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Isolation of New White Collar Mutants of *Neurospora crassa* and Studies on Their Behavior in the Blue Light-Induced Formation of Protoperithecia

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White collar (*wc*) mutants of *Neurospora crassa* are thought to be regulatory mutants blocked in the photoinduction of carotenogenesis. Eight new *wc* mutants have been isolated after UV mutagenesis; their morphology and linear growth rate are not altered, although blue light-induced carotenogenesis is completely blocked. All of the *wc* mutations fall into two complementation groups corresponding to the already-known *wc-1* and *wc-2* loci. It is shown that the *wc* mutations impair another blue light effect, the photoinduction of protoperithecia formation, as well as the low constitutive production of protoperithecia in the dark. These effects are not due to the lack of carotenoids since the albino mutants show a normal sexual development. The pleiotropic effects of the mutations in the *wc* genes indicate that they play a key role in the mechanisms of regulation of the blue light-induced responses of *N. crassa*.

In *Neurospora crassa*, the biosynthesis of carotenoids is constitutive in conidia but is regulated by blue light in mycelia (3, 25). The photoregulation of the carotenogenesis in the mycelium occurs by the de novo synthesis of some carotenogenic enzymes; this hypothesis is supported by results obtained in studies of inhibition with cycloheximide (9, 18) and the demonstration of an increase of the phytoene synthetase and geranylgeranyl pyrophosphate synthetase activities in cell-free extracts by an in vivo light treatment (11). It has been proposed that the control of protein synthesis is at the transcriptional level (23; U. Mitzka-Schnabel, E. Warm, and W. Rau; *in* H. Senger, ed., *Blue Light Effects in Biological Systems*, in press).

Regulatory mutants blocked in the pathway for light control and a genetic study of the systems would be very useful for elucidating the mechanism of photoregulation; that is, how a light signal can control an enzyme level. A group of mutants of carotenogenesis, called white collar (*wc*), have been known for some time in *N. crassa*. Their mycelium does not respond to the light stimulus but remains white, although their conidia show normal constitutive synthesis (16). The production of carotenoids in the conidia indicates that the structural genes for the carotenogenesis are functional but unexpressed in the mycelium, even after irradiation. On the basis of these considerations, Harding and Shropshire proposed that *wc* mutants are regulatory mutants which are blocked in the initial photoinduction steps (10). In agreement with such a proposal, it has been subsequently shown that the phytoene synthetase and geranylgeranyl pyrophosphate synthetase activities, which are increased by an in vivo light treatment in the wild type (WT), are not photoinduced in a *wc* mutant (11).

Three *wc* mutations have been reported: two of them map in a locus (*wc-1*) in linkage group VII; the other maps in a locus (*wc-2*) in linkage group I (17). As already pointed out by Harding and Shropshire (10), the light induction process for photoinduced carotenogenesis is likely to be under the control of several genes. Therefore, a systematic search for new *wc* mutants seems to be very useful in determining how many *wc* genes there are. Furthermore, it is of interest to see whether the *wc* genes are necessary also for the photoinduction of other blue light effects known in *Neurospora*. Blue light has five other effects in *N. crassa* besides the photoinduction of carotenoids: (i) inhibition and (ii) phase shift of the circadian rhythm of conidiation of the band mutant (6, 15, 21); (iii) photoinduction of protoperithecia formation (5); (iv) phototropism of perithecial beaks (1, 8); and (v) light-promoted conidiation of starved mycelia of the double mutant albino band (12). If the blue light-induced responses were controlled, at least in the initial steps, by the same pathway of photoregulation, then the *wc* mutants could be defective not only in photocarotenogenesis but also in the other effects. Very recently it has been shown that the phototropism of the perithecial beaks is blocked when *wc* strains are used as the protoperithecial parent (8).

The intent of our study was to isolate new *wc* mutants, to determine their complementation, and to check them along with the other three mutants already known for the photoinduction of protoperithecia formation.

MATERIALS AND METHODS

Strains and media. The *N. crassa* strains used in this work are listed in Table 1. The WT strains, *arg-1*, and *arg-10* were supplied by J. R. S. Fincham (Department of Genetics, University of Edinburgh, United Kingdom). Strains *wc-1* (allele P829 and P4723), *wc-2* (allele 234W), *al-1* (allele E54R11), *al-2* (allele Y254M220), and *al-3* (allele RP100) were obtained from the Fungal Genetics Stock Center (FGSC), Humboldt State University Foundation, Arcata, Calif. The other mutants have been isolated by us after UV mutagenesis. Media included the following: Vogel minimal medium (7); Westergaard and Mitchell crossing medium (24), modified in the trace elements composition (5); sorbose-glucose medium, namely Vogel medium supplemented with 1% (wt/vol) L-sorbose and 0.05% (wt/vol) glucose instead of 2% sucrose; sorbose medium, Vogel medium supplemented with 1% (wt/vol) L-sorbose and 0.1% (wt/vol) glucose. The media were supplemented with 1.5% (wt/vol) Bacto-Agar (Difco Laboratories, Detroit, Mich.). Arginine-requiring strains were usually grown in 0.3% (wt/vol) L-arginine (Calbiochem-Behring, La Jolla, Calif.) supplement-

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TABLE 1. Strains of *Neurospora crassa*[a]

Strain	Mating type	Locus	Allele no.	Linkage group	Origin	FGSC no.
R1	*A*	WT (ST A)	—	—	Fincham	—
R2	*a*	WT	—	—	Fincham	—
R3	*a*	*arg-1*	46004-10-1	I L	Fincham[b]	—
R4	*a*	*arg-10*	B317-9-9	VII R	Fincham[c]	—
R16	*A*	*arg-10*	B317-9-9	VII R	Cross between R4 and R1	—
R89	*a*	*wc-2*	ER44(t)	I R	UV mutagenesis of R2 (this work)[d]	4410
R97	*a*	*wc-1*	P829	VII R	FGSC[d]	143
R100	*a*	*wc-2*	ER33	I R	UV mutagenesis of R2 (this work)[d]	4408
R103	*a*	*wc-1*	ER57	VII R	UV mutagenesis of R2 (this work)[d]	4400
R110	*a*	*wc-1*	ER53	VII R	UV mutagenesis of R2 (this work)[d]	4398
R122	*a*	*wc-1*	MK1	VII R	UV mutagenesis of R2 (this work)[d]	4402
R126	*a*	*wc-1*	MK2	VII R	UV mutagenesis of R2 (this work)[d]	4404
R131	*a*	*wc-1*	P4723	VII R	FGSC[d]	3628
R133	*a*	*al-3*	RP100	V R	FGSC	2083
R134	*a*	*al-2*	Y254M220	I R	FGSC	911
R135	*A*	*al-1*	E54R11	I R	FGSC	899
R145	*a*	*wc-1*	ER45	VII R	UV mutagenesis of R2 (this work)[d]	4396
R160	*a*	*al-1*	E54R11	I R	Cross between R135 and R2	—
R162	*a*	*wc-2*	234W	I R	FGSC[d]	3818
R165	*a*	*wc-2*	ER24(t)	I R	UV mutagenesis of R2 (this work)[d]	4406
R184	*a*	*al-2*	ER7	I R	UV mutagenesis of R2 (this work)[d]	—
R196	*a*	*al-2*	MK8	I R	UV mutagenesis of R2 (this work)[d]	—
R200	*a*	*al-1*	ER2	I R	UV mutagenesis of R2 (this work)[d]	—

[a] The loci of the *wc* mutations isolated in the present work have been determined by complementation tests (this paper) and an interallelic recombination analysis (19). Strain R2 is the result of several generations of inbreeding to ST A (Fincham, personal communication).
[b] Isogenic with the WT as the result of 10 subsequential crosses with the WT (Fincham, personal communication).
[c] Isogenic with the WT as the result of nine subsequential crosses with the WT (Fincham, personal communication).
[d] The strain was obtained after subsequential crosses of the original strain with R16 and R3 (see text).

ed media. Unless otherwise specified, all chemicals were from Merck, Darmstadt, Federal Republic of Germany.

UV mutagenesis and isolation procedures. WT conidia were suspended in sterile double-distilled water at ca. 2×10^5 conidia per ml and treated with a Sylvania germicidal UV bulb (G8T5). The treatment yielded between 50 and 80% survivors. The mutagenized conidia were spread on sorbose-glucose agar medium (ca. 30 survivors per plate) and incubated in the dark at 34°C for 3 days. The developed colonies were then irradiated overnight with white fluorescent light (Sylvania; 10 W/m^2, as defined by a 4-mm filter [BG28] from Jenaer Glaswerk Schott and Gen, Mainz, Federal Republic of Germany) to induce carotenogenesis and examined. Colonies which had failed to become orange were picked and transferred to 5 ml of Vogel medium containing slants (13 by 160 mm) for a further phenotypic check (3 days at 34°C in the dark and then irradiation with white light overnight). This check is necessary, because *al* mutants also fail to produce carotenoids and are isolated along with the *wc* mutants. *Neurospora* strains grown in agar slants form a conidial band well separated from the mycelium under these conditions it is easy to recognize the *wc* phenotype (orange conidia but white mycelium, even after irradiation) and the *al* phenotype (white conidia and white mycelium).

Preliminary genetic crosses. The *wc* and *al* mutants, along with the other *wc* strains obtained from FGSC, have been subjected to a double set of crosses to eliminate possible heterokaryosis, to try to remove any additional mutation, and to obtain strains suitable for performing complementation tests with forced heterokaryons (see below). Crosses were carried out as described by Davis and De Serres (2) with random spore isolation and the *wc* strains as male parents. Each *wc* and *al* strain has been crossed first with the R16 strain (*arg-10 A*). The *wc arg-10 a* progeny were saved for complementation tests; the *wc* (or *al*) *arg-10*$^+$ *A* progeny were crossed with the R3 strain (*arg-1 a*). The *wc arg-1 a* progeny obtained from this second cross were saved for complementation tests; the progeny *wc* (or *al*) *arg-1*$^+$ *a* have been studied in this work (Table 1).

Complementation tests. Forced heterokaryons were obtained by inoculating together on minimal medium suspensions containing equal amounts (ca. 10^3 conidia) of conidia of the double mutants *wc arg-1* and *wc arg-10* obtained from preliminary crosses (see above). Each *wc* allele in *arg-1 a* background was forced with each of the various *wc* alleles in *arg-10 a* background, thereby obtaining heterokaryons in all possible combinations. The heterokaryons were grown in the dark at 34°C for 3 days and were then irradiated overnight with white light. Proof of complementation has been considered to be a visible production of carotenoids in the mycelium in comparison with the respective homokaryons. The complementation group of the *al* mutants has been determined by means of complementation tests, based on the construction of heterokaryons with the *al-1*, *al-2*, and *al-3* strains obtained from FGSC.

Extraction and measurement of carotenoids. The mycelia were grown for 44 h at 34°C on sorbose medium agar plates covered with circular dialysis membranes. Under these conditions the formation of conidia is prevented. The mycelia were irradiated with blue light of various fluence rates (photon density per unit time) for 60 s. The illumination procedures were as previously described (5). The extraction of carotenoids was done after 90 min of further incubation in the dark. Mycelia were scraped from the membranes and plunged into 10 ml of methanol. The supernatant obtained from centrifugation was collected, and the mycelia were treated with 5 ml of methanol plus 5 ml of *n*-hexane. The supernatant obtained from a second centrifugation was mixed with the former, and 15 ml of 0.02 M HCl was added. The mixture was allowed to stand overnight at ca. −30°C.

Samples of the *n*-hexane phase were read in a Perkin-Elmer 554 UV/VIS spectrophotometer at 465 nm. Dry weights were determined with mycelia grown in parallel. Dry weights were in the range of 10 to 15 mg per plate.

Measurement of growth. Linear growth rates were measured in race tubes (50 by 4 cm) as described by Davis and De Serres (2), using the same medium as in photoinduction experiments. The incubation was carried out in the dark, and the position of the mycelia was marked under red light.

Photoinduction experiments. All of the experimental procedures and growth conditions were as previously described (5) except for the following modifications. (i) Inoculations of various strains were made by spreading on each agar plate suspensions containing ca. 750 conidia; (ii) Protoperithecia developed in each plate were counted under a binocular stereoscopic microscope (Zeiss, Federal Republic of Germany) in nine fields of vision covering a total area of 1.76 cm^2. A template was used to mark and consider in each plate the same positions with regard to the edges. (iii) A container with a saturated solution of $(NH_4)_2SO_4$ was set in the incubator, to maintain the humidity at 81% (22).

RESULTS

Isolation and complementation of the mutants. Eight *wc* mutants [allele numbers ER24(t), ER33, ER44(t), ER45, ER53, ER57, MK1, and MK2] and three *al* mutants (allele numbers ER2, ER7, and MK8) have been isolated from 60,000 colonies screened after UV mutagenesis. The isolation procedures were based on the recognition of the phenotype of white mycelium and pigmented conidia. In Fig. 1, the fluence response curve of photocarotenogenesis of WT compared with the curve of a *wc* mutant is shown. The WT blue light fluence threshold is ca. 8 J/m^2; this value is very similar to that determined previously (5) for the photoinduction of protoperithecia formation (ca. 4.2 J/m^2). The *wc* mycelium clearly does not display an photoinduced production of carotenoids. The same lack of response is displayed by all of the other mutants, except those carrying mutations ER44(t) and ER24(t), when grown at 26°C. These mutations are temperature-sensitive (*ts*): the photoinduction of carotenoids is blocked when the growth temperature is 34°C, but it is reduced at 26°C (manuscript in preparation). In preliminary crosses (see above), all of the mutations segregated as single nuclear genes. Complementation tests based on a qualitative estimation of the light-induced carotenogenesis of forced heterokaryons (using *arg-1* and *arg-10* as forcing markers) indicated that all of the *wc* mutations (considering also those obtained from the FGSC) fall into two complementation groups corresponding to the already-known *wc-1* and *wc-2* loci (Table 1). In total, seven mutations are in the *wc-1* group (allele numbers ER57, ER53, MK1, MK2, ER45, P829, and P4723) and four are in the *wc-2* group [allele numbers ER33, ER44(t), ER24(t), and 234W]. This result is confirmed by a quantitative complementation analysis which will be published elsewhere. Furthermore, we have already shown with an interallelic recombination analysis that the mutations belonging to each of the two complementation groups are closely linked to the *wc* loci already mapped (19).

Measurements of growth of *wc* mutants. Before starting the study of sexual differentiation, we wanted to verify whether the mutants showed alterations in the vegetative phase. For this purpose, the linear growth rate of each mutant has been determined. Most of the measurements have been done, for practical reasons, at 21°C, but each mutant has also been checked at 26°C, the growth temperature used in the photoinduction experiments. The growth rates of the strains were

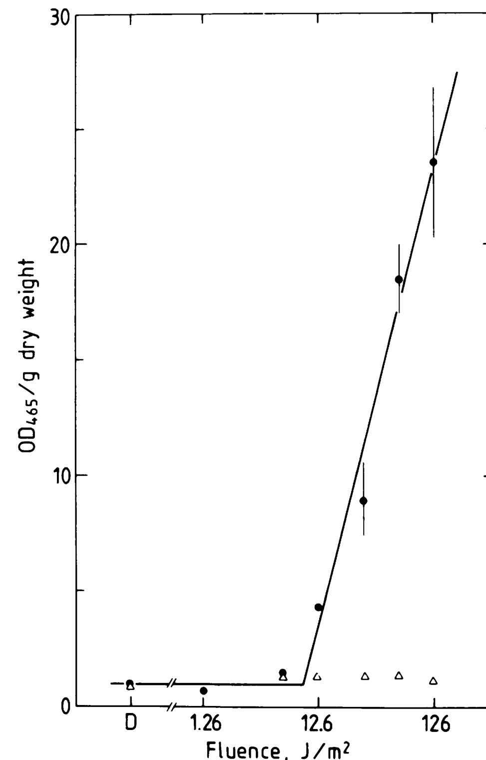

FIG. 1. Fluence-response curve of the photocarotenogenesis. D, Dark; (●) WT; average of four experiments; (△) *wc* mutant (allele MK1). The bars indicate the standard error of the mean. OD_{465}, optical density at 465 nm.

constant during the period considered by us (from 24 to 130 h after inoculation). The growth rates of the *wc* mutants were equal or very similar (± 7%) to the WT growth rate (0.30 cm/h at 21°C and 0.35 cm/h at 26°C, respectively.

Photoinduction of protoperithecia formation in *wc* mutants. The mutants have been characterized for the photoinduction of protoperithecia formation with a set of experiments, the results of which are shown in Table 2. The production of protoperithecia of each mutant was measured at various times (4, 5, and 6 days after inoculation), both in the dark and after irradiation with blue light (126 J/m^2 given in 60 s, 24 h before counting). The number of protoperithecia per square centimeter yielded by the various strains was normalized in each experiment to the corresponding WT value in the dark and was considered to be equal to 100. The analysis of the data shown in Table 2 leads to three observations: (i) the dark production of protoperithecia of the *wc* mutants is lower than that of the WT; exceptions are the *ts wc* strains (R89 and R165); (ii) the *wc* mutants do not show any significant increase in the production of protoperithecia after irradiation; the only exception is the *ts wc* strain R89; (iii) the *al* strains are photoinduced; one of them (R184) shows a lower production of protoperithecia with respect to the WT both in the dark and after irradiation, and another (R200) shows a higher production in both cases.

DISCUSSION

How many *wc* genes are there? In this work, eight *wc* mutant strains of *N. crassa* have been isolated after UV mutagenesis. Results obtained with complementation tests and an interallelic recombination analysis (19) indicate that

TABLE 2. Production of protoperithecia measured 4, 5, and 6 days after inoculation, with cultures incubated at 26°C in continuous dark or with blue light stimulation[a]

Strain	CG	Day 4		Day 5		Day 6	
		Dark	Blue light	Dark	Blue light	Dark	Blue light
R2	WT	100[b]	7,583 ± 1,599	100[c]	3,091 ± 386	100[d]	1,292 ± 275
R97	*wc-1*	0–6.4	0–0	1.0 ± 0.6	10.7 ± 7.6	2.5 ± 1.3	1.8 ± 0.6
R103	*wc-1*	0–6.4	6.4–12.5	3.5 ± 1.2	6.4 ± 1.8	2.8 ± 0.1	5.3 ± 1.9
R110	*wc-1*	4.9–4.9	0–4.9	0.5 ± 0.5	1.2 ± 0.6	0.5 ± 0.5	1.1 ± 0.6
R122	*wc-1*	0–4.9	0–4.9	2.1 ± 1.6	3.9 ± 0.8	10.4 ± 5.2	9.4 ± 6.8
R126	*wc-1*	0–4.9	0–0	0.9 ± 0.6	1.7 ± 1.5	2.7 ± 1.4	2.0 ± 0.2
R131	*wc-1*	0–4	0–4	8.1 ± 5	8.8 ± 3.1	11.5 ± 5.7	9.2 ± 4.1
R145	*wc-1*	7.9–32.4	4–12.2	28 ± 11	26.7 ± 15.5	34.6 ± 13	30 ± 21
R89	*wc-2 ts*	374–491	1,625–2,292	200 ± 58	907 ± 153	67–201	227–460
R100	*wc-2*	0–12.5	12.5–19.4	1.5 ± 0.2	1.0 ± 0.4	2.6 ± 1.6	0.9 ± 0.9
R162	*wc-2*	60–121	20–100	21 ± 7.4	19 ± 3.7	31 ± 20	28 ± 12
R165	*wc-2 ts*	327 ± 164	220 ± 111	160 ± 33	179 ± 28	270 ± 110	117 ± 21
R184	*al-2*	—	—	38 ± 8	1,839 ± 151	—	—
R196	*al-2*	—	—	168 ± 38	4,745 ± 618	—	—
R200	*al-1*	—	—	261 ± 108	7,548 ± 289	—	—

[a] Data shown are normalized values of one to three experiments with two replicates. Average ± standard error of the mean is given for more than one experiment. CG, Complementation group.
[b] 100 = 7.7 ± 1.8 protoperithecia per cm^2.
[c] 100 = 30.7 ± 6 protoperithecia per cm^2.
[d] 100 = 46 ± 12 protoperithecia per cm^2.

the *wc* mutations are alleles of the two already-known *wc-1* and *wc-2* genes. Therefore, no new *wc* gene has been found with this search. Assuming that every gene of *Neurospora* is equally susceptible to UV mutagenesis as generally believed (7) and that a hypothetical *wc-3* gene exists, we can determine the probability of not finding any mutation in the *wc-3* gene in a sample of eight mutants. Such a probability is the multinomial probability (13). The value obtained is so low (0.85%) that the existence of a third *wc* gene seems rather improbable.

Pleiotropism of *wc* mutations. We have characterized 11 independent *wc* mutations. When we started this work, the known physiological effect of those mutations was to abolish the photoinduction of carotenoids, causing the characteristic phenotype. We have shown that the *wc* mutations do not alter the growth rate in an appreciable fashion; this and the normal vegetative morphology of the strains are two good indications that the *wc* mutations do not cause alterations in the vegetative phase. On the contrary, the formation of protoperithecia, a very important step in sexual reproduction, is impaired by the *wc* mutations. We have determined the production of protoperithecia over 3 days to obtain the kinetics of differentiation in the dark and to check the response to light daily in the period considered. All of the strains having the *wc* mutations show a very low dark production which is not increased by light. This phenotype is due to the *wc* mutations and not to other unknown genetic elements present in the genetic background of the strains for the following reasons. (i) All of the *wc* mutants, except the two *ts*, show the same phenotype. (ii) The albino strains have the same pedigree as the *wc* strains, but they display a normal protoperithecial differentiation. The strain R184, which shows a lower production in the dark, still has a clear photoinduction. (iii) The strains which have the *ts wc* mutations [ER44(t) and ER24(t)] are, at the permissive temperature of 26°C, partially photoinducible in the synthesis of carotenoids. This means that at this temperature, the function specified by the *wc-2* gene is partially active. It is particularly significant that these *wc* strains show the ability to produce protoperithecia in the dark at WT levels and in one case to be photoinduced as well. Therefore, the results presented in this paper clearly indicate that the functions blocked by the *wc-1* and *wc-2* mutations are very important, not only for the blue light-induced carotenogenesis but also for the formation of the protoperithecia, both in the dark and after blue light irradiation. The impairment of sexual development is not an effect of the lack of carotenoids in the *wc* mutants since the albino mutants (devoid of carotenoids because of a block in the carotenoid biosynthetic pathway) show a normal induction of protoperithecia. In another paper, the possibility of inducing the formation of protoperithecia in *wc* mutants by treating the mycelia with suspensions of conidia is discussed (4). The induction obtained with conidia, whatever the mechanism of action, is very useful for our analysis because it excludes the possibility that the *wc* genes are structural genes necessary for the processes of morphogenesis of protoperithecia. Therefore, we can conclude that the *wc* mutations block the differentiation processes which cause the formation of protoperithecia in the dark and after blue light irradiation. The pleiotropic effect of the *wc* mutations suggests that the dark production of protoperithecia, the photoinduction of protoperithecia, and the photoinduction of carotenogenesis are controlled by the same regulation processes. It is not known yet whether the *wc* mutations also affect the low dark production of carotenoids.

Our analysis is a further confirmation of the hypothesis that the *wc* genes are regulatory genes necessary for all of the photoeffects of *Neurospora* (10). In *Phycomyces blakesleeanus*, which is a well-characterized fungus from a photobiological point of view, two genes called *mad*A and *mad*B are known. These have a pleiotropic effect analogous to that of the *wc* genes of *Neurospora*: they are necessary for all five blue light effects known in *Phycomyces* (20). This pleiotropism has been considered as a good indication that various photoresponses have their early regulation steps in common, including the photoreceptor (14, 20). Likewise, it can be supposed that the photoresponses of *Neurospora* impaired by the *wc* mutations share the same photoreceptor and the same pathway of photoregulation. We have suggested elsewhere that blue light primes the various responses by

regulating the transcription of specific mRNAs and that the *wc* gene products are necessary for this process (Degli-Innocenti and Russo, in press).

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LITERATURE CITED

1. **Backus, M. P.** 1937. Phototrophic response of perithecial necks in *Neurospora*. Mycologia **29**:383–386.
2. **Davis, R. H., and F. J. De Serres.** 1970. Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17A**:79–143.
3. **De Fabo, E. C., R. W. Harding, and W. Shropshire, Jr.** 1976. Action spectrum between 260 and 800 nanometers for the photoinduction of carotenoid biosynthesis in *Neurospora crassa*. Plant Physiol. **57**:440–445.
4. **Degli-Innocenti, F., J. A. A. Chambers, and V. E. A. Russo.** 1984. Conidia induce the formation of protoperithecia in *Neurospora crassa*. a further characterization of the white collar mutants. J. Bacteriol. **159**:808–809.
5. **Degli-Innocenti, F., U. Pohl, and V. E. A. Russo.** 1983. Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem. Photobiol. **37**:49–51.
6. **Feldman, J. F., and J. C. Dunlap.** 1983. *Neurospora crassa*: a unique system for studying circadian rhythms. Photochem. Photobiol. Rev. **4**:319–368.
7. **Fincham, J. R. S., P. R. Day, and A. Radford.** 1979. Fungal genetics. Blackwell Scientific Publications, Oxford.
8. **Harding, R. W., and S. Melles.** 1983. Genetic analysis of phototrophism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. Plant Physiol. **72**:996–1000.
9. **Harding, R. W., and H. K. Mitchell.** 1968. The effect of cycloheximide on carotenoid biosynthesis in *Neurospora crassa*. Arch. Biochem. Biophys. **128**:814–818.
10. **Harding, R. W., and W. Shropshire, Jr.** 1980. Photocontrol of carotenoid biosynthesis. Annu. Rev. Plant Physiol. **31**:217–238.
11. **Harding, R. W., and R. V. Turner.** 1981. Photoregulation of the carotenoid biosynthetic pathway in albino and white collar mutants of *Neurospora crassa*. Plant Physiol. **68**:745–748.
12. **Klemm, E., and H. Ninnemann.** 1978. Correlation between absorbance changes and a physiological response induced by blue light in *Neurospora*. Photochem. Photobiol. **28**:227–230.
13. **Lewontin, R. C., and T. Prout.** 1956. Estimation of the number of different classes in a population. Biometrics **12**:211–223.
14. **Lipson, E. D.** 1980. Sensory transduction in *Phycomyces* photoresponses, p. 110–118. *In* H. Senger (ed.), The blue light syndrome. Springer-Verlag, Berlin.
15. **Ninnemann, H.** 1979. Photoreceptors for circadian rhythms. Photochem. Photobiol. Rev. **4**:207–266.
16. **Perkins, D. D., M. Glassey, and B. A. Bloom.** 1962. New data on markers and rearrangements in *Neurospora*. Can. J. Genet. Cytol. **4**:187–205.
17. **Perkins, D. D., A. Radford, D. Newmeyer, and M. Björkman.** 1982. Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. **46**:426–570.
18. **Rau, W., I. Lindemann, and A. Rau-Hund.** 1968. Untersuchungen über die lichtabhängige carotinoidsynthese. Planta **80**:309–316.
19. **Russo, V. E. A., and F. Degli-Innocenti.** 1983. Recombination frequencies of mutations located in *wc*-1 and *wc*-2. Neurospora Newsl. **30**:11–12.
20. **Russo, V. E. A., P. Galland, M. Toselli, and L. Volpi.** 1980. Blue light induced differentiation in *Phycomyces blakesleeanus*, p. 563–569. *In* H. Senger (ed.), The blue light syndrome. Springer-Verlag, Berlin.
21. **Sargent, M. L., and W. R. Briggs.** 1967. The effects of light on a circadian rhythm of conidiation in *Neurospora*. Plant Physiol. **42**:1504–1510.
22. **Sargent, M. L., and S. H. Kaltenborn.** 1972. Effects on medium composition and carbon dioxide on circadian conidiation in *Neurospora*. Plant Physiol. **50**:171–175.
23. **Subden, R. E., and G. Bobowski.** 1973. Evidence for actinomycin D inhibition of transcription of carotenoid loci in *Neurospora*. Experientia 29:965–967.
24. **Westergaard, M., and H. K. Mitchell.** 1947. *Neurospora* V. A synthetic medium favouring sexual reproduction. Am. J. Bot. **34**:573–577.
25. **Zalokar, M.** 1955. Biosynthesis of carotenoids in *Neurospora*. Action spectrum of photoactivation. Arch. Biochem. Biophys. **56**:318–325.