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Regulation and Targeted Mutation of *opsA*, Coding for the NOP-1 Opsin Orthologue in *Fusarium fujikuroi*

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Opsins are widespread photoreceptor proteins involved in a diversity of light-driven processes in all major taxa that use the apocarotenoid retinal as a light-absorbing prosthetic group. Proteins from the opsin family are also found in filamentous fungi, but no function has been attributed to them. The fungus *Fusarium fujikuroi* contains two genes for presumptive retinal-binding opsins, which we call *carO* and *opsA*, and a gene for an opsin-related protein, called *hspO*. One report showed that *carO* is linked and co-regulated with the enzymatic genes of the carotenoid pathway, *carRA*, *carB*, and *carX*, but that its mutation produced no detectable phenotype. Sequence analyses suggest that OpsA, not CarO, is the orthologue of the *Neurospora* opsin NOP-1. mRNA levels for the three *Fusarium* opsin genes are induced by heat shock, while those for *carO* and *opsA* are induced by light. This photoinduction is lost in mutants of the white collar gene *wcoA*, which contains much higher *carO* and *opsA* mRNA levels than the wild type, indicating a down-regulation of both genes by WcoA. Conversely to *carO*, *opsA* mRNA levels are not enhanced in carotenoid-overproducing mutants. Targeted *opsA* mutants have no discernible external phenotype, but they exhibit a significant decrease in mRNA levels for structural genes of the carotenoid pathway. Similar reductions are produced by mutations in the enzymatic genes *carRA* and *carB*, but not in *carX*, responsible for retinal biosynthesis.

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Keywords: opsin; retinal; carotenoid; photoinduction; white collar

Introduction

Opsins are a widespread group of transmembrane photoreactive proteins that use the apocarotenoid retinal as their chromophore (reviewed by Spudich *et al.*[1] and Spudich[2]). Knowledge on this chromophore-retinal complex, also known as rhodopsin, started with the identification of bacteriorhodopsin (BR) and halorhodopsin in halophilic archaea, which mediate proton and chloride membrane transport, respectively, using light as the sole energy source.[3] New members of this protein family were subsequently discovered in all major microbial groups, where they play different light-dependent functions.[2] Research on these proteins, called type I rhodopsins, has associated many of them to specific photoreception roles, such as phototaxis in algae[4] and photoadaptation in eubacteria.[5] Structurally related to them are animal opsins, also called type II rhodopsins, responsible for vision in the retina.[6] The three-dimensional structure resolved for several opsin proteins revealed a conserved tertiary organization consisting of a seven-transmembrane domain.[2,7] The spatial organization includes an internal pocket with a highly conserved lysine residue to which retinal is covalently bound.

Putative opsin-encoding genes are also found in fungal genomes.[8] Some of them have been the subject of detailed investigation. This is the case of *nop-1* from *Neurospora crassa*,[9] discovered from an expressed sequence tag library, which stood out as the first opsin-like sequence found in a fungus. NOP-1 binds retinal *in vitro*[10] and shows a photochemical reaction cycle.[11] Targeted mutation of *nop-1* revealed no apparent phenotypic alteration except for a light-dependent change of colony morphology on oligomycin-supplemented medium.[9] Analyses on *nop-1* mRNA levels indicated a role associated to airborne-induced production of the asexual spores, the conidia, as indicated by its parallel induction with the conidiation-specific

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Abbreviations used: ORP, opsin-related protein; BR, bacteriorhodopsin; PPO, predictable photoactive opsin; WC, white collar; RT, reverse transcriptase.

gene *con-10* and the lower *nop-1* mRNA amounts in regulatory mutants affected in different stages of conidia development.[12]

OPS from *Leptosphaeria maculans*, another fungal opsin,[13] differs from NOP-1 and resembles BR in that it exhibits a fast photocycle and an efficient light-driven proton-pumping activity.[11,14,15] Also, in contrast to *nop-1*, *ops* mRNA levels are barely affected by light. Despite their high sequence similarity, the biochemical properties of NOP-1 and OPS suggest different functions, involved in light sensing and proton pumping, respectively. As for other fungal opsins, the biological role for OPS in *L. maculans* has not been elucidated and the effect of *ops* mutation has not been investigated.

NOP-1 and OPS are just two examples from the diversity of opsin proteins identified in low numbers in ascomycete and basidiomycete species as new fungal proteomes become available.[8] In some cases, despite their typical opsin tertiary structure, these proteins lack the conserved retinal-binding lysine present in the seventh transmembrane domain of photoactive opsins to which retinal is covalently linked. These proteins, called opsin-related proteins or ORPs,[1] presumably lack light-dependent functions. To this group belongs a subfamily of heat-shock chaperones,[16] represented by HSP30 from *Saccharomyces cerevisiae* or similar proteins in other fungi (e.g., Cvhsp30/1 and Cvhsp30/2 from *Coriolus versicolor*).[17] Other presumptive opsins contain the conserved lysine residue, but retinal binding and photoreactivity have not been demonstrated; we shall refer to them as predictable photoactive opsins or PPOs here.

The opsin chromophore retinal (C20) is produced through the oxidative cleavage of β-carotene (C40), a reaction mediated by enzymes from the carotene oxygenase family.[18] Recently, a retinal-forming oxygenase encoded by gene *carX* has been described in *Fusarium fujikuroi* (*Gibberella fujikuroi* mating group C).[19] This fungus stands out for its capacity to produce gibberellins,[20,21] which are growth-promoting plant hormones[22] with agricultural applications. Surface cultures of *F. fujikuroi* become orange under light because of the accumulation of an acidic apocarotenoid, neurosporaxanthin, and minor amounts of carotenoid precursors.[23] Neurosporaxanthin biosynthesis is mediated in this fungus by at least three enzymes, encoded by genes *carRA*, *carB*, and *carT*.[24,25] CarRA is a bifunctional protein with phytoene synthase and carotene cyclase activities, while CarB is a desaturase, able to introduce up to five conjugated double bonds in the aliphatic carotene chain (Fig. 1). The sequential activities of these enzymes produce the monocyclic torulene (C40), which is cleaved by the CarT oxygenase to yield β-apo-4′-carotenal (C35), the aldehyde precursor of neurosporaxanthin.[25] The cyclase activity of CarRA also yields the bicyclic β-carotene, a substrate of the CarX oxygenase.[19]

Biosynthesis of neurosporaxanthin by *F. fujikuroi* is induced by light in wild-type strains,[23,26] but large amounts of carotenoids are accumulated in the dark by deregulated mutants (*carS*).[23,27] Carotenoid content correlates with mRNA levels from the enzymatic genes *carRA*, *carB*, and *carT*,[24,25,28] as well as with those from *carX*, coding for the retinal-forming enzyme.[29] Accordingly, these mRNA levels are low in the dark, increase rapidly upon illumination, and decrease afterward (photoadaptation), while they are high irrespective of light in the *carS* mutants.

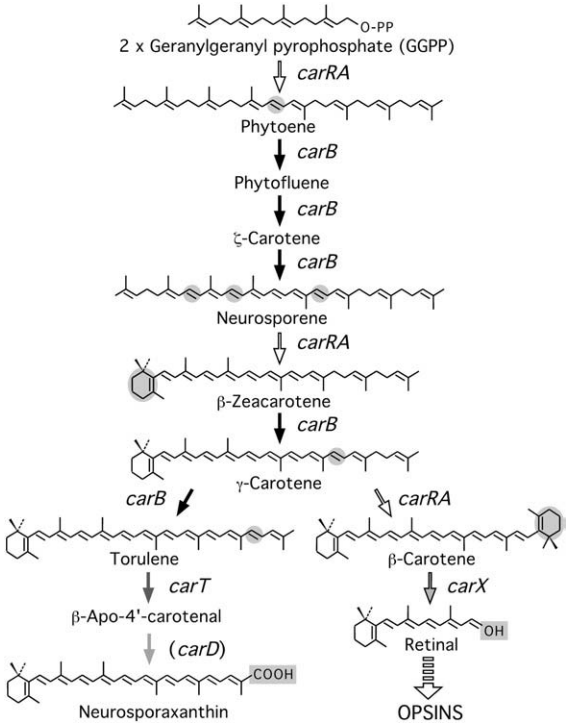

Fig. 1. Biosynthesis of the opsin chromophore retinal from the diterpenoid precursor GGPP and its relation with the neurosporaxanthin biosynthetic pathway in *F. fujikuroi*. The genes responsible for each step are indicated. Identification of the *carD* gene is currently in progress. Chemical modifications on the intermediate products are shaded.

Light-induced carotenogenesis has been thoroughly investigated in a *Fusarium* relative, the ascomycete *N. crassa*, in which neurosporaxanthin was first described.[30] The photoresponse in this fungus is mediated by the white collar (WC) complex,[31] which is formed by the PAS-mediated interaction of the WC-1 and WC-2 proteins.[32] Activation by light of this heterodimer allows it to bind the promoters of target genes and stimulate their transcription.[33] Light detection in the WC complex is achieved by WC-1, which absorbs blue light through an FAD molecule,[34] bound to a special PAS domain known as LOV from "light, oxygen and voltage."[35] Similar WC proteins are responsible for photocarotenogenesis and other photoresponses in the zygomycetes *Phycomyces blakesleeanus*[36] and *Mucor circinelloides*.[37] Against the findings in *Neurospora* and other fungi, mutation of the only *wc-1*-like gene in *F. fujikuroi* did not prevent stimulation by light of the carotenoid