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Photoperception and De-Etiolation [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1983 **303**, 489-501
doi: 10.1098/rstb.1983.0109

Phil. Trans. R. Soc. Lond. B **303**, 489–501 (1983)
Printed in Great Britain

Photoperception and de-etiolation

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In seedlings or sprouts of higher plants, photomorphogenesis is the strategy of development if and as long as abundant light is available, and scotomorphogenesis (etiolation) is the developmental strategy of choice as long as light is not yet, or no longer, available. The transition from scotomorphogenesis to photomorphogenesis (called de-etiolation) can be considered a process in which a single, well defined environmental factor causes a plant to change its pattern of gene expression. The present article focuses on the question: what is the photosensory system, including photoreception and signal transduction, through which a plant can detect those light conditions that justify the (gradual) shift from scotomorphogenesis to photomorphogenesis, i.e. de-etiolation, which implies a strong and partly irreversible investment of matter and energy? The significance of phytochrome for signal reception, the mode of signal expression, and the time course of signal transduction in phytochrome-mediated responses are reviewed briefly. The emphasis is on amplification of the phytochrome signal by red, blue and ultraviolet light (measured as responsivity amplification) because these recent findings may lead to a better understanding of the responses of plants under natural light conditions.

Introduction

Every seedling or sprout of terrestrial plants is genetically endowed with the ability to follow two different strategies of development, depending on the ambient light conditions. These are photomorphogenesis and scotomorphogenesis (figure 1). Photomorphogenesis is the strategy of development if and as long as light is available, and scotomorphogenesis (etiolation) is the developmental strategy of choice as long as light is not yet, or no longer, available. The adaptive value of having different strategies is obvious. Scotomorphogenesis is the appropriate strategy of survival under conditions where light is lacking, whereas photomorphogenesis is the appropriate strategy of development under conditions of light affluence.

The plants in figure 1 illustrate extreme cases (dark compared with full sunlight). The appearance of a plant under conditions where light is *sometimes* limiting may show features of etiolation (e.g. elongated internodes) even though photomorphogenesis dominates the scene.

The transition from scotomorphogenesis to photomorphogenesis (called de-etiolation) can be considered a process in which a single, well defined environmental factor causes a plant to change its pattern of gene expression (Mohr 1972).

Photomorphogenesis and scotomorphogenesis can be distinguished not only on the level of the organism but also in intracellular morphogenesis of organelles. As an example, in the presence of light a proplastid develops into a green mature chloroplast whereas development in darkness follows a different strategy, leading to an etioplast. Upon irradiation with white light, rapid 'de-etiolation' of the etioplast takes place, i.e. the light-mediated transformation of the etioplast into a mature chloroplast occurs.

De-etiolation is, in part at least, reversible (re-etiolation). An example of re-etiolation is

dark-mediated plastid 'senescence', which takes place if a (mustard) plant is kept in darkness for more than 2 days. Dark-mediated plastid senescence, including loss of pigment and breakdown of fine structure, can be prevented by red light pulses, which operate through phytochrome (Biswal *et al.* 1983). Another example of re-etiolation is the phytochrome-controlled synthesis of phytochrome. During phytochrome-mediated de-etiolation the synthesis of phytochrome becomes suppressed (Gottmann & Schäfer 1983). However, this suppression persists only as long as P_{fr} is present (V. Otto, personal communication).

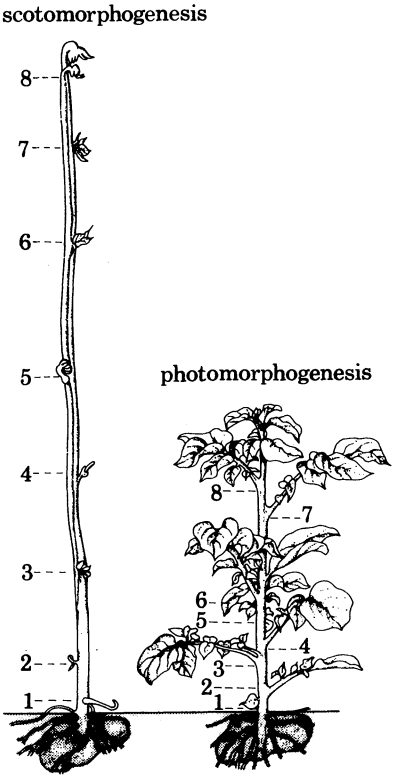

FIGURE 1. Illustrations of alternative developmental strategies. Genetically identical potato plants (*Solanum tuberosum* L.) were grown in the dark (scotomorphogenesis) or in natural daylight (photomorphogenesis) (after Pfeffer 1904). The numbers indicate the position of the corresponding leaves along the main axis to document the constancy of the phyllotactic pattern in light and dark (see Mohr 1978).

In order to respond properly in the delicate interplay between scotomorphogenesis and photomorphogenesis a plant has to sense the light conditions in its environment precisely. This paper will focus on the question: What is the photosensory system, including photoreception and signal transduction, through which a plant can detect those light conditions that justify the (gradual) shift from scotomorphogenesis to photomorphogenesis, i.e. de-etiolation, which implies a strong and partly irreversible investment of matter and energy?

SENSOR PIGMENTS

With regard to de-etiolation (excluding phototropism) higher plants possess three sensor pigments: phytochrome, a blue u.v.-A light photoreceptor ('cryptochrome') and a specific u.v.-B photoreceptor (see Mohr 1983). The action spectrum related to the latter photoreceptor shows

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a single intense peak at 290 nm and no action at wavelengths longer than 350 nm (Yatsuhashi *et al.* 1982). It could be that the u.v.-B photoreceptor occurs widely but has been overlooked so far owing to experimental difficulties in working with u.v.-B. In any case, at least some plants (e.g. milo, *Sorghum vulgare* Pers.) are obviously capable of sensing the light conditions throughout the Sun's spectrum, i.e. as far as the sunlight leads to electronic excitations (290–800 nm) (Drumm-Herrel & Mohr 1981).

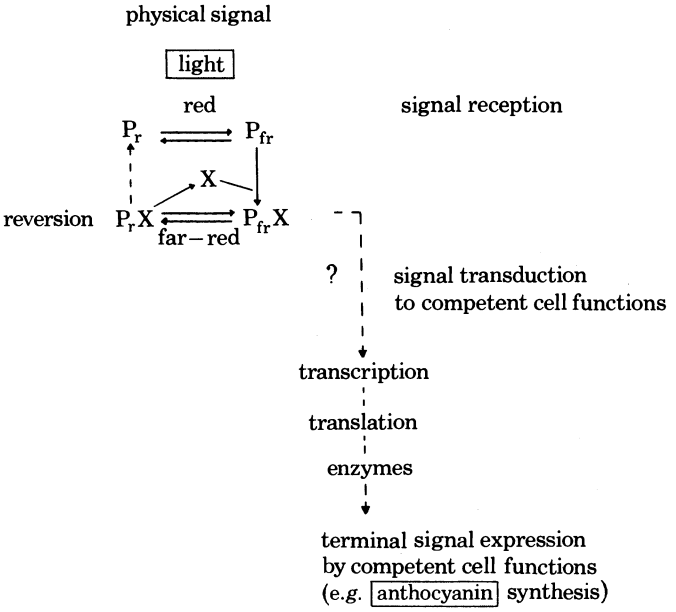

FIGURE 2. A scheme to illustrate the sequence of events occurring in the light-mediated synthesis of flavonoid compounds. The basic observation is that the environmental (physical) signal light leads to synthesis of particular pigment molecules, e.g. anthocyanin, in particular cells. The core of the explanatory argument is that terminal signal expression (i.e. the appearance of the final response, in the present case cyanidin, or flavone glucosides) is due to the induction of competent genes, i.e. to the onset of transcription. The premises that signal reception is taking place through phytochrome and that competent cells in a de-etiolating plant respond to the amount of P_{fr} (rather than to the P_{fr}/P_{tot} or P_{fr}/P_r ratio) are well substantiated (see Schmidt & Mohr 1982). The P_{fr} 'receptor', X, is largely hypothetical, and the mechanism of signal transduction from P_{fr} to the competent genes is still an enigma. On the other hand, the sequence of events between the induced appearance of RNA and the appearance of flavonoid compounds is well documented.

MODE OF SIGNAL EXPRESSION IN PHYTOCHROME-MEDIATED RESPONSES

As far as we know today, phytochrome is involved as a photoreceptor in all processes of de-etiolation in higher plants. For this brief review of what we know about the mode of action of phytochrome in bringing about de-etiolation we consider two 'biochemical model systems' of de-etiolation that have been investigated intensively in Freiburg, namely light-mediated synthesis of flavone glucosides in cell suspension cultures of parsely and light-mediated synthesis of juvenile anthocyanin in the epidermal cells of mustard (*Sinapis alba* L.) cotyledons. Regarding the 'molecular' mechanism of phytochrome action there is hardly any doubt that signal expression, i.e. the appearance of the flavonoid pigments, is due to the induction of competent genes (figure 2). Experimental evidence in favour of this scheme has been summarized repeatedly (see, for example, Hahlbrock *et al.* 1976; Schröder *et al.* 1979; Kreuzaler *et al.* 1983; Mohr 1982; Mohr & Schopfer 1977) and need not be repeated here. However, some recent data

obtained in crucial experiments to test the above scheme (figure 2) further will be briefly presented.

1. The claim that phytochrome-mediated induction of enzymes of the flavonoid pathway is due to synthesis *de novo* of enzyme protein was confirmed by immunotitration studies on phenylalanine ammonia-lyase and chalcone synthase (figure 3). The data show that under all experimental conditions enzyme activity of an extract is proportional to the amount of immunoresponsive material. No indication was found with the present assay of enzymically inactive but immunoresponsive material in extracts prepared from dark-grown seedlings.

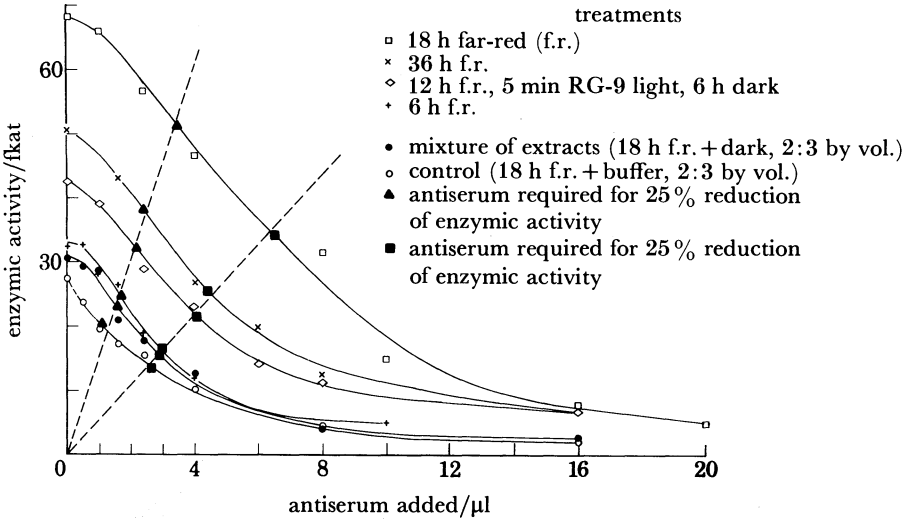

FIGURE 3. Immunotitration of chalcone synthase in extracts from mustard (*Sinapis alba* L.) cotyledons. Antiserum (kindly supplied by Professor K. Hahlbrock) was raised in rabbits by injections of highly purified chalcone synthase isolated from parsley cell suspension cultures (for details see Schröder *et al.* 1979). Control serum had no inactivating effect. Chalcone synthase activity is hardly detectable in extracts from dark-grown cotyledons. However, a strong phytochrome-mediated activity increase is observed in continuous far-red light with a peak around 18 h after the onset of light (36 h dark germination). The column headed 'treatments' designates the treatment of the seedling before extraction, and the preparation of mixed extracts to test for immunoresponsive material in an extract from dark-grown seedlings. The assay for chalcone synthase (see Heller & Hahlbrock 1980) was performed after the addition of antiserum, incubation (30 min at 30 °C, 20 min in the cold), and centrifugation (10 min at 12000 rev. min^{-1}). The broken lines show that the amount of antiserum required to reduce enzyme activity by 25 or 50% is directly proportional to the activity of the original extract. Results shown are per cotyledon pair. (Data obtained by R. Brodenfeldt.)

2. The Hahlbrock group (Kreuzaler *et al.* 1983) has shown in parsley cell suspension cultures that light-mediated changes in the amount of chalcone synthase mRNA coincides with the light-mediated changes of chalcone synthase synthesis *in vivo* and *in vitro*. The data are consistent with the hypothesis that induction of chalcone synthase by light is due to a transient increase in the rate of synthesis of chalcone synthase mRNA.

3. Regarding de-etiolation of the plastid, i.e. etioplast–chloroplast photoconversion, it was shown by Apel (1979) in barley and by Tobin (1981) in duckweed that light (via phytochrome) mediates specifically the appearance in the cytosol of a prominent mRNA species which codes for the apoprotein of the light-harvesting chlorophyll *a*/*b* protein, LHCP, located within the thylakoid membrane. While continuous chlorophyll synthesis is required for the incorporation of the apoprotein into the thylakoid membrane, appearance of the mRNA in the cytosol is

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exclusively phytochrome-controlled. It seems that in the absence of chlorophyll *a* the apoprotein of LHCP is unstable and subjected to rapid degradation (Apel & Kloppstech 1980).

Link (1982) has demonstrated with mustard seedlings, by means of hybridization of mRNA to cloned plastid DNA, that the phytochrome-mediated appearance of a prominent protein of the thylakoid membrane (32 kDa 'shielding' polypeptide of photosystem II) is due to a rise of the level of the pertinent mRNA transcribed from plastid DNA.

In brief: the mechanism of signal expression in 'positive' responses such as phytochrome-mediated flavonoid biosynthesis and plastid genesis seems to be settled: phytochrome causes – in some way or other – activation of competent genes.

Signal expression in 'negative' responses might become understandable along the same lines. As examples, rapid phytochrome-mediated *decreases* in mRNA activities have been observed in the phytochrome-mediated decline of synthesis of protochlorophyllide oxidoreductase (Apel 1981) and phytochrome apoprotein (Gottmann & Schäfer 1983). Presumably, phytochrome causes – in some way or other – the inactivation of competent genes.

Signal perception in phytochrome-mediated responses

In crucial experiments on phytochrome-mediated anthocyanin synthesis in mustard cotyledons, evidence was obtained that a mustard seedling responds to the amount of P_{fr} and not to the P_{fr}/P_{tot} ratio (Schmidt & Mohr 1982). Thus, the unsolved problem is signal transduction. At least in flavonoid synthesis and the synthesis of some plastidal proteins, 'signal transduction' means the transduction of the P_{fr} signal to the competent genes. Unexpected data were obtained in studies of the time course of signal transduction and with regard to signal amplification during transduction.

Time course of signal transduction in P_{fr}-mediated responses

It was found in phytochrome action on chlorophyll (Chl) synthesis (Oelze-Karow & Mohr 1982) as well as in phytochrome-mediated anthocyanin synthesis (Schmidt & Mohr 1983) that P_{fr} operates in two steps. The principal findings – formulated for phytochrome action on chlorophyll synthesis – are the following: a brief red light pretreatment (pulse) operating through phytochrome stimulates the synthesis of Chl *a* and *b* in milo (*Sorghum vulgar* Pers.) shoots placed in continuous saturating white light. The red effect is fully reversible by a far-red (756 nm) light pulse for 45 min. Thereafter the escape from reversibility is fast, being completed within 2 h. The major finding has been that physiologically active phytochrome is required continuously during these first 45 min if the onset of the loss of photoreversibility is to begin 45 min after the red light treatment. Thus, the initial action of P_{fr} consists of two distinct processes: the first is to overcome the lag before signal transduction; the second is the actual signal transduction (probably to the pertinent genes). The duration of the lag before the onset of signal transduction depends on the level of P_{fr} established by the initial light pulse. The duration increases with increasing P_{fr} levels from non-detectable to 45 min. Above approximately 15% P_{fr} ($P_{fr}/P_{tot} \approx 0.15$), the duration of the lag before signal transduction remains constant at 45 min. We suggest explaining these novel findings in terms of interaction of large ligands (P_{fr}) with lattice-like chains (McGhee & von Hippel 1974), following in principle the argument that we have advanced previously to account for threshold phenomena in P_{fr} action (Mohr

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& Oelze-Karow 1976) and for the shape of the P_{fr}-effect curve in light-mediated anthocyanin synthesis (Drumm & Mohr 1974). Corresponding phenomena are observed in light-mediated decrease of phytochrome content in the milo shoot. The same lag (45 min) was measured before the onset of destruction. Moreover, it was found that P_{fr} is required continuously to overcome the lag before the onset of loss (M. Sauter, personal communication).

Signal amplification

It has been noticed (Mohr *et al.* 1979) that the effectiveness of P_{fr} in mediating anthocyanin or phenylalanine ammonia-lyase synthesis in mustard cotyledons can be increased strongly by a light pretreatment of the seedling before competence. The effect (called 'increase of

Table 1. Induction (or lack of induction) of anthocyanin synthesis in the mesocotyl of milo seedlings by light of different qualities (after Drumm & Mohr 1978)

(Treatment was started 60 h after sowing; anthocyanin was measured 87 h after sowing.)

treatment	amount of anthocyanin (A_{510})
27 h dark	0
27 h white light†	1.85
27 h red light	0
27 h far-red light	0
3 h white light‡	0.19
3 h blue-u.v.	0.19
3 h white light + 5 min red light	0.19
3 h white light + 5 min 756 nm light	0.06
3 h white light + 5 min 756 nm light + 5 min red light	0.20
3 h blue-u.v. + 5 min red light	0.19
3 h blue-u.v. + 5 min 756 nm light	0.05
3 h blue-u.v. + 5 min 756 nm light + 5 min red light	0.19

Photoequilibria of the phytochrome are of the order of $\phi_{red} = 0.8$ and $\phi_{756} < 0.01$ (see Schäfer *et al.* 1975). A 5 min light pulse virtually suffices under the present circumstances to establish the photoequilibrium $\phi_\lambda = [P_{fr}]_\lambda/[P_{tot}]$.

† Xenon arc light, similar to sunlight, 250 W m^{-2}.

‡ Seedlings were kept in the dark for 24 h before extraction of anthocyanin.

effectiveness of P_{fr}'or 'increase of responsivity to P_{fr}', or in short, 'responsivity amplification', we prefer this last expression) can best be described in terms of 'signal amplification': a given amount of P_{fr} (signal) leads to a much higher response! The light that brings about 'signal amplification' in the mustard seedling is absorbed by phytochrome. Thus a light pretreatment, operating through phytochrome, leads to a strong albeit transient signal amplification in P_{fr}-mediated anthocyanin synthesis.

In mustard, phytochrome causes a signal amplification with regard to P_{fr}. In other cases, however, amplification of the P_{fr} signal is caused by light absortion in cryptochrome or in the u.v.-B photoreceptor, or both.

The first example is milo seedlings (*Sorghum vulgare* Pers., cv. Wieder-hybrid). The mesocotyl of the milo seedling does not produce anthocyanin in complete darkness. As described originally

by Downs & Siegelman (1963) even long-term red or far-red light does not lead to any anthocyanin synthesis, whereas white light causes rapid pigmentation (table 1). In this case phytochrome can only act once a blue u.v. light effect has occurred. On the other hand, the expression of the blue u.v. light effect is controlled by phytochrome. In experiments with dichromatic irradiation, i.e. simultaneous irradiation with two kinds of light to modulate the level of P_{fr} strongly on a constant background of blue u.v. light, it was found that the blue u.v. light photoreaction as such is not affected by the presence or virtual absence of P_{fr} (Drumm

TABLE 2. INDUCTION (OR LACK OF INDUCTION) AND REVERSION OF ANTHOCYANIN SYNTHESIS IN THE COLEOPTILES OF *TRITICUM AESTIVUM* L. CV. SCHIROKKO, WITH LIGHT OF DIFFERENT QUALITIES (FOR DETAILS SEE DRUMM-HERREL & MOHR 1981) AND DIFFERENT LENGTHS OF TIME

(At the time indicated by a point (●) seedlings received either a saturating r.l. or RG-9 light pulse. Anthocyanin contents were measured at the age of 98 h. R.l., red light, 6.7 W m^{-2}, $\phi = 0.8$; RG-9 light, long-wavelength far-red light, 10 W m^{-2}, $\phi < 0.01$. As a gauge for responsivity to phytochrome we consider the extent of the reversible response, ΔR, defined by $\Delta R = R_1 - R_2$, where R_1 is the response obtained if the light treatment is terminated with a saturating red light pulse, and R_2 is the response obtained if the light treatment is terminated with a saturating long-wavelength far-red (RG-9) light pulse to return almost all P_{fr} to P_r (for definition of ϕ_λ see legend to table 1). (After Mohr & Drumm-Herrel (1983).)

	amount of anthocyanin (A_{546})		
treatment†	R_1	R_2	ΔR
5 d D (dark)		0.004	
2 d D+10 h s.l.●+40 h D	**0.169**	0.043	**0.126**
2 d D+12 h w.l.●+38 h D	0.092	0.030	0.062
3 d D+2 h D●+24 h D	0.004	0.004	0.000
3 d D+2 h r.l.●+24 h D	0.004	0.004	0.000
3 d D+2 h b.l.●+24 h D	0.004	0.004	0.000
3 d D+2 h u.v. (WG345)●+24 h D	0.008	0.004	0.004
3 d D+2 h u.v. (PG218)●+24 h D	0.012	0.004	0.008
2 d D+12 h f.r.●+38 h D	0.005	0.005	0.000
2 d D+12 h r.l.●+38 h D	0.004	0.004	0.000
2 d D+12 h b.l.●+38 h D	0.010	0.004	0.006
2 d D+12 h u.v. (WG345)●+38 h D	0.021	0.005	0.016
2 d D+12 h u.v. (PG218)●+38 h D	**0.160**	0.071	**0.089**
3 d D+2 h u.v.-B●+24 h D	0.033	0.012	0.021

† S.l., sunlight (changing fluxes due to clouds); w.l., fluorescent white light, 12000 lx; b.l., blue light, 7 W m^{-2}; u.v. (WG345), u.v.-A, 9.3 W m^{-2}; u.v. (PG218), u.v.-A with a small amount of u.v.-B, 12.6 W m^{-2}; u.v.-B, λ_{max} at 310 nm, 3 W m^{-2}.

& Mohr 1978). Our present interpretation of these facts is that phytochrome (P_{fr}) is always the effector molecule that causes anthocyanin synthesis through the activation of competent genes, whereas the blue–u.v. effect must be considered a transient 'responsivity amplification' (half-life approximately 6 h) (Drumm & Mohr 1978). In anthocyanin synthesis in the milo mesocotyl there is no P_{fr} signal transduction (and thus no responsivity) without the operation of a blue–u.v. photoreceptor.

The second example is wheat seedlings (*Triticum aestivum* L., cv. Schirokko). Anthocyanin synthesis in the wheat coleoptile takes place readily in natural sunlight and in fluorescent white light (table 2). Short-term and long-term treatments with red and far-red light proved ineffective. Surprisingly, blue and pure u.v.-A have very little effect, whereas u.v. light with a small content of u.v.-B is very effective in eliciting anthocyanin synthesis. After a u.v.

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treatment the response was found to be controlled by phytochrome (figure 4). In fact, at least the major effect of the u.v. treatment is to establish responsivity to phytochrome. In contrast to milo, u.v. cannot be replaced by blue light in its responsivity amplification function. Thus the function of a separate u.v.-B photoreceptor is indicated.

The responsivity amplification for P_{fr} brought about by u.v.-B is only short-lived. It is completely lost if 12 h darkness is inserted between the u.v.-B treatment and a red light pulse (H. Drumm-Herrel, personal communication).

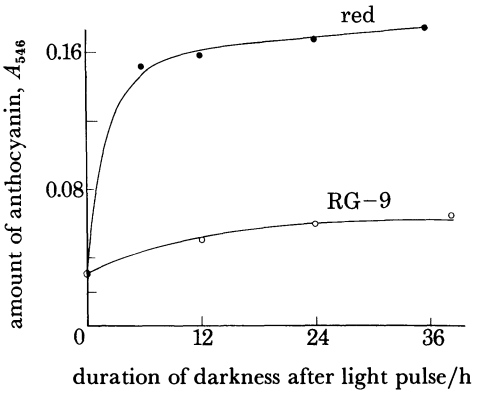

FIGURE 4. Anthocyanin accumulation in wheat (*Triticum aestivum* L.) coleoptiles in darkness after a u.v. pretreatment. The pretreatment (2 days dark, then 12 h u.v. (PG218) was terminated either with a saturating red light ($\phi_{red} = 0.8$, see table 1) or with a saturating RG-9 light pulse ($\phi_{756} < 0.01$, see table 1). (After Mohr & Drumm-Herrel (1983).)

The three species whose behaviours have been described briefly differ greatly with respect to the events underlying the same phenomenon, namely light-mediated synthesis of juvenile anthocyanin. Clearly, phytochrome is involved in the photoresponse in all cases. P_{fr}-mediated activation of competent genes seems to be at the core of the response in all cases. The different species vary with regard to the light-dependent 'mechanisms' that they use to establish responsivity towards P_{fr}. While the mustard seedling has some residual responsivity towards P_{fr} even in the dark and can perform a strong responsivity amplification via phytochrome, milo depends on light absorption in a blue u.v.-A photoreceptor (cryptochrome) to establish P_{fr} responsivity, whereas wheat even requires light absorption in a u.v.-B photoreceptor to become responsive to P_{fr} for anthocyanin synthesis.

A model for the process of responsivity amplification cannot be suggested at present. Increased responsivity was suggested to be due to a much more rapid signal transduction from P_{fr} to the responsive genes. This conjecture could not be supported in pertinent experiments (Schmidt & Mohr 1983). At present we are testing the hypothesis that responsivity amplification is due to an increase of some (proteinaceous?) factor in the cell nucleus that interacts with the P_{fr} signal.

This biochemical approach to the mechanism of signal transduction requires a response that takes place in many or most cells of an organ. We have chosen P_{fr}-mediated formation of Calvin cycle enzymes (ribulose 1,5-bisphosphate carboxylase, EC 4.1.1.39, and NADP-dependent glyceraldehyde 3-phosphate dehydrogenase, EC 1.2.1.13) in the shoot, predominantly in the primary leaf of the milo seedling. There is a small residual responsivity to P_{fr} that can be increased by blue–u.v. light approximately 60-fold within 6 h (figure 5). The high responsivity

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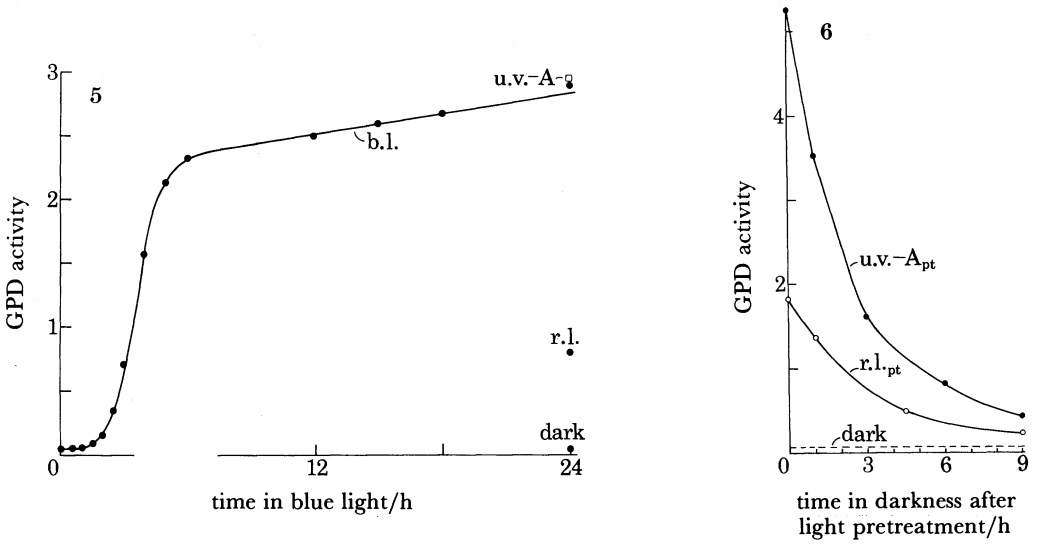

FIGURE 5. Blue-light-mediated responsivity increase in the milo (*Sorghum vulgare* Pers.) shoot with regard to phytochrome-mediated synthesis of NADP-dependent glyceraldehyde 3-phosphate dehydrogenase (GPD, EC 1.2.1.13). As a gauge for responsivity to phytochrome we consider the extent of the reversible response, ΔR (for definition see table 2). ΔR refers to the amount of total phytochrome, $[P_{tot}]$, present in the milo shoot at the time of the red and RG-9 light pulse treatment. We consider the ratio $\Delta R/[P_{tot}]$ the most precise gauge for the effectiveness of P_{fr} (or, expressed another way, for the responsivity towards P_{fr}). Dark-grown seedlings were pretreated with blue light (bl. 7 W m^{-2}) of different durations. At 72 h after sowing the pretreatment was terminated with a red or RG-9 light pulse. Until enzyme assay (96 h after sowing) seedlings were kept in darkness. U.v.-A (9.3 W m^{-2}) is as effective as bl., whereas red light (rl., 6.8 W m^{-2}) is much less effective. Dark, level of responsivity in dark-grown seedlings. (Data obtained by R. Oelmüller.)

FIGURE 6. Decrease of responsivity in darkness after a 24 h treatment with u.v.-A, terminated with an RG-9 light pulse to return almost all P_{fr} back to P_r (see table 2). The experimental system was the same as in figure 5. U.v.-A (9.3 W m^{-2}) and blue light (7 W m^{-2}) were adjusted so as to give approximately the same effect with regard to responsivity amplification (see figure 5). Red light (r.l., 6.8 W m^{-2}) of corresponding fluence rate is far less effective. Red or RG-9 light pulses were given after different durations of darkness but always at 72 h after sowing. Enzyme assay was performed 24 h after the light pulse, i.e. always 96 h after sowing. U.v.-A_{pt}, plants pretreated with 24 h of u.v.-A; r.l.$_{pt}$, plants pretreated with 24 h of red light. The low endogenous level of responsivity (dark) does not change during the period of examination. (Data obtained by R. Oelmüller.)

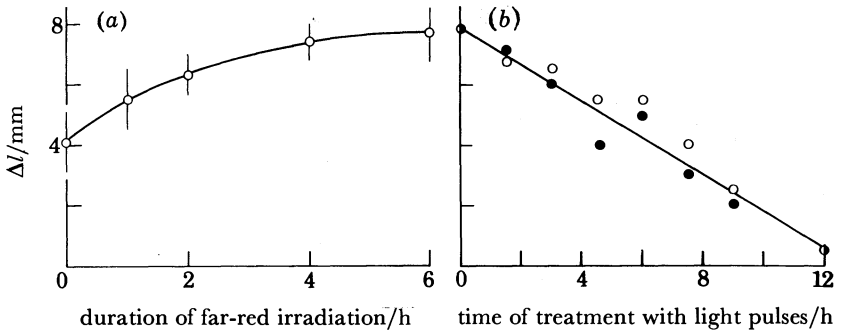

FIGURE 7. Experiments with mustard (*Sinapis alba* L.) seedlings. (*a*) Difference in hypocotyl length (Δl) caused within 24 h by a 5 min RG-9 light pulse compared with a 5 min red light pulse is plotted as a function of the duration of a continuous far-red light pre-treatment. The light pulses were always given 48 h after sowing. (*b*) A plot of Δl as a function of the dark interval between the pretreatment and the test light pulses. The pretreatment was 48 h dark plus 6 h white light. The white light was terminated with 5 min RG-9 light (○) or 5 min red light (●). Data obtained by B. Heim & C. Ebert.)

is only maintained as long as the blue or u.v.-A light is on. If we turn off the light, responsivity decays with a short half life (figure 6). In view of these data the level of responsivity to P_{fr} in the light must be considered a quasi-steady state that results from responsivity amplification by light and a light-independent decay reaction that decreases responsivity. Similar phenomena (increase of responsivity in light, decrease of responsivity after transferring to darkness) were also observed in control by phytochrome of hypocotyl elongation growth in mustard (figure 7). In mustard a phytochrome-mediated 'high-irradiance reaction', characterized by dependence on time (figure 7) and fluence rate (Beggs *et al.* 1981), leads to the responsivity amplification as tested by light pulses (figure 7). A contribution of a specific (i.e. non-phytochrome) blue light effect on responsivity could not be demonstrated (Beggs *et al.* 1981).

As shown in figure 7*b*, the decay of responsivity observed in the dark is independent of the P_{fr} level established at the onset of darkness. This suggests that continuous light or a rapid sequence of light pulses is required to cause an increase of responsivity.

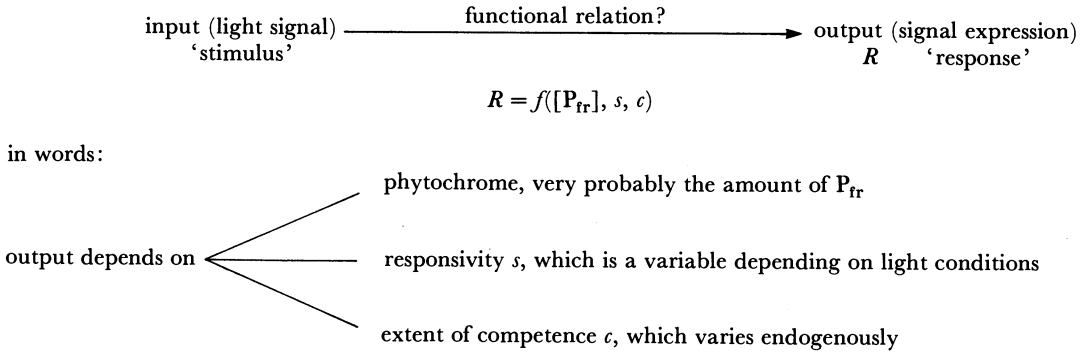

FIGURE 8. A scheme to account for the complexity of the functional relation between light signal and degree (extent) of response (R). Further explanation in text. The term 'competence' requires a comment: in developmental biology 'competence' means that a cell or a tissue is able to respond to a specific (inductive) stimulus with a specific response. As an example, an epidermal cell of a mustard cotyledon acquires competence towards P_{fr} with respect to anthocyanin synthesis approximately 27 h after sowing (25 °C). Competence disappears approximately 36 h later.

CONCLUSION

We conclude that de-etiolation, i.e. the gradual replacement of scotomorphogenesis by photomorphogenesis (see figure 1) is caused by phytochrome. In mustard the plant simply responds to the amount of P_{fr} established at the beginning of a dark period. However, the relation between amount of P_{fr} and the extent of response is a complex function because the responsivity of a plant towards P_{fr} depends on the light–dark conditions. Responsivity amplification by light can occur through phytochrome-mediated high-irradiance reaction and by light absorbed in a blue-u.v.-A light photoreceptor (cryptochrome) or in a u.v.-B photoreceptor, or both. Moreover, competence must also be considered. The changing spatial and temporal pattern of competence (Mohr 1978) is a crucial variable in any P_{fr} response function. Thus the output (extent of response) not only depends on the amount of P_{fr} but also on variables such as responsivity and competence (figure 8).

As an example, the perception of shade could be due to a reduced quantity of blue light below a leaf canopy – leading to a decrease of responsivity – *and* to a decrease of the amount of P_{fr} due to the strong depletion of red light, but relatively weak depletion of far-red light,

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by green leaves. Regarding light–dark transitions in adult plants we suggest that such transitions may not be sensed predominantly by a lowering of P_{fr} in darkness – because P_{fr} in totally de-etiolated plants seems to be quite stable (Gottmann & Schäfer 1983) – but by a relatively rapid decrease of responsivity.

In continuous light of constant quality and quantity an almost constant level of P_{fr} and a quasi-steady-state level of responsivity can probably be assumed after a couple of hours (see figures 5–7). However, under changing light conditions, in experiments with repeated light pulse treatments, or in light–dark experiments, the functional relation between the amount of P_{fr} and the extent of response becomes exceedingly complex. Thus quantitative experiments on 'molecular mechanisms' of signal transduction or signal expression should be performed either in continuous light or in darkness after single inductive light pulses (provided that responsivity is high enough).

An interpretation of action spectra obtained for de-etiolation responses under continuous irradiation will decisively depend on our knowledge about kinetics and wavelength dependence of responsivity towards P_{fr}. We suggest that the phenomena of 'high-irradiance reactions' are mainly due to responsivity amplification.

The research was supported by the Deutsche Forschungsgemeinschaft (SFB 206). We are greatly indebted to our research associates for placing unpublished data at our disposal.

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Discussion

R. J. Ellis (*Department of Biological Sciences, University of Warwick, U.K.*). I should like to make a comment concerning the way that we think of phytochrome action with respect to changes in gene expression, and especially to changes in transcription. There is now evidence for a number of species and from a number of laboratories that light acting via phytochrome causes a large increase in the rate of transcription of certain genes. The point that I wish to make is that the evidence that we have so far does *not* suggest that light acts to switch genes on. I say this because wherever it has been looked for it turns out that these genes are transcribed in plants raised from seeds in total darkness, albeit at lower rates. For example, it is difficult to detect the mRNA for the small subunit of ribulose bisphosphate carboxylase in dark-grown *Pisum* plants if 5 μg total RNA is analysed by dot–blot hybridization, but when 30 μg poly $(A)^+$ RNA is analysed by Northern blotting, this dark level of mRNA is readily seen; this was first shown by S. Smith in his Ph.D work (1980, University of Warwick), and subsequently Sasaki *et al.* (*J. biol. Chem.* **256**, 2315–2320 (1980)) showed that this dark mRNA is translatable *in vitro*. So I suggest that we must not think of light acting via phytochrome on transcription in an absolute sense; rather light has a *kinetic* role: it causes the amplification of processes already occurring in absolute darkness. I think that this is an important distinction to make, especially for those of us trying to devise ways of demonstrating a relevant effect of added phytochrome in isolated subcellular systems.

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H. Mohr. I agree with Professor Ellis that evidence from thoses cases that have been analysed so far on the molecular level are compatible with the concept that phytochrome increases the rate of transcription rather than switches genes on. The conclusion that phytochrome causes the amplification – sometimes one hundred or more times – of molecular processes already occurring in absolute darkness is certainly compatible with the currently available evidence. However, this is not the only interpretation. An alternative explanation of the 'leakiness' in darkness would be that in a few cells of an organ or a cell suspension culture transcription of a particular gene takes place in complete darkness whereas in most cells phytochrome is required to switch the gene on. The existence of responsivity amplification as an essential feature of phytochrome action – as described in the present paper – is compatible with Professor Ellis's suggestion that light via phytochrome 'causes the amplification of processes already occurring in absolute darkness' albeit at a low rate. In pursuing this problem we have found that the time course of competence of different genes towards phytochrome must be considered. It was observed in plastid genesis as well as in flavonoid biosynthesis that a dark level of an enzyme becomes detectable only once the particular transcription (or the particular gene) has become competent to the action of phytochrome. Thus we believe that the appearance of 'dark leakiness' of a particular transcription process has to do with those molecular changes that lead to competence.

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