those genes that are direct targets of the white collar orthologs, or are perhaps a target of another transcription factor directly downstream of the WCC (Chen et al. 2009). The *T. reesei* study in Table 1, by contrast, assesses gene expression changes following 72 h light exposure. While some genes identified in the study may be WCC targets (e.g., *env1*), others may be involved in the long-term adaptation to light and, consequently, may be unresponsive during the early photoresponse (Wolfers et al. 2015). Arguably, an ideal photoregulatable promoter is one that provides both an acute and prolonged light induction, but studies that track gene expression over an extended timecourse (from minutes to days post-induction) in a single organism are currently lacking.

Putative light-repressed promoters

Table 2 catalogs the most strongly light-repressed genes from the studies described above and, in doing so, highlights two major barriers to the development of a light-*repressible* promoter system. First, the magnitude to which genes tend to be repressed by light is considerably smaller than that of induction. For example, the greatest degree of light suppression observed in *Neurospora* and *T. atroviride* (the RNA-seq studies) was 10-fold and 16-fold, respectively; by comparison, the strongest degrees of induction were, respectively, 362-fold and 266-fold. Indeed, assessment of *Neurospora* light-regulated genes by microarray failed to detect the light-repressed category altogether due to the lower sensitivity of the technique (Chen et al. 2009; Wu et al. 2014). An exception to this appears to be *T. reesei* microarray dataset, for which the highest magnitudes of induction and repression were both around 30-fold. Nevertheless, it has yet to be determined if such a promoter can repress transcription enough to be experimentally useful, e.g., studies in which essentiality is to be tested. The analysis of light-repressed genes by RT-PCR and western blotting will be important follow-ups to this end. Table 2 also reveals a lack of correspondence between light-suppressed genes across the datasets. This suggests that selecting putative photorepressible genes in species outside of those in Table 2 may be difficult.

In summary, the existing transcriptomic datasets provide a good starting point in the search for light-regulatable promoters. Such data are, however, so-far limited in their phylogenetic scope (all are in the Ascomycota, mostly Sordariomycetes), and so comparable studies in representatives of the Basidiomycota or Mucormycotina will be needed for accurate promoter predictions in those clades. Furthermore, whole-genome data are lacking with respect to the differential regulation of genes by distinct light qualities,

Table 2 Top light-repressed genes across whole-genome transcriptional datasets

Gene	Description	L/D	Orthologs with a conserved light induction					
			Nc	*Bc*	*Tr*	*Ta*	*An*	*Af*
Neurospora crassa (Wu et al. 2014)								
NCU09873	Phosphoenolpyruvate carboxykinase, acu-6	−9.7	X	–	–	–	–	AFUA_6G07720
NCU09506	Hypothetical protein	−7.6	X	–			–	
NCU07307	Fatty acid synthase beta subunit dehydratase, cel-2	−7.3	X	–	–	Ta_226146	–	–
NCU02344	Fungal cellulose binding domain, gh61–12	−7.2	X	–	TR_73643	–	–	–
NCU05126	Hypothetical protein	−7.2	X	–	–	Ta_299408	–	–
NCU03963	5′-Methylthioadenosine phosphorylase, nic-7	−7	X	–	–	–	–	–
NCU07308	Fatty acid synthase alpha subunit reductase, cel-1	−6.6	X	–	–	Ta_85662	AN9407	–
NCU09497	Fatty acid desaturase, fam-3	−6.5	X	–	–	Ta_296851 Ta_297121	AN7204	–
NCU09764	Hypothetical protein, gh61-14	−6.4	X	–	–	–	–	–
NCU07375	MFS phosphate transporter, pho-7	−6.3	X	–	–	–	–	–
Botrytis cinerea (Schumacher et al. 2014)								
BC1G_10514	Similar to TF Cys6	−6.7	–	X	–	–	–	–
BC1G_10537	BcSTC5, sesquiterpene cyclase 5	−4.9	–	X	–	–	–	–
BC1G_10512	No annotation	−4.0	–	X	–	–	–	–
BC1G_09971	Similar to amino acid transporter	−4.0	–	X	TR_57185	–	AN2043	–
BC1G_10515	Hypothetical protein	−3.9	–	X	–	–	–	–
BC1G_02024	No annotation	−3.8	–	X		–	–	–
BC1G_02022	Predicted protein	−3.3	–	X		–	–	–
BC1G_13472	Hypothetical protein	−3.3	–	X		–	–	–
BC1G_13471	Similar to U1biquitin-specific peptidase	−2.8	–	X	–	–	–	–
BC1G_06003	No annotation	−2.7	–	X		–	–	–
Trichoderma reesei (Tisch et al. 2011)								
TR_124198	Unknown protein	−29.7	–	–	X	–	–	–
TR_108232	Unique protein	−11.0	–	–	X	–	–	
TR_64869	Cytochrome P450 subfamily	−8.3	–	–	X	–	–	–
TR_23292	Zinc-binding oxidoreductase	−7.3	NCU07042	–	X	–	–	–
TR_105449	Cyclin C-dependent kinase CDK8	−6.7	–	–	X	–	–	–
TR_49274	GH16 β-1,3/4-glucanase	−6.6	–	–	X	–	–	–
TR_58366	AAA ± type ATPase	−6.4	–	–	X	–	–	–
TR_123955	Hypothetical protein bearing a cerato-platanin domain, related to *N. crassa* SnodProt1	−6.1	–	–	X	–	–	AFUA_2G12630
TR_74282	C-rich protein (no hydrophobin, Q174-related)	−6.1	–	–	X	–	–	–
TR_73623	Monooxygenase, aromatic ring catabolism	−5.7	NCU07737	–	X	–	–	–
Trichoderma atroviride (García-Esquivel et al. 2016)								
Ta_301894	Eukaryotic translation initiation factor 3 subunit 2	−16.7	–	–	–	X	–	–
Ta_85568	PTH11-like, GPCR receptor	−15.1	NCU09823	–	–	X	AN6413	–
Ta_244768	Hypothetical protein similar to alanine racemase	−12.3	–	–	–	X	–	–
Ta_296851	Hypothetical protein similar to oleate delta-12 desaturase	−10.7	NCU09497 NCU02209	–	–	X	AN7204	–
Ta_298755	Hypothetical protein similar to high-affinity iron permease CaFTR2	−8.7	–	–	TR_80639	X	–	AFUA_5G03800
Ta_147947	Cysteine synthase related to N. crassa CYS-17	−8.3	–	–	–	X	–	–
Ta_46589	Predicted protein	−7.0	–	–	–	X	–	–
Ta_301399	Tetrahydroxynaphthalene reductase	−6.8	NCU01904	–	–	X	–	–

Table 2 (continued)

Gene	Description	L/D	Orthologs with a conserved light induction					
			Nc	*Bc*	*Tr*	*Ta*	*An*	*Af*
Ta_143500	Hypothetical protein similar to phenol 2-monooxygenase	−6.4	–	–	–	X	–	–
Ta_40957	Hypothetical protein similar to DEAD box RNA helicase Hca4	−6.0	NCU04439 NCU09349	–	–	X	–	–
Aspergillus nidulans (Ruger-Herreros et al. 2011)								
AN1052	veA	−9	–	–	–	–	X	–
AN8647	ALS family protein, nitA	−6.3	–	–	–	–	X	–
AN1008	Putative nitrate transporter (crnA)	−5.2	–	–	–	–	X	–
AN5558	Alkaline protease (prtA)	−5.0	NCU06055	–	–	Ta_302419 Ta_198568	X	–
AN3304	GABA transporter	−4.6	–	–	–	–	X	–
AN0231	Conidiophore-specific phenol oxidase (ivoB)	−4.4	NCU09199	–	–	–	X	–
AN8063	Acid phosphatase activity	−3.9	–	–	–	–	X	–
AN9076	Putative adhesion function	−3.9	–	–	–	–	X	–
AN2926	60S ribosomal protein Nsa2	−3.9	–	–	–	–	X	–
AN8539	GNAT acetyltransferase. sidG	−3.8	–	–	–	–	X	–
Aspergillus fumigatus (Fuller et al. 2013)								
AFUA_2G03730	Ctr copper transporter family	−4.8	–	–	–	–	–	X
AFUA_4G14380	Glutathione S-transferase	−3.7	–	–	–	–	–	X
AFUA_3G06720	ThiJ/PfpI family protein	−3.4	–	–	–	–	–	X
AFUA_1G02290	Hypothetical protein	−3.3	–	–	–	–	–	X
AFUA_4G03940	Ferric-chelate reductase, fre7	−3.2	–	–	–	–	AN8683	X
AFUA_8G01860	NmrA-like family protein	−3.2	–	–	–	–	–	X
AFUA_6G00430	IgE-binding protein	−2.7	–	–	–	–	–	X
AFUA_6G06470	Heat shock protein Hsp30-like	−2.7	–	–	–	–	–	X
AFUA_5G02330	Major allergen and cytotoxin AspF1	−2.7	–	–	–	–	–	X
AFUA_1G05790	GPI anchored serine-rich protein	−2.5	–	–	–	–	–	X

A summary of the most light-repressed genes in the indicated organism. The predicted protein sequence of each gene in the first column was used in a BLASTP search (NCBI) against the predicted protein database of the other five fungi represented in the table. Putative homologs that appear in the light-regulated dataset of those fungi are provided (columns 5–9)

either directly with blue versus red light, or by proxy using white light against strains bearing knockouts of blue (WCC) vs. red (phytochrome) light photoreceptors. Only for *Neurospora* do such data exist, and in this case, red light fails to drive detectable transcriptional changes and the response to white light is apparently unaltered in a phytochrome knockout mutant (Froehlich et al. 2005; Chen et al. 2009). In contrast, many fungi that harbor phytochrome orthologs do indeed exhibit red-light-driven responses (Idnurm and Heitman 2005a; Fuller et al. 2015); therefore, one exciting possibility in those fungi is the development of a multichromatic system (e.g., one gene-of-interest is induced in blue light, a different gene by red light, and both in white!) which would be particularly useful for epistasis or synthetic lethality studies.

Is a light-regulatable promoter system right for you?

There are some minimal requirements in place for a light-regulatable promoter system to get off the ground. First and foremost, your fungus must overtly respond to light. The details of the photosensory cascade may not be important, however (e.g., whether the WCC serves as an activator or a repressor), so long as light-responsive promoters meet your experimental need, e.g., they are *tight* and *robust* (Dasgupta et al. 2016). Some important fungal lineages have apparently lost their suite of photoreceptors over the course of their evolution, including the hemiascomycete yeasts (including the important model *Saccharomyces* pathogen *Candida* sp.) and various ascomycete and basidiomycete dermatophytes (e.g., *Trichophyton*, *Microsporum*, *Malessezia*) (Dunlap and Loros 2006; Idnurm et al. 2010). A few technical requirements exist as well. For example, the experimenter must be able to control the light environment, which may include specialized incubators that are fitted with a programmable light source. White fluorescent lights are typically sufficient as their emissions span the major biologically active spectra (e.g., blue and red wavelengths). However, multiple LEDs may be needed if dual-wavelength systems were developed. Molecular biology experiments may further demand that un-induced (dark) samples be harvested in the absence of stimulating light. In the case of fungi that are unresponsive to the red spectrum (e.g., *Neurospora*), sample collection can be performed under a red safe light, such as those used for film development; for most fungi, however, samples harvesting should be performed in complete darkness with infrared oculars.

Two important considerations, depending upon the experiment, center around the long-term exposure of fungus to light. First is the issue of photoadaptation which, as described above, may limit the ability to constitutively overexpress a protein under constant illumination. This can be bypassed by using photoadaptation-deficient strains as demonstrated with *Δvvd* in *Neurospora* (Hurley et al. 2012). However, photoadaptation has been observed in fungi that lack a clear *vvd* ortholog, including *Aspergillus* (Fig. 2a) and *Phycomyces*, and the mechanism by which it occurs remains obscure (Ruger-Herreros et al. 2011; Rodríguez-Romero and Corrochano 2006; Olmedo et al. 2013; Fuller et al. 2013). Second is the potential of phototoxicity mediated by light itself. In *S. cerevisiae* for instance—a fungus with no intrinsic photosensory capability—high intensity light exposure can modulate cellular respiration and induce oxidative stress (Robertson et al. 2013). In agreement, light reduces the growth rate of the plant fungal pathogen *Botrytis cinerea*, but this can be overcome through the addition of the antioxidant ascorbate to the medium (Canessa et al. 2013). Therefore, the secondary impact of reactive oxygen species may represent an important experimental variable between light and dark cultures, or may alter cellular metabolism and interfere with the expression of the target gene. Both the photoadaptation and phototoxicity issues may be bypassed by exposing the fungus to periodic light pulses, however, rather than placing it under constant illumination. Figure 2b demonstrates that 3 min of white light is sufficient to induce *phr1* expression in *A. fumigatus*, and this induction remains detectable for several hours (Fuller, Dunlap, and Loros,

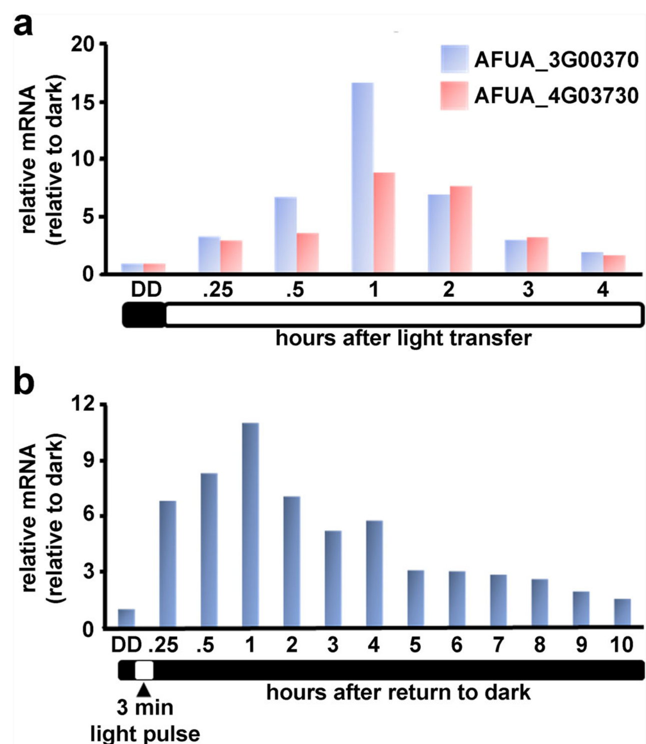

Fig. 2 Kinetics of light-regulated gene expression in *A. fumigatus*. **a** Photoadaptation of two genes under constant white light illumination. **b** The initial induction and eventual decline of *phr1* (photolyase) transcript after a 3 min white light pulse. Both panels represent previously unpublished qRT-PCR data, with actin serving as a normalizing gene

previously unpublished). Therefore, a short light pulse every 4 h or so may be sufficient to keep expression levels high on a long-term basis (depending on the experimental demand) and effectively minimize off-target effects by light itself (Schwerdtfeger and Linden 2001).

A major advantage of using light as an inducing signal, in principle, is that it allows for *metabolic flexibility*, i.e., it can be used irrespective of the medium. Indeed, the light induction of certain conserved genes (e.g., the white collar, photolyases, or *vvd* orthologs) seems to occur on various carbon sources, such as glucose, glycerol, and microcrystalline cellulose (Wu et al. 2014; Tisch et al. 2011). However, in other cases, the light response may vary qualitatively and quantitatively with the medium. In *A. nidulans*, for example, the influence of light on secondary metabolism depends on the glucose concentration: At 1% glucose, light represses sterigmatocystin production; at 2% glucose, it induces production (Atoui et al. 2010). The degree to which light stimulates conidiation in *A. nidulans*, and likely the expression of developmental gene promoters, also depends upon the glucose concentration (Atoui et al. 2010). Similarly, *Neurospora ccg-1*, which is the ortholog of the highly light-induced *T. atroviride grg-1* and *A. nidulans ccgA* genes listed in Table 1, is only weakly light-induced in the *Neurospora* RNA-seq experiment because the gene is also glucose-repressed (McNally and Free 1988). Broadly speaking, light is well known to influence primary metabolic pathways in fungi, and so long-term/secondary effects of culture illumination may impact phenotypes beyond the direct induction of the target gene (Tisch and Schmoll 2010; Tisch et al. 2014).

In summary, regulatable promoter systems are important tools to probe fungal gene function as well as optimize protein/metabolite production in industry. Perhaps the greatest advantage of a light-regulatable promoter over nutritional or chemical systems is the ease at which the signal can be applied and removed—literally with a flip of a switch! The *pvvd* system of *Neurospora* further demonstrates the potential *tightness*, *robustness*, and *tunability* of light-regulatable promoters, but these should be weighed against the need to stringently control the light environment as well as the global impact of light on the metabolism and development on your organism.

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Compliance with ethical standards

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References

Alejandre-Durán E, Roldán-Arjona T, Ariza RR, Ruiz-Rubio M (2003) The photolyase gene from the plant pathogen *Fusarium oxysporum* f. sp. lycopersici is induced by visible light and alpha-tomatine from tomato plant. Fungal Genet Biol 40:159–165

Amaar YG, Moore MM (1998) Mapping of the nitrate-assimilation gene cluster (*crnA-niiA-niaD*) and characterization of the nitrite reductase gene (niiA) in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr Genet 33(3):206–215

Antunes MS, Hodges TK, Carpita NC (2016) A benzoate-activated promoter from *Aspergillus niger* and regulation of its activity. Appl Microbiol Biotechnol 100:5479–5489. https://doi.org/10.1007/s00253-016-7373-3

Atoui A, Kastner C, Larey CM, Thokala R, Etxebeste O, Espeso EA, Fischer R, Calvo AM (2010) Cross-talk between light and glucose regulation controls toxin production and morphogenesis in *Aspergillus nidulans*. Fungal Genet Biol 47:962–972. https://doi.org/10.1016/j.fgb.2010.08.007

Avelar GM, Schumacher RI, Zaini PA, Leonard G, Richards TA, Gomes SL (2014) A rhodopsin-guanylyl cyclase gene fusion functions in visual perception in a fungus. Curr Biol 24:1234–1240. https://doi.org/10.1016/j.cub.2014.04.009

Ballario P, Talora C, Galli D, Linden H, Macino G (1998) Roles in dimerization and blue light photoresponse of the PAS and LOV domains of *Neurospora crassa* white collar proteins. Mol Microbiol 29:719–729

Bayram O, Braus GH, Fischer R, Rodriguez-Romero J (2010) Spotlight on *Aspergillus nidulans* photosensory systems. Fungal Genet Biol 47:900–908. https://doi.org/10.1016/j.fgb.2010.05.008

Berrocal-Tito G, Sametz-Baron L, Eichenberg K, Horwitz BA, Herrera-Estrella A (1999) Rapid blue light regulation of a *Trichoderma harzianum* photolyase gene. J Biol Chem 274:14288–14294

Bottin A, Kämper J, Kahmann R (1996) Isolation of a carbon source-regulated gene from *Ustilago maydis*. Mol Gen Genet 253:342–352

Brych A, Mascarenhas J, Jaeger E, Charkiewicz E, Pokorny R, Bölker M, Doehlemann G, Batschauer A (2016) White collar 1-induced photolyase expression contributes to UV-tolerance of *Ustilago maydis*. Microbiology 5:224–243. https://doi.org/10.1002/mbo3.322

Canessa P, Schumacher J, Hevia MA, Tudzynski P, Larrondo LF (2013) Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: characterization of the white collar complex. PLoS One 8:e84223. https://doi.org/10.1371/journal.pone.0084223

Castellanos F, Schmoll M, Martínez P, Tisch D, Kubicek CP, Herrera-Estrella A, Esquivel-Naranjo EU (2010) Crucial factors of the light perception machinery and their impact on growth and cellulase gene transcription in *Trichoderma reesei*. Fungal Genet Biol 47:468–476. https://doi.org/10.1016/j.fgb.2010.02.001

Castrillo M, Avalos J (2014) Light-mediated participation of the VIVID-like protein of *Fusarium fujikuroi* VvdA in pigmentation and development. Fungal Genet Biol 71:9–20. https://doi.org/10.1016/j.fgb.2014.08.004

Chen CH, Ringelberg CS, Gross RH, Dunlap JC, Loros JJ (2009) Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. EMBO J 28:1029–1042. https://doi.org/10.1038/emboj.2009.54

Chen CH, DeMay BS, Gladfelter AS, Dunlap JC, Loros JJ (2010) Physical interaction between VIVID and white collar complex regulates photoadaptation in *Neurospora*. Proc Natl Acad Sci U S A 107:16715–16720. https://doi.org/10.1073/pnas.1011190107

Cheng P, Yang Y, Gardner KH, Liu Y (2002) 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 22:517–524

Corrochano LM, Garre V (2010) Photobiology in the Zygomycota: multiple photoreceptor genes for complex responses to light. Fungal Genet Biol 47:893–899. https://doi.org/10.1016/j.fgb.2010.04.007

Corrochano LM, Kuo A, Marcet-Houben M, Polaino S, Salamov A, Villalobos-Escobedo JM, Grimwood J, Álvarez MI, Avalos J, Bauer D, Benito EP, Benoit I, Burger G, Camino LP, Cánovas D, Cerdá-Olmedo E, Cheng JF, Domínguez A, Eliáš M, Eslava AP, Glaser F, Gutiérrez G, Heitman J, Henrissat B, Iturriaga EA, Lang BF, Lavín JL, Lee SC, Li W, Lindquist E, López-García S, Luque EM, Marcos AT, Martin J, McCluskey K, Medina HR, Miralles-Durán A, Miyazaki A, Muñoz-Torres E, Oguiza JA, Ohm RA, Olmedo M, Orejas M, Ortiz-Castellanos L, Pisabarro AG, Rodríguez-Romero J, Ruiz-Herrera J, Ruiz-Vázquez R, Sanz C, Schackwitz W, Shahriari M, Shelest E, Silva-Franco F, Soanes D, Syed K, Tagua VG, Talbot NJ, Thon MR, Tice H, de Vries RP, Wiebenga A, Yadav JS, Braun EL, Baker SE, Garre V, Schmutz J, Horwitz BA, Torres-Martínez S, Idnurm A, Herrera-Estrella A, Gabaldón T, Grigoriev IV (2016) Expansion of signal transduction pathways in fungi by extensive genome duplication. Curr Biol 26:1577–1584. https://doi.org/10.1016/j.cub.2016.04.038

Dasgupta A, Chen CH, Lee C, Gladfelter AS, Dunlap JC, Loros JJ (2015) Biological significance of photoreceptor photocycle length: VIVID photocycle governs the dynamic VIVID-white collar complex pool mediating photo-adaptation and response to changes in light intensity. PLoS Genet 11:e1005215. https://doi.org/10.1371/journal.pgen.1005215

Dasgupta A, Fuller KK, Dunlap JC, Loros JJ (2016) Seeing the world differently: variability in the photosensory mechanisms of two model fungi. Environ Microbiol 18:5–20. https://doi.org/10.1111/1462-2920.13055

de Graaff LH, van den Broeck HC, van Ooijen AJ, Visser J (2005) Regulation of the xylanase-encoding *xlnA* gene of *Aspergillus tubigensis*. Mol Microbiol 12:479–490

Drepper T, Krauss U (2011) Lights on and action! Controlling microbial gene expression by light. Appl Microbiol Biotechnol 90:23–40

Druzhinina IS, Kubicek CP (2017) Genetic engineering of *Trichoderma reesei* cellulases and their production. Microb Biotechnol 10:1485–1499. https://doi.org/10.1111/1751-7915.12726

Dunlap JC, Loros JJ (2006) How fungi keep time: circadian system in *Neurospora* and other fungi. Curr Opin Microbiol 9(6):579–587

Exley GE, Colandene JD, Garrett RH (1993) Molecular cloning, characterization, and nucleotide sequence of *nit-6*, the structural gene for nitrite reductase in *Neurospora crassa*. J Bacteriol 175:2379–2392

Fischer R, Aguirre J, Herrera-Estrella A, Corrochano LM (2016) The complexity of fungal vision. Microbiol Spectr 4:1–22. https://doi.org/10.1128/microbiolspec.FUNK-0020-2016

Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White Collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. Science 297:815–819

Froehlich AC, Noh B, Vierstra RD, Loros J, Dunlap JC (2005) Genetic and molecular analysis of phytochromes from the filamentous fungus *Neurospora crassa*. Eukaryot Cell 4:2140–2152

Fuller KK, Ringelberg CS, Loros JJ, Dunlap JC (2013) The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. MBio ;4. pii: e00142–13. doi: https://doi.org/10.1128/mBio.00142-13.

Fuller KK, Loros JJ, Dunlap JC (2015) Fungal photobiology: visible light as a signal for stress, space and time. Curr Genet 61:275–288. https://doi.org/10.1007/s00294-014-0451-0

Fuller KK, Dunlap JC, Loros JJ (2016) Fungal light sensing at the bench and beyond. Adv Genet 96:1–51. https://doi.org/10.1016/bs.adgen.2016.08.002

García-Esquivel M, Esquivel-Naranjo EU, Hernández-Oñate MA, Ibarra-Laclette E, Herrera-Estrella A (2016) The Trichoderma atroviride cryptochrome/photolyase genes regulate the expression of blr1-independent genes both in red and blue light. Fungal Biol 120:500–512. https://doi.org/10.1016/j.funbio.2016.01.007

García-Martínez J, Brunk M, Avalos J, Terpitz U (2015) The CarO rhodopsin of the fungus *Fusarium fujikuroi* is a light-driven proton pump that retards spore germination. Sci Rep 5:7798. https://doi.org/10.1038/srep07798

Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S, Dow S, Lucau-Danila A, Anderson K, André B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Güldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kötter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR, Wilhelmy J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418:387–391. https://doi.org/10.1038/nature00935

Giles NH, Case ME, Baum J, Geever R, Huiet L, Patel V, Tyler B (1985) Gene organization and regulation in the qa (quinic acid) gene cluster of *Neurospora crassa*. Microbiol Rev 49:338–358

Hartman JL 4th, Garvik B, Hartwell L (2001) Principles for the buffering of genetic variation. Science 291:1001–1004

Hedtke M, Rauscher S, Röhrig J, Rodríguez-Romero J, Yu Z, Fischer R (2015) Light-dependent gene activation in *Aspergillus nidulans* is strictly dependent on phytochrome and involves the interplay of phytochrome and white collar-regulated histone H3 acetylation. Mol Microbiol 97:733–745. https://doi.org/10.1111/mmi.13062

Heintzen C, Loros JJ, Dunlap JC (2001) The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. Cell 104:453–464

Helmschrott C, Sasse A, Samantaray S, Krappmann S, Wagener J (2013) Upgrading fungal gene expression on demand: improved systems for doxycycline-dependent silencing in *Aspergillus fumigatus*. Appl Environ Microbiol 79:1751–1754. https://doi.org/10.1128/AEM.03626-12

Hu W, Sillaots S, Lemieux S, Davison J, Kauffman S, Breton A, Linteau A, Xin C, Bowman J, Becker J, Jiang B, Roemer T (2007) Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. PLoS Pathog 3(3):e24

Hunt SM, Thompson S, Elvin M, Heintzen C (2010) VIVID interacts with the WHITE COLLAR complex and FREQUENCY-interacting RNA helicase to alter light and clock responses in *Neurospora*. Proc Natl Acad Sci U S A 107:16709–16714. https://doi.org/10.1073/pnas.1009474107

Hurley JM, Chen CH, Loros JJ, Dunlap JC (2012) Light-inducible system for tunable protein expression in *Neurospora crassa*. G3 (Bethesda) 2:1207–1212. https://doi.org/10.1534/g3.112.003939

Idnurm A, Heitman J (2005a) Photosensing fungi: phytochrome in the spotlight. Curr Biol 15:R829eR832

Idnurm A, Heitman J (2005b) Light controls growth and development via a conserved pathway in the fungal kingdom. PLoS Biol 3:e95

Idnurm A, Heitman J (2010) Ferrochelatase is a conserved downstream target of the blue light-sensing white collar complex in fungi. Microbiology 156:2393–2407. https://doi.org/10.1099/mic.0.039222-0

Idnurm A, Rodríguez-Romero J, Corrochano LM, Sanz C, Iturriaga EA, Eslava AP, Heitman J (2006) The *Phycomyces madA* gene encodes a

blue-light photoreceptor for phototropism and other light responses. Proc Natl Acad Sci U S A 103:4546–4551

Idnurm A, Verma S, Corrochano LM (2010) A glimpse into the basis of vision in the kingdom Mycota. Fungal Genet Biol 47(11):881–892. https://doi.org/10.1016/j.fgb.2010.04.009

Kamada T, Sano H, Nakazawa T, Nakahori K (2010) Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. Fungal Genet Biol 47:917–921. https://doi.org/10.1016/j.fgb.2010.05.003

Lamb TM, Vickery J, Bell-Pedersen D (2013) Regulation of gene expression in *Neurospora crassa* with a copper responsive promoter. G3 3: 2273–80. doi: https://doi.org/10.1534/g3.113.008821.

Larrondo LF, Colot HV, Baker CL, Loros JJ, Dunlap JC (2009) Fungal functional genomics: tunable knockout-knock-in expression and tagging strategies. Eukaryot Cell 8:800–804. https://doi.org/10.1128/EC.00072-09

Lee BY, Han SY, Choi HG, Kim JH, Han KH, Han DM (2005) Screening of growth- or development-related genes by using genomic library with inducible promoter in *Aspergillus nidulans*. J Microbiol 43: 523–528

Lee MK, Kwon NJ, Lee IS, Jung S, Kim SC, Yu JH (2016) Negative regulation and developmental competence in *Aspergillus*. Sci Rep 6: 28874. https://doi.org/10.1038/srep28874

Liu T, Wang T, Li X, Liu X (2008) Improved heterologous gene expression in *Trichoderma reesei* by cellobiohydrolase I gene (*cbh1*) promoter optimization. Acta Biochim Biophys Sin Shanghai 40:158–165

Losi A, Gärtner W (2011) Old chromophores, new photoactivation paradigms, trendy applications: flavins in blue light-sensing photoreceptors. Photochem Photobiol 87:491–510. https://doi.org/10.1111/j.1751-1097.2011.00913.x

Malzahn E, Ciprianidis S, Káldi K, Schafmeier T, Brunner M (2010) Photoadaptation in *Neurospora* by competitive interaction of activating and inhibitory LOV domains. Cell 142:762–772. https://doi.org/10.1016/j.cell.2010.08.010

McNally MT, Free SJ (1988) Isolation and characterization of a *Neurospora* glucose-repressible gene. Curr Genet 14:545–551

Mehra A, Shi M, Baker CL, Colot HV, Loros JJ, Dunlap JC (2009) A role for casein kinase 2 in the mechanism underlying circadian temperature compensation. Cell 137:749–760. https://doi.org/10.1016/j.cell.2009.03.019

Meyer V, Wanka F, van Gent J, Arentshorst M, van den Hondel CA, Ram AF (2011) Fungal gene expression on demand: an inducible, tunable, and metabolism-independent expression system for *Aspergillus niger*. Appl Environ Microbiol 77:2975–2983. https://doi.org/10.1128/AEM.02740-10

Meyer V, Fiedler M, Nitsche B, King R (2015) The cell factory *Aspergillus* enters the big data era: opportunities and challenges for optimising product formation. Adv Biochem Eng Biotechnol 149:91–132. https://doi.org/10.1007/10_2014_297

Möller M, Stukenbrock EH (2017) Evolution and genome architecture in fungal plant pathogens. Nat Rev Microbiol 15:771. https://doi.org/10.1038/nrmicro.2017.143

Navarro-Sampedro L, Yanofsky C, Corrochano LM (2008) A genetic selection for *Neurospora crassa* mutants altered in their light regulation of transcription. Genetics 178:171–183. https://doi.org/10.1534/genetics.107.079582

Olmedo M, Ruger-Herreros C, Luque EM, Corrochano LM (2013) Regulation of transcription by light in *Neurospora crassa*: a model for fungal photobiology? Fungal Biol Rev 27:10–18

Osmani SA, Mirabito PM (2004) The early impact of genetics on our understanding of cell cycle regulation in *Aspergillus nidulans*. Fungal Genet Biol 41:401–410

Ouyang S, Beecher CN, Wang K, Larive CK, Borkovich KA (2015) Metabolic impacts of using nitrogen and copper-regulated promoters to regulate gene expression in *Neurospora crassa*. G3 5: 1899–908. doi: https://doi.org/10.1534/g3.115.020073

Pachlinger R, Mitterbauer R, Adam G, Strauss J (2005) Metabolically independent and accurately adjustable *Aspergillus* sp. expression system. Appl Environ Microbiol 71:672–678

Powers-Fletcher MV, Kendall BA, Griffin AT, Hanson KE (2016) Filamentous fungi. Microbiol Spectr 4:doi: https://doi.org/10.1128/microbiolspec.DMIH2-0002-2015

Purschwitz J, Müller S, Kastner C, Fischer R (2006) Seeing the rainbow: light sensing in fungi. Curr Opin Microbiol 9:566–571

Purschwitz J, Müller S, Kastner C, Schöser M, Haas H, Espeso EA, Atoui A, Calvo AM, Fischer R (2008) Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. Curr Biol 18: 255–259

Purschwitz J, Müller S, Fischer R (2009) Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the white collar protein LreB. Mol Gen Genomics 281:35–42

Robertson JB, Davis CR, Johnson CH (2013) Visible light alters yeast metabolic rhythms by inhibiting respiration. Proc Natl Acad Sci U S A 110:21130–21135. https://doi.org/10.1073/pnas.1313369110

Roche CM, Loros JJ, McCluskey K, Glass NL (2014) *Neurospora crassa*: looking back and looking forward at a model microbe. Am J Bot 101:2022–2035. https://doi.org/10.3732/ajb.1400377

Rodríguez-Romero J, Corrochano LM (2006) Regulation by blue light and heat shock of gene transcription in the fungus Phycomyces: proteins required for photoinduction and mechanism for adaptation to light. Mol Microbiol 61:1049–1059

Rodriguez-Romero J, Hedtke M, Kastner C, Müller S, Fischer R (2010) Fungi, hidden in soil or up in the air: light makes a difference. Annu Rev Microbiol 64:585–610. https://doi.org/10.1146/annurev.micro.112408.134000

Romero B, Turner G, Olivas I, Laborda F, De Lucas JR (2003) The *Aspergillus nidulans alcA* promoter drives tightly regulated conditional gene expression in *Aspergillus fumigatus* permitting validation of essential genes in this human pathogen. Fungal Genet Biol 40:103–114

Rørth P, Szabo K, Bailey A, Laverty T, Rehm J, Rubin GM, Weigmann K, Milán M, Benes V, Ansorge W, Cohen SM (1998) Systematic gain-of-function genetics in *Drosophila*. Development 125:1049–1057

Rosales-Saavedra T, Esquivel-Naranjo EU, Casas-Flores S, Martínez-Hernández P, Ibarra-Laclette E, Cortes-Penagos C, Herrera-Estrella A (2006) Novel light-regulated genes in *Trichoderma atroviride*: a dissection by cDNA microarrays. Microbiology 152:3305–3317

Ruger-Herreros C, Rodríguez-Romero J, Fernández-Barranco R, Olmedo M, Fischer R, Corrochano LM, Canovas D (2011) Regulation of conidiation by light in *Aspergillus nidulans*. Genetics 188:809–822. https://doi.org/10.1534/genetics.111.130096

Salinas F, Rojas V, Delgado V, Agosin E, Larrondo LF (2017) Optogenetic switches for light-controlled gene expression in yeast. Appl Microbiol Biotechnol 101:2629–2640. https://doi.org/10.1007/s00253-017-8178-8

Sanz C, Rodríguez-Romero J, Idnurm A, Christie JM, Heitman J, Corrochano LM, Eslava AP (2009) *Phycomyces* MADB interacts with MADA to form the primary photoreceptor complex for fungal phototropism. Proc Natl Acad Sci U S A 106:7095–7100. https://doi.org/10.1073/pnas.0900879106

Schmoll M, Franchi L, Kubicek CP (2005) Envoy, a PAS/LOV domain protein of *Hypocrea jecorina* (Anamorph *Trichoderma reesei*), modulates cellulase gene transcription in response to light. Eukaryot Cell 4:1998–2007

Schumacher J, Simon A, Cohrs KC, Viaud M, Tudzynski P (2014) The transcription factor BcLTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*. PLoS Genet 10: e1004040. https://doi.org/10.1371/journal.pgen.1004040

Schwerdtfeger C, Linden H (2001) Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. Mol Microbiol 39:1080–1087

Schwerdtfeger C, Linden H (2003) VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J 22: 4846–4855

Shi M, Collett M, Loros JJ, Dunlap JC (2010) FRQ-interacting RNA helicase mediates negative and positive feedback in the Neurospora circadian clock. Genetics 184:351–361

Shoji JY, Maruyama J, Arioka M, Kitamoto K (2005) Development of *Aspergillus oryzae thiA* promoter as a tool for molecular biological studies. FEMS Microbiol Lett 244:41–46

Smith KM, Sancar G, Dekhang R, Sullivan CM, Li S, Tag AG, Sancar C, Bredeweg EL, Priest HD, McCormick RF, Thomas TL, Carrington JC, Stajich JE, Bell-Pedersen D, Brunner M, Freitag M (2010) Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for neurospora white collar complex. Eukaryot Cell 9:1549–1556. https://doi.org/10.1128/EC.00154-10

Sopko R, Huang D, Preston N, Chua G, Papp B, Kafadar K, Snyder M, Oliver SG, Cyert M, Hughes TR, Boone C, Andrews B (2006) Mapping pathways and phenotypes by systematic gene overexpression. Mol Cell 21:319–330. https://doi.org/10.1016/j.molcel.2005.12.011

Stajich JE (2016) Fungal evolution: *Mucor* and *Phycomyces* see double. Curr Biol 26:R775–R777. https://doi.org/10.1016/j.cub.2016.06.049

Sun J, Phillips CM, Anderson CT, Beeson WT, Marletta MA, Glass NL (2011) Expression and characterization of the *Neurospora crassa* endoglucanase GH5-1. Protein Expr Purif 75:147–154. https://doi.org/10.1016/j.pep.2010.08.016

Tagua VG, Pausch M, Eckel M, Gutiérrez G, Miralles-Durán A, Sanz C, Eslava AP, Pokorny R, Corrochano LM, Batschauer A (2015) Fungal cryptochrome with DNA repair activity reveals an early stage in cryptochrome evolution. Proc Natl Acad Sci U S A 112: 15130–15135. https://doi.org/10.1073/pnas.1514637112

Tisch D, Schmoll M (2010) Light regulation of metabolic pathways in fungi. Appl Microbiol Biotechnol 85:1259–1277. https://doi.org/10.1007/s00253-009-2320-1

Tisch D, Schmoll M (2013) Targets of light signalling in *Trichoderma reesei*. BMC Genomics 14:657. https://doi.org/10.1186/1471-2164-14-657

Tisch D, Kubicek CP, Schmoll M (2011) The phosducin-like protein PhLP1 impacts regulation of glycoside hydrolases and light response in *Trichoderma reesei*. BMC Genomics 12:613. https://doi.org/10.1186/1471-2164-12-613

Tisch D, Schuster A, Schmoll M (2014) Crossroads between light response and nutrient signalling: ENV1 and PhLP1 act as mutual regulatory pair in *Trichoderma reesei*. BMC Genomics 15:425. https://doi.org/10.1186/1471-2164-15-425

Vogt K, Bhabhra R, Rhodes JC, Askew DS (2005) Doxycycline-regulated gene expression in the opportunistic fungal pathogen *Aspergillus fumigatus*. BMC Microbiol 5:1

Wanka F, Cairns T, Boecker S, Berens C, Happel A, Zheng X, Sun J, Krappmann S, Meyer V (2016) Tet-on, or Tet-off, that is the question: advanced conditional gene expression in *Aspergillus*. Fungal Genet Biol 89:72–83. https://doi.org/10.1016/j.fgb.2015.11.003

Wang W, Shi XY, Wei DZ (2014) Light-mediated control of gene expression in filamentous fungus *Trichoderma reesei*. J Microbiol Methods 103:37–39. https://doi.org/10.1016/j.mimet.2014.05.017

Wang B, Zhou X, Loros JJ, Dunlap JC (2015) Alternative use of DNA binding domains by the *Neurospora* white collar complex dictates circadian regulation and light responses. Mol Cell Biol 36:781–793. https://doi.org/10.1128/MCB.00841-15

Waring RB, May GS, Morris NR (1989) Characterization of an inducible expression system in *Aspergillus nidulans* using *alcA* and tubulin-coding genes. Gene 79:119–130

Weirauch MT, Yang A, Albu M, Cote AG, Montenegro-Montero A, Drewe P, Najafabadi HS, Lambert SA, Mann I, Cook K, Zheng H, Goity A, van Bakel H, Lozano JC, Galli M, Lewsey MG, Huang E, Mukherjee T, Chen X, Reece-Hoyes JS, Govindarajan S, Shaulsky G, Walhout AJM, Bouget FY, Ratsch G, Larrondo LF, Ecker JR, Hughes TR (2014) Determination and inference of eukaryotic transcription factor sequence specificity. Cell 158:1431–1443. https://doi.org/10.1016/j.cell.2014.08.009

Wolfers S, Kamerewerd J, Nowrousian M, Sigl C, Zadra I, Kürnsteiner H, Kück U, Bloemendal S (2015) Microarray hybridization analysis of light-dependent gene expression in Penicillium chrysogenum identifies bZIP transcription factor PcAtfA. J Basic Microbiol 55:480–9. https://doi.org/10.1002/jobm.201400588

Wu C, Yang F, Smith KM, Peterson M, Dekhang R, Zhang Y, Zucker J, Bredeweg EL, Mallappa C, Zhou X, Lyubetskaya A, Townsend JP, Galagan JE, Freitag M, Dunlap JC, Bell-Pedersen D, Sachs MS (2014) Genome-wide characterization of light-regulated genes in *Neurospora crassa*. G3 4:1731–45. doi: https://doi.org/10.1534/g3.114.012617

Yu Z, Armant O, Fischer R (2016) Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling. Nat Microbiol 1:16019. https://doi.org/10.1038/nmicrobiol.2016.19

Zhang JZ (2003) Overexpression analysis of plant transcription factors. Curr Opin Plant Biol 6:430–440

Zhang G, Liu P, Wei W, Wang X, Wei D, Wang W (2016) A light-switchable bidirectional expression system in filamentous fungus *Trichoderma reesei*. J Biotechnol 240:85–93. https://doi.org/10.1016/j.jbiotec.2016.11.003