Comments

The above methods can be applied to isolate xanthosomes as energetically coupled assemblies from most of the chromophytic algae, including brown algae *(Sphacelaria, Halopteris, Cutleria, Cladosiphon, Analipus, Ecklonia),* diatoms *(Pheodactylum, Cyclotella),* chrysophytes *(Chromulina, Ruttnera),* raphidophytes *(Heterosigma),* prymnesiophytes *(Prymnesium, Isochrysis),* and dinoflagellates *(Amphidinium, Glenodinium, Prorocentrum).* However, it should be borne in mind that chromophytic algae vary extensively in the stability of xanthosomes and in the readiness with which xanthosomes are separated from the thylakoid membranes. For instance, in xanthosomes obtained from the brown algae *Scytosiphon* or *Undaria,* a significant part of the fucoxanthin if blue shifted even if prepared carefully.

Generally the saccharose esters are effective in separating xanthosomes from thylakoid lamellae, and the samples obtained with these detergents show better signs of intactness in absorption spectra, in fine structures, and in energetic coupling than those obtained with *n*-decylmaltoside (Sigma, St. Louis, MO) or *n*-octylthioglucoside (Sigma), though the latter detergents may yield better results depending on the materials. It should be noted that other classes of detergents, including Triton X-100, Nonidet P-40, LDAO, Deriphat 160, and Zwittergent 3-14, act in such a manner as to disintegrate the energetic coupling of xanthosomes and cause the accompanying blue shift of fucoxanthin. As we did not make an extensive survey of detergents, these methods should be considered as only a starting point.

[38] Photoregulated Carotenoid Biosynthetic Genes of *Neurospora crassa*

By GIORGIO MORELLI, MARY ANNE NELSON, PAOLA BALLARIO, and GIUSEPPE MACINO

Introduction

Plants and algae, as well as many fungi and bacteria, are able to direct the *de novo* synthesis of carotenoids, and in many cases this synthesis has been demonstrated to be under light control.[1] In the filamentous fungus

[1] T. W. Goodwin, "The Biochemistry of the Carotenoids," 2nd Ed., Vol. 1. Chapman & Hall, London, 1980.

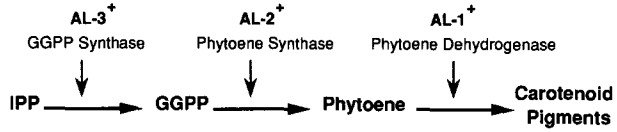

FIG. 1. Model for carotenoid biosynthesis in *Neurospora crassa*. AL-3$^+$, AL-2$^+$, and AL-1$^+$ refer to the wild-type albino genes. IPP, Isopentenyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate.

Neurospora crassa, carotenoid biosynthesis is under blue light control in the vegetative mycelium, but it proceeds even in the absence of light induction in the asexual spores or conidia.[2] When grown in the light, wild-type *Neurospora* is a uniform bright orange, while in the dark it produces white mycelia and orange conidia.

Since carotenoids are not required for growth of *Neurospora,* the isolation of strains harboring mutations in regulatory and structural genes of the carotenoid biosynthetic pathway proved to be straightforward. Two regulatory mutants, known as *white collar (wc-1* and *wc-2)* because they produce carotenoids normally in the conidia but fail to produce them in the underlying mycelium, have been isolated.[3,4] The *wc* mutations exert a pleiotropic effect on all the known light-induced phenomena in *N. crassa,* which include stimulation of carotenoid biosynthesis,[2] shifting and photosuppression of the circadian rhythm,[5] production of the female reproductive structures or protoperithecia,[6] and phototropism of the perithecial beaks.[7] The sweeping control exerted by the *wc* genes leads one to conclude that the products of these two loci are involved in the initial steps of photoinduction. Indeed, one of the *wc* genes might encode the *Neurospora* photoreceptor, which has not yet been identified.

Carotenoids are very hydrophobic and thus difficult to use as substrates in the cell-free extracts that are utilized for the characterization of enzymatic activities. However, such experiments, coupled with the analysis of mutant strains, have led to the generally accepted scheme of carotenoid biosynthesis in *Neurospora* that is diagrammed in Fig. 1.[3] Three *albino* mutants are known in *Neurospora crassa* (*al-1, al-2,* and *al-3*), each affected in a structural gene of the carotenogenic pathway. The *al-1* mutant

[2] R. W. Harding and W. Shropshire, *Annu. Rev. Plant Physiol.* **31,** 217 (1980).
[3] R. W. Harding and R. V. Turner, *Plant Physiol.* **68,** 745 (1981).
[4] F. Degli Innocenti and V. E. A. Russo, *in* "Blue Light Effects in Biological Systems" (H. Senger, ed.), p. 213. Springer-Verlag, Berlin, 1984.
[5] J. F. Feldman, *Annu. Rev. Plant Physiol.* **33,** 583 (1982).
[6] F. Degli Innocenti and V. E. A. Russo, *Photochem. Photobiol.* **37,** 49 (1983).
[7] R. W. Harding and S. Melles, *Plant Physiol.* **72,** 996 (1983).

strain accumulates phytoene and is thought to be defective in phytoene dehydrogenase.[8] The *al-2* mutant has been shown to be defective in phytoene synthase, whereas *al-3* mutants are almost completely lacking the enzymatic activity required for geranylgeranyl pyrophosphate (GGPP) biosynthesis.[3]

In our investigation of the light regulation of carotenogenesis, we chose to clone one of the structural genes involved in the pathway, namely, the GGPP synthase or *al-3*$^+$ gene.[9] Another group has cloned the structural gene for phytoene dehydrogenase, *al-1*$^+$.[10] The transcription of the *al-1*$^+$ and *al-3*$^+$ genes is induced by light treatment, and this induction requires functional *white collar* gene products.[9,10] Analyses of the structural *albino* and regulatory *white collar* genes should yield much information about this photoinduction pathway.

Walking to the Albino-1 Gene

When one wishes to isolate a gene encoding a nonselectable phenotype (like the *albino* genes of *Neurospora*), it is often efficacious to isolate a nearby gene whose function is selectable and then use that gene as the starting point in a "walk" to the gene of interest. That is the approach which was used to isolate the *al-1*$^+$ gene of *Neurospora;* a cosmid carrying a nearby gene of selectable phenotype (*hom,* homoserine-requiring) also contained the *al-1*$^+$ gene.[10] When that cosmid was transformed into a *hom al-1* strain, many of the *hom*$^+$ transformants produced carotenoids, demonstrating the presence on the cosmid of the intact *al-1*$^+$ gene.

Walk to Albino-3 Failed

We attempted to walk to the *al-3*$^+$ gene from the nearby *inl* (inositol-requiring) gene, using a cosmid library prepared by Vollmer and Yanofsky.[11] We now know that the *al-3*$^+$ gene is not present in that library, and so our attempts had no chance of success. Not wanting to relinquish our search for *al-3*$^+$, we undertook a more laborious (but ultimately successful) scheme for its isolation.

[8] A. H. Goldie and R. E. Subden, *Biochem. Genet.* **10,** 275 (1973).
[9] M. A. Nelson, G. Morelli, A. Carattoli, N. Romano, and G. Macino, *Mol. Cell. Biol.* **9,** 1271 (1989).
[10] T. J. Schmidhauser, F. R. Lauter, V. E. A. Russo, and C. Yanofsky, *Mol. Cell. Biol.* **10,** 5064 (1990).
[11] S. J. Vollmer and C. Yanofsky, *Proc. Natl. Acad. Sci. U.S.A.* **83,** 4869 (1986).

Identification of *al-3*$^+$ Transformants

Strains and Vectors

The *Neurospora* strains were obtained from the Fungal Genetics Stock Center (FGSC; University of Kansas, Kansas City, KS): *al-3* (RP100; FGSC No. 2082) and *qa-2 aro-9* (M246 Y325M6; FGSC No. 3958). Double *qa-2* (quinate utilization) *aro-9* (aromatic cluster) mutants require an aromatic amino acids supplement for growth. The *al-3 qa-2 aro-9* triple mutant was constructed using standard procedures.[12] The *al-3* mutant that was used has a leaky phenotype, characterized by the production of extremely light orange conidia. Methods for culturing *N. crassa* were standard.[12]

The plasmid library that was used is based on the pRAL1 vector, which contains the *qa-2*$^+$ gene for selection in *Neurospora* and the chloramphenicol resistance gene for selection in *Escherichia coli*.[13] The plasmid clone bank consists of 10 pools of about 1800 different plasmids each, and it has proved quite useful in the sib selection of various genes.

Spheroplast Preparation

The method used for preparation of *Neurospora* spheroplasts is based on that of Schweizer *et al.*[14] The *al-3 qa-2 aro-9* strain is grown with aromatic amino acid supplements in *Neurospora* growth flasks, and the conidia are harvested with sterile water. The conidia are inoculated into 0.5X Vogel minimal medium,[15] 2% sucrose plus supplements at 10^9 conidia per 100 ml, and germinated by shaking at 30° for 4 to 6 hr. Germinated conidia are harvested by centrifuging for 20 min at 8000 rpm in a Sorvall SA rotor (in sterile centrifuge bottles), then washed 3 times with 50 ml sterile 1 *M* sorbitol (centrifuging for a few minutes each time at 4000 rpm in 50-ml conical polypropylene tubes). The final pellet is resuspended in 10 ml sterile 1 *M* sorbitol. Novozym 234 (BioLabs, Novo Alle', Denmark) is dissolved in sterile 1 *M* sorbitol and added to the germinated conidia at a final concentration of 0.5 mg/ml. Digestion is carried out at 30° with gentle shaking and allowed to continue until the efficiency of spheroplast formation is about 80 to 90% (as visualized with a phase-contrast microscope). The spheroplasts are diluted with cold sterile 1 *M* sorbitol to 50 ml and washed gently 3 times with the same (centrifuging for

[12] R. H. Davis and F. J. de Serres, this series, Vol. 17, p. 79 (1970).
[13] R. A. Akins and A. M. Lambowitz, *Mol. Cell. Biol.* **5**, 2272 (1985).
[14] M. Schweizer, M. E. Case, C. C. Dykstra, N. H. Giles, and S. R. Kushner, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5086 (1981).
[15] H. J. Vogel, *Am. Nat.* **98**, 435 (1964).

a few minutes each time at 2000 rpm in 50-ml conical polypropylene tubes). At this point the spheroplasts are counted and washed a final time with cold sterile 1 *M* sorbitol, 50 m*M* Tris-HCl, pH 8, 50 m*M* $CaCl_2$. To the pellet is added, per 10^8 spheroplasts, the following: 0.7 ml of 1 *M* sorbitol, 50 m*M* Tris-HCl, pH 8, 50 m*M* $CaCl_2$; 0.2 ml of 40% polyethylene glycol (PEG) 4000, 50 m*M* Tris-HCl, pH 8, 50 m*M* $CaCl_2$; and 10 μl dimethyl sulfoxide (DMSO). The spheroplasts are either used immediately or stored in small aliquots at $-70°$.

Transformation Protocol

DNA that is to be used for transformation of *Neurospora* must be free of contaminating RNA. We carry out a CsCl purification of the plasmids, followed by precipitation with PEG. Specifically, we adjust the DNA solutions to final concentrations of 10% PEG 4000 and 0.5 *M* NaCl and incubate them on ice for at least 1 hr, followed by centrifugation and two 70% ethanol washes. The transformation protocol that we use is based on that of Vollmer and Yanofsky.[11] Transformations with each of the 10 plasmid banks[13] are carried out separately. Five microliters (5 μg) of DNA is mixed with 2 μl of 50 m*M* spermidine and 5 μl heparin solution (5 mg/ml heparin, 1 *M* sorbitol, 50 m*M* Tris-HCl, pH 8, 50 m*M* $CaCl_2$) and incubated on ice for 20 min. Then 100 μl of *al-3 qa-2 aro-9* spheroplasts are added, mixed in gently, and stored on ice for 30 min. One milliliter of 40% PEG 4000, 50 m*M* Tris-HCl, pH 8, 50 m*M* $CaCl_2$ is added and mixed in gently, after which the mixture is incubated at room temperature for 20 min. The entire transformation mixture is transferred to 5 ml of regeneration mix (0.5 *M* $MgSO_4$, 0.5X Vogel's, 2% sucrose, 1% glucose) and shaken gently at room temperature overnight. Aliquots of the regenerated spheroplasts are spread onto selective plates (lacking the aromatic amino acids supplement that is required for the growth of *qa-2 aro-9* double mutants) and incubated at 30° for 4 to 5 days. The transformant *(qa-2$^+$)* colonies are allowed to conidiate and then are screened for the production of carotenoids.

Results

We identified a population of plasmids containing the GGPP synthase *(al-3$^+$)* gene by the ability of this population to complement an *al-3* mutation and cause the synthesis of orange rather than white mycelia and conidia.[9] About 10,000 stable *qa-2$^+$* transformants were screened for each of the 10 plasmid pools. Primary transformants producing orange conidia (clearly more pigmented than the conidia produced by the *al-3* mutant strain) were observed when plasmid pools 7 and 9 were used in the trans-

formations. However, stable *al-3*$^+$ transformants were quite rare, occurring with frequencies of 1 in every 5000 to 10,000 *qa-2*$^+$ transformants obtained with the 7 and 9 plasmid pools.

Cloning of Geranylgeranyl Pyrophosphate Synthase (*al-3*$^+$) Gene

When complementation of a particular mutation has been detected after transformation in *Neurospora,* two general methods have been used to isolate the vector carrying the gene of interest. Sib selection (the repeated subdivision of a genomic library) can be used to enrich progressively for and ultimately isolate the gene of interest.[11,13] Alternatively, the complementing sequences can be recovered from the *Neurospora* transformant strain. Transforming DNA is always integrated into the host genome, preventing the recovery of the plasmid by direct retransformation of *E. coli.* However, in many cases the integrated plasmid remains associated with the bacterial sequences necessary for selection and propagation in *E. coli,* so that it is possible to rescue the transforming plasmid from the recipient *N. crassa* strain. This is accomplished by digestion of recipient chromosomal DNA with an appropriate restriction enzyme and ligation to obtain a circular vector for transformation into *E. coli.* In some cases, the integrated sequences are heavily methylated, and plasmids can be recovered only on transformation into *E. coli* strains with defective methylcytosine restriction systems.[16]

Attempts were made to use the sib selection procedure for the isolation of the *al-3*$^+$ containing plasmid from plasmid pools 7 and 9, but those attempts failed (see discussion below). Only with the selection of plasmids in *E. coli* were we able to isolate the *al-3*$^+$ gene. The gene was isolated after selection in a strain with functional methylcytosine restriction systems, suggesting that in this case the transforming DNA had not been extensively methylated.

Preparation of al-3$^+$ *Transformant DNA*

Homokaryotic derivatives of the stable *qa-2*$^+$ *al-3*$^+$ transformants were isolated by successive single colony isolations under restrictive conditions, and a transformant producing apparently normal levels of carotenoids (named AL3-1) was chosen for further study. We attempted to isolate some or all of the original transforming vector from this transformant as follows. The mycelia produced after overnight growth of AL3-1 in 100 ml liquid culture are filtered and frozen in liquid nitrogen; about 1 g of dry frozen

[16] M. J. Orbach, W. P. Schneider, and C. Yanofsky, *Mol. Cell. Biol.* **8**, 2211 (1988).

mycelia is recovered. The frozen mycelia are ground to a powder in liquid nitrogen, resuspended in 15 ml of 50 m*M* Tris-HCl, pH 8, 20 m*M* EDTA, 1% sodium dodecyl sulfate (SDS), and incubated at 65° for 30 min. After the addition of 4.5 ml of 5 *M* potassium acetate, the mixture is put on ice for 1 hr, followed by centrifugation for 10 min at 12,000 rpm. The DNA in the supernatant is precipitated with 2 volumes of ethanol. After 1 hr at room temperature, the DNA is recovered by centrifugation at 12,000 rpm for 20 min. The pellet is washed with 70% ethanol and resuspended in 2 ml of TE (10 m*M* Tris-HCl, pH 8, 1 m*M* EDTA). Three hundred micrograms of DNase-free RNase is added, and the solution is incubated at 37° for 30 min. The solution is brought to 0.3 *M* sodium acetate, and the DNA is precipitated with 1 volume of 2-propanol.

Isolation of Chloramphenicol-Resistant Plasmids from AL3-1 Transformant

Southern analysis of AL3-1 DNA suggested that the transforming vector sequence was probably integrated at a single site (and in only one copy).[9] Also, the results of restriction analysis led us to believe that the vector sequence had not been rearranged. Since pRAL1 contains unique *Hind*III and *Sal*I sites that lie outside the essential regions of the vector,[13] we used these two restriction enzymes in an attempt to rescue plasmids in *E. coli* containing both vector and flanking sequences.

Ten micrograms of AL3-1 DNA is digested with *Hind*III or *Sal*I, diluted in ligation buffer to 1 μg/ml (the dilute solution is used to avoid intermolecular annealing), and ligated at 4° overnight with T4 DNA ligase (1 U/ml). The ligated DNAs are concentrated to 200 μl with 2-butanol and then ethanol precipitated. A 1-μg sample of ligated DNA is used to transform 5×10^{10} competent *E. coli* HB101 cells to chloramphenicol resistance.

A 5.2-kbp plasmid (pNC6) was isolated from *Sal*I-digested AL3-1 DNA, and a 10.8-kbp plasmid (pNC3) was isolated from the *Hind*III-digested sample. The pNC3 and pNC6 plasmids (both of which contain functional *Neurospora qa-2*$^+$ genes) were used to transform *al-3 qa-2 aro-9* spheroplasts to prototrophy (*qa-2*$^+$); no *al-3*$^+$ transformants were obtained, indicating that an intact *al-3*$^+$ gene is not contained in either pNC3 or pNC6. The structures of these plasmids are shown in Fig. 2.

Isolation of Plasmid Containing Intact al-3$^+$ *Gene*

Restriction fragments from pNC3 and pNC6 were used as probes in the isolation of the original transforming plasmid (pNC39) from pool 9.[9] pNC39 transformed *al-3 qa-2 aro-9* spheroplasts to *qa-2*$^+$*al-3*$^+$ at high frequency; about 20% of the primary heterokaryotic transformants pro-

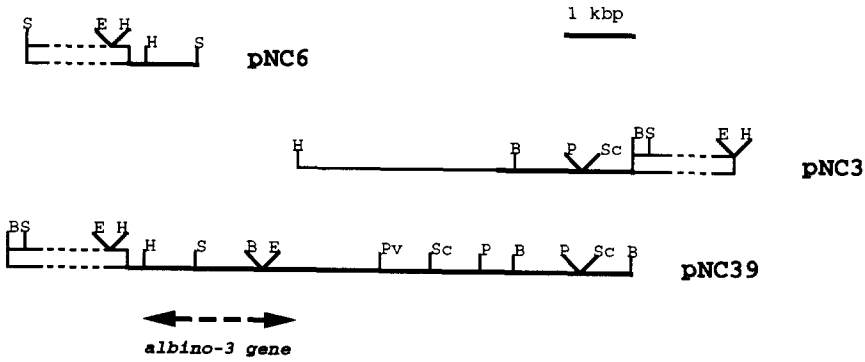

FIG. 2. Structures of the pNC3, pNC6, and pNC39 plasmids. *Neurospora* insert sequences are indicated by lines; the thick lines denote sequences that are present in the original pNC39 insert, whereas light lines denote chromosomal sequences flanking the inserted vector. pNC6 contains only sequences that are also present in the transforming plasmid pNC39, and pNC3 contains also chromosomal flanking sequences. The *N. crassa* sequences are drawn approximately to scale (see bar). Vector (pRAL1) sequences are indicated by open boxes and have been compressed. The position of the *al-3*$^+$ gene is shown. Restriction sites: S, *Sal*I; E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; Pv, *Pvu*II; Sc, *Sac*I; P, *Pst*I.

duced dark orange conidia. The color of the remaining transformants ranged from light orange to white; analysis of homokaryotic derivatives of the transformants showed that about 80% contained functional *al-3*$^+$ genes. The pNC39 plasmid was present at low frequencies (10^{-4}) in plasmid pool 9; its low representation might partially explain why our attempts at sib selection of the *al-3*$^+$ gene (above) failed. Also, poor expression of the *al-3*$^+$ gene in the primary heterokaryotic transformants reduced the likelihood of a successful sib selection.

Comparative restriction analysis of the pNC3, pNC6, and pNC39 plasmids (Fig. 2) has confirmed that pNC39 was the vector used to generate the AL3-1 transformant, and that the vector sequences were not rearranged during the transforming event. Conventional subcloning techniques, followed by transformation of *al-3 qa-2 aro-9* spheroplasts to *qa-2*$^+$ and screening for orange (*al-3*$^+$) colonies, were used to further localize the *al-3*$^+$ gene within pNC39[9,17]; its position is indicated in Fig. 2.

Mapping to Confirm Identity of *al-3*$^+$ Gene

To rule out the possibility that we had cloned an unlinked suppressor of the *al-3* mutation rather than the *al-3*$^+$ gene itself, we determined the map position of the pNC39 insert using RFLP (restriction fragment length

[17] A. Carattoli, N. Romano, P. Ballario, G. Morelli, and G. Macino, *J. Biol. Chem.* **266**, 5854 (1991).

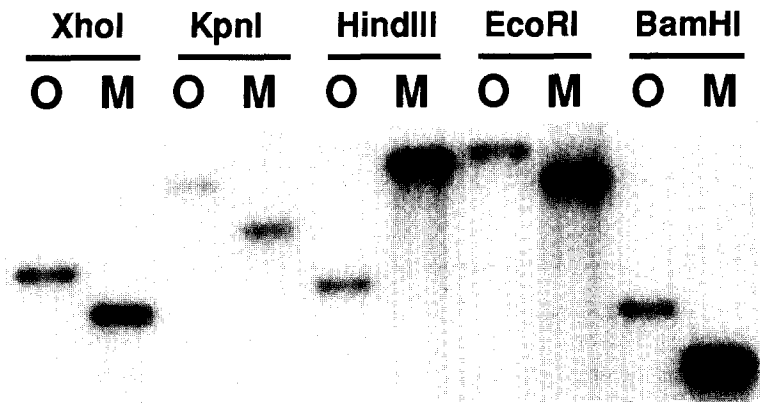

FIG. 3. Restriction fragment length polymorphisms (RFLPs) used to map the *al-3*$^+$ gene. A 4-kbp *Eco*RI–*Bam*HI fragment of pNC39 was used to probe digests of genomic *Neurospora* DNA from two strains, Mauriceville-1c *A* (M) and multicent-2-*a* (Oak Ridge, O). The restriction enzymes used to digest DNA from the two mapping strains are indicated above the lanes.

polymorphism) mapping. The technique used was that devised by Metzenberg *et al.*[18] This technique takes advantage of the large number of restriction site polymorphisms in two strains of *N. crassa*. Segregation of both conventional genetic markers and RFLPs is analyzed, and cosegregation of RFLPs within the cloned DNA and these markers is evidence of genetic linkage. A 4-kbp *Eco*RI–*Bam*HI fragment of pNC39 was used in this analysis; sites for five restriction enzymes were tested, and all were polymorphic in the two *Neurospora* mapping strains, Mauriceville and Oak Ridge (Fig. 3). The segregation of these sites among the progeny of a Mauriceville × Oak Ridge cross was analyzed, and the pNC39 fragment was mapped to linkage group V, between the *cyh-2* and *inl* genes, as expected based on conventional mapping of *al-3* mutants.[9]

Photoinduction Experiments

Culture Conditions and Light Treatment

Neurospora crassa strains are cultured on vegetative growth medium (Vogel minimal medium, 2% sucrose, 1.5% agar)[15] for 8 days at 30°. The conidia are harvested with sterile distilled water and filtered through cheesecloth. Conidial stocks (4×10^8/ml) are stored at −20° in distilled

[18] R. L. Metzenberg, J. N. Stevens, E. U. Selker, and E. Morzycka-Wroblewska, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2067 (1985).

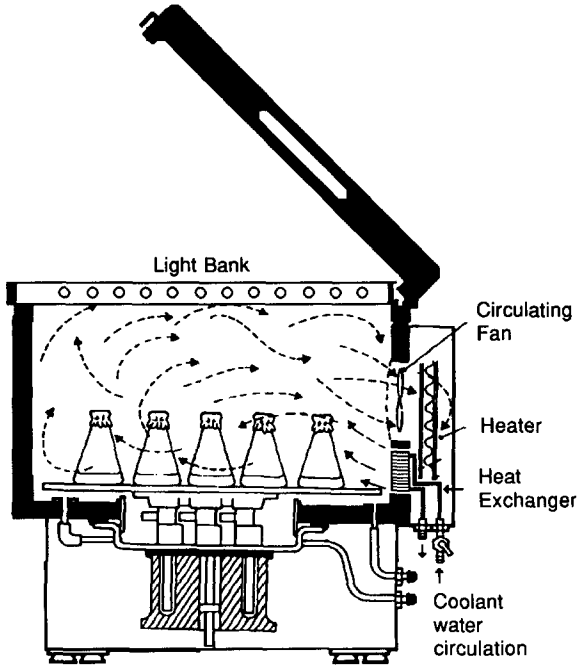

FIG. 4. System used for photoinduction experiments. The light bank also serves as the chamber lid, as there is a plexiglass sheet between the lights and the chamber.

water. For the photoinduction experiments, conidia are inoculated at 4×10^5/ml in 100 ml Vogel minimal medium plus 2% sucrose (in 250-ml Erlenmeyer flasks). The submerged mycelia are grown in the dark with agitation (200 rpm) in a New Brunswick Scientific G25 incubator shaker at 30° for 20 hr at which time the cells were at the end of log phase; the mycelia consist of a fairly homogeneous network of short hyphae at this stage.

The cultures are illuminated directly in the growth flasks with a light bank located at the top of the shaker (Fig. 4). A heat exchanger is used to maintain constant temperature during the period of illumination. The light bank consists of 12 Sylvania GRO-LUX F 18W-GRO lamps; these lamps generate an energy fluence rate of 8 W/m^2 in the blue region. The light-induced cultures and the dark-grown controls are collected by filtration on filter paper disks and immediately frozen in liquid nitrogen.

RNA Extraction

Total RNA is extracted from frozen mycelia that had been powdered in a Waring blendor with liquid nitrogen. Frozen powdered mycelia

(0.4 g) are dissolved in 8 ml GT buffer (5 *M* guanidinium thiocyanate, 25 m*M* sodium citrate, pH 7, 0.5% sarcosyl, 2 m*M* EDTA, 5% 2-mercaptoethanol)[19] and incubated at 50° for 15 min. Cell debris is removed by centrifugation at 8000 rpm for 15 min in a Sorvall SS34 rotor. The supernatant is placed in a polyallomer ultracentrifuge tube on a CsCl cushion (4.95 *M* CsCl, 0.1 *M* sodium acetate, pH 5, 5 m*M* EDTA),[20] and centrifuged (34,000 rpm for 18 hr at 20°) in a Beckman SW41T rotor. The supernatant is carefully aspirated, and the RNA pellet is gently resuspended in 400 μl of 7 *M* urea, 2% sarcosyl. A half-volume of phenol is added, and the solution is mixed by vortexing; the urea prevents the formation of phases. Then a half-volume of chloroform is added, and the phases are separated by microcentrifugation. The RNA is precipitated with 0.3 *M* sodium acetate, pH 5.2, and 3 volumes of ethanol and pelleted by microcentrifugation; finally, the RNA is resuspended in 200 μl distilled water.

Northern Hybridizations

For Northern blot analysis, total RNA is denatured and electrophoresed on 1.2% agarose–1.9% formaldehyde gels[21] with 20 m*M* morpholinopropanesulfonic acid, 5 m*M* sodium acetate, 1 m*M* EDTA, pH7, running buffer. The separated RNAs are transferred to nylon membranes or nitrocellulose.[22] Samples are loaded in duplicate and hybridized with either an *al-3*-specific oligonucleotide probe (27 nucleotides long) or a control probe from the *N. crassa* β-tubulin gene.[23] The ^{32}P-labeled oligonucleotide probe is prepared by 5′-end-labeling with T4 polynucleotide kinase,[24] and the ^{32}P-labeled β-tubulin probe is made with the random oligomer-primer method.[25] When oligonucleotide probes are used, filters are hybridized with 5×10^6 counts/min (cpm)/ml of probe at 50° for 18 hr in 5× SSC, 5× Denhardt's solution, 50 m*M* sodium phosphate, pH 7, 0.5% SDS, 50 μg/ml denatured herring sperm DNA (1× SSC is 0.15 *M* NaCl, 15 m*M* sodium citrate, pH 7; 1× Denhardt's is 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone). Unhybridized oligonucleotide probe is removed by washing in 1× SSC, 0.1% SDS at 50° for 30 min. In

[19] J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, and W. J. Rutter, *Biochemistry* **18,** 5294 (1979).
[20] V. Glisin, R. Crkvenjakov, and C. Byus, *Biochemistry* **13,** 2633 (1974).
[21] H. Lehrach, D. Diamond, J. M. Wozney, and H. Boedtker, *Biochemistry* **16,** 4743 (1977).
[22] P. S. Thomas, this series, Vol. 100, p. 255 (1983).
[23] M. J. Orbach, E. B. Porro, and C. Yanofsky, *Mol. Cell. Biol.* **6,** 2452 (1986).
[24] T. Maniatis, E. F. Fritsch, and J. Sambrook, "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982.
[25] A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132,** 6 (1983).

hybridizations with random primer-labeled probes, filers are hybridized with 2×10^6 cpm/ml of probe at 42° for 18 hr in 6× SSPE, 1× Denhardt's solution, 50% formamide, 5% dextran sulfate, 0.1% SDS, 200 μg/ml denatured herring sperm DNA (1× SSPE is 0.18 *M* NaCl, 10 m*M* sodium phosphate, pH 7.7, 1 m*M* EDTA); the filters are washed in 0.1× SSC, 0.1% SDS at 48° for 30 min. The filters are autoradiographed with preflashed Hyperfilm-MP film (Amersham) at −70°; signals are increased with intensifying screens (Cronex Quanta III, Du Pont Corp.).

Results of Transcript Analyses

The expression of the *al-3* gene in light-induced and dark-grown mycelial cultures has been examined.[9,17] Since the *white collar* genes are thought to encode regulatory products necessary for the expression of the *albino* genes,[3,4] *al-3* expression was analyzed in *wc-1* and *wc-2* mutants as well as in a wild-type strain. The results of those analyses demonstrated that photoinduction causes a dramatic increase in the transcription of the *al-3* gene, and that functional *white collar* products are required for this photoinduced response.[9,17] Similar regulation of the expression of the *al-1* gene has also been shown.[10]

Conserved Domains in *Neurospora crassa* Geranylgeranyl Pyrophosphate Synthase and Other Prenyltransferases

GGPP synthase is a member of the prenyltransferase family. Prenyltransferases carry out the major synthetic steps in isoprenoid metabolism and produce various prenyl compounds that are the precursors of a variety of products including carotenoids, steroids, chlorophylls, heme *a*, prenylated tRNAs, glycosyl carrier lipids, plant hormones, and side chains of quinones.[26] We have analyzed *N. crassa* GGPP synthase and other prenyltransferases for conserved regions,[17,27] and a similar comparison (of yeast hexaprenyltransferase and farnesyl diphosphate synthases) has been made by Ashby and Edwards[28]; three conserved domains have been identified in these analyses. Domain I (LXXDDXXDXSXXRRGXP) contains many conserved highly charged amino acids (where X is any amino acid and the other amino acids are indicated by single-letter code). The second domain is more variable in the different prenyltransferases.[17] Domain III (GXXFQIXDDYLD) also contains many highly charged residues. Do-

[26] C. D. Poulter and H. C. Rilling, *in* "Biosynthesis of Isoprenoid Compounds" (J. W. Porter and S. L. Spurgeon, eds.), p. 162. Wiley, New York, 1981.

[27] V. Ilardi, F. Luti, G. Macino, and G. Morelli, manuscript in preparation (1992).

[28] M. N. Ashby and P. A. Edwards, *J. Biol. Chem.* **265**, 13157 (1990).

mains I and III both contain "DDXXD" motifs; these aspartate residues could serve in the binding of Mg^{2+} or Mn^{2+} cations, which is required for the activities of some prenyltransferases.[29] The positively charged amino acids in domains I–III may be involved in the enzyme activity[17]; site-directed mutagenesis could be used to test the functions of these conserved residues. The genes encoding prenyltransferases in other organisms could be isolated using oligonucleotides corresponding to the amino acid sequences of the conserved domains.

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