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Genetic Analysis of Phototropism of *Neurospora crassa* Perithecial Beaks Using White Collar and Albino Mutants

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ABSTRACT

Positive phototropism of perithecial beaks in the fungus *Neurospora crassa* has been demonstrated. The effect was shown to be mediated by blue light. When mutants (*white collar-1* and *white collar-2*) which are blocked in the light induction of enzymes in the carotenoid biosynthetic pathway were used as the protoperithecial parent in crosses, the resulting perithecial beaks did not show a phototropic response. However, when wild type, *albino-1*, *albino-2*, or *albino-3* strains were used as the protoperithecial parent, phototropism occurred.

The results show that both photoinduced carotenogenesis and phototropism in *N. crassa* are controlled by the *white collar-1* and *white collar-2* loci. Thus, the sensory transduction pathways for the two photoresponses must have some steps in common. The results further support the proposal that the *white collar* strains are regulatory mutants blocked in the light induction process, whereas the *albino-1*, *albino-2*, and *albino-3* strains can carry out light induction but have the *albino* phenotype because they are each defective for a different enzyme in the carotenoid biosynthetic pathway.

Blue light-induced responses both in higher plants and microorganisms have been extensively studied (see reviews 27, 28). In the fungus *Neurospora crassa*, blue light has been shown to regulate a number of important processes including carotenoid biosynthesis (8, 24), photosuppression and phase shifting of the circadian rhythm of conidiation (5), photoinduction of protoperithecia formation (15), and under certain conditions promotion of conidiation (16). In *Neurospora sitophila*, positive phototropism of perithecial beaks (also referred to as perithecial necks) has also been demonstrated (1), but it was not determined whether this phototropic effect is a blue light response.

Genetic studies have been carried out with *N. crassa* (9, 17, 19, 20), *Phycomyces blakesleeanus* (2, 3, 18, 25), and *Trichoderma* (11) which are aimed at eventually identifying the blue light photoreceptor(s) (designated 'cryptochrome' [6]). In *N. crassa*, photoinduced carotenoid biosynthesis was investigated using *wc* and *al* mutants (9). The *wc* phenotype is defined as albino mycelia with normal pigmentation in the conidia, while the *al* strains have a reduced level of carotenoid pigment in both the mycelia and conidia (21–23). Evidence has been presented that the *wc* phenotype is characteristic of regulatory mutants blocked in the light induction process, while the *al* mutants (*al-1*, *al-2*, and *al-3*) can carry out light induction but are each defective for a different enzyme in the carotenoid biosynthetic pathway (9). From this proposal, it was predicted that the *wc* mutants may be blocked in other blue light-mediated responses in *Neurospora* (9). The present study was undertaken to test this hypothesis. Inasmuch as a phototropic response of *N. sitophila* perithecial beaks has been previously demonstrated (1), it was decided to determine whether such a response occurs in *N. crassa*.

In the present study, phototropism of perithecial beaks in *N. crassa* was demonstrated. Furthermore, this response was blocked in perithecial beaks derived from crosses in which *wc* mutants were used as the protoperithecial (maternal) parent. No defect in phototropism was observed when the protoperithecial parent was wild type or *al*.

MATERIALS AND METHODS

Strains. The wild type strains were obtained from FGSC[3], Humboldt State University Foundation, Arcata, CA, and included Em5297*a* (FGSC 352), 74-OR23-1*A* (FGSC 987), and 74-OR8-1*a* (FGSC 988) and will be designated as Em*a*, 74*A*, and 74*a*, respectively, throughout the remainder of this report. The mutant strains used are listed in Table I and will be referred to by locus and allele. Under our conditions (7), the *al* strains *al-1A* (ALS4), *al-2A* (MN58p), and *al-3*^{ros}*A* (Y234M470) all produced visually detectable carotenoid pigment in the mycelia following irradiation, but at a lower level than wild type strains. The *al-3A* (RP100) strain produced only a trace amount of pigment following irradiation. No pigment could be observed in the mycelia of *al-1A* (RES6) and *al-2A* (Y254M165) strains. A detailed description of

Table I. *Neurospora crassa Mutants Used in This Investigation*

Locus[a]	Allele	Mating Type	FGSC Number	Mutant Obtained from
al-1	RES6	*A*	2152	RES[b]
al-1	ALS4	*A*	1526	FGSC
al-2	Y254M165	*A*	904	FGSC
al-2	MN58p	*A*	2666	FGSC
al-3	RP100	*A*	2082	FGSC
al-3^{ros}	Y234M470	*A*	908	FGSC
wc-1	P829	*A*	128	FGSC
wc-1	P829	*a*	143	FGSC
wc-1	P4723	*a*	3628	DDP[c]
wc-2	234(w)	*a*	—[d]	DDP[c]

[a] See abbreviations for definition of different loci listed.
[b] Dr. R. E. Subden.
[c] Dr. D. D. Perkins.
[d] Perkins stock 9896-5, identical in *wc* genotype to FGSC 3818, which is a sibling.

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[3] Abbreviations: FGSC, Fungal Genetics Stock Center; *al*, *albino*; *al-3*^{ros}, *albino-3*^{rosy}; *wc*, *white collar*.

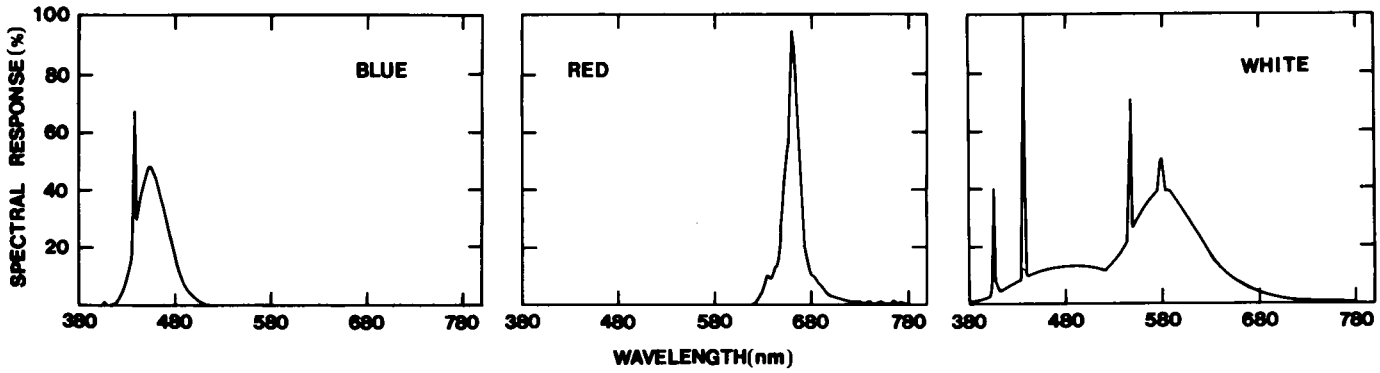

FIG. 1. Spectral distribution of light energy as a function of wavelength for blue, red, and white light irradiation treatments measured at the position of the perithecial beaks. The 100% value corresponds to 6 $\mu w \cdot cm^{-2}$ per nm interval for the blue and white light treatments and to 14 $\mu w \cdot cm^{-2}$ per nm interval for the red light treatment.

Table II. *Effect of Varying the Daily Irradiation Time on Phototropism of Perithecial Beaks*

74*A* was used as the protoperithecial parent and 74*a* as the fertilizing parent (inoculated 3 d apart). White light treatments (332 $\mu w \cdot cm^{-2}$) were given on a daily basis for the indicated times starting 3 d after inoculation of the fertilizing parent.

Daily Irradiation Time	Orientation of Perithecial Beaks			No. of Perithecial Beaks Scored
	Toward light	Away from light	Neutral	
min		%		
0	8	13	79	157
0.5	36	9	55	115
1	79	1	20	144
3	78	3	19	210
5	87	1	12	444

all known chromosomal loci of *Neurospora crassa* has been published recently (23).

Culture and Irradiation Conditions. Crosses were carried out in Petri dishes 100 mm in diameter and 80 mm high. Into each dish was poured 25 ml of 1.9% corn meal agar, dextrose free (Becton, Dickinson and Co.[4], Cockeysville, MD). The protoperithecial parent was inoculated under room lights uniformly over the surface of the agar using an aqueous suspension of conidia. The plates were then kept in the dark at 25°C for 3 or 5 d as indicated. Under red safelight (7), a conidial suspension of the fertilizing parent was applied as a thin line along the diameter of the plate by lightly touching the agar with the edge of a sterile razor blade which had been dipped into the suspension. The plates were then placed back in the dark at 25°C.

For light treatments, the plates were placed in black boxes which had an opening 4 cm wide × 2 cm high. This procedure was carried out under red safelight (7) unless otherwise indicated. Each plate was positioned in the box so that the direction of irradiation was perpendicular to the line of perithecia, and when multiple irradiation treatments were used, the perithecia were always irradiated from the same direction. The forward edge of each dish was 60 cm from the light source. Irradiation treatments consisted either of a single 5 min light treatment given at varying times after inoculation of the fertilizing parent or light treatments were given daily for 5 min starting 3 d after inoculation of the fertilizing parent.

[4] Reference to brand or firm name does not constitute endorsement by the Smithsonian Institution over others of a similar nature not mentioned.

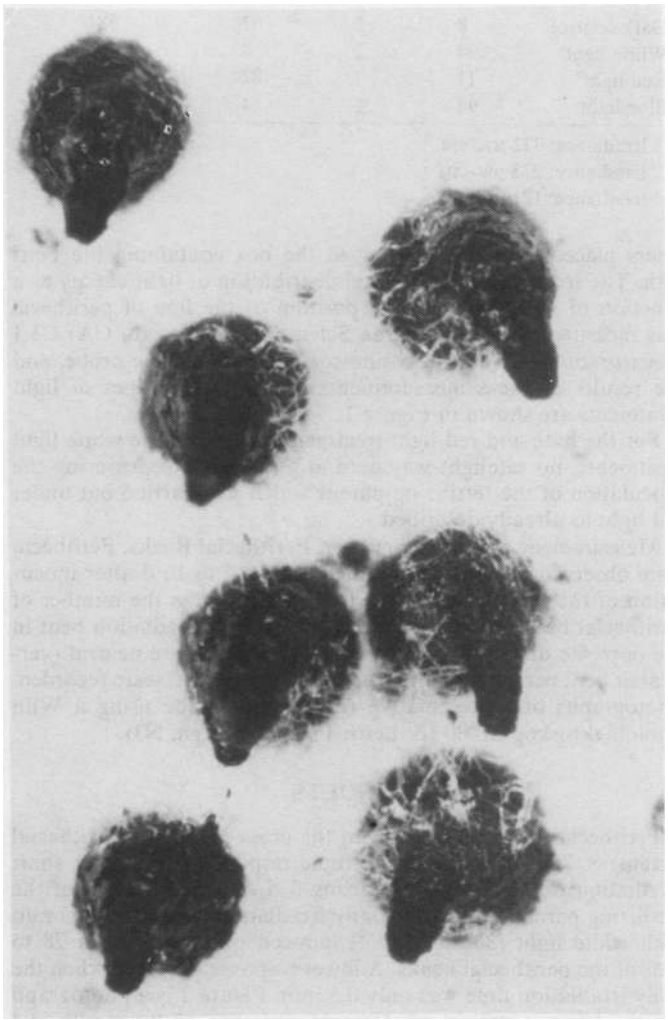

FIG. 2. Phototropism of perithecial beaks derived from the cross 74*A* (protoperithecial parent) × 74*a*. The direction of irradiation was from the bottom of the figure toward top.

Three different light sources were used in these experiments: (a) white light—two General Electric F40 CW cool-white fluorescent lamps; (b) blue light—two Sylvania F48T12/246/VHO fluorescent lamps with two blue Roscolene 863 filters (Kliegl Brothers, Long Island City, NY) placed over the opening to the box containing the Petri dish; (c) red light—two Sylvania F48T12/236/VHO fluorescent lamps with two layers of red Roscolene 823

Table III. *Effect of Different Light Sources on Phototropism of Perithecial Beaks*

74*A* was used as the protoperithecial parent and 74*a* as the fertilizing parent (inoculated 5 d apart). The indicated light treatments were given for 5 min daily starting 3 d after inoculation of the fertilizing parent. No safe light was used at any time except during inoculation of the fertilizing parent when red light was used as described in "Materials and Methods." See Figure 1 for the spectral distribution of light energy as a function of wavelength measured at the position of the perithecia for each of the three irradiation treatments.

Light treatment	Orientation of Perithecial Beaks			No. of Perithecial Beaks Scored
	Toward light	Away from light	Neutral	
	%			
Dark control	8	5	87	581
White light[a]	94	2	4	658
Red light[b]	11	7	82	441
Blue light[c]	94	2	4	817

[a] Irradiance: 332 $\mu w \cdot cm^{-2}$.
[b] Irradiance: 273 $\mu w \cdot cm^{-2}$.
[c] Irradiance: 121 $\mu w \cdot cm^{-2}$.

filters placed over the opening to the box containing the Petri dish. The irradiance and spectral distribution of light energy as a function of wavelength at the position of the line of perithecia was measured using a Gamma Scientific (San Diego, CA) C3.1 Spectroradiometer with a cosine-corrected fibre optic probe, and the results of these measurements for the three types of light treatments are shown in Figure 1.

For the blue and red light treatments and for some white light treatments, no safelight was used at any time except during the inoculation of the fertilizing parent which was carried out under red light as already described.

Measurement of Phototropism of Perithecial Beaks. Perithecia were observed in a dissecting microscope 14 to 16 d after inoculation of the fertilizing parent, and for each cross the number of perithecial beaks bent toward the direction of irradiation bent in the opposite direction, and the number which were neutral (vertical or bent perpendicular to the direction of light) were recorded. Photographs of representative results were made using a Wild Photomakroskop M400 (E. Leitz, Inc., Rockleigh, NJ).

RESULTS

Perithecial beaks derived from the cross 74*A* (protoperithecial parent) × 74*a* showed a phototropic response when given short irradiation treatments daily starting 3 d after inoculation of the fertilizing parent (Table II). Daily irradiation times of 1 to 5 min with white light (332 $\mu w \cdot cm^{-2}$) induced phototropism in 78 to 87% of the perithecial beaks. A lower response occurred when the daily irradiation time was only 0.5 min. Figure 2 is a photograph which shows a typical phototropic response of the perithecial beaks.

The perithecial beaks were most sensitive to light during the period 5 to 10 d after inoculation of the fertilizing parent. During this period, a single 5-min white light irradiation (332 $\mu w \cdot cm^{-2}$) induced phototropism in 30 to 48% of the perithecial beaks (scored 15 d after inoculation of the fertilizing parent). Irradiation at either earlier or later times produced a lower response (data not presented).

To determine whether the phototropic effect is a blue light response, the perithecial beaks were irradiated with either blue or red light for 5 min daily starting 3 d after inoculation of the fertilizing parent. Phototropism of the perithecial beaks was shown to be induced by blue light (Table III), and red light had no significant effect on phototropism of the beaks.

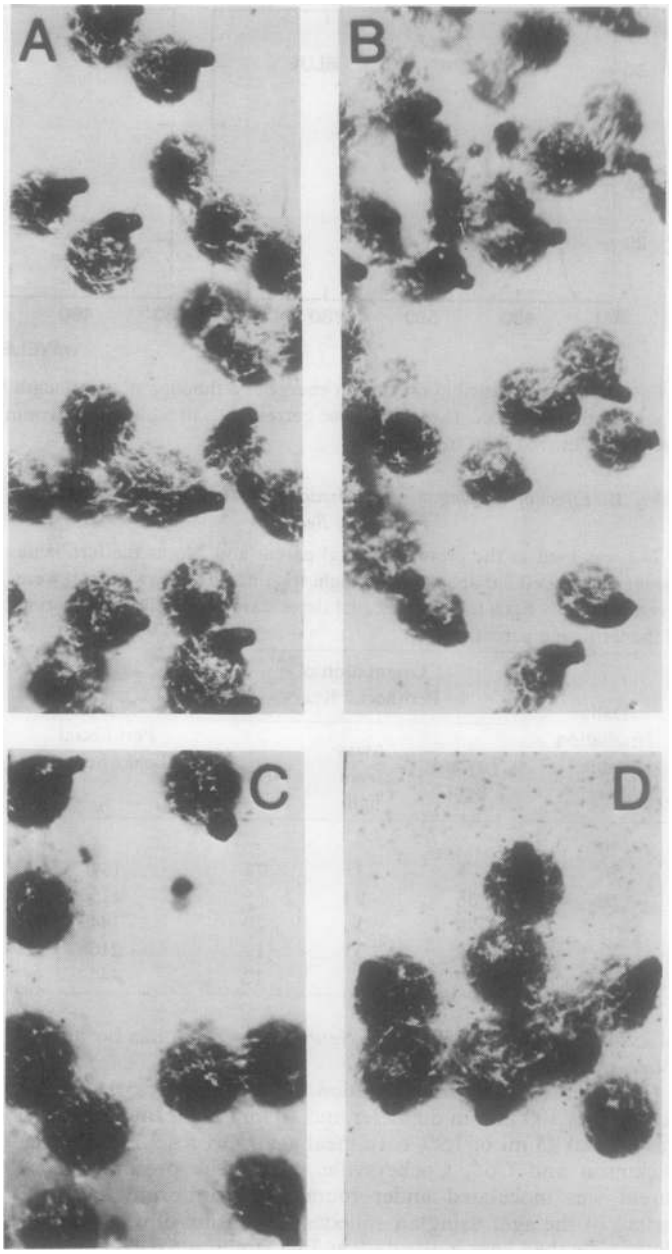

FIG. 3. The effect on phototropism of perithecial beaks when wild type (74) *versus wc* strains were used as the protoperithecial parent in crosses. Perithecial beaks were derived from the following crosses (the protoperithecial parent is listed first in each case): A, 74*A* × *wc-2a* [234(w)]; B, 74*a* × *wc-1A* (P829); C, *wc-2a* [234(w)] × 74*A*; D, *wc-1A* (P829) × 74*a*. The direction of irradiation in each case was from right to left in the figure.

A series of crosses was carried out between the 74 wild type strain and various *al* and *wc* mutants, and the resulting perithecial beaks were irradiated with white light (332 $\mu w \cdot cm^{-2}$) for 5 min daily starting 3 d after inoculation of the fertilizing parent. When the wild type strain or any of the *al* mutants were used as the protoperithecial parent, phototropism of the resulting perithecial beaks occurred (Table IV). However, phototropism of the beaks did not occur when the *wc-1* or *wc-2* mutants were used as the protoperithecial parent (Table V). These results are also shown by the photographs presented in Figure 3.

Table IV. *Phototropism of Perithecial Beaks Formed in Crosses Using Wild Type or Various al Mutants as the Protoperithecial Parent*

The protoperithecial and fertilizing parents (inoculated 5 d apart) are indicated in the table. The perithecial beaks were irradiated with white light (332 $\mu w \cdot cm^{-2}$) for 5 min daily starting 3 d after inoculation of the fertilizing parent.

Parent		Orientation of Perithecial Beaks			No. of Perithecial Beaks Scored
Protoperithecial	Fertilizing	Toward light	Away from light	Neutral	
			%		
74*a*	*al-1A* (RES6)	89	3	8	995
74*a*	*al-1A* (ALS4)	91	2	7	340
74*a*	*al-2A* (Y254M165)	92	3	5	1554
74*a*	*al-2A* (MN58p)	96	1	3	358
74*a*	*al-3A* (RP100)	92	2	6	1119
74*a*	*al-3^{ros}A* (Y234M470)	97	1	2	378
74*a*	*wc-1A* (P829)	92	3	5	1276
74*A*	*wc-1a* (P829)	95	1	4	207
74*A*	*wc-1a* (P4723)	95	1	4	246
74*A*	*wc-2a* [234(w)]	94	3	3	423
al-1A (RES6)	74*a*	94	2	4	954
al-1A (ALS4)	74*a*	92	2	6	505
al-2A (Y254M165)	74*a*	85	6	9	88
al-2A (MN58p)	74*a*	94	3	3	458
al-3A (RP100)	74*a*	78	1	21	530
al-3^{ros}A (Y234M470)	74*a*	95	1	4	425

Table V. *Effect of Using wc Mutants as the Protoperithecial Parent on Phototropism of Perithecial Beaks*

For procedures used, see Table IV.

Parent		Orientation of Perithecial Beaks			No. of Perithecial Beaks Scored
Protoperithecial	Fertilizing	Toward light	Away from light	Neutral	
			%		
wc-1A (P829)	74*a*	10	8	82	505
wc-1a (P829)	74*A*	3	3	94	250
wc-1a (P4723)	74*A*	8	8	84	171
wc-2a [234(w)]	74*A*	18	18	64	375
wc-1A (P829)	*wc-2a* [234(w)]	9	8	83	287
wc-2a [234(w)]	*al-1A* (RES6)	11	17	72	212
wc-2a [234(w)]	*al-2A* (Y254M165)	20	13	67	126
wc-2a [234(w)]	*al-3A* (RP100)	14	18	68	149

In crosses where the resulting perithecial beaks showed a phototropic response, a dense band of spores which had shot toward the direction of the irradiation could be readily observed (Fig. 4). The band of spores was generally so dense that it could be seen without a microscope. This was not the case when a phototropic response did not occur, *i.e.* when a *wc* strain was used as the protoperithecial parent, because the spores were distributed on both sides of the line of perithecia as well as between perithecia.

The *wc* mutants are also blocked in induction of enzymes in the carotenoid biosynthetic pathway. Previously, this was demonstrated for *wc-1A* (P829) (9). Similar enzyme studies of the other *wc* strains used in the present investigation have been carried out, and as was previously found for *wc-1A* (P829), no light induction of enzymes required for the conversion of isopentenyl pyrophosphate to phytoene was detected (Harding, unpublished data).

DISCUSSION

In *N. crassa*, biochemical studies of the carotenoid biosynthetic pathway were carried out using *wc* and *al* mutants (9). Evidence was presented that the *wc* phenotype is characteristic of regulatory mutants blocked in the light induction process, whereas the *al* mutants can carry out light induction of enzymes but are each defective for a different enzyme in the carotenoid biosynthetic pathway.

Photoinduced carotenoid biosynthesis in *Neurospora* is regulated by blue light (4, 30). Other blue light-mediated effects in *Neurospora* could be completely independent of photoinduced carotenogenesis, or the photoinduction process for both responses may use common regulatory elements. If the latter is the case, then at least some *wc* mutants would be blocked in both light effects.

To test this hypothesis, another possible blue light-mediated effect in *N. crassa* was investigated. In *Neurospora sitophila*, perithecial beaks show a positive phototropic response (1). It was not determined whether this is a blue light effect. In an abstract which dealt with carotenoid biosynthesis in mutant strains of *N. crassa*, Haxo made the statement that the "phototropic response charac-

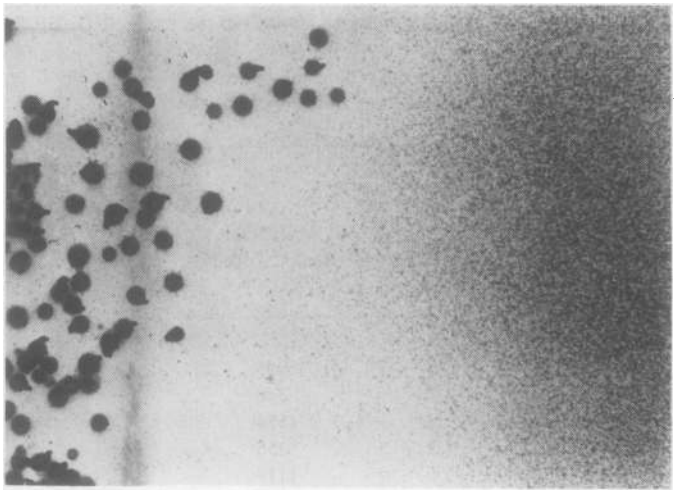

FIG. 4. Dense band of ascospores shot toward light. Perithecial beaks were derived from the cross 74*A* (protoperithecial parent) × *wc-1a* (P829).

teristic of the wild type strains is also displayed by albino mutants" (10). However, no data were presented. In the present study, it has been demonstrated that *N. crassa* perithecial beaks do show a phototropic response. Furthermore, the response was mediated by blue light, and no effect of red light on phototropism was detected.

In addition, phototropism of the perithecial beaks was blocked when *wc* strains were used as the protoperithecial parent. There was no defect in phototropism of perithecial beaks when wild type strains or *al* mutants were used as the protoperithecial parent even when the fertilizing parent was *wc*.

It has been previously shown using perithecial color mutants (*per-1*) that melanin production in perithecia and perithecial beaks is genetically controlled by the protoperithecial parent (12, 13). The present study shows that components of the phototropic sensory transduction pathway which are regulated by the *wc-1* and *wc-2* loci are also genetically controlled by the protoperithecial parent and not by the fertilizing parent.

The light induction process for photoinduced carotenogenesis and for phototropism of perithecial beaks would be expected to be under the control of several genes. So far two loci (*wc-1* and *wc-2*) have been found which affect both responses indicating that the two processes share common regulatory components. It is possible that other *wc* loci will be found which do not regulate phototropism *i.e.* the block in these *wc* mutants occurs after a branch point in the two sensory transduction pathways.

Both photoresponses are mediated by blue light, but a detailed action spectrum for phototropism of perithecial beaks has not been determined. However, the involvement of phytochrome in both responses can be ruled out by the present phototropism study and by extensive investigations of photoinduced carotenogenesis (4, 26, 30). In contrast to these results, phytochrome has been proposed to be a photoreceptor for photoinduced carotenogenesis in another fungus *Verticillium agaricinum* (29). However, Hsiao and Björn (14) have concluded that phytochrome is unlikely to be a photoreceptor in *V. agaricinum*.

The study of *wc* mutants may lead to identification of the blue light receptor which mediates a number of photoresponses. However, it is important to point out that one cannot assume that a *wc* mutant is a photoreceptor mutant. Any block in the light induction process required for photoinduction of enzymes in the carotenoid biosynthetic pathway would lead to the *wc* phenotype. Such a block could be due to either a defective photoreceptor or to other modified components of the sensory transduction pathway. Thus, the problem will be to determine which *wc* strains are photoreceptor mutants. One approach would be to look for wild type revertants of *wc* mutants and to determine whether any of these strains have altered action spectra compared to the wild type. In addition, the absence or modification in a *wc* mutant of a pigment which fits other criteria for the blue light photoreceptor would lead to the conclusion that cryptochrome had been identified.

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