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The White Collar protein WcoA of *Fusarium fujikuroi* is not essential for photocarotenogenesis, but is involved in the regulation of secondary metabolism and conidiation

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Abstract

The fungal proteins of the White Collar photoreceptor family, represented by WC-1 from *Neurospora crassa*, mediate the control by light of different biochemical and developmental processes, such as carotenogenesis or sporulation. Carotenoid biosynthesis is induced by light in the gibberellin-producing fungus *Fusarium fujikuroi*. In an attempt to identify the photoreceptor for this response, we cloned the only WC-1-like gene present in the available *Fusarium* genomes, that we called *wcoA*. The predicted WcoA polypeptide is highly similar to WC-1 and contains the relevant functional domains of this protein. In contrast to the *Neurospora* counterpart, *wcoA* expression is not affected by light. Unexpectedly, targeted *wcoA* disruptant strains maintain the light-induced carotenogenesis. Furthermore, the *wcoA* mutants show a drastic reduction of fusarin production in the light, and produce less gibberellins and more bikaverins than the parental strain under nitrogen-limiting conditions. The changes in the production of the different products indicate a key regulatory role for WcoA in secondary metabolism of this fungus. Additionally, the mutants are severely affected in conidiation rates under different culture conditions, indicating a more general regulatory role for this protein.

Keywords: *Gibberella*; WC proteins; Light regulation; Nitrogen regulation; Carotenoid; Gibberellin; Bikaverin; Fusarin

1. Introduction

The genus *Fusarium* groups a large number of phytopathogenic fungi characterized by the production of fusiform asexual spores, called conidia. The *Fusarium* species display a complex secondary metabolism, which includes the production of different mycotoxins (Desjardins, 2006). Some secondary metabolites produced by these fungi have found commercial applications. A representative example are the gibberellins, a group of tetracyclic terpenoids synthesized by *Fusarium fujikuroi* (Tudzynski, 2005; Avalos et al., 2007), formerly known as the mating population C of the *Gibberella fujikuroi* species complex. The gibberellins are growth-promoting plant hormones (MacMillan, 1997), rarely produced by microorganisms (MacMillan, 2001), whose inducing effects on plant development have found applications in agriculture and brewing industry. The simple growth and high gibberellin biosynthetic activity of *F. fujikuroi* have made this fungus a major source for biochemical information on the pathway and the obvious choice for large-scale gibberellin production (Tudzynski, 1999; Avalos et al., 1999).

In addition to gibberellins, *F. fujikuroi* produces other secondary metabolites, which include carotenoids, (Avalos and Cerdá-Olmedo, 1986, 1987), bikaverins (Giordano et al., 1999) and fusarins (Barrero et al., 1991). Carotenoids are terpenoid pigments widely spread in nature, where they are synthesized by all plants and algae, and by many bacteria and fungi (Britton et al., 1998; Sandmann and Misawa, 2002). As terpenoids, carotenoids and gibberellins

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share their first biosynthetic steps up to geranylgeranyl pyrophosphate, from which both pathways bifurcate as independent branches. Bikaverins and fusarins are polyketides, and their syntheses start from acetate units through the activity of a multifunctional enzyme called polyketide synthase. The genes responsible for the biosynthesis of these compounds are well known for the carotenoid (Linnemannstöns et al., 2002a; Prado-Cabrero et al., 2007a) and gibberellin pathways (Tudzynski, 2005), and only partially for the ones of bikaverins and fusarins. The major polyketide synthase gene for bikaverin has been identified in *F. fujikuroi* (Linnemannstöns et al., 2002b) but not the one for fusarins, although it is known in other *Fusarium* species (Song et al., 2004).

The synthesis of these secondary metabolites is subject to different regulations. Thus, gibberellin and bikaverin productions are induced by the absence of nitrogen in the medium, mainly through the activation by the key nitrogen regulator AreA (Mihlan et al., 2003). Additionally, bikaverin biosynthesis is regulated by pH (Giordano et al., 1999). Fusarin production depends on temperature (Barrero et al., 1991), but the effects of nitrogen limitation and pH have not been investigated. In contrast, carotenoid biosynthesis of *F. fujikuroi* is mostly dependent on illumination. The surface cultures of this fungus acquire an orange pigmentation when grown under light because of the accumulation of a mixture of carotenoids, which includes neutral intermediates like γ-carotene and torulene, and the acidic apocarotenoid neurosporaxanthin, end product of the pathway (Avalos and Cerdá-Olmedo, 1987).

Light is a key environmental signal that controls many aspects of the life of microorganisms (Herrera-Estrella and Horwitz, 2007). Some fungi, as the ascomycete *Neurospora crassa* and the zygomycete *Phycomyces blakesleeanus* (Linden, 2002; Cerdá-Olmedo, 2001), stand out as models for photobiology research. These fungi exhibit a variety of responses to light, which include the modulation of different developmental processes and the stimulation of carotenoid biosynthesis (Corrochano and Cerdá-Olmedo, 1992; Linden et al., 1997). In both cases, the control is carried out through the transcriptional induction of relevant gene sets. This activation is achieved in *N. crassa* by the White Collar proteins (Talora et al., 1999), identified because of the lack of carotenoids in light-grown mycelia in the mutants of the genes *wc-1* and *wc-2* (Harding and Turner, 1981). Both proteins contain a PAS domain, that mediates their interaction to form a heterodimer known as WC complex (Ballario et al., 1996), and a DNA-binding zinc-finger domain. The WC complex is transiently activated by light and binds regulatory elements of light-regulated target genes to activate their transcription (Liu et al., 2003). The photoreceptor of the complex is WC-1, which contains a special PAS domain, called LOV from "light, oxygen and voltage" (Cheng et al., 2003), that binds a light-absorbing FAD molecule (He et al., 2002).

The WC complex controls all known photoresponses in *N. crassa*. The identification of these proteins led to find similar light-regulatory systems in other fungi (Corrochano, 2007), as the ones from the ascomycete *Trichoderma atroviride* (Casas-Flores et al., 2004) or the basidiomycete *Cryptococcus neoformans* (Idnurm and Heitman, 2005). The photoresponses vary between different species, e.g., the WC system governs light-induction of conidiation in the first case (Casas-Flores et al., 2004), and light repression of sexual cell fusion and its subsequent filamentation in the second (Idnurm and Heitman, 2005; Lu et al., 2005). The number of examples is currently increasing and putative WC orthologs are found in the fungal genome sequences that are becoming available. Recently, WC proteins have also been described in zygomycetes. The *madA* mutants of *P. blakesleeanus*, identified by genetic analyses and affected in most of the photoresponses of this fungus, turned out to lack a functional WC-1-like protein (Idnurm et al., 2006). Moreover, the zygomycetes display a unique diversification of these regulatory proteins: three WC genes have been described in *Mucor circinelloides* (Silva et al., 2006), and a second one has been found in *P. blakesleeanus* (Idnurm et al., 2006).

The similar light-regulation mechanisms found in taxonomically distant species point to the WC proteins as universal photoreceptor systems in fungi, already present in early ancestors common to ascomycetes, basidiomycetes and zygomycetes. To extend the knowledge of this regulation to the fungus *F. fujikuroi*, we cloned the only presumptive *wc-1* ortholog found in the available *Fusarium* genomes, which we called *wcoA*, and we obtained targeted disruptant strains for this gene. Unexpectedly, the *wcoA* mutants conserve the photoinduction of carotenoid biosynthesis but exhibit a pleiotropic phenotype. This includes alterations in the patterns of gibberellin, bikaverin and fusarin productions, as well as in conidia formation, pointing to an unusually complex regulatory role for the WcoA protein in *F. fujikuroi*.

2. Materials and methods

2.1. Strains and culture conditions

FKMC1995 is a wild-type strain of *F. Fujikuroi* (*Gibberella fujikuroi* mating population C; O'Donnell et al., 1998). SF4 is a carotenoid overproducing strain obtained by exposition to *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Prado-Cabrero et al., 2007a). Unless otherwise stated, experiments were done on DG minimal medium (Avalos et al., 1985) with L-asparagine instead of sodium nitrate as nitrogen source. When required, the medium was supplemented with 200 mg hygromycin ml^{-1}. For analysis of carotenoids and fusarins in the mycelia, the strains were grown on DG minimal agar for 7 days as described by Prado et al. (2004). Incubations were at 22 or 30 °C, either in the dark or under illumination (5 W m^{-2} white light at 22 °C and 25 W m^{-2} at 30 °C). For gibberellin and bikaverin analyses, the strains were grown as submerged shake cultures in 500 ml flasks with 250 ml of high nitrogen broth (ICI medium,