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Photoregulation of the Carotenoid Biosynthetic Pathway in Albino and White Collar Mutants of *Neurospora crassa*

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ABSTRACT

The conversion of isopentenyl pyrophosphate to phytoene in *Neurospora crassa* requires both a soluble and a particulate fraction. Soluble and particulate enzyme fractions obtained from light-treated and dark-grown wild type, *albino-1*, *albino-2*, *albino-3*, and *white collar-1* strains were mixed in various combinations, and the activity for conversion of [1-^{14}C]isopentenyl pyrophosphate to phytoene was assayed. From such experiments it can be concluded that: (a) *albino-3* is defective in the soluble fraction; (b) *albino-2* is defective in the particulate fraction; (c) the *in vivo* light treatment increases the enzyme activity in the particulate fraction; (d) this light effect occurs in wild type, *albino-1*, and *albino-3* strains; and (e) enzyme activity is present in the particulate fraction obtained from the *white collar-1* mutant, but the *in vivo* light treatment does not cause an increase in this activity. To measure directly the level of particulate enzyme activity, [^{14}C]geranylgeranyl pyrophosphate was used as a substrate. This compound, which is not available commercially, was synthesized enzymically using extracts of pea cotyledons. Particulate enzyme fractions obtained from wild type, *albino-1*, and *albino-3* strains incorporate [^{14}C]geranylgeranyl pyrophosphate into phytoene, and this activity is higher in extracts obtained from light-treated cultures. The particulate fraction obtained from the *white collar-1* mutant also incorporates [^{14}C]geranylgeranyl pyrophosphate into phytoene, but the *in vivo* light treatment does not cause an increase in this activity. No incorporation occurs when particulate fractions obtained from either dark-grown or light-treated *albino-2* cultures are assayed. The soluble enzyme fraction obtained from the *albino-3* mutant was shown to be almost totally defective in enzyme activity required for the biosynthesis of [^{14}C]geranylgeranyl pyrophosphate from [1-^{14}C]isopentenyl pyrophosphate. An *in vivo* light treatment increases the level of this activity in wild type, *albino-1*, *albino-2*, and *albino-3* strains, but not in the *white collar-1* mutant. A model is presented to account for all of the results obtained in this investigation. It is proposed that the *white collar-1* strain is a regulatory mutant blocked in the light induction process, whereas the *albino-1*, *albino-2*, and *albino-3* strains are each defective for a different enzyme in the carotenoid biosynthetic pathway.

Light is an important environmental factor in the regulation of carotenoid biosynthesis in a wide variety of organisms (1, 6, 19, 20, 24). In several organisms such as *Neurospora crassa* (2, 25), *Fusarium aquaeductuum* (18), and *Mycobacterium* sp. (7, 21), formation of carotenoid pigments has been shown to be a blue light-mediated response.

Based on inhibitor studies with several bacteria and fungi (see 1, 6, 19, 20, 24), it has been proposed that light induces the *de novo* synthesis of one or more of the enzymes required for the production of carotenoids. In *Mycobacterium* sp., additional evidence in support of this hypothesis has been obtained (4, 8). The enzyme required for the conversion of GGPP[2] to prephytoene pyrophosphate was shown to be absent in dark-grown cells and to be induced by an *in vivo* light treatment. Also, the level of the prenyltransferase which catalyzes the formation of GGPP is increased severalfold by the light treatment. Addition of chloramphenicol to the cells immediately after irradiation blocks the light-induced increase in activity of these enzymes.

Several studies have been conducted to determine which enzymes are regulated by light in *Neurospora*. Phytoene, a colorless 40-carbon compound, has been shown to be a precursor of carotenoid pigments in a number of organisms (see review 17). Phytoene accumulates in dark-grown *Neurospora* cultures (5), and thus, it has been proposed that one or more of the enzymes after phytoene in the pathway are photoinduced (5). This proposal still seems reasonable, but has not been verified by using phytoene as a substrate in cell-free studies.

Direct evidence that the biosynthesis of phytoene in *Neurospora* is photoregulated has been obtained (10, 12, 23). First it was shown that phytoene levels in an *al-1* mutant are increased by an *in vivo* light treatment (10). Subsequently, cell-free studies showed that phytoene biosynthesis in *Neurospora* is photoregulated (12, 23). In one such investigation, the enzyme activity required for the conversion of GGPP to phytoene was shown to be membrane-bound, and the level of this activity is increased by an *in vivo* light treatment (12). In another investigation, it was demonstrated that the over-all enzyme activity which catalyzes the conversion of IPP to phytoene is increased 9-fold by an *in vivo* light treatment (23). This increase is inhibited by cycloheximide. A similar light-induced increase in activity was demonstrated with an *al-1* mutant. This activity is absent in both dark-grown and light-treated *al-2* and *al-3* cultures.

Phytoene biosynthesis from mevalonic acid (12) and from IPP (23) requires both a soluble and a particulate fraction. The present investigation makes use of this fact to ask the following questions: (a) does light regulate a particulate enzyme activity (as reported in a previous investigation [12]); (b) which fraction (soluble or particulate) is defective in the *al-2* strain; and (c) which fraction is defective in the *al-3* strain? In addition, a procedure for the direct assay of the particulate enzyme activity using [^{14}C]GGPP as a substrate is presented.

Phytoene biosynthesis in another type of albino strain, originally designated *wc* (15, 16), was also investigated. The *wc* mutant has albino mycelia but normal pigmentation in the conidia. The *al-1*, *al-2*, and *al-3* strains have a reduced level of carotenoid pigment

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[2] Abbreviations: GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate; *al*, *albino*; *wc*, *white collar*; FGSC, Fungal Genetics Stock Center; Em, wild type strain Em 5297a (FGSC 352); AMO-1618, 2′-isopropyl-4′-(trimethyl-ammonium chloride)-5′-methylphenyl piperidine-1-carboxylate; FPP, farnesyl pyrophosphate.

in both the mycelia and conidia. A proposal that the *wc* phenotype is characteristic of regulatory mutants is presented.

MATERIALS AND METHODS

Strains. The *al-1* (RES-6) mating type A strain (FGSC 2152) was obtained from Dr. R. E. Subden. All other strains were obtained from the Fungal Genetics Stock Center, Humboldt State University Foundation, Arcata, CA. 95521. These included the Em 5297a wild type (FGSC 352), *al-2* (Y254M165; FGSC 904), *al-3* (RP100; FGSC 2082), and *wc-1* (P829; FGSC 128).

Substrates for Enzyme Assays. [1-^{14}C]IPP was purchased from Amersham Corp.[3] [^{14}C]GGPP is not available commercially, so it was prepared enzymically using a procedure similar to that of Moore and Coolbaugh (13). Pea plants (*Pisum sativum* L. cv. Alaska) were grown in a greenhouse, and developing cotyledons were excised from seeds of approximately half-maximum fresh weight and stored frozen at −80 C. The cotyledons were ground in a mortar and pestle with 0.1 M K-phosphate (pH 7.5) (1 ml/g fresh weight). The homogenate was centrifuged at 10,000*g* for 15 min, and the resulting supernatant fraction was centrifuged at 100,000*g* for 2 h. The supernatant fraction was stored at −80 C. For the preparation of [^{14}C]GGPP, the following concentrations of the incubation components were used: 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM AMO-1618, 5 mM iodoacetamide, 70 mM K-phosphate (pH 7.5), and 20 μM [1-^{14}C]IPP (45×10^6 dpm). The inclusion of iodoacetamide at this concentration causes about a 50% decrease in the total production of acid-labile compounds. However, for reasons unknown to us, addition of this compound to the incubation mixture causes [^{14}C]GGPP to be the predominant product. The reaction was started by the addition of 106 mg protein (pea cotyledon 100,000*g* supernatant), and the total volume of the incubation mixture was 16 ml. The mixture was incubated for 3 h at 25 C under N_2, and 16 ml saturated NaCl solution were added. The resulting mixture was extracted with hexane and then butanol. The butanol extract was flash-evaporated to dryness (at 25 C or lower) and the residue dissolved in 10 mM $NaHCO_3$. The resulting solution was applied to a DEAE-Sephadex A-25[3] column (0.9 × 12 cm) which had been equilibrated with 0.1 M NH_4HCO_3. The column was then developed as described by Oster and West (14). The NH_4HCO_3 solutions used to equilibrate and elute the column were adjusted to pH 9.0. Fractions comprising each peak of radioactivity were pooled and lyophilized to remove NH_4HCO_3, dissolved in 0.1 M K-phosphate (pH 7.5), and stored at −20 C. To demonstrate the purity of the [^{14}C]GGPP thus obtained, an aliquot was hydrolyzed with Sigma Type III-R bacterial alkaline phosphatase. Following hydrolysis, the incubation mixture was extracted with hexane. Complete recovery of the radioactivity in the hexane extract was obtained. An aliquot of this fraction was chromatographed on GLC (183 × 0.34 cm i.d. column of 3% SE-30 on 80/100 mesh Gas-chrom Q; temperature program was 2 min 110 C, then 10 C·min^{-1} increase to 275 C). All of the radioactivity co-chromatographed with authentic geranylgeraniol. In addition, an aliquot of the [^{14}C]-GGPP fraction was chromatographed on silica gel thin layer plates using a procedure similar to that of Sagami *et al.* (22). Only one band of radioactivity was observed. The preparation is thus free of [^{14}C]GGP which has a higher R_F than [^{14}C]GGPP in this chromatography system.

Growth of Cultures and Preparation of Enzyme Extracts. In these experiments, two different treatments were used. In treatment L, cultures were grown for 6 days at 18 C, equilibrated for 2 h at 6 C, irradiated for 2 min with blue light, incubated 24 h at 6 C, frozen, lyophilized, ground to a powder, and stored at −80 C as previously described (23). Treatment D was exactly the same, except the blue light irradiation was omitted. The lyophilized powders were extracted with 0.1 M K-phosphate (pH 7.5, 10 ml/g powder) containing 1 mM DTT. The extract was centrifuged at 10,000*g* for 15 min. The 10,000*g* pellet was extracted and a second 10,000*g* supernatant fraction collected. The two supernatant fractions were combined and centrifuged at 100,000*g* for 1 h. Part of the resulting supernatant fraction was removed and stored at −80 C. This extract will be referred to as the soluble fraction. The 100,000*g* pellet consists of two distinct phases, namely a loose turbid layer on top of a transparent tightly packed pellet. The turbid layer was removed, diluted with buffer, and centrifuged at 100,000*g* for 1 h. The resulting pellet was suspended in buffer and stored at −80 C. This extract will be referred to as the particulate fraction.

Assay of Phytoene Formation. When [1-^{14}C]IPP was the substrate, the following concentrations of buffer and cofactors were used: 50 mM K-phosphate (pH 7.5), 0.5 mM DTT, and 15 mM $MgCl_2$. The concentration of [1-^{14}C]IPP was 10 μM (1×10^6 dpm). The reaction was started by the addition of *Neurospora* enzyme (soluble [0.3 mg], or particulate [0.02 mg], or a mixture of soluble [0.3 mg] and particulate [0.02 mg]). The total volume of the incubation mixture was 0.7 ml. Incubations were for 30 min at 25 C under N_2. The reactions were stopped with ethanol, the reaction mixtures extracted with hexane, and the level of [^{14}C]phytoene was measured as previously described (23). When [^{14}C]GGPP (16,800 dpm) was used as the substrate instead of [1-^{14}C]IPP, the concentration of DTT was 0.14 mM. The concentrations of each of the other components of the incubation mixture and the methods for measuring the production of [^{14}C]phytoene were unchanged. Protein concentrations of all enzyme extracts were determined by the method of Lowry *et al.* (11) using BSA as a standard.

Assay of FPP and GGPP Formation. Soluble enzyme fractions were assayed for the production of [^{14}C]FPP and [^{14}C]GGPP from [1-^{14}C]IPP. The incubation conditions were the same as those described above for measuring the biosynthesis of [^{14}C]phytoene from [1-^{14}C]IPP. The amount of soluble enzyme used was varied up to 0.3 mg/incubation mixture. At the end of the 30-min incubation, enzyme reactions were stopped by heating for 10 min at 60 C. Then 1.4 ml 1.5 M Tris-HCl (pH 8.3), plus 0.2 mg Sigma Type III-R bacterial alkaline phosphatase were added, and the resulting mixture was incubated for 6 h at 37 C. The reactions were stopped with ethanol and the reaction mixtures extracted with hexane. The level of [^{14}C]farnesol and [^{14}C]geranylgeraniol in the hexane extract was measured by using a gas-liquid chromatograph and a Packard model 894 Gas Proportional Counter. The GLC procedure was the same as already described.

RESULTS

Soluble extracts prepared from light-treated Em 5297a, *al-1*, and *al-3* cultures catalyze a low level of incorporation of [1-^{14}C]IPP into phytoene (570, 590, and 150 dpm, respectively). No significant incorporation by any of the other soluble extracts or by any of the particulate fractions occurs. When soluble and particulate fractions obtained from Em 5297a light-treated (L) and dark-grown (D) cultures are mixed, there is an increase in this incorporation: (a) soluble-D plus particulate-L, 2640 dpm; (b) soluble-D plus particulate-D, 220 dpm; (c) soluble-L plus particulate-L, 2570 dpm; (d) soluble-L plus particulate-D, 150 dpm. In all such mixing experiments, the soluble enzyme is present in excess relative to the particulate fraction. Thus, the level of incorporation of [1-^{14}C]IPP into phytoene is a measure of particulate enzyme activity. As shown by these results, the particulate fraction obtained from light-treated Em 5297a cultures has a higher activity than the corresponding fraction from dark-grown cultures.

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

The same type of experiment was carried out using mixtures of soluble and particulate fractions obtained from Em 5297a and *al-1* strains (Table I). For both Em 5297a and *al-1*, incorporation is higher when particulate fractions obtained from light-treated cultures are used.

In Table I, the level of incorporation of [1-^{14}C]IPP into phytoene by mixtures of enzyme fractions prepared from Em 5297a and *al-2* cultures is also presented. No incorporation occurs when *al-2* particulate fractions are added. However, when Em 5297a particulate fractions are employed, incorporation takes place, and the level of incorporation is higher when the particulate fraction obtained from light-treated Em 5297a cultures is used.

For *al-3* cultures, the defect in phytoene biosynthesis is in the soluble fraction (Table I). Little or no incorporation of [1-^{14}C]IPP into phytoene occurs when *al-3* soluble extracts are used in mixtures with Em 5297a particulate fractions. However, when *al-3* particulate fractions are used, incorporation occurs. In addition, the level of incorporation is higher when particulate fractions obtained from light-treated *al-3* cultures are added.

The level of incorporation of [1-^{14}C]IPP into phytoene by mixtures of enzyme extracts prepared from Em 5297a and *wc-1* cultures is presented (Table II). There is no light effect on the level of activity in the *wc-1* particulate fractions. When *wc-1* soluble extracts are used, the usual effect of the *in vivo* light treatment on Em 5297a particulate activity is observed.

Table I. *Incorporation of [1-^{14}C]IPP into Phytoene by Mixtures of Soluble and Particulate Fractions Obtained from Wild Type Em 5297a and Albinos*

Enzyme Fractions Mixed in Incubations				Incorporation of [1-^{14}C]IPP into Phytoene by Mixture of Soluble and Particulate Fractions[a]
Soluble		Particulate		
Strain	Treatment	Strain	Treatment	
				dpm
Em	D	*al-1*	L	3,650
Em	D	*al-1*	D	150
Em	L	*al-1*	L	4,170
Em	L	*al-1*	D	90
al-1	D	Em	L	2,490
al-1	D	Em	D	220
al-1	L	Em	L	3,620
al-1	L	Em	D	90
Em	D	*al-2*	L	10
Em	D	*al-2*	D	0
Em	L	*al-2*	L	0
Em	L	*al-2*	D	0
al-2	D	Em	L	2,490
al-2	D	Em	D	180
al-2	L	Em	L	3,160
al-2	L	Em	D	240
Em	D	*al-3*	L	4,240
Em	D	*al-3*	D	950
Em	L	*al-3*	L	4,340
Em	L	*al-3*	D	700
al-3	D	Em	L	120
al-3	D	Em	D	0
al-3	L	Em	L	130
al-3	L	Em	D	10

[a] The amount of incorporation by the individual components of each mixture has been subtracted.

Table II. *Incorporation of [1-^{14}C]IPP into Phytoene by Mixtures of Soluble and Particulate Fractions Obtained from Wild Type Em 5297a and wc-1*

Enzyme Fractions Mixed in Incubations				Incorporation of [1-^{14}C]IPP into Phytoene by Mixture of Soluble and Particulate Fractions[a]
Soluble		Particulate		
Strain	Treatment	Strain	Treatment	
				dpm
Em	D	*wc-1*	L	360
Em	D	*wc-1*	D	290
Em	L	*wc-1*	L	290
Em	L	*wc-1*	D	240
wc-1	D	Em	L	3,050
wc-1	D	Em	D	200
wc-1	L	Em	L	2,980
wc-1	L	Em	D	240

[a] The amount of incorporation by the individual components of each mixture has been subtracted.

All combinations of soluble plus particulate fractions prepared from light-treated and dark-grown *al-1*, *al-2*, *al-3*, and *wc-1* strains were tested for phytoene-synthesizing activity using [1-^{14}C]IPP as a substrate. As expected, little or no incorporation occurs when either *al-2* particulate or *al-3* soluble fractions are used in the mixtures (data not presented). When *wc-1* particulate fractions are mixed with soluble extracts obtained from the other strains, incorporation occurs in all cases, except with *al-3* soluble fractions (Table III). In no case is there evidence that the *in vivo* light treatment has significantly increased the enzyme activity in the *wc-1* particulate fraction. When *wc-1* soluble extracts are used, the *al-1* and *al-3* particulate fractions obtained from light-treated cultures catalyze a higher level of incorporation than those from dark-grown cultures (data not presented).

The enzyme which catalyzes the conversion of GGPP to phytoene (phytoene synthetase) was assayed directly in the particulate fractions using [^{14}C]GGPP as the substrate (Table IV). The particulate fractions from all of the strains except *al-2* catalyze the conversion of [^{14}C]GGPP to phytoene. A light effect on the level of this particulate activity is observed in the Em 5297a, *al-1*, and *al-3* strains but not with the *wc-1* mutant.

The soluble fractions were assayed for the biosynthesis of [^{14}C]FPP and [^{14}C]GGPP from [1-^{14}C]IPP. The soluble extract obtained from the wild type strain catalyzes the biosynthesis of large amounts of [^{14}C]FPP (treatment L, 214,000 dpm; treatment D, 195,000 dpm [0.14 mg protein/incubation mixture]). Similar results were obtained with each of the other strains. The activity for [^{14}C]GGPP biosynthesis is increased nearly 2-fold in the wild type strain by the *in vivo* light treatment (treatment L, 9950 dpm; treatment D, 5710 dpm [0.14 mg protein/incubation mixture]). A similar light effect on [^{14}C]GGPP biosynthesis is observed when *al-1* and *al-2* soluble extracts are assayed. The light treatment does not cause an increase in activity for [^{14}C]GGPP biosynthesis in *wc-1* soluble extracts (treatment L, 4480 dpm; treatment D, 5280 dpm [0.14 mg protein/incubation mixture]). No activity for [^{14}C]GGPP biosynthesis was detected when soluble extracts obtained from either light-treated or dark-grown *al-3* cultures were assayed at a concentration of 0.14 mg protein/incubation mixture. However, at a higher protein concentration (0.3 mg/incubation mixture), some [^{14}C]GGPP biosynthesis is catalyzed by the extract obtained from the light-treated *al-3* culture (1840 dpm).

Table III. *Incorporation of [1-^{14}C]IPP into Phytoene by Mixtures of Soluble and Particulate Fractions Obtained from al and wc-1 strains.*

Enzyme Fractions Mixed in Incubations				Incorporation of [1-^{14}C]IPP into Phytoene by Mixture of Soluble and Particulate Fractions[a]
Soluble		Particulate		
Strain	Treatment	Strain	Treatment	
				dpm
al-1	D	*wc-1*	L	340
al-1	D	*wc-1*	D	310
al-1	L	*wc-1*	L	290
al-1	L	*wc-1*	D	280
al-2	D	*wc-1*	L	450
al-2	D	*wc-1*	D	450
al-2	L	*wc-1*	L	400
al-2	L	*wc-1*	D	370
al-3	D	*wc-1*	L	0
al-3	D	*wc-1*	D	0
al-3	L	*wc-1*	L	0
al-3	L	*wc-1*	D	0
wc-1	D	*wc-1*	L	360
wc-1	D	*wc-1*	D	260
wc-1	L	*wc-1*	L	290
wc-1	L	*wc-1*	D	240

[a] The amount of incorporation by the individual components of each mixture has been subtracted.

Table IV. *Effect of an In Vivo Light Treatment on Incorporation of [^{14}C]GGPP into Phytoene by Neurospora Particulate Enzyme Fractions*

[^{14}C]GGPP (16,800 dpm) was used as the substrate. Incubation times were 30 min for *al-2* extracts and 15 min in all other cases.

Strain	Treatment	Amount of Particulate Fraction Protein Added per Incubation	Incorporation of [^{14}C]GGPP into Phytoene by Particulate Fractions
		mg	*dpm*
Em	L	0.014	1,310
Em	D	0.028	140
al-1	L	0.014	1,130
al-1	D	0.056	120
al-2	L	0.028	0
al-2	D	0.028	0
al-3	L	0.014	1,020
al-3	D	0.028	180
wc-1	L	0.056	420
wc-1	D	0.056	410

DISCUSSION

We have shown that the cell-free biosynthesis of phytoene from IPP requires both a soluble and a particulate fraction. The low level of this activity which is present in soluble extracts obtained from light-treated Em 5297a, *al-1*, and *al-3* cultures may be due to some solubilization of the particulate enzyme activity. These results also show that the *al-3* mutant is "leaky" and can produce low levels of phytoene.

Soluble and particulate fractions obtained from different strains were mixed, and the incorporation of [1-^{14}C]IPP into phytoene by these mixtures was assayed. These experiments show that the defect of the *al-3* mutant is in the soluble fraction, while that of the *al-2* mutant is in the particulate fraction. These experiments also show that the *in vivo* light treatment causes an increase in enzyme activity in the particulate fractions obtained from Em 5297a, *al-1*, and *al-3* strains but not in that from the *wc-1* mutant.

Mitzka-Schnabel and Rau (12) combined a GGPP-generating system (prepared from pumpkin seeds) with *Neurospora* particulate fractions to show that phytoene synthetase in wild type *Neurospora* is membrane-bound and regulated by light. We have confirmed these results by using purified [^{14}C]GGPP as the substrate. In addition, we have extended the investigation of phytoene synthetase to include studies of *al-1*, *al-2*, *al-3*, and *wc-1* mutants. As shown in Table IV, [^{14}C]GGPP is converted to phytoene by particulate fractions obtained from Em 5297a, *al-1*, *al-3*, and *wc-1* strains, and the *in vivo* light treatment causes an increase in activity in all of these strains except *wc-1*. No activity is present in the particulate fractions obtained from either light-treated or dark-grown *al-2* cultures. These results agree with those obtained in the experiments where soluble and particulate fractions were mixed and [1-^{14}C]IPP was used as a substrate (Tables I–III). Thus, we have demonstrated that *Neurospora* phytoene synthetase can be assayed by either procedure. Because [^{14}C]GGPP is not available commercially, the assay using [1-^{14}C]IPP as a substrate is more convenient and can be used in many investigations of the enzyme.

The soluble fractions obtained from all of the strains used in this investigation were shown to catalyze the biosynthesis of [^{14}C]FPP and [^{14}C]GGPP from [1-^{14}C]IPP. However, the *al-3* mutant has a reduced activity for the production of [^{14}C]GGPP, while the activity for the biosynthesis of [^{14}C]FPP is unaffected. In addition, the *in vivo* light treatment increases the activity for [^{14}C]GGPP production in all of the strains except *wc-1* but has no significant effect on [^{14}C]FPP biosynthesis in any of the strains. Based on these results, it is tentatively proposed that the biosynthesis of FPP and GGPP are catalyzed by different prenyltransferases, and that only one of these prenyltransferases (GGPP synthetase) is regulated by light. However, further studies will be necessary to determine whether this proposal is correct. Additional investigations will also be necessary to determine whether there is any light effect on the level of IPP isomerase, the first enzyme in the pathway from IPP to phytoene. However, inasmuch as there is no effect of the light treatment on the amount of [^{14}C]FPP produced from [1-^{14}C]IPP, regulation of the isomerase activity by light is unlikely.

Based on the results of this investigation, the model shown in Figure 1 is proposed. The *al-1*, *al-2*, and *al-3* loci are postulated to be structural genes each coding for a different enzyme required for carotenoid biosynthesis. The *al-1* mutant accumulates phytoene and has been proposed to be defective in phytoene dehydrogenase (3). In the present investigation, the *al-2* mutant has been shown to be defective in phytoene synthetase, a particulate enzyme. The *al-3* mutant is almost totally defective in a soluble enzyme activity required for GGPP biosynthesis. It had been previously concluded from *in vivo* labeling experiments that the *al-3* mutant is blocked in the conversion of GGPP to phytoene (9). Our results unequivocally rule out this possibility.

In the present investigation, phytoene synthetase activity has been shown to be increased by an *in vivo* light treatment in wild type, *al-1*, and *al-3* strains, but not in the *wc-1* mutant. In all strains except *wc-1*, the light treatment also increases the level of enzyme activity required for GGPP biosynthesis. It is predicted that the light treatment also increases the level of phytoene dehydrogenase in wild type, *al-2*, and *al-3* strains, but not in the *wc-1* mutant. However, this aspect of the proposal cannot be tested at this time because an assay for phytoene dehydrogenase in

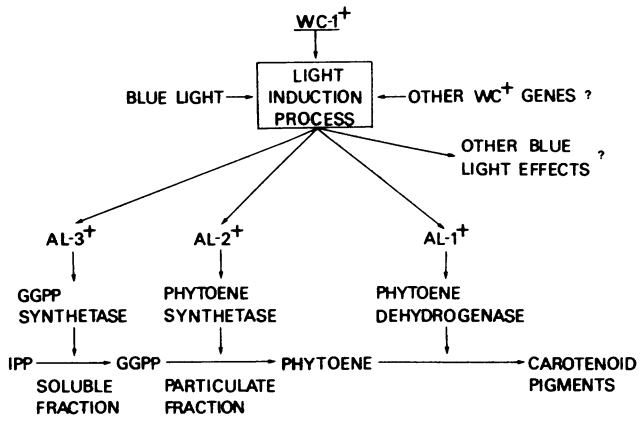

FIG. 1. A proposed model for photoinduced carotenoid biosynthesis in *N. crassa*.

Neurospora has not been developed.

The *wc-1* strain is proposed to be a regulatory mutant, *i.e.* an albino which is not defective in any structural genes but is blocked in the initial photoinduction process. This proposal is also consistent with the unusual phenotype of the *wc-1* mutant, *i.e.* albino mycelia and normal pigmentation in the conidia. An important point to keep in mind is that in wild type *Neurospora*, carotenoid pigment is produced by conidia in the dark, but light is required for carotenoid biosynthesis in the mycelia. Thus, the conidia can somehow bypass the light requirement for pigment production. For this reason, mutants which have normal pigmentation in the conidia and albino mycelia (*wc* mutants) are proposed to be blocked in the photoinduction process. The other albinos are postulated to have mutations in structural genes, and thus, carotenoid biosynthesis is reduced in both conidia and mycelia.

The mechanism of light induction is unknown. However, it would seem unlikely that this process is under the control of only one gene. Hence, it has been predicted that eventually other *wc* genes will be found, and that some *wc* mutants will be defective in the blue light photoreceptor (6). The possibility that *wc* mutants may be blocked in other blue light-mediated responses in *Neurospora* has also been previously discussed (6) and is currently under investigation.

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