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Glucose sensing and light regulation: A mutation in the glucose sensor RCO-3 modifies photoadaptation in *Neurospora crassa*

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ABSTRACT

Light regulates fungal gene transcription transiently leading to photoadaptation. In the ascomycete *Neurospora crassa* photoadaptation is mediated by interactions between a light-regulated transcription factor complex, the white-collar complex, and the small photoreceptor VVD. Other proteins, like the RCO-1/RCM-1 repressor complex participate indirectly in photoadaptation. We show that RCO-3, a protein with high similarity to glucose transporters, is needed for photoadaptation. The mutation in *rco-3* modifies the transcriptional response to light of several genes and leads to changes in photoadaptation without significantly changing the amount and regulation of WC-1. The mutation in *rco-3*, however, does not modify the capacity of the circadian clock to be reset by light. Our results add support to the proposal that there is a connection between glucose sensing and light regulation in *Neurospora* and that the fungus integrates different environmental signals to regulate transcription.

Introduction

Light can be both a signal and a source of stress. Fungi use light as a signal from the environment to regulate several aspects of their biology, including development and metabolism, and to anticipate fungal stress and DNA damage from an excess of UV radiation (Corrochano 2007; Fischer *et al.* 2016; Idnurm *et al.* 2010; Rodríguez-Romero *et al.* 2010). The ascomycete *Neurospora crassa* has been widely used as a model to understand the molecular mechanisms of fungal responses to light (Chen *et al.* 2010a; Dasgupta *et al.* 2015; Olmedo *et al.* 2013). These responses include the development of asexual spores and sexual structures (Park & Yu 2012; Springer 1993), phototropism of the perithecial beak (the tip of the sexual structure) (Harding & Melles 1983), biosynthesis of photoprotective pigments (carotenoids) (Avalos & Limón 2015; Corrochano & Avalos 2010), and entrainment of the circadian clock (Baker *et al.* 2012; Cha *et al.* 2015).

The responses to light in *Neurospora* are mediated by the activity of a light-regulated transcription factor complex, the White Collar Complex (WCC), and result in light-regulated changes in gene transcription (Chen *et al.* 2010a; Olmedo *et al.*

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2013). The mechanisms of the regulation of gene expression by light have been investigated in detail. The protein WC-1 has a LOV domain that binds the chromophore FAD and allows the protein to act as a blue light photoreceptor (Froehlich *et al.* 2002; He 2002). WC-1 interacts with WC-2 to form the WCC that has been detected in the promoters of several light-regulated genes in the dark. After light exposure, a conformational change allows the interaction between two WC-1 through their LOV domains, resulting in the binding of multiple WCCs to the promoters to activate transcription (Froehlich *et al.* 2002; He 2005a; Malzahn *et al.* 2010; Smith *et al.* 2010). One of these light-activated genes is *vvd*, which encodes a small blue-light photoreceptor containing a LOV domain similar to that of WC-1 (Heintzen *et al.* 2001). VVD then competes for binding between light-activated WC-1 proteins and provokes the dissociation of interacting WCCs, leading to the attenuation of light-induced transcription in a process known as photoadaptation (Chen *et al.* 2010b; Hunt *et al.* 2010; Malzahn *et al.* 2010).

VVD is a key regulator of photoadaptation, but other proteins play a role in the photoadaptation process although the mechanism is still under investigation (Navarro-Sampedro *et al.* 2008; Olmedo *et al.* 2010a). The RCO-1/RCM-1 repressor complex is the homologue of the Tup repressor complex in yeast and the mutations in the corresponding proteins in *Neurospora* disrupt photoadaptation for several genes (Olmedo *et al.* 2010a). One of the consequences of mutations in the RCO-1/RCM repressor complex is the reduction in the light-regulated expression of *vvd*, and a reduction in the amount of VVD that is available to interfere with the WCC during photoadaptation. The modification of the transcriptional response of *vvd* by mutations in the genes for the RCO-1/RCM-1 repressor complex is responsible, in part, for the alteration of photoadaptation in *rco-1* and *rcm* mutants (Ruger-Herreros *et al.* 2014). In addition, the RCO-1/RCM-1 complex plays a role in the regulation of the *Neurospora* circadian clock since a mutation in *rco-1* leads to changes in amplitude and period length (Olivares-Yañez *et al.* 2016). The circadian clock is an endogenous oscillator that allows organisms to anticipate the cyclic changes in their environment due to the rotational movement of the Earth. In *Neurospora*, the manifestation of circadian rhythms includes rhythmic expression of multiple transcripts (Hurley *et al.* 2014), rhythmic gating of the responses to light (Merrow *et al.* 2001), and rhythmic conidiation that can be monitored growing the mycelia in race tubes (Pittendrigh *et al.* 1959). The observation that the RCO-1/RCM-1 complex is required for the proper transcription of the clock regulatory gene *frq* supports the proposal that this repressor complex plays a role in the regulation of the *Neurospora* clock (Liu *et al.* 2015; Zhou *et al.* 2013).

Mutants in the *rco* genes were identified by their misregulation of the transcription of conidiation genes (Madi *et al.* 1994; Yamashiro *et al.* 1996). *Conidiation* (*con*) genes are expressed at different stages during the asexual reproduction cycle (Berlin & Yanofsky 1985; Sachs & Yanofsky 1991), and several *con* genes are induced by light. The regulation by light of *con-6* and *con-10* has been characterized in detail (Corrochano *et al.* 1995; Lauter & Yanofsky 1993; Olmedo *et al.* 2010b).

One of the *rco* genes, *rco-3*, encodes a protein with high similarity to glucose transporters of *Saccharomyces cerevisiae* but the pleiotropic phenotype of *rco-3* mutants has lead to the suggestion that RCO-3 might work as a nutrient sensor (Madi *et al.* 1997). Mutants in *rco-3* have an elevated expression of conidiation genes in vegetative mycelia, but the mechanism that allows a nutrient sensor to regulate the expression of developmentally-regulated genes remains to be identified. Conidiation is regulated by the carbon and nitrogen source, and it is stimulated when the fungus grows under carbon or nitrogen starvation (Springer 1993). In addition, the developmental transcriptional regulator CSP-1 participates in the coordination between metabolism and clock regulation by responding to changing levels of glucose concentration and repressing the expression of *wc-1* (Sancar *et al.* 2012). Here we show that the mutation in *rco-3* modifies the transcriptional response to light of several genes and leads to changes in photoadaptation without significantly changing the amount and regulation of WC-1. The mutation, however, does not modify the capacity of the clock to be reset by light. Our results add additional support to the proposal that there is a connection between glucose sensing and light regulation in *Neurospora* and that the fungus integrates different environmental signals to regulate transcription.

Materials and methods

Strains and culture conditions

We used the standard *Neurospora crassa* wild type strain 74-OR23-1VA (FGSC 2489, *mat*A), and the mutant strains FGSC 9513 (*rco-3*^{1} *mat a*) and FGSC 7854 (*vvd*^{P4246} *mat* A). *Neurospora* strains were obtained from the Fungal Genetics Stock Center (FGSC, http://www.fgsc.net) and were maintained by growth in slants of Vogel's minimal media with 1.5 % sucrose as carbon source. We followed standard procedures and protocols for strain manipulation and growth media preparation (Davis 2000). See also, the *Neurospora* protocol guide (http://www.fgsc.net/Neurospora/NeurosporaProtocolGuide.htm).

Light induction experiments

For all experiments except the race tubes, we grew pads of mycelia by inoculating 10^6 viable conidia into 25 ml of liquid Vogel's minimal medium containing 0.2 % Tween 80 as wetting agent in standard Petri plates (10 cm diameter). The carbon source in liquid Vogel's is 2 % sucrose. Mycelia pads were illuminated as previously described (Olmedo *et al.* 2010a) for the times indicated to measure regulation of gene expression by light and to detect changes in the phosphorylation status of the WC-1 protein. Briefly, cultures were incubated in the dark for 48 h (22 °C) inside a dark box and were then exposed to white light (containing 1 W m^{-2} of blue light) provided by a set of fluorescent bulbs. Light exposures with different intensities were obtained using an illumination chamber as in (Olmedo *et al.* 2010a). After light exposure and, when indicated, incubation in darkness, we collected mycelia using tweezers, dried them on filter paper and wrapped them in aluminium foil. Then we froze them in liquid nitrogen and stored at −80 °C. Control cultures were kept in the dark prior to collection. All the manipulations in the dark were performed under red light. Light intensities were measured with a calibrated photodiode.

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