Role of the White Collar 1 Photoreceptor in Carotenogenesis, UV Resistance, Hydrophobicity, and Virulence of *Fusarium oxysporum*\(^\text{\dag}\)

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Knockout mutants of *Fusarium oxysporum* lacking the putative photoreceptor Wc1 were impaired in aerial hyphae, surface hydrophobicity, light-induced carotenogenesis, photoreactivation after UV treatment, and upregulation of photolyase gene transcription. Infection experiments with tomato plants and immunodepressed mice revealed that Wc1 is dispensable for pathogenicity on plants but required for full virulence on mammals.

Light is a signal from the environment that regulates different aspects of fungal development. Photoreceptors perceive light and generate signals, the propagation of which stimulates cellular responses, such as carotenoid biosynthesis, spore formation, and phototropism, among others (20, 24). White collar 1 (WC-1) and WC-2 are two key elements involved in light signal transduction in *Neurospora crassa*, the best-studied fungal system (2, 21, 24). The genes that are orthologous to *N. crassa* wc-I in several ascomycetes, basidiomycetes, and zygomycetes have also been identified (6).

In this work, we have characterized the wc1 gene, an orthologue of *N. crassa* wc-1, in *Fusarium oxysporum*, a soilborne pathogen that causes economically important losses of a wide variety of crops (8) and has been reported as an emerging human pathogen (27, 28). The wc1 gene was cloned by PCR, using gene-specific primers based on the locus FOXG_03727.2 in the *F. oxysporum* genome sequence (https://www.broad.mit.edu/annotation/). *F. oxysporum* wc1 was expressed independently of light at very low levels, as determined by reverse transcription-PCR (RT-PCR) (data not shown). The predicted gene product showed various degrees of identity with Wc-1 proteins from *Fusarium graminearum* (91.1%; Broad Institute accession no. FG07941.1), *N. crassa* (58.0%; GenBank accession no. X94300), *Magnaporthe grisea* (55.3%; GenBank accession no. MG03538.4), and *Mucor circinelloides* (40.3%; GenBank accession no. AM048041). Similar to *N. crassa* WC-1, *F. oxysporum* Wc1 contained a putative activation domain at the N terminus, a LOV domain, two PAS dimerization domains, a nuclear localization sequence, and a Zn finger DNA binding domain. The LOV domain contained the 11 conserved amino acid residues suggested to accommodate the terminal adenine moiety of FAD (13) that is required for the binding of the WC-1/WC-2 complex to the *frq* gene (11), suggesting that *F. oxysporum* Wc1 has functions in light regulation that are similar to those of other Wc-1 photoreceptors.

To study the role of *wc1*, the gene was knocked out in *F. oxysporum* as reported previously (9). Several transformants carrying a copy of *wc1* disrupted with the hygromycin resistance marker (Δwc1) were identified by PCR and Southern hybridization. The absence of full-length *wc1* transcripts in the Δwc1 strain was confirmed by RT-PCR (data not shown), although the occurrence of truncated transcripts lacking the essential dimerization, nuclear localization signal, and DNA binding domains cannot be ruled out. Complementation of a Δwc1 strain was performed by cotransformation with the wc1 wild-type allele and the phleomycin resistance cassette, and the presence of the complementing wild-type allele (Δwc1+wc1) confirmed by PCR.

No significant differences were observed in the hyphal morphology and growth rate (determined as mycelial dry weight and sporulation rate) of the Δwc1 mutant and the wild-type strain grown in submerged culture (potato dextrose broth [PDB]) or on solid media (potato dextrose agar [PDA]), either in constant darkness or under photoperiods of 10 h dark/14 h white light. However, the Δwc1 strain did not produce aerial hyphae on PDA plates with cellophane sheets under photoperiods, suggesting a possible role of Wc1 in the hyphal development of *F. oxysporum* under these conditions. To test whether this phenotype was due to defects in surface hydrophobicity, water droplets were placed on the surface of fungal colonies grown either in the dark or under photoperiods. Drops remained for more than 4 h on the colony surface in all cases except for the Δwc1 mutant grown under photoperiods, where droplets rapidly soaked into the surface. To further investigate the light regulation of hydrophobicity by Wc1, the *F. oxysporum* genome was searched in silico, revealing four putative hydrophobin genes (*hyd1*, *hyd3*, *hyd4*, and *hyd5*) and an agglutinin-like gene (*alp1*). Similar expression profiles for *hyd3*, *hyd4*, *hyd5*, and *alp1* were observed by semiquantitative RT-PCR of the wild-type, the Δwc1 mutant, and the

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Δwc1+wc1 strain grown in the dark or under illumination for 30 or 60 min (data not shown). By contrast, the expression of hyd1 was not detected in dark-grown mycelia from the Δwc1 mutant and appeared significantly decreased in this strain after illumination (Fig. 1). In the wild-type and the Δwc1+wc1 strains, different hyd1 transcript sizes were observed, containing either both, one, or none of the introns, whereas only the completely spliced form was detected in the Δwc1 mutant (Fig. 1). Differential splicing of a putative hydrophobin gene of Trichoderma viride in response to light was reported previously (32). These results suggest that Wc1 could play a role in the light-independent regulation of hyd1 transcription and mRNA processing and that hyd1 is not related to the light-dependent hydrophilic phenotype of the Δwc1 mutant.

The light induction of carotenogenesis was studied by extracting carotenoids from mycelia grown for 7 days in the dark or under photoperiods and measuring them as described previously (31). The carotenoid levels in dark-grown mycelia of the wild type did not increase, whereas significant carotene levels were observed in the Δwc1 mutant in comparison with the recovery of the Δwc1+wc1 strain (Fig. 1). Thus, we conclude that Wc1 regulates the phr1-mediated UV light resistance in F. oxysporum.

Several phytopathogenic fungi have been reported to harbor genes orthologous to wc-1 in their genomes (18, 25), but their role in pathogenicity to plants has not been studied. We carried out tomato root infection assays with the different F. oxysporum strains, no light-transcriptional activation of phr1 was observed in the Δwc1 mutant (Fig. 1). Thus, we conclude that Wc1 regulates the phr1-mediated UV light resistance in F. oxysporum.

Transcriptional activation of photolyase genes mediated by white collar complexes was previously described in fungi (5, 18). We investigated the role of Wc1 in the expression of the F. oxysporum phr1 gene (1). The lethal effect of UV light on spore survival of the Δwc1 mutant (quantified as survival rate on PDA plates after irradiation of 10³ microconidia with a 245-nm lamp) was similar to its effect on spores of the wild type (Table 1), but no recovery of survival after photoreactivation (induced by 5 h of white-light illumination of UV-irradiated spores followed by 48 h of incubation at 28°C in the dark) was detected in the Δwc1 mutant in comparison with the recovery of the wild-type or the Δwc1+wc1 strain, indicating a defect in the photoreactivation mechanism. To confirm this hypothesis, we investigated the role of Wc1 in the expression of the Δwc1 mutant sug-
of mice, the fungus remains in the dark. On the other hand, no increased thermosensitivity was observed in the Δwc1 mutant compared with the thermosensitivity of the wild type, as determined by counting colonies originated from spores grown on PDA plates at 37°C. A possible explanation for the reduced virulence of the Δwc1 mutant could be the existence of Wc1-dependent signal transduction pathways in F. oxysporum, controlling the production of other secondary metabolites under dark conditions. According to this hypothesis, the Δwc1 mutant would produce different amounts of those compounds, leading to a decrease in pathogenicity on mice. A recent report on the effects of the inactivation of the white collar gene wcoA of F. fujikuroi showed that the deduced protein is involved in the light-independent regulation of the production of secondary metabolites, such as gibberellins and bikaverins (10). The identification of genes differentially expressed by the Δwc1 mutant during the infection of immunodepressed mice should further elucidate the molecular basis for the role of Wc1 in the virulence of F. oxysporum and other pathogenic fungi in mammalian hosts.

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