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Light-dependent Anthocyanin Synthesis: A Model System for the Study of Plant Photomorphogenesis

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I. Abstract

The biosynthesis of anthocyanins in plant tissues either requires light or is enhanced by it. Light-dependent anthocyanin synthesis has been extensively used as a model system for studies of the mechanism of photoregulation of plant development. Two components can be distinguished in the action of light on anthocyanin production. The first component is the red–far red reversible, phytochrome-mediated response induced by short irradiations; the amount of anthocyanin formed in response to a single, short irradiation is small. The second component is the response to prolonged exposures; the formation of large amounts of anthocyanin requires prolonged exposures to high fluence rates of visible and near-visible radiation (290 to 750 nm) and shows the typical properties of the "High Irradiance Reaction" (HIR) of plant photomorphogenesis. Phytochrome is involved in the photoregulation of the HIR response and is the only photoreceptor mediating the action of prolonged red and far red irradiations. The response to prolonged ultraviolet and blue radiation is probably mediated, at least in some systems, by two photoreceptors: phytochrome and cryptochrome, the latter being a specific ultraviolet-blue-light photoreceptor. The nature of the interaction between phytochrome and cryptochrome in the regulation of plant photomorphogenic responses is still unclear.

II. Riassunto

La biosintesi delle antociane nei tessuti vegetali è un processo metabolico regolato dalla luce. Alcune specie formano antociane solo quando sono esposte alla luce. Altre specie possono formare antociane al buio, ma velocità di sintesi e concentrazione finale del pigmento aumentano notevolmente quando l'organismo è esposto alla luce. Lo studio degli effetti della luce sulla biosintesi delle antociane è stato usato estensivamente come un sistema modello per lo studio della photoregolazione dello sviluppo dei vegetali. Nella azione della luce sulla biosintesi delle antociane si possono distinguere due tipi di risposta allo stimolo luminoso. Il primo tipo è la risposta a illuminazioni brevi (pochi minuti), caratterizata dalla fotoreversibilita degli effetti indotti dalla radiazione rossa (R, 600–670 nm) e rosso-estrema (FR, 710–750 nm) ed è mediato dal fitocromo; la quantità di antociane formate in risposta an una breve illuminazione è scarsa. Il secondo tipo è la risposta a illuminazioni prolungate (ore or giorni); la risposta massima per la formazione di antociane richiede esposizioni prolungate ad alte intensità di radiazione nella regione spettrale compresa tra l'ultravioletto vicino e l'estremo rosso (290–750 nm) ed ha le caratteristiche tipiche dei processi fotomorfogenici HIR. Il fitocromo è il fotorecettore responsabile per la fotoregolazione dei processi HIR ed

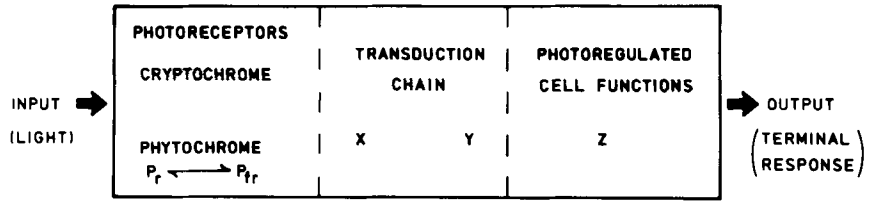


Fig. 1. A scheme to illustrate the sequence of steps involved in photomorphogenesis. Pr and Pfr: the two interconvertible forms of phytochrome. X and Y: hypothetical component of the transduction chain before and after photoreceptor-mediated changes.

è probabilmente l'unico fotorecettore responsabile per la fotoregolazione della risposta a irradiazioni prolungate con R e FR. La risposta a irradiazioni prolungate con UV e BL è probablimente regolata, in alcune specie, attraverso la cooperazione tra due fotorecettori, fitocromo e criptocromo; quest'ultimo è un fotorecettore specifico per la radiazione UV e BL. L'identità del criptocromo e le caratteristiche dell'interazione tra fitocromo e criptocromo nella fotoregolazione dei processi di sviluppo dei vegetali sono ancora poco conosciute.

III. Introduction

Light is the ultimate source of energy for virtually all life on earth. Photosynthesis is the photobiological process responsible for the transfer of energy from light to the biosphere, however it is not the only photobiological process occurring in plants. Through evolution, plants have developed systems regulating their developmental patterns in response to changes in the light conditions (direction, duration, intensity and spectral quality of radiation) of the environment. The non-photosynthetic action of light on plant development is known as photomorphogenic action and affects all phases of the life cycle of plants, from seed germination to seed formation (Mohr and Shropshire, 1983).

The final expression of photomorphogenic responses is the result of an orderly sequence of events that starts with the absorption of light by sensor pigments (photoreceptors) and ends with the expression of the response (light-dependent changes in membrane potentials, seed and spore germination, leaf expansion, stem elongation, flowering, plastid development, enzyme synthesis, pigment synthesis, etc.). The processes involved in the sequence are schematized in Figure 1.

The absorption of light causes changes in the operational state of the photoreceptors. Through a transduction chain, the signal perceived by the photoreceptors is transmitted to other cell functions (e.g., metabolic pathways involved in the response) causing changes in their state and even-

tually the response is expressed. To understand the mechanism of action of light in the photoregulation of plant development knowledge of all the processes occurring in the signal-to-response chain is required: (1) identification of the photoreceptors; (2) determination of the effects of light on the state of the photoreceptors; (3) identification of the nature of the transduction chain; (4) determination of the nature of the interaction between photoreceptors and the transduction chain and the nature of the first molecular changes induced in the responding system by the active form of the photoreceptor; (5) determination of the nature of the interaction between the transduction chain and cell processes subjected to photoregulation; and (6) understanding how the various processes at the levels of molecules, organelles, cells, tissues, and organs are integrated to produce the final response in the whole individual.

At present, we have some understanding of the first and second of these steps, at least insofar as phytochrome is concerned; for the others we have some vague working hypotheses, usually represented by the insertion of X's, Y's and Z's (Fig. 1) between the photoreceptors and the terminal response. The nature of cryptochrome [a photoreceptor involved in the photoregulation of responses elicited by ultraviolet (UV) and blue light (BL) radiation] is still unclear. The available data indicate that it might be a flavin derivative, perhaps a flavoprotein (Gressel, 1979; Mohr and Shropshire, 1983). The use of the term cryptochrome does not necessarily mean that there is only one specific UV–BL light photoreceptor. There is evidence suggesting that there might be at least three UV–BL light photoreceptors which may be found both in green plants and fungi (Briggs, 1983). The name cryptochrome, as used in this review, is a convenient term to denote the still unknown UV–BL light photoreceptor(s).

The involvement of phytochrome in the photoregulation of plant development is well established (Borthwick, 1972a, 1972b; Mohr, 1972; Schäfer, 1982); the properties of phytochrome are partially known (Mohr, 1972; Pratt, 1978, 1979, 1982; Quail, 1983; Smith, 1975; Smith and Kendrick, 1976; W. O. Smith, 1983). The expression of light-dependent, phytochrome-mediated responses requires the presence of Pfr, the physiologically active form of the pigment (Pr is the physiologically inactive form).

The nature of the transduction chain is unknown, but, since some very fast phytochrome-mediated responses to light are apparently linked to changes in membrane permeability, it seems possible that membrane-dependent processes might be components of the transduction chain (Hendricks and Borthwick, 1967; Quail, 1983; Roux, 1984; Satter and Galston, 1976). The rates of synthesis and activation of several enzymes (Z in Fig. 1) are affected by light and the action of light is phytochrome-mediated

(Lamb and Lawton, 1983; Mohr, 1972; Rau and Schrott, 1979; Schopfer, 1977; Schopfer and Apel, 1983; Smith, 1975).

In this article, I shall try to summarize the research on the photoregulation of light-dependent anthocyanin production and to discuss its contribution to the general understanding of plant photomorphogenic processes.

Anthocyanins (glycosylated anthocyanidins) are water-soluble, vacuolar pigments responsible for the the violet, blue, purple, red and scarlet colors of stems, leaves, flowers and fruits in the vast majority of higher plants (Swain, 1976). The Centrospermae are exceptions; their violet-red colors are due to a different group of water-soluble pigments, the betacyanins (Piattelli, 1976). The anthocyanins are part of a class of chemically related pigments, the flavonoids, which includes flavonols, flavanones, flavones, catechins, chalkones and anthocyanins (Swain, 1976). The basic chemical structure of the flavonoids is the $C_6C_3C_6$ structure of flavanone, derived from the condensation of 4-coumaroyl-CoA and malonyl-CoA, a reaction catalyzed by the enzyme flavanone (chalkone) synthase (Wong, 1976). The reactions leading to the formation of 4-coumaroyl-CoA are the deamination of phenylalanine to cinnamic acid (enzyme: phenylalanine ammonia lyase, PAL), hydroxylation of cinnamic acid to 4-coumaric acid (enzyme: cinnamate-4-hydroxylase, CAH) and formation of 4-coumaroyl-CoA (enzyme: 4-coumarate : CoA ligase). These reactions form the general phenylpropanoid pathway and are common to the pathways for the biosynthesis of cinnamate esters, flavonoids and lignins (Hahlbrock and Grisebach, 1979).

The flavonoids are rather abundant in nature. It has been estimated that about 1.5% of the carbon fixed annually in photosynthesis is used for the synthesis of flavonoids (Smith, 1972). The functions of flavonoids in plants are not completely understood: flower colors, together with scent and nectar, attract insects to plants and insure cross-fertilization; some leaf flavonoids are apparently involved in insect feeding responses as specific deterrents; flavonoids can act as a light screen against damaging UV radiation (Harborne, 1976).

The capability for anthocyanin synthesis is genetically determined. The amount of pigment formation in competent cells is affected by numerous environmental factors such as nutritional state, water conditions, wounding, infections, age, temperature and light. Light is particularly important because little or no anthocyanin is formed in darkness in most systems. Even in those systems that can produce some pigment in darkness, exposure to light results in a marked enhancement of the rate of synthesis and total production of anthocyanin.

Research on anthocyanin production has been developed along three

main lines: (a) applied aspects, i.e., studies directed to determine the best conditions to increase the deepness of fruit coloration, a very important factor for the marketing of the product; (b) studies of the metabolic pathways for the synthesis of anthocyanin and other flavonoids; and (c) research on the action of light on anthocyanin production as a model system for the study of the mechanism of photoregulation of plant development (Mancinelli and Rabino, 1978; Mohr, 1972), the aspect dealt with in this article.

IV. The Operational State of the Photoreceptors in vivo

The term "operational state of the photoreceptors" is one of convenience, used to indicate, in a general way, the state resulting from the interaction between the light and dark reactions in which a photoreceptor is involved.

The hypotheses about the state of the photoreceptors in vivo and about the relationship between their state and the expression of photoresponses are based on three groups of data: (a) results from physiological experiments; (b) results of in vivo assays of the photoreceptors; and (c) the known spectral and physicochemical properties of the photoreceptors as determined by studies of the isolated, purified photoreceptors. In this section, we shall summarize the present state of knowledge about the state of the photoreceptors in vivo. Since the identity of cryptochrome, the specific UV–BL light photoreceptor, is still unknown, the discussion in the following sections deals mostly with phytochrome.

The physiological functions and the general properties of phytochrome have been discussed in several publications (Mitrakos and Shropshire, 1972; Mohr, 1972; Pratt, 1978, 1979, 1982; Satter and Galston, 1976; Shropshire, 1972a; Shropshire and Mohr, 1983; Smith, 1975, 1981; Smith and Kendrick, 1976; W. O. Smith, 1983) to which the reader is referred for further detail.

IV.A. LIGHT AND DARK REACTIONS OF PHYTOCHROME

The light and dark reactions of phytochrome, as they are understood at present, are illustrated in Figure 2 (a, b, c).

Scheme *a* (Butler, 1972) shows the photoreversible transformation (photoconversion) between the two interconvertible forms of phytochrome, Pr (the physiologically inactive form; absorption maximum at 660–670 nm) and Pfr (the physiologically active form; absorption maximum at 725–735 nm). Scheme *b,* known as the "open photoreceptor model" (Fukshansky and Schäfer, 1983; Schäfer, 1975), shows the relationships between the light (photoconversion) and the dark (synthesis, destruction, dark reversion) reactions of phytochrome. Phytochrome de-

struction is a proteolytic process resulting in a loss of spectrophotometrically and immunologically detectable phytochrome (Pratt, 1978, 1979). Dark reversion is the non-photochemical reversion of Pfr to Pr (Frankland, 1972). Scheme *c*, known as the "cyclic model of phytochrome dynamics" (Fukshansky and Schäfer, 1983; Schäfer, 1975), includes the relationships between soluble or diffuse (Pr^s and Pfr^s) and bound (Pr^b and Pfr^b; pelletable, sequestered) forms of phytochrome (Fukshansky and Schäfer, 1983; Schäfer, 1975) and between the "labile" (characterized by fast destruction) and "stable" (characterized by slow destruction) pools of the pigment (Jabben and Holmes, 1983).

According to scheme *a*, the rate of change of Pfr is (Butler, 1972):

$$d[Pfr]/dt = k_1[Pr] - k_2[Pfr] \qquad \text{Eq. 1}$$

where t = time. Since total phytochrome, [P] = [Pr] + [Pfr], equation 1 can be rewritten as:

$$d[Pfr]/dt = k_1([P] - [Pfr]) - k_2[Pfr].$$

This equation indicates that the rate of photoconversion (cycling) between Pr and Pfr is a function of fluence rate and wavelength. The solution of this equation for a given time t is as follows (Butler, 1972; Fukshansky and Schäfer, 1983):

$$Pfr_t = \left(Pfr_0 - \frac{k_1}{k_1 + k_2} P_0\right) e^{-(k_1+k_2)t} + \frac{k_1}{k_1 + k_2} P_0$$

At photoequilibrium (dPfr/dt = 0; $k_1[Pr] = k_2[Pfr]$) the value of the Pfr/P ratio is a function of wavelength only (Butler, 1972; Butler et al., 1964), as shown by the stationary solution of equation 1 (the terms $\epsilon_{r\lambda}\phi_r$ and $\epsilon_{fr\lambda}\phi_{fr}$ are called the cross sections for photoconversion).

$$\frac{[P_{fr}]}{[P]} = \frac{\epsilon_{r\lambda}\phi_{fr}}{\epsilon_{r\lambda}\phi_r + \epsilon_{fr\lambda}\phi_{fr}}$$

The values of the Pfr/P ratios at photoequilibrium for purified phytochrome in solution are: 0.60 at 280 nm; about 0.65 (average) in the ultraviolet (UV–A) (320–390 nm) region; about 0.35 to 0.45 in the blue light (BL) (410–480 nm) region; about 0.80 in the red (R) (600–670 nm) region; 0.20 at 700 nm; 0.06 at 710 nm; 0.01 or less in the far-red (FR) (720–780 nm) region (Butler et al., 1964; Pratt and Butler, 1970).

Equation 1 is adequate to describe the changes in the state of phytochrome under conditions in which synthesis, destruction and reversion do not play a major role, for example for phytochrome solution kept at low temperature and, at least as a first approximation, in vivo under brief irradiations. However, it is not adequate to describe the changes in the state of phytochrome under prolonged light treatments because the dark

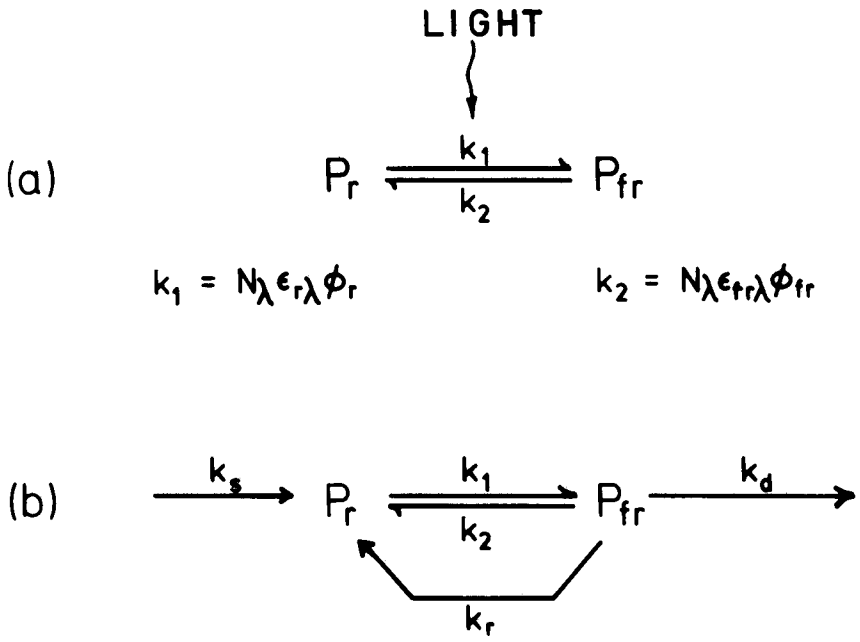


Fig. 2. Basic schemes showing the relationships between the two forms of phytochrome, Pr and Pfr.

(a) Photoconversion only. N_λ: photon fluence rate at wavelength λ. $\epsilon_{r\lambda}$ **and** $\epsilon_{fr\lambda}$: molecular extinction coefficients of Pr and Pfr at wavelength λ. ϕ_r **and** ϕ_{fr}: quantum efficiency for the Pr → Pfr and Pfr → Pr photoconversions. k_1: rate constant for Pr → Pfr photoconversion. k_2: rate constant for Pfr → Pr photoconversion.

(b) Open photoreceptor model. k_d: rate constant for Pfr destruction. k_r: rate constant of dark reversion. k_s: rate constant for synthesis of phytochrome.

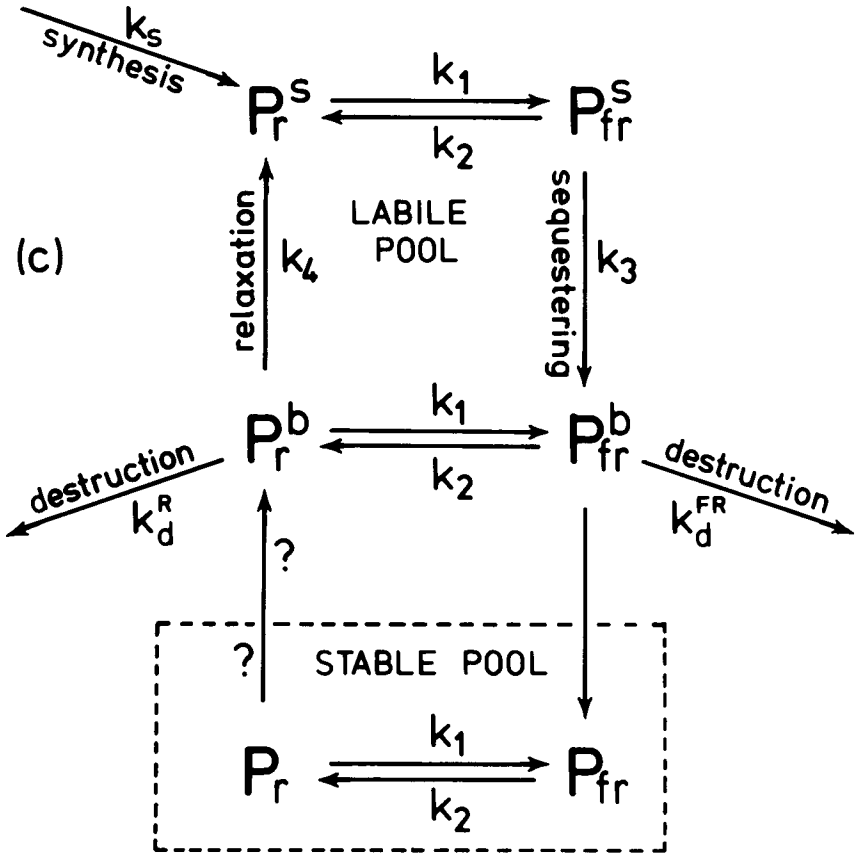
reactions play an increasing role as the length of the irradiation is increased. As an example let's modify equation 1 by adding destruction and dark reversion.

$$d[Pfr]/dt = k_1[Pr] - k_2[Pfr] - k_r[Pfr] - k_d[Pfr] \qquad \text{Eq. 2}$$

The stationary solution (dPfr/dt = 0) of this equation,

$$\frac{[Pfr]}{[P]} = \frac{k_1}{k_1 + k_2 + k_r + k_d}$$

clearly indicates that, at photoequilibrium, the Pfr/P ratio is a function of both wavelength and fluence rate, and not of wavelength only as in the stationary solution of equation 1. The solutions of the systems of equations including all the factors of scheme *c* are much more complex and the reader can find a detailed analysis in a recent article (Fukshansky and Schäfer, 1983).



(c) Cyclic photoreceptor model. **Pr^s and Pfr^s**: soluble, diffuse forms of Pr and Pfr. **Pr^b and Pfr^b**: bound (sequestered, pelleted) forms of Pr and Pfr. **k_d^r and k_d^{fr}**: rate constants for phytochrome destruction. **k_3**: rate constants for sequestering and pelletability. **k_4**: rate constant for relaxation.

In conclusion, in vivo, the rate of change of Pfr and the values of the Pfr/P photoequilibrium ratios are a function of both the rate of photoconversion (fluence rate and wavelength dependence) and the rates of the dark reactions (destruction, reversion, synthesis). In vivo, depending on the ratio between the rates of the light and dark reactions, [Pfr] and the Pfr/P ratio values could be much lower than those calculated and measured for phytochrome in vitro, especially at low fluence rates of irradiation and/or at wavelengths where the molecular absorption coefficients of Pr and Pfr are low, as shown by the calculated data of Table I. This has been confirmed experimentally: the values of the Pfr/P ratio in vivo are much more dependent on fluence rate under BL (low values of phy-

Table I

Effect of fluence rate of irradiation at 450 and 660 nm and of phytochrome destruction on the value of the Pfr/P photoequilibrium ratio

Fluence rates ($W\ m^{-2}$)	Pfr/P photoequilibrium ratio			
	450 nm		660 nm	
	A	B	A	B
0.03	0.01	0.08	0.35	0.69
0.1	0.04	0.17	0.58	0.77
0.3	0.09	0.26	0.71	0.79
1.0	0.19	0.31	0.77	0.80
3.0	0.27	0.34	0.79	0.80
10.0	0.32	0.34	0.80	0.80
30.0	0.34	0.35	0.80	0.80
100.0	0.34	0.35	0.80	0.80

A: half-life for phytochrome destruction = 30 minutes.
B: half-life for phytochrome destruction = 240 minutes.
Values of the Pfr/P photoequilibrium ratio were calculated using the formula $Pfr/P = k_1/(k_1 + k_2 + k_d)$ and values of photoconversion cross sections from Butler et al. (1964). Values of Pfr/P photoequilibrium ratios for phytochrome in solution are 0.35 at 450 nm and 0.80 at 660 nm (Butler et al., 1964).

tochrome photoconversion cross sections) than under R (high values of phytochrome photoconversion cross sections) (Jabben et al., 1982). One important consequence of these observations is that in vivo photoequilibrium values of the Pfr/P ratios under irradiation with sources of different spectral composition and fluence rate should be checked experimentally; assuming that they might be equal to those observed in vitro might be wrong and might result in misinterpretation of physiological data.

IV.B. FACTORS AFFECTING PHYTOCHROME PHOTOCONVERSION *IN VIVO*

The rate of phytochrome photoconversion in vivo is affected by age. In mustard seedlings, the rate of the Pr → Pfr photoconversion increases while the rate of the Pfr → Pr photoconversion decreases with increasing seedling age (Schäfer and Mohr, 1980). The value of Pfr/P at photoequilibrium under prolonged exposure to FR changes with seedling age (Schäfer et al., 1972).

The addition of FMN enhances the rate of the Pr → Pfr photoconversion and decreases the rate of the Pfr → Pr photoconversion in phytochrome solutions exposed to BL radiation (Sarkar and Song, 1982), resulting in an increase of the value of the Pfr/P at photoequilibrium. It is not known if a similar interaction between flavins and phytochrome also occurs in

vivo, but it has been reported that addition of flavins enhances the phytochrome-mediated opening of excised bean hooks exposed to BL radiation (Klein, 1959).

Light attenuation due to screening by foreign pigment (carotenoids, chlorophyll) affects the rate of phytochrome photoconversion and may cause changes in the value of the Pfr/P ratio at photoequilibrium and a shift of the action peaks of various photoresponses (Beggs et al., 1980; Holmes and Wagner, 1982; Jose and Schäfer, 1978; Shropshire, 1972b). In addition, since the rate of phytochrome destruction under continuous irradiation is a function of Pfr/P and fluence rate (see section IV.c), light attenutation due to foreign pigment screening might also affect the rate of destruction. As a consequence of light attenuation from foreign pigment screening, the extent of the differences in the state of the photoreceptors (e.g., Pfr/P ratio, rates of phytochrome photoconversion and destruction) under irradiation with different spectral regions might vary considerably in time due to the combination of several factors: (a) the carotenoid and chlorophyll content increases with increasing duration of the irradiation; (b) the amount of carotenoids and chlorophylls formed under irradiation with different spectral regions is different; (c) the screening effects are more pronounced in the spectral regions of maximum absorption of the screening pigments, i.e., BL and R for screening by chlorophyll and BL for screening by carotenoids; (d) foreign pigment screening would affect phytochrome only under long wavelength (R & FR) irradiation and both cryptochrome and phytochrome under short wavelength (UV & BL) irradiation; and (e) differences in the values of phytochrome photoconversion cross sections for different wavelength regions (Butler et al., 1964; Schäfer, 1981). Unfortunately, since accurate spectrophotometric measurements of phytochrome are next to impossible in chlorophyll-containing tissues (Pratt, 1978, 1983), one can only make an educated guess (Holmes and Fukshansky, 1979) about the actual state of phytochrome in light-grown, chlorophyll-rich tissues.

To avoid the problems created by foreign pigment screening in light-grown systems, researchers have used inhibitors that reduce the carotenoid and chlorophyll content. An inhibitor that has been used quite extensively in the last few years is the herbicide Norflurazon (NF). It inhibits carotenoid synthesis and, in the absence of carotenoids, chlorophyll is subjected to photodestruction; NF-treated, light-grown seedlings contain very little carotenoids and chlorophylls (Bartels and Hyde, 1970). In vivo spectrophotometric measurements of phytochrome have been made in NF-treated, light-grown, chlorophyll-less seedlings (Heim et al., 1981; Jabben, 1980; Jabben and Deitzer, 1978a, 1978b, 1979; Kilsby and Johnson, 1982). The results of these measurements, together with data from immunoassay of plant extracts (Hunt and Pratt, 1980; Shimazaki et al.,

1981, 1983) have shown that the phytochrome content of light-grown seedlings is much lower, about 5% or less, than that of dark-grown ones.

Apparently the rates of the Pr → Pfr and Pfr → Pr photoconversions are the same in light-grown and etiolated seedlings (Jabben and Holmes, 1983). No significant differences in spectral and immunochemical properties were found for phytochrome extracted from light-grown and etiolated pea shoots (Shimazaki et al., 1981), but some differences were found for phytochrome extracted from light-grown and etiolated oats seedlings (Shimazaki et al., 1983; Tokuhisa and Quail, 1983). Immunochemical assays of phytochrome are much more sensitive than spectrophotometric methods and are not affected by the presence of chlorophyll. Unfortunately, until recently, the immunochemical assay did not discriminate between Pr and Pfr (Hunt and Pratt, 1979, 1980; Pratt, 1983). Monoclonal antibodies showing preferential binding for Pfr (MAC 50) and Pr (MAC 49 and MAC 52) have recently been obtained (Thomas et al., 1984).

Spectrophotometric measurements of phytochrome in NF-treated, light-grown, chlorophyll-less seedlings (Heim et al., 1981; Jabben, 1980; Jabben and Deitzer, 1978a, 1978b; Jabben and Holmes, 1983; Jabben et al., 1980; Kilsby and Johnson, 1982) have contributed to improve our knowledge of the state (e.g., content and rate of destruction) of phytochrome in light-grown plants. Norflurazon has been used in the physiological study of photomorphogenesis in light-grown systems to eliminate the effects of carotenoid and chlorophyll screening on the state of the photoreceptors (Beggs et al., 1980, 1981; Holmes and Wagner, 1982; Mancinelli, 1984; Mancinelli and Schwartz, 1984). However, the use of NF might create other problems. According to some reports (Gorton and Briggs, 1980; Jabben and Deitzer, 1979), NF does not seem to affect either phytochrome itself or the response system involved, but, according to other reports, NF inhibits phytochrome accumulation (Shimazaki et al., 1983) and can affect the response system (seed germination: Widell, 1983; Widell et al., 1981; leaf expansion, stem elongation and hook opening: Pardo and Schiff, 1980). The effects of NF on the spectral sensitivity of photomorphogenic responses (section VII.d; Figs. 9, 10) might be a consequence of differences in the degree of carotenoid and chlorophyll screening between water-grown and NF-treated systems, but other factors cannot be ruled out. In conclusion, Norflurazon must be used with caution in photomorphogenic studies of light-grown systems and never without reference data for its effects on the photoreceptors and on the response system.

IV.C. FACTORS AFFECTING THE DARK REACTIONS OF PHYTOCHROME (SYNTHESIS, REVERSION, DESTRUCTION) IN VIVO

The phytochrome content of dark-grown seedlings increases with age for several days after sowing (Correll and Shropshire, 1968; Mancinelli

and Tolkowsky, 1968; Quail et al., 1973a, 1973b; Schäfer, 1978). The increase in phytochrome content is the result of de novo synthesis and the newly synthesized phytochrome is in the Pr form (Quail et al., 1973a, 1973b). (Note: some of the phytochrome present in seeds is in the Pfr form and remains in this form for several hours after sowing; Rollin, 1972; Spruit and Mancinelli, 1969.) The rate of phytochrome synthesis is low. From the data of Quail et al. (1973a, 1973b), the apparent rate constant of phytochrome accumulation is 3×10^{-4} min^{-1}. The rate of phytochrome accumulation in seedlings first increases and then decreases with age (Schäfer, 1978). Exposure to light causes a decline in phytochrome content, but, upon return to darkness, the phytochrome content increases again (Hunt and Pratt, 1980; Quail et al., 1973a, 1973b; Schäfer, 1978). The rate of phytochrome synthesis is apparently decreased by exposure to light (Colbert et al., 1983; Gottmann and Schäfer, 1982).

The decrease in phytochrome content, following exposure to light, is caused by the destruction (also called decay) of the pigment. The early data on phytochrome destruction based on spectrophotometric measurements (Butler et al., 1963; Butler and Lane, 1965; Frankland, 1972) have been confirmed later with immunological assays (Pratt, 1978, 1979). Low temperatures, anaerobiosis, EDTA, 2-mercaptoethanol, azide, ethylene and inhibitors of protein synthesis decrease the rate of phytochrome destruction, while high temperature increases it (Butler et al., 1963; Butler and Lane, 1965; Frankland, 1972; Furuya et al., 1965; Kidd and Pratt, 1973; Stone and Pratt, 1978). The rate of phytochrome destruction is affected by seedling age, increasing for four to five days after sowing and then remaining approximately constant (Schäfer, 1978). Phytochrome destruction is a first order reaction (Frankland, 1972).

Even though the mechanism of phytochrome destruction is still unclear, we know that it consists of at least three processes: (A) Pfr destruction, the first to be recognized (Butler and Lane, 1965; Butler et al., 1963; Frankland, 1972; Pratt, 1979); in seedlings, the rate constants of Pfr destruction vary from about 0.001 min^{-1} to about 0.050 min^{-1}, depending on the species and other factors (e.g., seedling age); (B) slow Pr destruction (turnover) with a rate one to two orders of magnitude smaller than that of Pfr destruction (Quail et al., 1973b); and (C) Pfr-induced Pr destruction, proceeding from Pr after cycling through Pfr (Chorney and Gordon, 1966; Dooskin and Mancinelli, 1968; Jabben, 1980; Stone and Pratt, 1979). The rate constant for Pfr-induced Pr destruction is approximately equal to that for Pfr destruction (Stone and Pratt, 1979). In seedlings exposed to R light for a few minutes and then returned to darkness (Butler et al., 1963), most of the loss of phytochrome is due to Pfr destruction. In seedlings exposed to a R pulse + FR pulse sequence and then returned to darkness (Dooskin and Mancinelli, 1968; Stone and Pratt, 1979), most

of the loss of phytochrome is probably due to Pfr-induced Pr destruction. In seedlings exposed to continuous or intermittent irradiation (Mancinelli et al., 1974, 1975; Schäfer et al., 1975, 1976) the loss of phytochrome is probable due to both Pfr and Pfr-induced Pr destructions, with the relative contribution of the two processes probably depending on the rate of cycling between Pr and Pfr and on the value of the Pfr/P ratio. The rate of phytochrome destruction in vivo under continuous irradiation is a function of wavelength and fluence rate (Frankland, 1972; Schäfer et al., 1975, 1976) and shows a linear relationship with the value of the Pfr/P ratio (Kendrick and Frankland, 1968).

The kinetics of Pfr destruction show two phases: following exposure to a saturating R pulse, there is a rapid loss of Pfr, about 80–90% in 2 hours, followed by a much slower decay of the rest (Brockman and Schäfer, 1982). Slow Pfr destruction is predominant in light-grown seedlings (Heim et al., 1981; Jabben and Deitzer, 1978b; Jabben and Holmes, 1983; Jabben et al., 1980). These findings have led to the suggestion (Brockmann and Schäfer, 1982; Jabben and Holmes, 1983) that there might be two pools of Pfr, a labile one (*l*Pfr), characterized by fast destruction, with a half-life varying from about 30 to 60 minutes, depending on the species, and a stable one (*s*Pfr), characterized by slow destruction, with a half-life of several hours.

The idea of two different populations of phytochrome is not a new one. It was first proposed by Hillman (1967, 1972) to explain a discrepancy (the well known *Pisum* and *Zea* paradoxes) between what could be inferred on the state of phytochrome from the results of physiological experiments and what was shown by spectrophotometric measurements in vivo. Newly synthesized phytochrome is labile phytochrome. Most of the phytochrome in etiolated or re-etiolated seedlings is in the labile form. In light-grown plants, from 50 to 80% of the phytochrome, depending upon the species, is in the stable form. The difference in the distribution ratio of phytochrome between the two pools in different species is probably a reflection of differences in the rate of synthesis of Pr (Jabben and Holmes, 1983). Spectrophotometric data suggest that the total amount of stable phytochrome in light-grown plants is approximately constant (Brockmann and Schäfer, 1982; Heim et al., 1981; Jabben et al., 1980; Jabben and Holmes, 1983). It has been suggested that *s*Pfr might be the predominant physiologically effective form of phytochrome in light-grown plants (Jabben and Holmes, 1983).

Some reasonable deductions may be made on the basis of the hypotheses that there are labile and stable pools of Pfr and that the value of the *s*Pfr/*l*Pfr is higher in light-grown than in dark-grown seedlings. The first is that fluence rates required to reach the photoequilibrium value of the Pfr/P ratio (Table I) might be lower in light-grown than in dark-grown seedlings.

The second is that the rate of escape of phytochrome-mediated responses from R–FR reversibility might be different in dark-grown and light-pretreated seedlings. In dark-grown seedlings, *l*Pfr is predominant, is rapidly lost and, consequently, R–FR reversibility should be lost rapidly. In light-grown seedlings, *s*Pfr is predominant, is lost slowly and, consequently, R–FR reversibility should be lost slowly. The third is that the differences in the extent of the response brought about by intermittent light treatments with short and long dark intervals between successive exposures should be smaller in light-grown than in dark-grown seedlings. The hypothesis and the deduction based on it are consistent with data from physiological experiments (Downs et al., 1957; Wildermann et al., 1978) which show that the effectiveness of the Pfr present at the end of a prolonged exposure to light is maintained over a prolonged period in darkness.

Insofar as the Pfr → Pr dark reversion is concerned, it is known that it is absent in monocots and in the Centrospermae, while it seems to be present in most dicots (Frankland, 1972). However, in contrast to etiolated dicot seedlings, there is no evidence for dark reversion in light-grown *Cucurbita* and *Brassica napus* seedlings. It has been suggested that, in light-grown plants, the dark reversion probably does not play a major role in the regulation of Pfr levels (Jabben and Holmes, 1983; Jabben et al., 1980).

IV.D. INTRACELLULAR DISTRIBUTION OF PHYTOCHROME

Immunocytochemically detectable phytochrome is uniformly distributed (diffuse) throughout the cytosol of unirradiated cells. Following exposure to R, phytochrome rapidly aggregates into small (1 μm), discrete loci in the cytoplasm (Mackenzie et al., 1975, 1978; Pratt, 1979; Quail, 1983). This process has been termed "sequestering." Over 90% of the phytochrome extractable from unirradiated tissues is soluble. Exposure to R of dark-grown seedlings or of homogenates from dark-grown seedlings causes a large increase in the proportion of phytochrome that can be recovered in a pelleted form (Pratt, 1978, 1979; Quail, 1975, 1983). The kinetics of conversion from diffuse to sequestered state and from soluble to pelletable state (Fig. 2, scheme *c*), after the Pr → Pfr photoconversion, are very similar, with half-lives of 2 to 5 seconds at 25°C. The conversion from sequestered or pelleted to diffuse or soluble states, after Pfr → Pr photoconversion, takes a much longer time, with half-lives of about 25 minutes (Quail, 1983).

There is an apparent correlation between phytochrome destruction and the pigment being in the sequestered or pelletable state (Fig. 2, scheme *c*, destruction starting from Pr^b and Pfr^b; Boisard et al., 1974; Mackenzie et al., 1978; Stone and Pratt, 1979). According to Schäfer et al. (1975,

1976), the kinetics of destruction are consistent with the intercalation of a "binding" reaction between Pfr formation and phytochrome destruction. An action spectrum for light-dependent pelletability under prolonged irradiation (Fuad and Yu, 1977) bears a close resemblance to some action spectra for HIR responses. Some preliminary results indicate that Pfr/P ratios in pelleted phytochrome extracted from tissue exposed to BL and FR might be higher than those measured in vivo in the same tissue (Rabino and Mancinelli, 1980). At present, there is no unequivocal evidence that either pelletability or sequestering are the result of a binding of phytochrome to specific receptors and the physiological significance of these two processes is still unclear (Pratt, 1978, 1979; Quail, 1983).

IV.E. CRYPTOCHROME

The identity of cryptochrome, the specific UV/BL light photoreceptor, has not been established, even though the evidence that it might be a flavin derivative (i.e., flavoprotein) is becoming stronger and stronger (Ninnemann, 1980; Schmidt, 1980; Senger, 1980, 1982). There is also some preliminary evidence that there might be more than one cryptochrome (Briggs, 1983). Because so little is known about cryptochrome, not much can be said about its state in the cell. Prolonged exposures to high fluence rates of BL radiation cause an irreversible bleaching of flavin and the loss of a BL-mediated photoresponse (Munoz and Butler, 1975). Exogenously supplied flavins enhance the opening of excised bean hooks exposed to BL radiation (Klein, 1959). For a general review of the present state of knowledge on UV–BL light photoreceptors the reader is referred to the following recent publications: Gressel, 1979; Ninnemann, 1980; Schäfer and Haupt, 1983; Schmidt, 1980; Senger, 1980, 1982; Wellmann, 1983.

IV.F. RESPONSIVENESS OF BIOLOGICAL SYSTEMS TO LIGHT

The photosensitivity of several photomorphogenic responses is affected by various factors (see sections V, VI and VII for details). Some of these effects can be attributed to changes in the state (e.g., content, rate of photoconversion, rate of destruction, value of the *s*Pfr/*t*Pfr ratio) of the photoreceptors. The interpretations of these observations, based solely on the known properties of the photoreceptors, are not sufficient to provide a satisfactory explanation. For example, in young mustard seedlings (Steinitz et al., 1976), exposed to continuous irradiations started at the time of sowing, anthocyanin production does not start until about 27 hours after sowing. Since the phytochrome system is apparently active (Schäfer et al., 1972; Schmidt and Mohr, 1981a, 1981b; Steinitz et al., 1976) long before the system becomes fully "competent" for anthocyanin

production, the only reasonable explanations for these observations are: (a) that the transduction chain does not become operative until about 27 hours after sowing; and (b) the metabolic pathway for anthocyanin biosynthesis does not become inducible until about 27 hours after sowing. Nothing is known about the nature of the changes that must occur to make the system fully competent for anthocyanin production. These changes are dependent upon some "internal" factor(s) and independent of external factors such as light or nutrients (Steinitz et al., 1976).

V. General Characteristics of the Action of Light on Anthocyanin Synthesis

Small amounts of anthocyanin can be formed in response to short light exposures (few minutes). Anthocyanin formation induced by short light exposures (pulses) shows the typical properties of phytochrome-mediated responses: R–FR reversibility and validity of the reciprocity law (Downs, 1964; Downs and Siegelman, 1963; Ku and Mancinelli, 1972; Lange et al., 1971; Mohr, 1972; Siegelman and Hendricks, 1957; Smith, 1975).

The formation of large quantities of anthocyanin requires prolonged irradiations. Anthocyanin production brought about by prolonged irradiations shows the typical properties of the "high irradiance reaction" (HIR) of plant photomorphogenesis: (1) the full expression of the response requires prolonged exposures (hours to days) to high fluence rates of visible and near visible radiation (290–750 nm); (2) the extent of the response is a function of duration and fluence rate of the irradiations, but does not follow the Bunsen-Roscoe reciprocity law; and (3) the response does not show R–FR photoreversibility (Borthwick et al., 1969; Hartmann, 1966; Mancinelli, 1980b; Mancinelli and Rabino, 1978; Mohr, 1972; Smith, 1975).

The time-course of light-dependent anthocyanin accumulation shows a lag phase between the onset of the light treatments and the beginning of anthocyanin production. The lag phase of light-dependent anthocyanin production is probably a reflection of the lag phase in the light-dependent increase of activity of the enzymes of the flavonoid biosynthesis pathway (Acton et al., 1980; Hahlbrock and Grisebach, 1979; Mohr, 1972; Smith, 1972, 1975). The duration of the lag phase is different in different systems: about two to five hours in most young, dark-grown (kept in darkness until the onset of the light treatments) seedlings (e.g., maize, Duke and Naylor, 1976; mustard, Lange et al., 1971; radish, Bellini and Martelli, 1973; red cabbage, Ku and Mancinelli, 1972; sorghum, Downs and Siegelman, 1963; turnip, Grill and Vince, 1969, Schneider and Stimson, 1972); about 20 hours in apple skin sections (Siegelman and Hendricks, 1958) and about 30 hours in *Spirodela oligorrhiza* (Thimann and Radner, 1958).

Table II

Red–far red reversible and continuous irradiation responses in dark-grown and light-pretreated systems. From data of Mancinelli (1984) and Mancinelli and Schwartz (1984)

Light treatments	Anthocyanin production			
	Cabbage	Rye	Tomato	*Spirodela*
A. Pretreatment: dark				
10 min R + D[a]	0.188	0.061	0.008	0.377
10 min R + 10 min FR + D[a]	0.136	0.024	0.005	0.105
R–FR reversible response	0.052	0.037	0.003	0.272
Continuous WL[b]	0.862	0.179	0.264	3.087
B. Pretreatment: 8 h WL (rye) or 24 h WL (all others)				
10 min R + D[a]	0.478	0.149	0.159	1.753
10 min R + 10 min FR + D[a]	0.343	0.073	0.103	1.085
R–FR reversible response	0.135	0.076	0.056	0.668
Continuous WL[b]	0.602	0.076	0.184	2.280

Absorbance values reported are average values from several experiments.

[a] D (dark): 24 hours for cabbage and tomato and 48 hours for rye and *Spirodela.* Values reported corrected by subtraction of absorbance values of extracts prepared at end of pretreatments.

[b] Continuous WL: 24 hours for cabbage and tomato and 48 hours for rye and *Spirodela.* Values reported are the differences between "pretreatment + 24 or 48 hours WL" and "pretreatment + 24 or 48 hours D." Pretreatments terminated by a 10 minute exposure to FR.

Apparently much longer lag phases can be encountered if the light treatments are started before the biological system has attained competence for anthocyanin production. For example, in mustard seedlings, exposed to continuous irradiation started at sowing time, there is no detectable increase in anthocyanin until about 27 hours after sowing. Exposure to light pretreatments can shorten the duration of the lag phase in young seedlings (Lange et al., 1971; Schmidt and Mohr, 1981a), with the exception of those cases in which the light pretreatments are terminated before the system has attained competence for anthocyanin production (Schmidt and Mohr, 1981a, 1981b). The duration of the lag phase in apple skin sections can be reduced from 20 to 10 hours by preincubation in darkness in a sucrose solution (Siegelman and Hendricks, 1958). The duration of the linear phase (increase rate constant with time) of light-dependent anthocyanin is also different in different systems, varying from about 18 to 24 hours in young, dark-grown seedlings of mustard (Wagner and Mohr, 1966), to about 50 hours in apple skin sections (Siegelman

Table III

Action of intermittent R and FR treatments on anthocyanin production in cabbage and mustard seedlings. From data of Ku and Mancinelli (1972) and Mancinelli et al. (1974)

Light treatment	Anthocyanin production	
	Cabbage	Mustard
4 × (3 min R + 357 min D)	0.50	0.36
4 × (3 min R + 3 min FR + 354 min D)	0.27	0.18
4 × (3 min FR + 357 min D)	0.25	0.17
4 × (3 min FR + 3 min R + 354 min D)	0.52	0.37
8 × (3 min R + 177 min D)	0.51	0.43
8 × (3 min R + 3 min FR + 174 min D)	0.35	0.31
8 × (3 min FR + 177 min D)	0.36	0.30
8 × (3 min FR + 3 min R + 174 min D)	0.54	0.45
24 × (1 min R + 59 min D)	0.46	0.43
24 × (1 min R + 1 min FR + 58 min D)	0.48	0.54
24 × (1 min FR + 59 min D)	0.44	0.47
24 × (1 min FR + 1 min R + 58 min D)	0.47	0.45
144 × (1 min R + 9 min D)	0.64	0.58
144 × (1 min R + 1 min FR + 9 min D)	1.05	0.96
144 × (1 min FR + 9 min D)	0.74	0.67
144 × (1 min FR + 1 min R + 9 min D)	0.64	0.54
144 × (2 min R + 8 min D)	0.59	0.52
144 × (2 min FR + 8 min D)	0.79	0.69
144 × (1 min [R+FR][a] + 9 min D)	0.65	0.63

Values reported are mean absorbancies of extracts prepared 24 hours after the beginning of the light treatments, corrected by subtraction of the values of the dark controls (0.28 for cabbage and 0.05 for mustard).

[a] [R+FR]: R & FR applied simultaneously.

and Hendricks, 1958) and to about five days in *Spirodela oligorrhiza* (Thimann and Radner, 1958). In red cabbage seedlings, there is a positive correlation between the increase in anthocyanin content and the increase in activity of flavanone synthase, the enzyme that catalyzes the formation of the $C_6C_3C_6$ basic flavonoid structure (Hrazdina and Creasy, 1979).

VI. Anthocyanin Production Induced by Short Irradiations (Inductive Response)

The production of small quantities of anthocyanin can be induced by a single light pulse (few minutes) in several dark-grown systems (Table

II). The effect of the R pulse can be reversed by a FR pulse applied immediately after R (Table II) (Grill, 1965; Ku and Mancinelli, 1972; Lange et al., 1971; Mohr, 1972; Mohr and Drumm-Herrel, 1981; Siegelman and Hendricks, 1957). The response induced by a single, short light pulse obeys the reciprocity law (Lange et al., 1971; Steinitz et al., 1979). The R–FR reversibility of the response, indicative of phytochrome involvement, can also be observed when one uses multiple short irradiations, separated by 3 to 6 hour dark intervals (Table III) (Lange et al., 1971; Mancinelli et al., 1974). The extent of the inductive, R–FR reversible response is small, when compared to the extent of the response brought about by prolonged irradiations (Table II), but significant.

In seedlings of sorghum, tomato and turnip and in apple skin sections, there is no inductive, R–FR reversible anthocyanin production, unless the R and FR pulses are applied at the end of a light pretreatment in the seedlings (Downs and Siegelman, 1963; Drumm and Mohr, 1978; Drumm-Herrel and Mohr, 1982a; Grill, 1965; Mancinelli and Schwartz, 1984; Mohr and Drumm-Herrel, 1981, 1983) or during the dark interval between the preinductive and inductive light treatments in apple skin sections (Downs, 1964). In general, a light pretreatment enhances the inductive, R–FR reversible response (Table II). The light-dependent enhancement of the inductive, R–FR reversible response is affected by the duration and spectral region of the light pretreatments, the duration of the dark interval between the end of the pretreatment and the application of the light pulse, and the age of the seedlings (Grill, 1965; Mohr and Drumm-Herrel, 1981; Mohr et al., 1979; Schmidt and Mohr, 1981b). For example, in sorghum and wheat seedlings, a R–FR reversible induction of anthocyanin production can be obtained only after exposure to UV–BL radiation (Drumm and Mohr, 1978; Mohr and Drumm-Herrel, 1981, 1983). Light pretreatments affect the time course of the responsiveness to light (Schmidt and Mohr, 1981b). The factors responsible for the light-dependent enhancement of the inductive response, observed also for light-dependent inhibition of hypocotyl elongation (Beggs et al., 1981), are not clearly known. Schmidt and Mohr (1981b) have suggested that the effects of light pretreatments on the photosensitivity of the response might be, at least in part, a consequence of time and light-dependent changes in the responsiveness of cell functions upon which phytochrome acts. Perhaps, changes in the state of the transduction chain (changes in the availability of action sites for Pfr? Mohr et al., 1979; X in Fig. 1) might be the main factor responsible for the change in the responsiveness of cell functions to Pfr. At present, this suggestion cannot be verified experimentally because the nature of the transduction chain and the identity of the action site for Pfr are still unknown. Another factor that might

be involved in the light-dependent enhancement of the inductive response is the difference in the values of the stable to labile Pfr ratio between dark-grown and light-pretreated seedlings (see section IV.c). In dark-grown seedlings, after exposure to a saturating R pulse, *l*Pfr would be predominant; but *l*Pfr is rapidly lost, therefore its action would be limited in time and the result would be a small induction response. In light-grown seedlings, after exposure to a R pulse, *s*Pfr would be predominant; *s*Pfr is lost slowly and its action would be extended in time; therefore the result would be a larger induction response.

A good correlation exists between the extent of anthocyanin production and levels of Pfr established by a light pulse (Drumm and Mohr, 1974; Schmidt and Mohr, 1982; Steinitz et al., 1979). In conclusion, there is ample evidence for the involvement of phytochrome in the photoregulation of anthocyanin production under inductive conditions.

VII. Anthocyanin Production under Prolonged Irradiation (HIR Response)

VII.A. FLUENCE RATE DEPENDENCE

The fluence rate-dependence of HIR anthocyanin production has been demonstrated in many systems (Bregeaut and Rollin, 1965; Creasy, 1968; Downs and Siegelman, 1963; Johnson, 1980; Klein et al., 1957; Lange et al., 1971; Mancinelli and Rabino, 1975, 1978; Mohr, 1972; Rabino et al., 1977; Schneider and Stimson, 1972; Wagner and Mohr, 1966). There is some discrepancy with respect to the extent of the fluence rate-dependence under continuous irradiation with different spectral regions, especially under continuous R. In mustard (Wagner and Mohr, 1966) and cabbage (Rabino et al., 1977) seedlings, anthocyanin production is strongly fluence rate-dependent under continuous BL and FR and weakly fluence rate-dependent under continuous R. However, Lange et al. (1971) and Johnson (1980) reported a strong fluence rate-dependence of anthocyanin production under continuous R in mustard seedlings. Anthocyanin production in bean seedlings exposed to continuous R is fluence rate-dependent (Klein et al., 1957). Insofar as theoretical considerations are concerned, the models of Hartmann (1966), Schäfer (1975) and Fukshansky and Schäfer (1983) predict a weak fluence rate-dependence for the extent of the response under continuous R; another model (Johnson and Tasker, 1979; Wall and Johnson, 1983) predicts a strong fluence rate-dependence under continuous R. A strong fluence rate-dependence under continuous R irradiation has been found for other HIR responses, such as the photoinhibition of hypocotyl elongation in mustard (Beggs et al., 1980; Holmes and Schäfer, 1981) and radish (Jose and Vince-Prue, 1977b) seedlings.

VII.B. CONTINUOUS VERSUS INTERMITTENT LIGHT TREATMENTS

The full expression of HIR responses requires prolonged exposures, but not necessarily continuous ones. Downs and Siegelman (1963) reported that intermittent light treatments (cycle duration = 20 minutes; light ON from 10 to 100% of the duration of the cycle) were very effective in bringing about anthocyanin production; the efficiency of the response [(anthocyanin accumulation)/(min of light) = 11.0 for the "2 minutes L + 18 minutes D" cycle and 3.2 for continuous irradiation], was higher for cyclic than for continuous irradiation; however, total pigment accumulation was higher under continuous than under intermittent irradiation. Later on, it was reported that, for anthocyanin production in cabbage and mustard seedlings, continuous irradiations could be fully substituted for by intermittent irradiations extended over the same duration and of equal total radiation fluence (Ku and Mancinelli, 1972; Mancinelli and Rabino, 1975; Rabino et al., 1977). To obtain the same extent of the response under cyclic and continuous irradiations, the duration of the cycles had to be kept short (about 5 minutes or less) under FR and BL, but could be extended to about 60 minutes under R (Mancinelli and Rabino, 1975; Rabino et al., 1977). These observations were recently confirmed; Schäfer et al. (1981) and Heim and Schäfer (1982) have reported that the action of continuous R on anthocyanin formation and hypocotyl elongation in mustard seedlings can be fully substituted for by hourly, 5 minute R pulses, but hourly BL and FR pulses substituted only partially for continuous BL and FR; the effects of cycles shorter than 60 minutes was not tested. Under intermittent irradiation, the extent of the response is dose-dependent (dose = fluence rate × time) rather than fluence rate-dependent (see section VII.c for further detail) as observed under continuous irradiation (Mancinelli and Rabino, 1975, 1978; Rabino et al., 1977). Heim and Schäfer (1982) reported a strong fluence rate-dependence for the response (paralleling the fluence rate-dependence of Pfr levels and Pfr/P ratios), under both continuous and pulsed R treatments. However, under the experimental conditions used in their experiments (only one duration, 5 minutes, was used for the hourly R pulses), it is not possible to distinguish between dose- and fluence rate-dependence. The response elicited by intermittent light treatments shows R–FR reversibility (Lange et al., 1971; Mancinelli et al., 1974; Schäfer et al., 1981). The extent of the R–FR reversibility of anthocyanin production under intermittent irradiations is a function of the duration of the dark interval between successive irradiations (Table III) (Mancinelli et al., 1974). Under 6 and 3 hour cycles, intermittent R (or FR–R) is more effective than intermittent FR (or R–FR); under 60 minute cycles, the differences are much reduced; under the 10 minute cycles, the "144 × (1 minute R + 1 minute FR + 8 minutes

D)" treatment is much more effective than all other 10 minute cyclic irradiations. These various observations indicate that a continuous exposure to light is not an obligatory requirement for the full expression of the HIR responses.

VII.C. RECIPROCITY FAILURE

The inductive, R–FR reversible, phytochrome-mediated responses induced by a single, short light pulse obey the Bunsen-Roscoe reciprocity law (Mancinelli and Rabino, 1978; Mohr, 1972; Shropshire, 1972a), but the responses brought about by continuous irradiation do not (Downs and Siegelman, 1963; Mancinelli and Rabino, 1975, 1978; Mohr, 1972; Schneider and Stimson, 1972; Shropshire, 1972a; Siegelman and Hendricks, 1957). Under conditions of validity of the reciprocity law, the extent of the light-dependent response is a function of the radiation dose: treatments $I \times t$ and $nI \times t/n$ (I = intensity; t = time) produce the same extent of the response. Validity of the reciprocity law indicates that the extent of the response is a function of the concentration of the product of a single photochemical reaction (e.g., [Pfr]). Non-conformity with the reciprocity law (reciprocity failure) can be a consequence of: (1) changes in the concentration of the photoreceptor with I and/or t (e.g., decrease of [P] during exposure as a consequence of phytochrome destruction); (2) interaction between the products of two or more photochemical processes; or (3) interaction between photochemical and non-photochemical processes, with the dark reactions being the limiting step for the response.

The reciprocity failure of the HIR can be reasonably explained on the basis of the known properties of phytochrome (Mancinelli and Rabino, 1975, 1978). Let us consider, as an example, the effects of two light treatments within the ranges of durations and fluence rates used in HIR research: (*A*) "24 hours L at 50 μW/cm^2," and (*B*) "3 hours L at 400 μW/cm^2 + 21 hours D." These two treatments are equal in terms of total radiation fluence and time allowed for the expression of the response; however, the extent of the response is much larger for treatment *A* than for treatment *B* (Downs and Siegelman, 1963; Mancinelli and Rabino, 1975; Rabino et al., 1977; Siegelman and Hendricks, 1957). The two treatments, *A* and *B*, are not equal in terms of phytochrome: under treatment *A*, there is continuous formation of Pfr due to phytochrome photoconversion and, even though [Pfr] decreases in time as a consequence of phytochrome destruction, there is some Pfr throughout the 24 hour period; but, under treatment *B*, as a consequence of Pfr destruction and dark reversion, there is essentially no Pfr left within a short time after the end of the 3 hour exposure. Thus, the reciprocity failure of the HIR could be a consequence of the differences in the levels of Pfr maintained

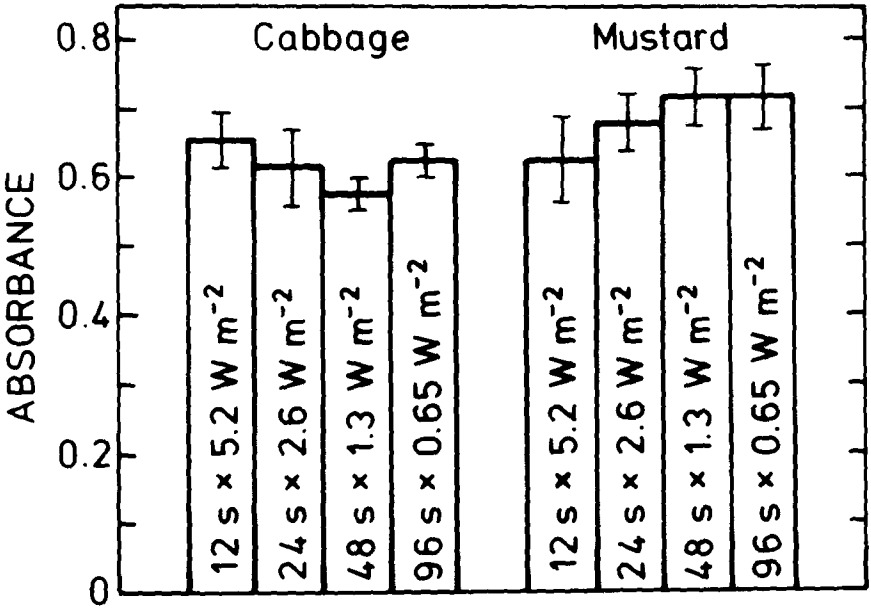


Fig. 3. Action of intermittent FR on anthocyanin production in cabbage and mustard seedlings demonstrating validity of reciprocity. Duration of the cycles was 6 minutes; the treatment was extended over a 48 hour period (480 cycles). Duration of the irradiations in each cycle and fluence rates are given inside the bars. From Mancinelli and Rabino, 1975.

over the 24 hour period between treatments *A* and *B*. As discussed previously (section VII.b), continuous irradiations can be replaced by intermittent ones extending over the same duration and of equal total radiation fluence. For example, treatment *A* above can be replaced by treatment (*C*) "480 × (15 seconds L at 600 μW/cm^2 + 165 seconds D)," equal to treatments *A* and *B* in terms of total radiation fluence and time allowed for the expression of the response. In terms of the state of phytochrome, treatment *C* should be similar to treatment *A* because there would be some Pfr present throughout the 24 hour period and the variations in [Pfr] during the short dark intervals between successive irradiations should be small.

Fluence rate and duration of the light pulses in the intermittent treatments can be easily changed maintaining the same total radiation dose. Results of experiments of this type in cabbage, mustard and tomato seedlings showed that anthocyanin production under intermittent light treatments was a function of dose (fluence rate × time) rather than of fluence rate and followed the reciprocity law (Fig. 3) (Mancinelli and Rabino, 1975; Rabino et al., 1977). Since intermittent light treatments can replace fully or almost fully continuous light treatments, and since the extent of

the response under intermittent light treatments is a function of radiation dose and follows the reciprocity law, one wonders if we should continue to consider the fluence rate-dependence and reciprocity failure of the HIR as basic properties of this class of photoresponses. This could have considerable consequences on the development of hypotheses on the relationships between state of the photoreceptors and photoregulation of the HIR. Further research is needed to clarify this point and to determine if the validity of the reciprocity law under intermittent irradiations is a general property of HIR responses.

Independently of the results of such verification, the use of intermittent light treatments can be a very useful tool in the study of the characteristics of the HIR. The comparison of the efficiency of intermittent light treatments with different durations of the dark interval between successive irradiations could help to determine the relative roles played by labile and stable Pfr (see section IV.c). The comparison of the efficiency of intermittent and continuous irradiation might help in determining the importance of transient forms of the photoreceptor in the photoregulation of the HIR.

VII.D. SPECTRAL SENSITIVITY AND FACTORS AFFECTING IT

Measurements of the spectral sensitivity of a photoresponse are very important in photomorphogenesis research because they provide a non-destructive method for determining the general absorption properties of the photoreceptors, the first necesary step to be taken toward the identification of the photoreceptors (Hartmann and Cohnen-Unser, 1972; Schäfer et al., 1983; Shropshire, 1972b). Demonstrating that a particular pigment is the photoreceptor for a given photoresponse can be a difficult and time-consuming task (numerous steps are required and several criteria must be met), as proved by the fact that more than 100 years after the discovery of phototropism [Darwin (1880), in "The power of movement in plants"; moreover, a theory to explain the bending of plants toward light was proposed earlier by de Candolle in his "Physiologie végétale" (1832)], the nature of the phototropic photoreceptor(s) has not yet been definitively established.

The spectral sensitivity of anthocyanin production under prolonged irradiation is different in different species (Mancinelli, 1980b; Mancinelli and Rabino, 1978; Smith, 1975). The data of Figure 4 provide a good example of the range of variation in the wavelength dependence of anthocyanin production in different systems; from these and other published data (Mancinelli and Rabino, 1978), one can distinguish three main groups of HIR spectral sensitivity.

Group I. The UV–BL, R and FR regions are all effective in bringing

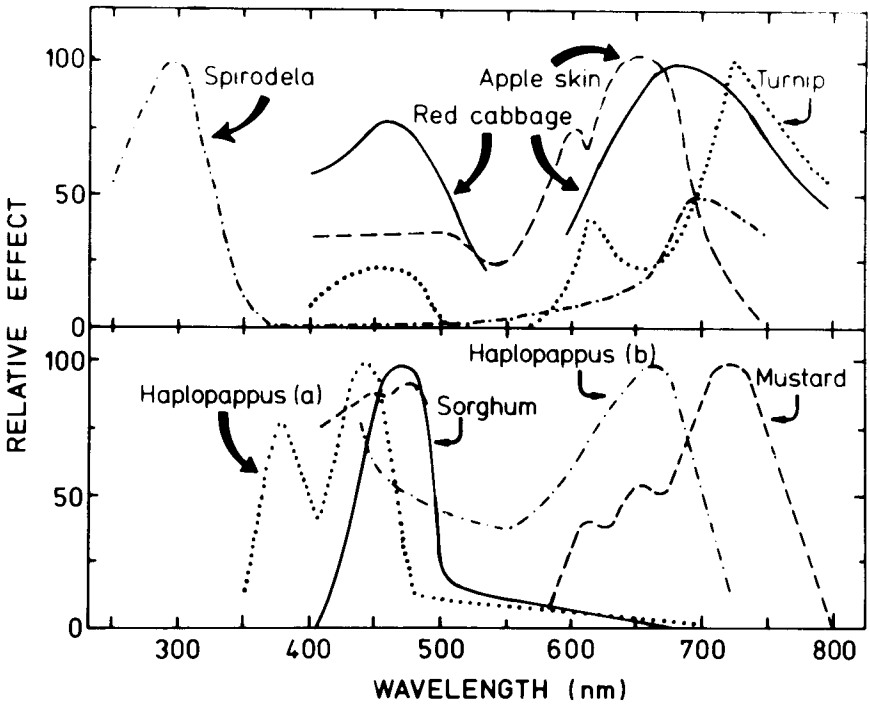


Fig. 4. Wavelength dependence of anthocyanin production under prolonged irradiations in different species. Redrawn from original data. Apple skin (Siegelman and Hendricks, 1958); *Haplopappus* (Lackmann, 1971; a, intact seedlings; b, isolated cotyledons); mustard (Mohr, 1957); red cabbage and turnip (Siegelman and Hendricks, 1957); sorghum (Downs and Siegelman, 1963); *Spirodela oligorrhiza* (Ng et al., 1964).

about anthocyanin production; FR and BL are generally the most effective regions. The systems that have a *group I* spectral sensitivity are the seedlings of white mustard, black mustard, leaf mustard, red cabbage, bean and buckwheat (Harraschain and Mohr, 1963; Kandeler, 1958; Klein et al., 1957; Ku and Mancinelli, 1972; Mancinelli and Walsh, 1979; Mohr, 1957; Mohr and Van Ness, 1963; Rabino et al., 1977; Siegelman and Hendricks, 1957; Tokhever and Voskresenskaya, 1971; Wagner and Mohr, 1966) and cultures of carrot (Alfermann, 1972).

Group II. For this group, the peak of action for anthocyanin production is in the R region; the UV–BL region is also quite effective, while the effectiveness of FR is very small or nil. The systems that have a *group II* spectral sensitivity are: apple skin sections, leaf disks of red cabbage, *Spirodela polyrrhiza,* isolated cotyledons of *Haplopappus gracilis* (Lackmann, 1971; Mancinelli, 1977, 1980a; Siegelman and Hendricks, 1958). Anthocyanin production in the systems with a *group II* spectral sensitivity is dependent on photosynthetic activity, as shown by the fact that inhib-

itors of photosynthesis, such as CMU and DCMU, inhibit the response; additions of sucrose to the CMU and DCMU-containing media restore anthocyanin production (Creasy, 1968; Downs, 1964; Downs et al., 1965; Mancinelli, 1977, 1980a). The requirement for photosynthetic activity is not a general characteristic of anthocyanin production. In the young seedlings of several species, anthocyanin production is independent of photosynthetic activity (Duke et al., 1976; Mancinelli et al., 1975, 1976; Mancinelli and Rabino, 1978). Actually, the development of the photosynthetic apparatus may be detrimental to anthocyanin production in young seedlings, perhaps as a consequence of competition for a common precursor (e.g., phenylalanine) amongst different metabolic pathways, as suggested by the observation that inhibition of the development of the photosynthetic apparatus results in an enhancement of anthocyanin production (Mancinelli et al., 1975, 1976; Wagner et al., 1967).

Group III. The only effective region for anthocyanin production under continuous irradiation is the UV–BL. The systems that have a *group III* spectral sensitivity are the seedlings of corn, sorghum, wheat and *Haplopappus gracilis,* tissue cultures of *Vitis vinifera* (some action in the green region reported; FR region not tested), callus and cell suspension cultures of *Haplopappus gracilis* (Downs and Siegelman, 1963; Drumm and Mohr, 1978; Drumm-Herrel and Mohr, 1981; Duke and Naylor, 1976; Lackmann, 1971; Mohr and Drumm-Herrel, 1981, 1983; Slabecka-Szweykowska, 1955; Wellmann, 1983; Wellmann et al., 1976). In several of these systems, the UV–B portion (290–320 nm) of the ultraviolet spectrum is very effective. Studies with these systems have provided most of the data suggesting an interaction between cryptochrome and phytochrome (Downs and Siegelman, 1963; Mohr and Drumm-Herrel, 1981, 1983; Wellmann, 1983).

The spectral sensitivity of anthocyanin production in some other systems seems to be intermediate between the groups described above. Examples: *Spirodela oligorrhiza,* with peaks of action at 300 and 700 nm (Ng et al., 1964); rye and tomato seedlings with maximum action in the UV–Bl region as in *group III,* but with some action in the R and FR (Drumm-Herrel and Mohr, 1982a, 1982b; Mancinelli and Schwartz, 1984; Mancinelli and Walsh, 1979; Rabino et al., 1977).

The experimental conditions used in studies of the wavelength dependence of anthocyanin production in different species (Fig. 4) vary quite widely. Let's mention just one factor, the duration of the irradiation: it varies from the 4 hours (terminated by a short exposure to R and followed by a 24 hour incubation in dark) used in red cabbage seedlings (Siegelman and Hendricks, 1957) to the 12 hours used in apple skin (Siegelman and Hendricks, 1958) to the several days used in bean seedlings (Klein et al., 1957). The significance of the differences in the spectral sensitivity of

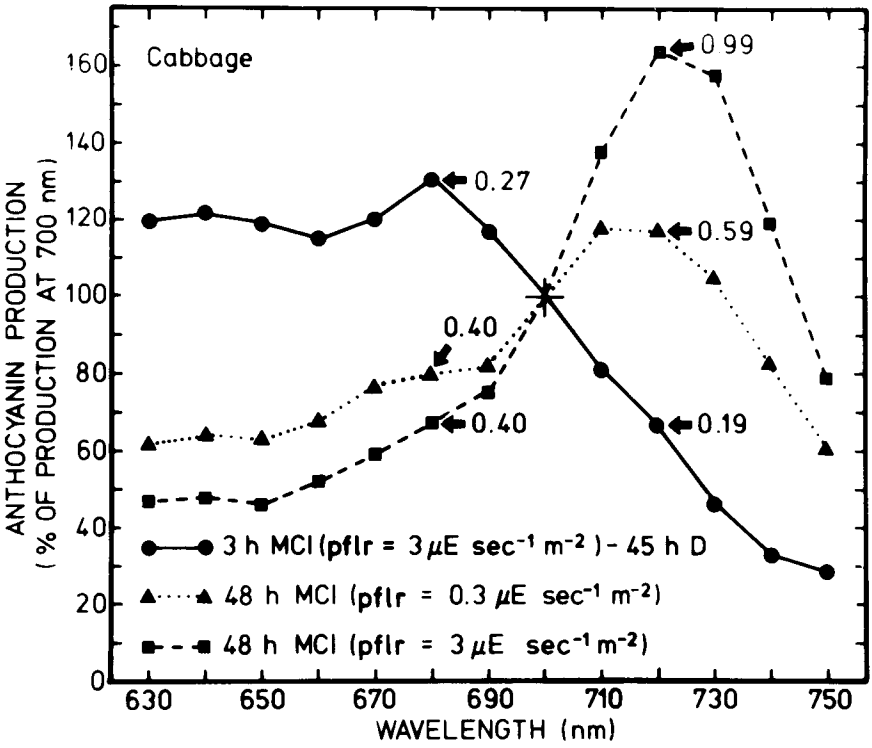


Fig. 5. Wavelength dependence of anthocyanin production in cabbage seedlings exposed to prolonged irradiation. MCI: monochromatic irradiation; pflr: photon fluence rate; E: Einstein (mole of photons). Numbers are the mean absorbance values of extracts from seedlings exposed to 680 and 720 nm monochromatic radiation.

anthocyanin production in different species (Fig. 4) is difficult to evaluate because the results of various studies indicate that the spectral sensitivity of anthocyanin production and other HIR responses in a given species can be affected by various factors: duration, fluence rate and mode of application (e.g., continuous versus intermittent with short and long cycles) of the light treatments, age, previous exposure to light, temperature and nutritional conditions (Alfermann, 1972; Beggs et al., 1980; Black and Shuttleworth, 1974; Evans et al., 1965; Grill, 1967; Grill and Vince, 1966; Jose and Vince-Prue, 1977b; Mancinelli and Rabino, 1978; Mancinelli and Walsh, 1979; Rabino et al., 1977; Wagner and Mohr, 1966). Let's use a few examples to illustrate the effect of various factors on the spectral sensitivity of anthocyanin production.

The position of the peak of action for anthocyanin production in cabbage seedlings exposed to continuous monochromatic irradiation (MCI) in the long wavelength region shifts from the R to the FR, depending on

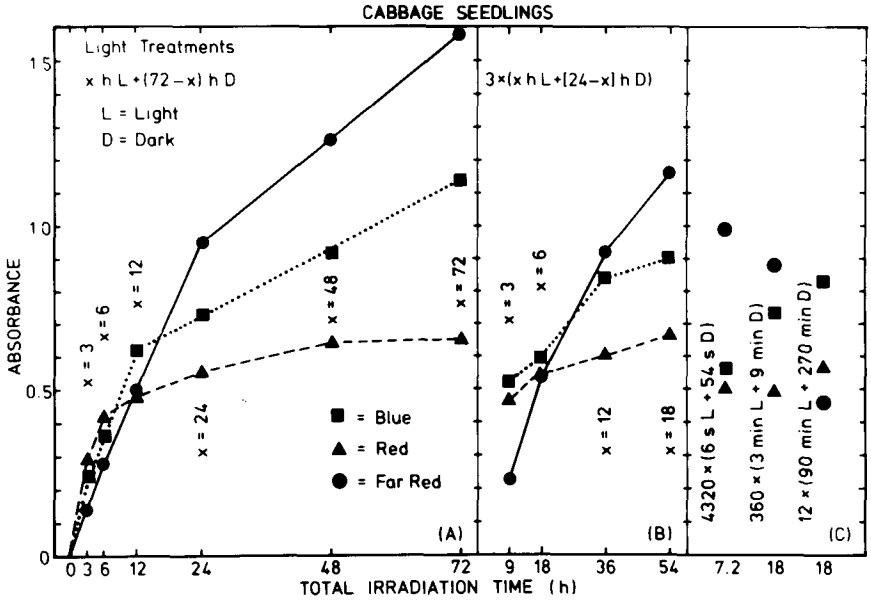


Fig. 6. Effects of duration and mode of application of blue, red and far red light treatments on anthocyanin production in cabbage seedlings. Redrawn from original data of Mancinelli and Walsh, 1979.

the duration of the light treatments (Fig. 5). Under the "3 hour MCI + 45 hour D" treatment, the wavelength dependence of anthocyanin production is similar to that reported by Siegelman and Hendricks (1957; treatment was 4 hour MCI + 5 minute R + 24 hour D); but, under the "48 hour MCI" treatment, the wavelength dependence of anthocyanin production in cabbage seedlings becomes much more similar to that reported for turnip (Grill and Vince, 1970; Siegelman and Hendricks, 1957) and mustard (Mohr, 1957). In both cabbage and tomato seedlings, the values of the FR/BL and FR/R effectiveness ratios increase with the duration of continuous irradiation (Figs. 6A, 7A) and of daily light treatments (Figs. 6B, 7B). Under short-cycled, intermittent light treatments (Figs. 6C, 7C), the values of the FR/R and FR/BL effectiveness ratios decrease with increasing duration of the dark interval between successive irradiations.

The spectral sensitivity of anthocyanin production is affected by seedling age (Fig. 8), as observed for other HIR responses (Black and Shuttleworth, 1974; Evans et al., 1965). In the case of anthocyanin production in tomato (Fig. 8), the values of the R/BL and R/UV effectiveness ratios show a marked decrease with increasing age.

Exogenously applied sugars, phenylalanine and acetate enhance antho-

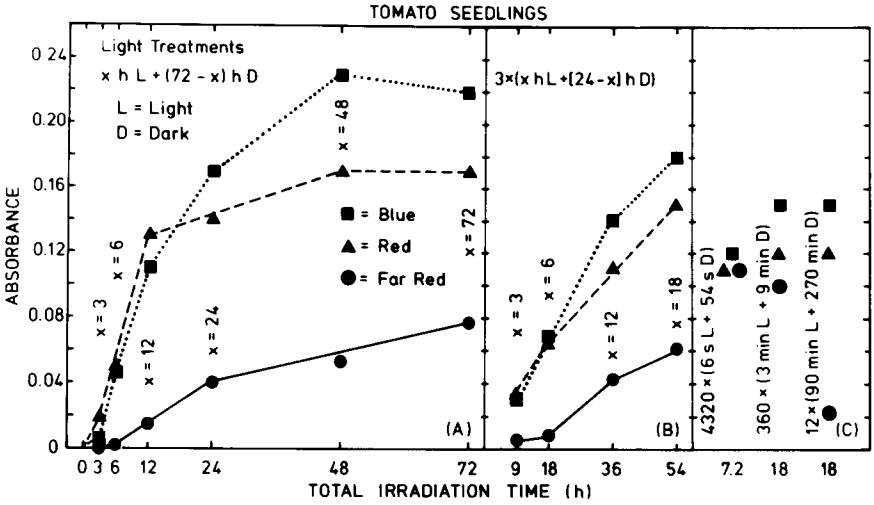


Fig. 7. Effects of duration and mode of application of blue, red and far red light treatments on anthocyanin production in tomato seedlings. Redrawn from original data of Mancinelli and Walsh, 1979.

cyanin production in cabbage and turnip seedlings (Grill and Vince, 1966; Huang-Yang, 1976) and in *Spirodela oligorrhiza* (Thimann et al., 1951). Sucrose affects the extent and spectral sensitivity of anthocyanin production in *Spirodela polyrrhiza* and cabbage leaf disks (Fig. 9). Norflurazon, an inhibitor used quite commonly to obtain chlorophyll-poor, light-grown systems (section IV.b), causes a considerable change in the spectral sensitivity of anthocyanin production in cabbage and tomato seedlings and in *Spirodela polyrrhiza* (Figs. 9, 10).

The spectral sensitivity of anthocyanin production in cabbage leaf disks cut from the leaf of adult, greenhouse-grown plants, with no action in the FR (Fig. 9), is quite different from that of the young seedlings of the same species (Fig. 8), where FR is the most effective region.

The extent and the spectral sensitivity of anthocyanin production are affected by previous exposures to light (Fig. 10), as observed for other HIR reponses (Beggs et al., 1980; Drumm et al., 1975; Holmes and Schäfer, 1981; Jose and Vince-Prue, 1977a). Note that the differences in the spectral sensitivity of anthocyanin production between cabbage and tomato are much more pronounced in the dark-grown than in the light-pretreated seedlings (Fig. 10).

The majority of the data available for the spectral sensitivity of anthocyanin production (Fig. 4) and other HIR responses (summarized in Mancinelli and Rabino, 1978) provide information for species X under condition X, for species Y under condition Y, for species Z under condition

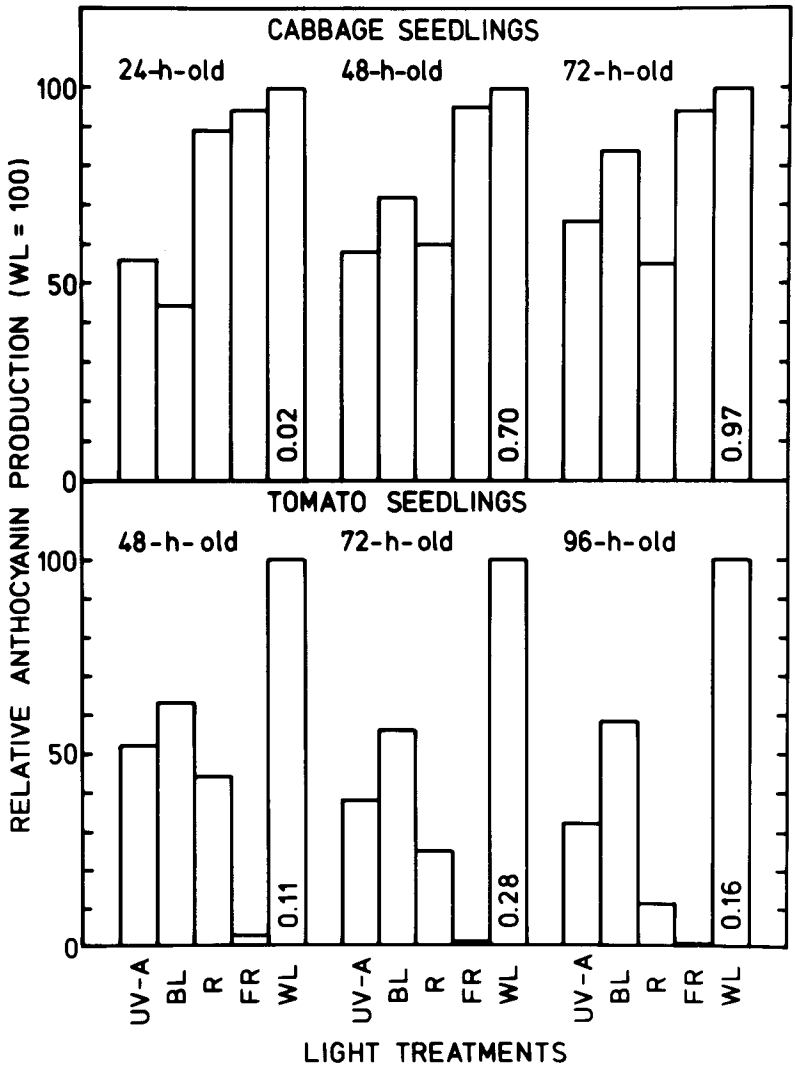


Fig. 8. Effect of seedling age on the spectral sensitivity of anthocyanin production in cabbage and tomato seedlings. Duration of the light treatments: 24 hours. Numbers inside the bars are mean absorbance values of extracts prepared from seedlings exposed to WL. Incubation medium: water. Redrawn from data of Mancinelli, 1984 and Mancinelli and Schwartz, 1984.

Z, etc., without providing comparative data for species X under conditions Y and Z, for species Y under conditions X and Z, etc. The observed effects of various factors on the spectral sensitivity of the HIR suggest that a great deal of caution should be exercised when using data such as those

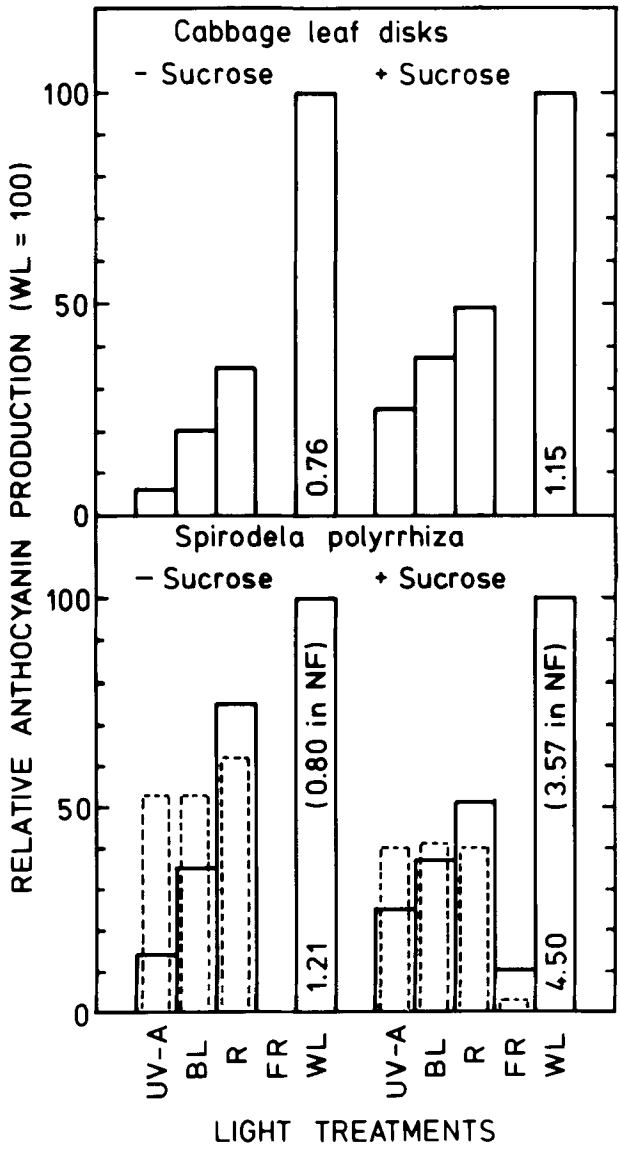


Fig. 9. Effects of sucrose and Norflurazon on the spectral sensitivity of anthocyanin production in cabbage leaf disks and *Spirodela*. Duration of the light treatments: 48 hours. Numbers inside the bars are mean absorbance values of extracts prepared from samples exposed to WL. Incubation media: water, 10 μM Norflurazon (NF, dashed-line bars) and 1% (w/v) sucrose. Redrawn from data of Mancinelli, 1984.

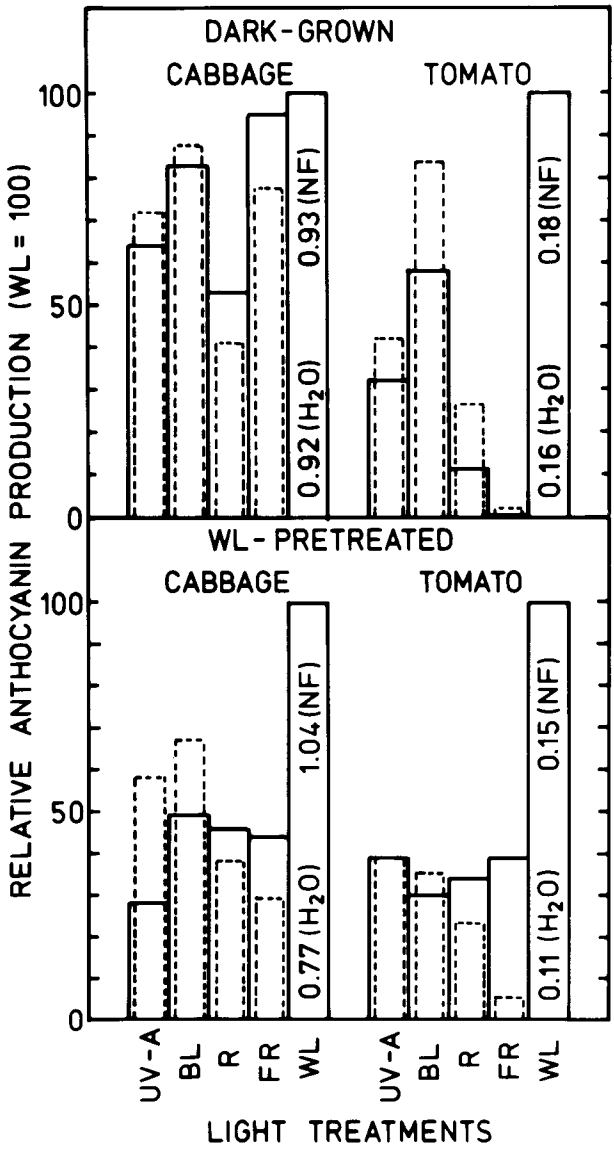


Fig. 10. Effects of white light pretreatments and Norflurazon on the spectral sensitivity of anthocyanin production in cabbage and tomato seedlings. Duration of light treatments: 24 hours. Numbers inside bars are average absorbance values of extracts prepared from seedlings exposed to WL. Incubation media: water (solid-line bars) and 5 μM Norflurazon (NF, dashed-lined bars). Redrawn from data of Mancinelli, 1984 and Mancinelli and Schwartz, 1984.

of Figure 4 to draw conclusions on the nature and state of the photoreceptors in different species, but they are not yet sufficient to allow a definitive evaluation of the significance of the reported differences. On this basis, the classification of HIR responses into three groups of spectral sensitivity must be considered valid only as a first approximation and subject to many limitations: it will have to be revised continuously as more data become available.

VIII. Photoreceptors and the Photoregulation of Anthocyanin Production and Other HIR Responses

In this section, we shall try to provide some plausible hypotheses to explain the properties (spectral sensitivity, fluence rate-dependence, differences in photosensitivity) of anthocyanin production and other HIR responses. Many factors must be considered, not all of them photoreceptor-related.

Many steps separate the final expression of the response from the initial light absorption act (Fig. 1). The availability of action sites for the photoreceptors might or might not be a limiting factor, depending on the species or the state of the system (e.g., age, previous exposure to light or not, temperature, etc.). (Note: the identity of the action sites is still unknown; the existence of action sites is a basic postulate: the photoreceptors must interact with some cell component to exert their action.) In different species, the rates of biochemical reactions involved in the same response might show differing sensitivities to temperature, nutritional conditions, pH, etc. In different species, or in the same species under different conditions, there might be differences in the degree of competition for a common precursor between the metabolic pathway of the response under study and other biochemical processes. In conclusion, numerous factors can affect the final expression of the response, independently of the primary effect of light on the photoreceptors.

The concentration of phytochrome is different in different parts of the seedling and in different species (Briggs and Siegelman, 1965). Rates of phytochrome destruction are different in different species (Frankland, 1972; Schäfer et al., 1975, 1976). Concentration of phytochrome, rates of phytochrome photoconversion and destruction, labile to stable Pfr ratio are affected by several factors (see section IV) and the extent of the changes may be different in different species.

In 1959, Hendricks and Borthwick (1959a, 1959b) suggested that phytochrome was involved in the photoregulation of HIR responses and that the characteristics of the HIR were a manifestation of the continuous excitation of phytochrome. Their suggestion provided the basis for further elaboration by several researchers, resulting in the development of several

models for phytochrome action in the photoregulation of the HIR (Borthwick et al., 1969; Fukshansky and Schäfer, 1983; Gammerman and Fukshansky, 1974; Hartmann, 1966; Johnson and Tasker, 1979; Mancinelli and Rabino, 1975; Mohr, 1972; Schäfer, 1975; Smith, 1975). At present, there seems to be no doubt that phytochrome, besides being responsible for the photoregulation of the inductive, R–FR reversible responses, is also involved in the photoregulation of the HIR and that it is the only photoreceptor involved in the photoregulation of HIR responses elicited by prolonged exposures to R and FR radiation.

It has been suggested that the action of prolonged exposures to UV and BL might be mediated by phytochrome only (Hartmann, 1966; Hartmann and Cohnen-Unser, 1973), but more and more evidence is accumulating for the involvement of a specific UV–BL light photoreceptor (Drumm and Mohr, 1978; Koornneef et al., 1980; Mohr and Drumm-Herrel, 1981, 1983; Schäfer and Haupt, 1983). It is not easy to demonstrate the action of cryptochrome in systems in which phytochrome is also active, because (a) phytochrome absorbs UV–BL radiation (Butler et al., 1964; Pratt and Butler, 1970), (b) Pfr may be required for the expression of cryptochrome-mediated responses (Drumm and Mohr, 1978; Mohr and Drumm-Herrel, 1981, 1983; Schäfer and Haupt, 1983), and (c) the identity of cryptochrome is not yet known. The responses to UV–BL may be mediated by phytochrome or by cryptochrome or by both, either interacting or not.

The nature of the processes involved in the interaction between cryptochrome and phytochrome is unknown. Several alternatives can be considered: (a) a direct interaction, perhaps in the nature of an energy transfer, as with that shown by Sarkar and Song (1982) between flavin and phytochrome in solution; (b) the response is controlled by both photoreceptors, independently of each other; (c) the response is controlled by phytochrome, but a cryptochrome-mediated action of light can affect the sensitivity of the response to Pfr, as suggested by studies on the photoregulation of anthocyanin production in sorghum, wheat, rye and tomato (Downs and Siegelman, 1963; Drumm and Mohr, 1978; Mohr and Drumm-Herrel, 1981, 1983); or (d) the response is controlled by cryptochrome (e.g., phototropism), but Pfr can affect the sensitivity of the cryptochrome-mediated response (Briggs and Chon, 1966; Chon and Briggs, 1966).

VIII.A. SPECTRAL SENSITIVITY

In the species in which anthocyanin production under continuous irradiation shows a *group I* HIR spectral sensitivity (action in the UV, BL, R and FR), phytochrome action seems to be predominant in the photoregulation of the response. No action of UV–BL on anthocyanin produc-

tion in cabbage and mustard seedlings has been found that cannot be explained on the basis of the known properties of phytochrome (Mohr and Drumm-Herrel, 1981; Mancinelli and Walsh, 1979; Rabino et al., 1977). Cryptochrome might be involved in the photoregulation of anthocyanin production in turnip seedlings exposed to prolonged BL radiation (Grill and Vince, 1965, 1966; Vince and Grill, 1966). The effect of BL pretreatments on the sensitivity of the response to light pulses (Grill, 1965) is very similar to that observed in tomato (Drumm-Herrel and Mohr, 1982a, 1982b).

The spectral sensitivity characteristics of anthocyanin production in the systems with a *group II* HIR spectral sensitivity (action in the UV, BL, R, with maximum action in the R; nil or limited action in the FR) are possibly a reflection of two factors: (a) a low phytochrome content; consequently, the level of Pfr maintained under FR might be too low for significant action; and (b) the requirement for photosynthetic activity for the expression of the response. Perhaps the spectral sensitivity of *group II* reflects the spectral sensitivity of photosynthesis more than that of the photomorphogenic pigments. Not enough detail for anthocyanin production in these systems is available to permit an educated guess about the possible involvement of cryptochrome in the mediation of the response to UV–BL radiation.

In the systems with a *group III* HIR spectral sensitivity (action in the UV and BL, no action in R and FR), there is a clear indication that cryptochrome is involved in the photoregulation of anthocyanin production under continuous UV–BL irradiations. For example, in sorghum, a species used in many studies (Downs and Siegelman, 1963; Drumm and Mohr, 1978; Drumm-Herrel and Mohr, 1981; Mohr and Drumm-Herrel, 1981, 1983), the UV–BL light photoreaction is independent of phytochrome, as suggested by the observation that the reduction of the level of Pfr by simultaneous irradiation with FR has no effect on the response (Drumm and Mohr, 1978). However, the final expression of the UV–BL light effect requires Pfr and the development of the sensitivity of anthocyanin production to Pfr is obligatorily dependent upon a previous exposure to UV–BL. The fact that prolonged exposures to long wavelength radiation (R & FR), even after exposure to UV–BL radiation, are not very effective in bringing about anthocyanin production suggests that the cryptochrome-mediated development of sensitivity to Pfr might be of a transient nature; perhaps, to maintain sensitivity to Pfr, UV–BL must be applied continuously or very frequently.

In rye and tomato seedlings, two species with a spectral sensitivity intermediate between *groups I* and *III* (Drumm-Herrel and Mohr, 1982a, 1982b; Mancinelli and Walsh, 1979; Mohr and Drumm-Herrel, 1981, 1983; Rabino et al., 1977), the sensitivity to Pfr is strongly enhanced by

a previous exposure to light; UV–BL pretreatments are more effective than R and FR ones in establishing sensitivity toward Pfr (Drumm-Herrel and Mohr, 1982a, 1982b; Mohr and Drumm-Herrel, 1981, 1983). Apparently, in these two species, the action of light in the development of sensitivity to Pfr for anthocyanin production can be mediated through both photoreceptors, with cryptochrome being the most effective of the two. In mustard seedlings, a species with a *group I* spectral sensitivity, the light-dependent enhancement of sensitivity to Pfr for anthocyanin production seems to be mediated exclusively, or mostly, by phytochrome (Mohr and Drumm-Herrel, 1981). The reader is referred to two recent reviews (Schäfer and Haupt, 1983; Wellmann, 1983) for a detailed discussion of the action of UV–BL radiation in photomorphogenesis.

In conclusion, it seems that phytochrome is involved in the photoregulation of anthocyanin production in all the species studied so far. In some species, phytochrome seems to be the only photomorphogenic receptor involved in the photoregulation of the response. In others, both cryptochrome and phytochrome are involved: Pfr can exercise its action only after the system has been activated by a cryptochrome-mediated process and, at the same time, the expression of the cryptochrome-mediated effect requires Pfr.

VIII.B. FLUENCE RATE-DEPENDENCE

The fluence rate-dependence of the HIR has not been satisfactorily explained. Apparently, it may be explained intuitively, in a relatively simple way, as a consequence of the interaction between the light and dark reactions of phytochrome (see section IV): under prolonged irradiations, the value of the Pfr/P ratio at photoequilibrium and the level of Pfr are a function of both. Starting from this simple observation and with the addition of a reaction between phytochrome and the unknown reaction partner X (Fig. 1), various models have been developed (Hartmann, 1966; Fukshansky and Schäfer, 1983; Johnson and Tasker, 1979; Schäfer, 1975).

In Hartmann's (1966) model, the fluence rate-dependence of the HIR is explained as the consequence of the fluence rate-dependence for the formation of a short-lived, overcritically excited form of Pfr. In Schäfer's (1975) model, the fluence rate-dependence of the HIR is explained as a consequence of the fluence rate-dependence of the formation of a short-lived PfrX complex, decaying to PfrX′ with a half-life of about 1 minute; PfrX is considered the effector for HIR responses and PfrX′, which is wavelength and fluence rate-independent and is longer-lived than PfrX, is considered the effector for the inductive, R–FR reversible responses. In a recent modification of this model (Fukshansky and Schäfer, 1983) the X is gone; PrX, PfrX, PrX′ and PfrX′ have been replaced by *Pr*,

Pfr, (Pr) and (Pfr), respectively. The stationary solutions for *Pr*, *Pfr*, (Pr) and (Pfr) are the same as those for PrX, PfrX, PrX′ and PfrX′ in the old model. Apparently the rate constants used are also the same as in the old model. In the modified model, *Pfr* (fluence rate- and wavelength-dependent) is a transient form, decaying to (Pfr) with a half-life of about 1 minute, and considered to be the effector for HIR responses, while (Pfr), which is wavelength- and fluence rate-independent and is longer-lived than *Pfr*, is considered to be the effector for the inductive, R–FR reversible responses. According to these two models, the formation of *Pfr* and PfrX under prolonged irradiation should be strongly fluence rate-dependent under FR and weakly fluence rate-dependent under R.

The predictions of these two models are not consistent with the results of physiological experiments which show a strong fluence rate-dependence of anthocyanin production and other HIR responses under continuous R (Beggs et al., 1980; Holmes and Schäfer, 1981; Johnson, 1980; Jose and Vince-Prue, 1977b; Klein et al., 1957; Lange et al., 1971). In another model (Johnson and Tasker, 1979; Wall and Johnson, 1983), the fluence rate-dependence of the HIR is considered a function of the cycling rate between Pr and Pfr; this model predicts a fluence rate-dependence of the physiological responses under continuous R, but its validity has been criticized (Fukshansky and Schäfer, 1983) because of a possible error in the equation for the rate of cycling. An action of cycling can not be ruled out, but further analysis is required.

One common aspect of these various models is that they predict that the fluence rate-dependence of the HIR is a function of a fluence rate-dependent, short-lived component (overcritically excited Pfr, Hartmann, 1966; transient PfrX, Schäfer, 1975; transient *Pfr*, Fukshansky and Schäfer, 1983; cycling rate between Pr and Pfr, Johnson and Tasker, 1979). This prediction does not agree with results of physiological experiments which show that continuous irradiations can be fully or almost fully replaced by intermittent ones, extended over the same duration and of equal total radiation fluence (Heim and Schäfer, 1982; Ku and Mancinelli, 1972; Mancinelli and Rabino, 1975; Rabino et al., 1977; Schäfer et al., 1981), and that the extent of the response under intermittent light treatments is a function of dose (fluence rate × time), not of fluence rate (Mancinelli and Rabino, 1975; Rabino et al., 1977). Under intermittent light treatments, the transient, short-lived effector would be present only during the brief exposures and for only a short time thereafter. Thus, either the transient effector has a much longer half-life than that used in the models or the transient effector plays only a minor role in the photoregulation of the HIR.

Large discrepancies exist between the predictions of the models (Schäfer, 1975) and the results of physiological experiments insofar as action

in the UV–BL region is concerned. Obviously, any theoretical model dealing with the mechanism of photoregulation of the HIR under continuous UV–BL irradiation must take into consideration the possible involvement of cryptochrome and the interaction between cryptochrome and phytochrome. This complicates the theoretical treatment and cannot be attempted at present because of the scarcity of knowledge about the nature of cryptochrome and of the interaction between cryptochrome and phytochrome.

In conclusion, it seems that one can explain the fluence rate-dependence of the HIR, in an intuitive way, on the basis of the interaction between the light (photoconversion) and dark (synthesis, destruction, reversion, interaction with X) reactions of phytochrome. However, the theoretical development of this intuitive interpretation has failed to provide a satisfactory explanation for the fluence rate-dependence of the HIR, fully consistent with the results of physiological studies. In addition, it must be noted that in even the most recent developments in the theoretical models (Fukshansky and Schäfer, 1983; Wall and Johnson, 1983) little attention is paid to the possible effects of changes in the labile to stable Pfr ratios and in the rates of phytochrome photoconversion, synthesis and destruction (see section IV).

VIII.C. CHANGES IN RESPONSE PHOTOSENSITIVITY

The extent and spectral sensitivity of anthocyanin production and other photoresponses can be affected by numerous factors, as shown in previous sections. As we have said before (see section VIII), changes in the photosensitivity of the response might be brought about not only by changes in photoreceptor state (e.g., changes in phytochrome content, changes in the rates of photoconversion, destruction, reversion and synthesis, changes in the stable to labile Pfr ratio), but also by changes in other factors, such as the state of the transduction chain and the state of the metabolic pathways involved in the response.

The effects of the duration of light treatments on the sensitivity to R, BL and FR (Fig. 6, A; R > BL > FR for 3 to 6 hour irradiations; BL > R = FR for 12 hour irradiations; FR > BL > R for 24 hour and longer irradiations) of anthocyanin production in cabbage seedlings correlate quite well with the changes in [P] and [Pfr] under continuous irradiation with BL, R and FR (Rabino et al., 1977). Under the short-cycled intermittent light treatments (Fig. 6C), FR is more effective than R and BL when the cycles are short and less effective when the cycles are long. This is probably a reflection of the decay of Pfr. When the dark interval between successive irradiations is long, [Pfr] under intermittent FR might be lower than that required for the full expression of the response. Note that even

though the spectral sensitivity of anthocyanin production in cabbage and tomato is quite different, the trend of change in the relative efficiency of BL, R, and FR is similar in the two species (Figs. 6, 7); this suggests that the factors responsible for the changes in the relative efficiency of BL, R and FR might be the same in the two species.

Exposure to light pretreatments affects the spectral sensitivity of anthocyanin production (Fig. 10). Light-pretreated seedlings have a higher stable to labile phytochrome ratio and a lower phytochrome content than dark-grown seedlings (section IV). It does not seem unreasonable that, as a consequence of these changes, the optimal (insofar as the photoreceptors are concerned) conditions for anthocyanin production might shift from one spectral region to another.

Mustard seedlings exposed to light from sowing onward do not start producing anthocyanin until about 27 hours after sowing, even though the phytochrome system is fully functional immediately after sowing (Mohr et al., 1979; Steinitz et al., 1976). This behavior is probably a reflection of time and light-dependent changes in the responsiveness of cell functions (state of the transduction chain?) on which phytochrome acts (Schmidt and Mohr, 1981b). Perhaps, some factors required for the coupling of phytochrome to the successive steps might be missing or inactive during the early phases of seedling growth.

In young cabbage and tomato seedlings the capability for anthocyanin production, after reaching a maximum, decreases with increasing seedling age (Mancinelli, 1984; Mancinelli and Schwartz, 1984). A decrease in the pool of metabolic precursors might, perhaps, be responsible for the decreased capability for anthocyanin production.

Light pretreatments have a differential effect on the sensitivity of anthocyanin production (Table II) and other photoresponses to short and prolonged light treatments applied immediately after the end of the light pretreatment: the inductive, R–FR reversible response is enhanced while the response to prolonged irradiations is decreased (Beggs et al., 1980, 1981; Drumm et al., 1975; Mohr and Drumm-Herrel, 1981, 1983; Mohr et al., 1979; Schmidt and Mohr, 1981b). It has been suggested that the decrease in the extent of the response to continuous irradiation in light-pretreated seedlings might be a consequence of the light-dependent decrease in phytochrome content (Beggs et al., 1980; Drumm et al., 1975); it is difficult to explain why a decrease in phytochrome content should result in a differential action on the extent of the inductive, R–FR reversible (increase) and HIR (decrease) responses. A possible interpretation for this differential effect (Mohr et al., 1979) is that there might be differences in the initial action of Pfr under inductive and HIR conditions, as suggested in some of the models for phytochrome action discussed previously (Fukshansky and Schäfer, 1983; Schäfer, 1975).

IX. Concluding Remarks

Studies of light-dependent anthocyanin synthesis have provided a great deal of information about several of the basic properties of photomorphogenic responses, especially those requiring prolonged exposures to high fluence rates of visible and near visible radiation, the HIR responses. During the six years since the publication of a review on the HIR (Mancinelli and Rabino, 1978), the factual knowledge about this class of photoresponses has considerably increased. However, the capability for explaining the properties (spectral sensitivity, fluence rate-dependence, changes in photosensitivity) of the HIR, as determined in physiological studies, on the basis of the known properties of the photoreceptors, has not increased in a way comparable to the increase in factual knowledge.

At present, there is very little doubt that phytochrome, besides being involved in the photoregulation of the inductive, R–FR reversible responses elicited by short exposures to light, is also involved in the photoregulation of the HIR. There is also very little doubt that phytochrome is the only photoreceptor involved in the photoregulation of HIR responses elicited by prolonged exposures to R and FR radiation. The results of several studies suggest that another photomorphogenic pigment, cryptochrome, may be involved in the photoregulation of responses elicited by prolonged exposures to UV and BL radiation. In all the systems in which a specific, cryptochrome-mediated effect on anthocyanin production in response to prolonged UV and BL radiation has been reasonably proved (Mohr and Drumm-Herrel, 1981, 1983), it has also been found that the final expression of the UV–BL-light-mediated response requires Pfr. The task of demonstrating a specific cryptochrome-mediated action of UV–BL radiation in systems in which phytochrome is also active is rather difficult and the situation is not helped by the fact that up to now cryptochrome cannot be measured directly and its identity is still unknown.

The effect of changes in phytochrome content, rates of phytochrome photoconversion and destruction, labile to stable phytochrome ratios on the expression of the response are still poorly understood. There are several reasons for this: (a) spectrophotometric measurements of phytochrome in vivo have several limitations [low sensitivity, possibility of artifactual data due to differences in light scattering of the samples, screening by other pigments, non-homogeneous pigment distribution, fluorescence induced in the sample by the spectrophotometer measuring beams and theoretical difficulties in the interpretation of the measurements (Fukshansky, 1978; Fukshansky and Kazarinova, 1980; Pratt, 1983)]; the immunological assays, although more sensitive than the spectrophotometric ones, can only partially discriminate between Pr and Pfr (Thomas et al.,

1984); (b) the nature of the first molecular changes induced by Pfr in the responding system is unknown; (c) the nature of the cell components which interact directly with Pfr is unknown; (d) the structure of phytochrome is not completely known (e.g., knowledge of the amino acid sequence is limited to about 1% of the length of the protein; the interaction between the protein moiety and the chromophore has not been defined); and finally, (e) the physiological importance and the mechanism of phytochrome destruction, sequestering and pelletability are still unclear.

Insofar as the results of physiological experiments are concerned, many of the data obtained in different species are not easily comparable because of large differences in the experimental conditions used and it is difficult, at present, to determine if the differences in the basic characteristics of the HIR (spectral sensitivity, fluence rate dependence, etc.) of different response-species combinations are species-related, response-related or experimental-conditions-related.

The path for future research is clear. To understand the mechanism of action of the photomorphogenic receptors at the molecular level, we need to improve our knowledge of: (a) the structure of the protein moiety of phytochrome, the interaction between protein moiety and chromophore, the differences between Pr and Pfr and the differences between the phytochrome of dark-grown and light-grown plants; (b) the identity of cryptochrome, the effects of light on the state of cryptochrome in the cell, etc., and (c) the nature of the cell components that interact directly with the active forms of the photoreceptors and the nature of the first molecular change induced in the responding system.

At the theoretical level, it is necessary to develop models for phytochrome action that are fully consistent with all the available experimental data. The present models are not much help in either understanding the mechanism of action of phytochrome or in planning future experiments. At the technical level, we need further development of the techniques for phytochrome assays; reliability and sensitivity of in vivo spectrophotometric assays probably cannot be improved much, although it may be possible to improve the interpretation of data with a development of the theoretical treatment of spectrophotometric assays. The advantages to the development of immunological assays capable of full discrimination between Pr and Pfr are obvious. At the genetic level, extending studies such as those of Koornneef et al. (1980) in *Arabidopsis thaliana* to other species might prove very helpful in providing evidence for the involvement of different photoreceptors in the photoregulation of plant development. There is still a great deal to be done at the physiological level: (a) physiological studies must be carried out to test the physiological significance of the hypotheses suggested by theoretical models; (b) physiological studies must be carried out to test and evaluate the physiological significance of

changes in phytochrome content, in the rates of the photoconversion and destruction processes and in the labile to stable phytochrome ratios; and (c) we need improve our knowledge of the comparative aspects of photomorphogenesis in different species, for different responses and for etiolated and light-grown systems.

In conclusion, while some progress has been made in the past few years, our understanding of plant photomorphogenic processes has not improved much and there is no shortage of research problems that must be dealt with.

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XII. List of Abbreviations

L: light; **D:** dark.

UV: ultraviolet; **UV-A:** 320–400 nm; **UV-B:** 290–320 nm.

BL: blue, 400–480 nm.

R: red, 600–690 nm.

FR: far red, 700–760 nm.

HIR: high irradiance reaction.

Pr: red absorbing form of phytochrome.

Pfr: far red absorbing form of phytochrome.

P: total phytochrome = Pr + Pfr.

CMU: monuron; 3-(p-chlorophenyl)-1,1-dimethylurea.

DCMU: diuron; 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

FMN: flavinmononucleotide.

NF: Norflurazon (SAN–9789); 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-m-tolyl)-3-(2H)-pyridazinone.