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PHOTOREGULATION OF CAROTENOID BIOSYNTHESIS IN PLANTS

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Abstract—Photoregulation of carotenoid synthesis is widespread in the plant kingdom. This paper concentrates on the results and problems concerning the mechanism of photoinduction. Photoinduction consists of initiating light-reactions and a subsequent *de novo* synthesis of carotenogenic enzymes. The acting photoreceptors so far known are phytochrome in higher plants and in bacteria and fungi very probably a flavin (or flavoprotein) or a porphyrin-like compound. The first steps of the biosynthetic pathway of carotenoids under photocontrol seem to be the synthesis of geranyl-geranyl-pyrophosphate and of phytoene. Results concerning the possible involvement of *m*-RNA in the mechanism of photoinduction are discussed.

INTRODUCTION

Carotenoids are the most widespread pigments in the plant kingdom and play an important role in plant life. Not as widespread as the ability to synthesize carotenoids is the regulation of this synthesis by light, but many organisms are known to show such a phenomenon. Among higher plants only in Angiosperms does carotenogenesis seem to be photoregulated especially during the development of the young plant. In this case the regulation of carotenoid synthesis is only a part of the photocontrolled development of the seedling. In algae the carotenoid pattern of many genera and species has been studied in detail, but only in a few cases was photoregulation observed.[1] In a number of fungi—such as *Phycomyces* or *Mucor*—light increases the rate of carotenoid synthesis quantitatively but only in some species is illumination obligatory for distinct colouring. In these species biosynthesis of pigments is strictly "induced"; the species of fungi so far known to show photoinduction of carotenogenesis are listed in Table 1. Among bacteria such strict photocontrol has only been detected in species of 3 genera, which have been studied thoroughly in this respect: *Mycobacterium*,[3] *Myxococcus* and *Flavobacterium*.[4] In recent years valuable reviews on the results on photoregulation of carotenoid synthesis in different groups of plants have been published: for green plants by Goodwin,[1,5] for non-photosynthetic organisms in detail by Batra[3] and by Weeks *et al.*[4] Therefore the purpose of this paper is not so much to survey the literature, but rather to concentrate on the *mechanism* of the photoregulation. I shall try to summarize results *and* problems.

PHOTOREGULATION—GENERAL ASPECTS

With regard to the characteristics of the over-all-reaction of photoregulation, and to the time course of light-induced carotenogenesis, 3 types of photocontrol may be summarized.

1. *Bacteria and fungi*

Microorganisms which obligatorily need light for a massive production of carotenoids synthesize only traces of pigments when grown in the dark. Only a brief exposure to light already induces substantial carotenogenesis although higher doses of irradiation are necessary for optimum production or for saturation of the photoreaction. In all species studied the time course of subsequent carotenogenesis is very similar to that observed in *Fusarium aquaeductuum* (Fig. 1): Following a lag-period after photoinduction the amount of pigments in the cells increases rapidly for a certain time, and thereafter net pigment synthesis ceases. Both length of the lag-period and of the time interval of carotenogenesis are specific for different organisms. For instance in *Flavobacterium dehydrogenans* the competence for carotenogenesis is established only for one generation during bacterial growth.[4] Addition of an inhibitor of protein synthesis prior to illumination completely blocks the synthesis of carotenoids; when the inhibitor was added at different times after illumination the inhibitory effect was reduced with time.[7–9] From these results it has been concluded that as a consequence of photoinduction the carotenogenic enzymes are synthesized *de novo*. Thus photoregulation in these organisms shows all features of a "classical" induction mechanism.

2. *Angiosperms*

The investigation of photoinduced carotenogenesis in seedlings of angiosperms is complicated by the fact, that under the light regimes used development of proplastids or etioplasts to chloroplasts—including development of thylakoids and synthesis of chlorophyll—also takes place and carotenoid synthesis may not be independent of these transformations.

Dark-grown etiolated seedlings contain some carotenoids, mainly xanthophylls.[10] After a brief exposure to red light the amount of carotenoids is increased severalfold during a subsequent dark period.[11,12] Although sufficient data are not available to permit a decision about what type of photocontrol is involved, we may assume that it is an induction mechanism as in bacteria and fungi. A different mechanism appears to be involved when etiolated seedlings of white mustard are exposed to continuous far-red light (Fig. 2).[13] Only after prolonged illumination and after a lag-period of 3 hr the rate of carotenoid synthesis is increased. As soon as the light is turned off pigment production is reduced to the dark rate which is different from the type of photoinduction mentioned earlier, although a slight after-effect may persist. Furthermore, a second illumination causes an immediate increase of the rate of carotenogenesis without lag-period, which also differs from fungal photoregulation. Similar observations have also been found in other photoregulated reactions in the mustard seedling mediated by prolonged illumination (for Ref. see[14]). A possible mechanism for this kind of photoregulation will be discussed in a following section.

Table 1. Species of fungi in which carotenogenesis is strictly photoregulated (for Ref. see[2])

Organism	Authors
Aspergillus giganteus mut. alba	Zurzycka (1963)
Cephalosporium diospyri	Codner and Platt (1959)
Dacryopinax spathularia	Goldstrohm and Lilly (1965)
Fusarium aquaeductuum	Rau and co-workers (1959–1974)
Fusarium coeruleum	Rau *et al.* (unpublished data)
Fusarium oxysporum	Carlile (1956); Rau *et al.* (unpubl. data)
Neurospora crassa	Went (1901); Haxo (1949); Zalokar (1954); Harding and Mitchell (1968); Rau *et al.* (1968)
Neurospora sitophilia	Ishii and Akagi (1948)
Pyronema confluens	Carlile and Friend (1956)
Sphaerobolus stellatus	Friederichsen and Engel (1957)
Syzygites megalocarpus	Wenger and Lilly (1966)
Verticillium agaricinum	Valadon and Mummery (1971)

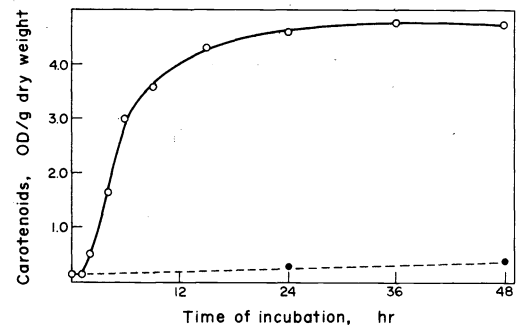

Fig. 1. Time course of carotenogenesis in *Fusarium aquaeductuum* in the dark ●---● and after a brief exposure to light ○—○.

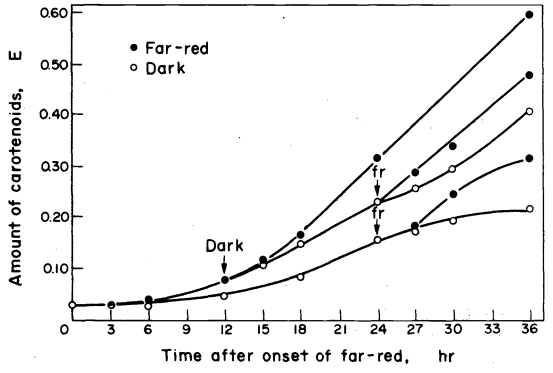

Fig. 2. Time course of carotenoid accumulation in the mustard seedling (*Sinapis alba* L.) in the dark and under the control of continuous far-red light. Initial onset of far-red light: 24 h after sowing (from Schnarrenberger and Mohr[13]).

3. *Algae*

Photoregulation of carotenoid synthesis in algae seems to be very rare; the only well documented case in wild type strains is that of *Euglena*.[15] In many species the synthesis of the so called "secondary" carotenoids—which are produced under conditions of nitrogen deficiency—is also independent of illumination;[16] only in *Acetabularia* has an influence on light been reported.[17] Although in the wild type of *Chlorella* only variations in the carotenoid pattern are induced by illumination, 2 mutants with photocontrolled carotenoid production have been described in the literature. Mutant 5/520—studied in detail by Claes[18,19]—synthesizes a series of acyclic polyenes in the dark; in the light cyclic carotenes are formed. In a chlorophyll-free yellow mutant synthesis of carotenoids is enhanced by blue light.[20] Both types of photocontrolled carotenogenesis show a common feature: Pigment production is maintained only during illumination indicating that the mechanism of photoregulation probably involves a light-mediated conversion of pigment precursors.

4. *Photoreactions and dark reactions*

Regardless of the type of mechanism of photocontrol, at least in bacteria, fungi, and in certain higher plants two types of reactions seem to be involved in the sequence of events during photoinduction: Light reactions and dark reactions. In all organisms so far investigated photoinduction seems to establish the ability of the particular organism to synthesise the whole set of carotenoids. This ability is under genetic control and therefore characteristic of the organism. However variations of the carotenoid synthesis following photoinduction are widespread and may be due to changes in nutrition, aeration of the culture, time after photoinduction etc. For instance, *Flavobacterium dehydrogenans*, when incubated after photoinduction in an optimal growth medium, synthesizes essentially one carotenoid, decaprenoxanthin; but under conditions of nutritional imbalance, which somehow reduce biosynthetic processes, precursor carotenoids accumulate.[4] In Table 2 the amounts of the different carotenoids reported to be present in *Neurospora crassa* by different investigators are listed along with data from this laboratory. The results clearly demonstrate that in the very same organism which is most often used for experiments on photoinduction pigment levels may vary quite considerably. Most striking are differences in the ratio of neutral pigments to neurosporaxanthin and in the portion of 3,4-dehydrolycopene, lycopene, torulene, γ- and β-carotene; each of these pigments more or less is an end product of the biosynthetic pathway.[26] The differences may be due to different growth conditions, to the use of different strains, and also to variations on the light regime used by the investigators. Results obtained with a *Neurospora crassa* "slime"-mutant, which lacks a cell wall and is therefore growing like sphaeroplasts are also entered in Table 2. This mutant shows the same characteristics of photoinduction as the wild type but exhibits some differences on the pattern of pigments synthesized, for instance no β-carotene is present and the percentages of neurosporaxanthin, lycopene and 3,4-dehydrolycopene seem to be increased.[25] It should be emphasized that the strain from which the "slime" mutant was derived[27] differs from the wild type strains listed in Table 2.

Regardless of the reasons for variations in the pattern of pigments formed it should be emphasized that such differences are *not* connected to the mechanism of photoinduction. During the reactions of photoinduction the competence for carotenoid synthesis is set up; in contrast, the variation of carotenoid levels are a result of the *realisation* of this competence reflecting modifications of the various steps of the biosynthetic pathway. Since it is my intention to concentrate on the mechanism of photoregulation, 3 topics appear to be of particular importance:

Table 2. Carotenoid content of *Neurospora crassa* as found by different investigators. For Ref. see[21–25]

	Zalokar	Jensen	Harding	Davies	Mitzka and Rau wild-type	Mitzka and Rau "slime"
			μg/g dry weight			
Total	755	‡	‡	‡	449	92
Phytoene	219	‡	‡	465	242	31
Neutral carotenoids	200	129	20	167	107	19
Neurosporaxanthene	336	15	244	‡	100	42
			% of neutral car.			
3,4-Dehydrolycopene	(9)†	2	24	1	6	19
Lycopene	13	§	15	6	6	14
Torulene	§	12	5	1	1	3
Neurosporene	16	22	10	9	14	18
γ-Carotene	34	5	18	32	26	10
ζ-Carotene	17	32	26	22	27	27
β-Carotene	4	16	§	13	4	§
β-Zeacarotene	‡	‡	§	6	2	1
Phytofluene	7	11	‡	11	15	8

†Tentatively identified as "spirilloxanthene" by Zalokar but this was excluded later by Liaaen-Jensen.
‡Data not given.
§Not detected.

1. The nature of the photoreceptor and the "primary reactions" induced by illumination.
2. Reactions of the biosynthetic pathway of carotenoids under photocontrol.
3. Mechanism of photoinduction of carotenogenic enzymes.

PHOTORECEPTORS AND THE PRIMARY REACTIONS

1. *Phytochrome*

In seedlings of angiosperms carotenogenesis induced by a brief illumination with red light can be reversed by a short light-period of far-red.[11] Although no action spectra are available for carotenoid synthesis the characteristics of this photoinduction closely resemble those of other photoregulations of this type.[28] Therefore, by analogy, there is little doubt that phytochrome is the acting photoreceptor and the mechanism is that of the "classical" phytochrome reaction.

The increased carotenoid accumulation in dark-grown seedlings under the influence of continuous far-red light[13] is also mediated by phytochrome; the radiation maintains a low but constant level of active phytochrome P_{fr} in the cotyledons over an extended period of time. Other developmental responses by seedlings to continuous illumination showed action of "blue light" in addition to the far-red action. It is therefore under discussion whether or not in such "high irradiance reactions" phytochrome is the only acting photoreceptor.[2]

2. *Porphyrin-like action spectra*

Mycobacterium marinum[8] and *Myxococcus xanthus*[29] have similar action spectra of carotenogenesis (Fig. 3). From the shape of these spectra, and by comparison with absorption spectra of porphyrin containing fractions of bacterial cell homogenates, it seems very likely that the photoreceptor is a porphyrin. In *Mycobacterium marinum* mesoporphyrin or coproporphyrin,[30] in *Myxococcus xanthus* protoporphyrin IX are favorite candidates.

3. *Flavin-like action spectra*

Many authors (for Ref. see[31]) had previously reported on the spectral dependence of carotenoid synthesis in fungi but Zalokar[31] was the first to determine an action spectrum of carotenogenesis in the spectral region between 400 and 500 nm for non-conidiating cultures of *Neurospora crassa*; prevention of conidiation is important because in conidia carotenoid production is not light-dependent. More detailed action spectra of carotenogenesis in *Fusarium aquaeductuum*[6] and *Mycobacterium sp.*[30] (Fig. 4) show a maximum at 370–380 nm and 3 peaks or at least shoulders between 400 and 500 nm; light with a wave length longer than 520 nm is ineffective. The shape of the action spectrum of carotenogenesis resembles action spectra of different developmental and movement responses in various plants, among which the phototropic reaction is the most prominent (Fig. 5).[2] Arguments for and against either of the two candidates commonly suggested to act as photoreceptor in phototropism, namely flavins and carotenoids, have been discussed in detail by Song *et al.*[32] The authors arrived at the conclusion that a flavin or very probably a flavoprotein is the photoreceptor and we also favour their interpretations for the photoinduction of carotenoid synthesis.

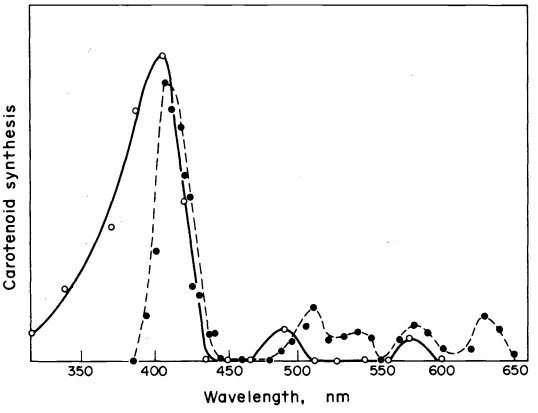

Fig. 3. Action spectra of photoinduced carotenoid synthesis in *Mycobacterium marinum* ○——○ (From Batra and Rilling[8]) and in *Myxococcus xanthus* ●---● (From Burchard and Hendricks[29]).

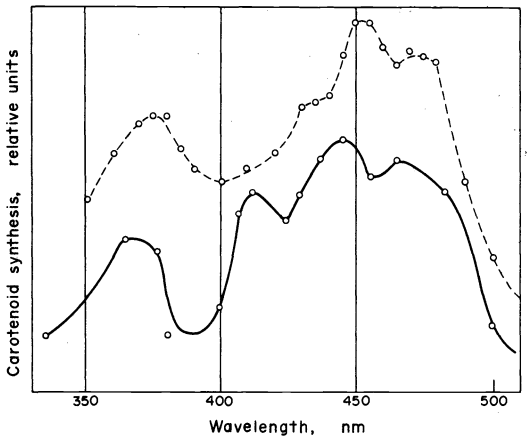

Fig. 4. Action spectra of photoinduced carotenoid synthesis in *Fusarium aquaeductuum* O---O (From Rau[6]) and in *Mycobacterium* sp. O——O (From Howes and Batra[30]).

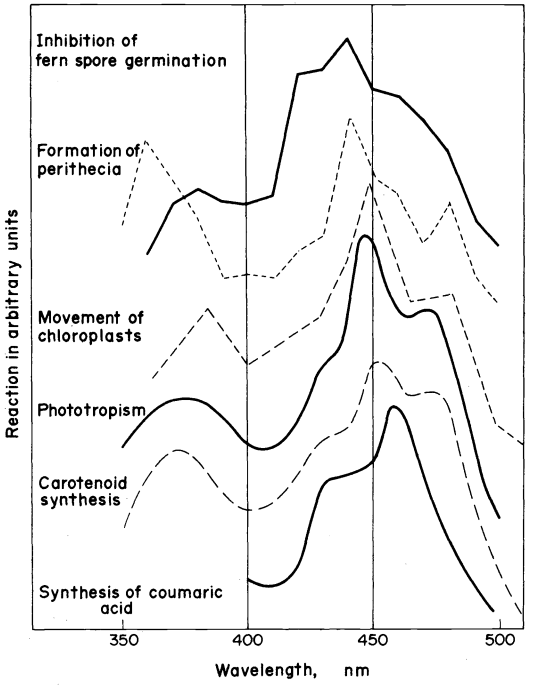

Fig. 5. Comparison of the action spectrum of carotenoid synthesis in *Fusarium aquaeductuum* with action spectra of different developmental and movement responses in various plants induced by "blue-light" (For ref. see[2]).

4. *Primary reactions*

At present, primary reactions caused by illumination of the photoreceptor are only partly understood. Photoconversion of phytochrome has been studied in detail and the characteristics of this reaction as well as hypotheses on subsequent steps in the sequence of photoinduced reactions have been reviewed *in extenso*.[28]

In cases where a porphyrin or flavin acts as the photoreceptor we have much less information on this problem. The photoreaction has been found to be independent of the temperature,[31,33,7,8] indicating that a photochemical reaction is involved. For a brief exposure to illumination the Bunsen–Roscoe-law of reciprocity proved to be valid.[34,35,8,6,36] The following data are taken for evidence that the primary reaction is a photooxidation step:

(a) The presence of oxygen is essential for *optimum* photoinduction but induction in *Fusarium aquaeductuum* and *Neurospora crassa* takes place to some extent also without oxygen. Under anaerobic conditions light saturation of photoinduction is reached at a relatively low dosage independent of light intensity and time of illumination. Mycelia subsequently supplied with O_2 are susceptible to an additional photoinduction. We therefore concluded that O_2 functions as an electron acceptor keeping the photoreceptor in a proper state of oxidation.[37] In contrast Rilling[35] and Batra[38] concluded from their results on *Mycobacteria* that O_2 participates directly in the primary photooxidation process.

(b) Strong reducing substances such as dithionite applied to mycelia after illumination (Fig. 6) inhibit photoinduced carotenoid synthesis completely and specifically. On the other hand treatment with hydrogenperoxide in the dark may substitute for light in the induction of carotenogenesis.[30]

(c) Red light is not absorbed by the endogenous photoreceptor. After addition of dyes absorbing red light photoinduction takes place also in red light. It is emphasized that only *redox* dyes—such as methylene blue or toluidine blue—proved to trigger carotenoid synthesis after illumination, indicating that these dyes may act as artificial photoreceptors.[40]

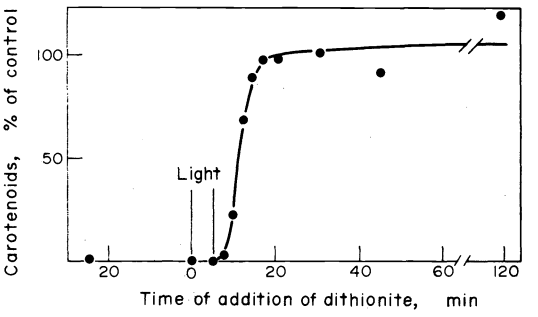

Fig. 6. Effect of dithionite (5×10^{-3} M) applied to mycelia of *Fusarium aquaeductuum* at various times before or after photoinduction on carotenogenesis. The inhibitor was removed 30 min after addition by rinsing the mycelia with buffer (From Theimer and Rau[39]).

BIOSYNTHETIC STEPS UNDER PHOTOCONTROL

Zalokar[21] and Rilling[35] have found in *Neurospora crassa* and in *Mycobacterium* respectively that after photoinduction the rate of synthesis of each of the carotenoids was increased; a detailed study in *Fusarium aquaeductuum* (Fig. 7) led to similar results.[41] But in addition it was demonstrated that the different pigments were synthesized in a sequence which corresponded closely to the proposed biosynthetic pathway. These data clearly show that the production of the whole set of carotenoids of an organism is under photocontrol. But then another question arises: which is the first step of the biosynthetic pathway photoinduced. Investigations of this problem are hampered by the fact, that in all organisms so far examined dark-grown cultures contain small amounts of carotenoids, especially phytoene; moreover fungi synthesize carotenoids in the dark at a little rate and Zalokar's[21] data indicated that for a short period after illumination the amount of phytoene is decreased rather than increased.

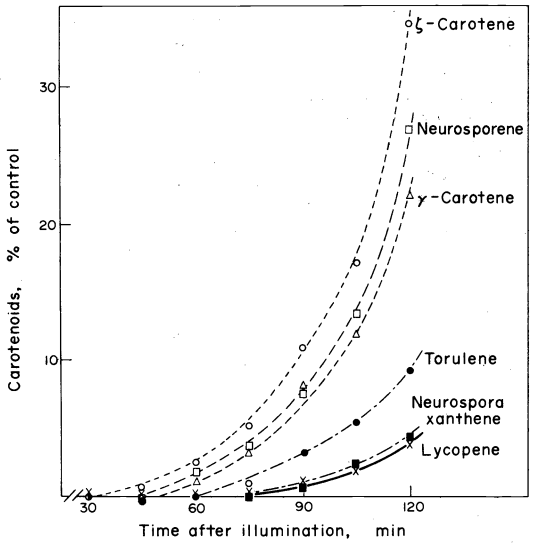

Fig. 7. Time course of synthesis of individual carotenoids in *Fusarium aquaeductuum* after a brief (10 min) exposure to light. Carotenoid content of control samples was determined when carotenogenesis has finished (36 hr) (From Bindl, Lang and Rau[41]).

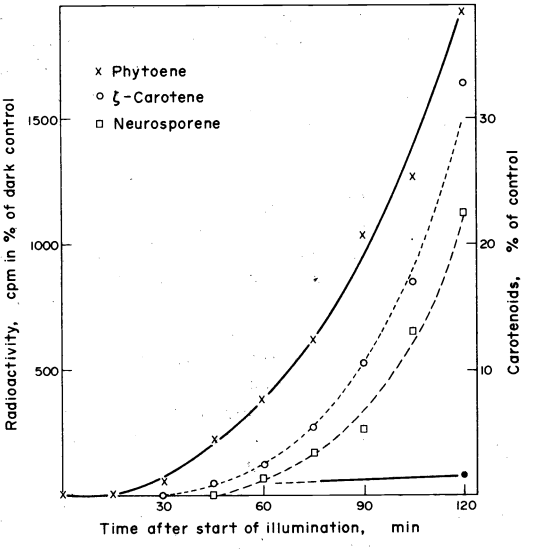

Fig. 8. Incorporation of 2-^{14}C-mevalonic acid into phytoene by *Fusarium aquaeductuum* in the dark ——● and after a 10 min exposure to light ×——×. For comparison the kinetics of accumulation of ζ-carotene and neurosporene from Fig. 7 are inserted.

Therefore it has been suggested that in fungi phytoene is accumulated in the dark; thus the enzyme system responsible for the production of phytoene would have to be constitutive in dark grown fungal cells, whereas in bacteria it is not. Recently Rilling[42] was able to prove in a cell-free system from *Mycobacterium* that the prephytoene synthetase is absent in dark-grown cells and is de novo synthesized as a consequence of photoinduction. But enzymatically active cell-free systems of strictly photoregulated fungi have not yet been reported and—as mentioned before—phytoene is present in dark-grown cultures. Using *Fusarium* we tried to show photoinduced phytoene synthesis by measuring the incorporation of labelled mevalonic acid (MVA) into phytoene in the dark and after illumination. The time course of incorporation (Fig. 8) clearly demonstrates that there is only a very small rate of synthesis in the dark which is increased dramatically after photoinduction. Comparison with the kinetics of accumulation of ξ-carotene and neurosporene once more indicates that pigment synthesis precisely follows the suggested sequence (Fig. 8). Chromatographic analyses showed that 99.5% of newly synthesized phytoene is the *cis*-isomer.[43] From these data we conclude that in fungi as well as in bacteria phytoene synthesis is photoinduced. One might argue that in our experiments increased labelling of phytoene after illumination was only a consequence of the conversion of phytoene accumulated in the dark to coloured carotenoids and a subsequent re-filling the phytoene pool with labelled molecules. But repeating our experiments in the presence of diphenylamine at concentrations at which the synthesis of coloured carotenoids is almost completely blocked yielded the results shown in Fig. 9. ^{14}C-MVA was incorporated only into phytoene in amounts similar to those found in the absence of diphenylamine and nearly no labelling of other carotenoids occurred.[43]

The latter experiment also gave an additional information concerning the problem, whether or not lycopersene is an intermediate in the biosynthesis of carotenoids in fungi. On thin layer chromatographs no label could be

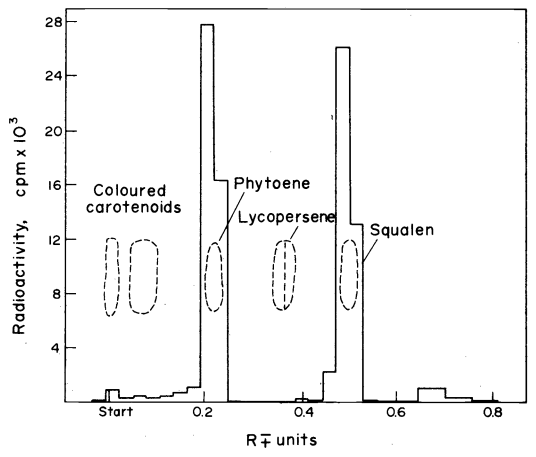

Fig. 9. Radiochromatogram scan of a thin layer chromatogram of the products synthesized in *Fusarium aquaeductuum* from 2-^{14}C-mevalonate after photoinduction in the presence of diphenylamine. Dotted lines indicate spots of authentic compounds as reference standards.

detected in the spots with a R_f-value of authentic lycopersene.

The enzymatic reaction leading to the immediate precursor of phytoene has also been examined by Rilling.[42] In a cell-free system of *Mycobacterium sp.* he demonstrated the presence of geranyl-geranyl-pyrophosphate synthetase (prenyltransferase) in dark-grown cells, and a severalfold increase of the enzyme activity by photoinduction. But no significant effects of light on earlier steps in the carotenogenic pathway have been found. To summarize, in bacterial geranyl-geranyl-pyrophosphate synthetase appears to be the first enzyme of carotenoid synthesis under photocontrol, which, however, is to some extent present in dark-grown cells and hence partly constitutive. In fungi this problem remains to be solved.

Also the question of synthesis of trace amounts of

carotenoids in the dark-grown cells is still unanswered. A possible—yet very hypothetical—explanation would be that the enzymes in the dark-grown cells, and those synthesized after photoinduction, belong to differently controlled isoenzyme systems.[39]

MECHANISM OF PHOTOINDUCTION OF CAROTENOGENIC ENZYMES

From results with various bacteria and fungi[3,4] and also in seedlings[13] on the inhibition of photoinduced carotenoid synthesis by inhibitors of protein synthesis, it has been concluded that as a consequence of illumination the carotenogenic enzymes are synthesized de novo. Additional data confirm this interpretation. Pigment synthesis following photoinduction starts only after a lag-period of certain length which appears to be specific for different organisms; the only exception is *Flavobacterium dehydrogenans* for which no apparent lag-period was found.[4] It seems very likely that the lag-period reflects protein biosynthesis because inhibition decreases quantitatively when the time of addition of inhibitors of protein synthesis after illumination is delayed. In a very recent paper Valadon[44] has shown that in *Verticillium agaricinum* light induces an activation of protein synthesis accompanied by an increase of the number of poly-ribosomes. Subden and Turian detected a new protein band after illumination in *Neurospora crassa*.[45,46] The most direct evidence for an enzyme synthesis comes from Rilling's results with the cell-free system mentioned before.[42] Therefore photoinduced *de novo* production of carotenogenic enzymes seems well documented. But two further problems need to be dealt with: (1) The level of regulation of *de novo* synthesis within the cell and (2) the mechanism of the induction of the enzymes.

1. *The level of regulation*

Regardless of the possibility that photoinduced carotenogenesis may be regulated by changes of enzyme *activity* two major regulation mechanisms have to be taken into consideration: regulation of transcription or regulation of translation. Carotenoid accumulation in mustard seedlings is relatively insensitive to actinomycin D—a well known inhibitor of transcription—but is sensitive to cycloheximide and puromycin.[13] From these data and from the fact that there is no lag-period after a second illumination (Fig. 2) photoregulation of transcription was doubted. As for other light responses in seedlings a model of "photomodulation" rather than an induction mechanism was suggested.[47] For photoinduction in bacteria and fungi, several authors assumed regulation on the transcription level, as is well established for substrate induction of enzymes in bacterial and animal systems, in terms of a gene derepression. However, only few results are available to support this conclusion. In *Mycobacterium sp.* proflavin—an inhibitor of transcription in some organisms—was found to inhibit photoinduction of carotenogenesis, but the specificity of this effect was not tested further.[48] In *Flavobacterium dehydrogenans* actinomycin D added prior to illumination prevented carotenogenesis although it inhibited incorporation of labelled uracil to only about 60%. Furthermore incorporation of ^{14}C-uracil was increased by light whereas the incorporation of thymidine was not affected.[4] Actinomycin D also inhibits photoinduced carotenoid synthesis but only to some extent in *Verticillium agaricinum*[49] and in *Neurospora crassa*.[50] Effects of photoinduction on the rate of RNA synthesis have been also reported by Valadon.[44,51] In *Fusarium aquaeductuum* we have recently shown that distamycin A, another inhibitor of transcription, completely blocks photoinduced pigment production. The specificity of this inhibition was tested by incorporation experiments with labelled uridine.[52]

Regulation on the transcription level would imply a *de novo* synthesis of specific messenger-RNA. On photoinduced synthesis of anthocyanins or chlorophyll respectively Dittes and Mohr[53] and Harel and Bogorad[54] have so far failed to obtain any evidence for a photoregulated synthesis of a specific *m*-RNA. In *Fusarium aquaeductuum* we have obtained evidence for a light-mediated synthesis of *m*-RNA by means of incorporation of labelled uridine, isolation of polysomes and separation of RNA fractions by affinity chromatography.[55,56]

2. *Possible mechanism of the induction of the carotenogenic enzymes*

Addition of cycloheximide at various times after illumination results (Fig. 10) in a differential inhibition of

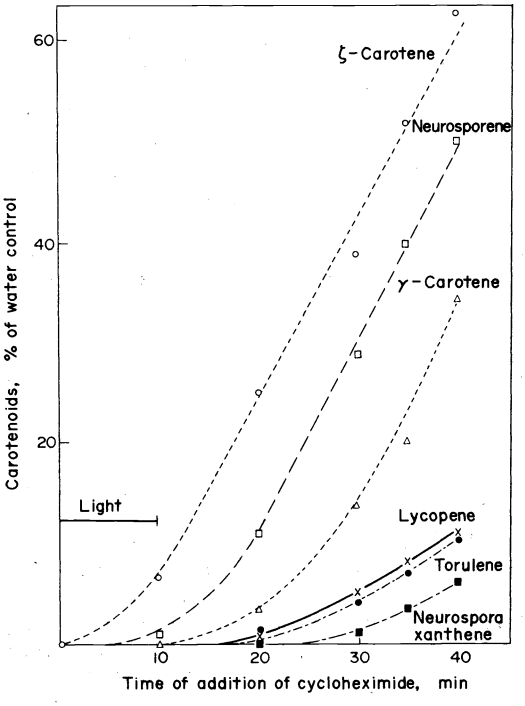

Fig. 10. The amounts of individual carotenoids synthesized in *Fusarium aquaeductuum* during a 36 hr incubation as a function of time of addition of cycloheximide after illumination (From Bindl, Lang and Rau[41]).

the synthesis of the various carotenoids of *Fusarium*, indicating that after photoinduction carotenogenic enzymes are synthesized sequentially.[41] Mainly two alternatives may be discussed for the induction mechanism of carotenogenic enzymes: (1) Only the first enzyme of the pathway—the "key-enzyme"—is photoregulated and the subsequent enzymes of the pathway are induced by their substrates, *or* (2) light triggers a *concurrent* induction of each of the different enzymes of the pathway. The data obtained from an experiment designed to provide evidence for either alternative are given in Fig. 11 After illumination the mycelia were kept under anaerobic conditions which results in an inhibition of carotenoid synthesis, but does *not* block the synthesis of

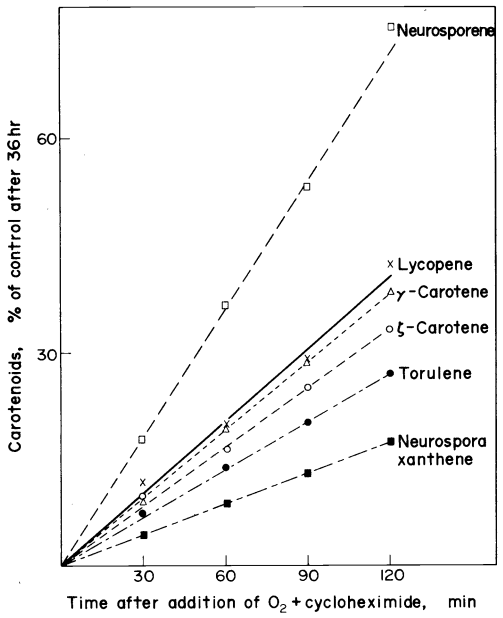

Fig. 11. Kinetics of synthesis of the individual carotenoids in *Fusarium aquaeductuum* after the following pretreatment: 10 min illumination under oxygen, 3 hr incubation under anaerobic conditions, subsequent simultaneous addition of oxygen and cycloheximide (From Lang and Rau[57]).

carotenogenic enzymes. Subsequent addition of oxygen in the presence of cycloheximide preventing any further synthesis of enzymes results in a strictly concurrent formation of the different carotenoids without any lag-period.[57] Obviously each of the carotenogenic enzymes had already been synthesized during anaerobic conditions without any concomitant synthesis of carotenoids. These data may be interpreted to mean that light induces carotenogenic enzymes as a coupled group. Similar findings on the photoregulation of phenolic compounds have previously led Zucker[58] to introduce the term "PAL-Operon" for a similar regulatory mechanism.

In summary, the data so far available are in good agreement with the hypothesis that the mechanism of photoinduction of carotenoid synthesis in fungi involves gene derepression and *de novo* synthesis of carotenogenic enzymes. Whether the genetic information is expressed en bloc by derepression of a "carotenoid operon" remains to be investigated by future experiments.

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