PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Is P<latex>$_{fr}$</latex> the Active Form of Phytochrome?

H. Smith

Phil. Trans. R. Soc. Lond. B 1983 **303**, 443-452
doi: 10.1098/rstb.1983.0105

Phil. Trans. R. Soc. Lond. B **303**, 443–452 (1983)
Printed in Great Britain

Is P_{fr} the active form of phytochrome?

By H. Smith
Department of Botany, University of Leicester, University Road, Leicester LE1 7RH, U.K.

A selective but critical assessment of the published relations between spectrophotometric measurements of phytochrome parameters *in vivo* and physiological responses is presented. Although a number of correlations between response and the apparent concentration of P_{fr} (i.e. $[P_{fr}]$) have been reported, these are counterbalanced by authenticated cases of lack of correlation. The reported relations between $[P_{fr}]$ and response are not uniform, nor are they subject to ready interpretation. It seems useful to consider the responses of dark-grown and light-grown plants to be in principle different, with partly de-etiolated plants exhibiting intermediate responses. Dark-grown plants show high sensitivity to small changes in $[P_{fr}]$ at low $[P_{fr}]$, whereas light-grown plants show high sensitivity at high $[P_{fr}]$. Current data are not easily interpretable in terms of P_{fr} being the only active component of the phytochrome system.

Introduction

The concept of P_{fr} as the active form of phytochrome is central to current thought on the cellular mechanism of action of the photoreceptor. It forms the basis of the sophisticated analysis of the molecular properties of phytochrome *in vitro*, which seeks to define differences between P_r and P_{fr} sufficient to account for the presumed biological activity of P_{fr} (see for example Quail, Furuya and Rüdiger, this symposium), and lies at the heart of almost every attempt to interpret complex physiological data. Indeed, most papers on phytochrome begin with an assertion similar to: 'It has been generally agreed that P_{fr} is the physiologically active form of phytochrome' (quoted from the initial sentences in Whitelam & Johnson (1981) and Schmidt & Mohr (1982)). The view is so deep-seated that authors in general do not feel it is necessary to provide any supporting evidence, and assertions such as the one quoted above are normally followed by unhelpful references to the text-books written by Mohr (1972) and Smith (1975). 'P_{fr} as the active form', therefore, seems to have passed from the status of a theory to that of a central dogma. My objective in this article is selectively to examine the evidence for this 'central dogma', with particular reference to the action of phytochrome in light-grown plants. First, however, I wish to draw attention to the loose nature of the concept, as it is normally formulated. The statement 'P_{fr} as the active form' is imprecise unless it also means 'P_{fr} as the *only* active form'; in this article, the exclusivity expressed in the latter formulation is assumed to hold.

Correlations between measured phytochrome levels and physiological responses

The concept of P_{fr} as the active form was generated by the earliest experiments on phytochrome-mediated responses, which demonstrated that red light induces developmental effects whereas far-red has no effect except after red; this generalization came from physiological responses observed in dark-imbibed seeds, dark-grown seedlings, and in light-grown plants given brief light periods during a photoperiodically inductive dark period (Borthwick *et al.*

1952, 1954; Parker *et al.* 1946, 1949). Because only a small quantity of red light is sufficient to elicit substantial developmental changes, it was natural to assume that the form produced by that light – i.e. P_{fr} – actively induces the changes via some physiological amplification mechanism. The logical alternative, that P_r acts to inhibit the developmental changes, was justifiably ruled out on the grounds that a large proportion of an active inhibitor would need to be removed before significant response would be detected.

Because it has not yet been possible to associate a biological activity with purified phytochrome *in vitro*, the only evidence on whether or not P_{fr} possesses unique biological activity not exhibited by other forms of phytochrome comes from correlations between spectrophotometrically determined phytochrome levels *in vivo* and physiological responses. There are many problems inherent in this limitation in experimental approach; for example:

(*a*) it is only possible to measure the tissue average of phytochrome content;
(*b*) only relative measures of phytochrome can be obtained;
(*c*) the measurements are affected by the optical properties of the tissues, which may change during the treatments given;
(*d*) some parameters of phytochrome are not measurable, e.g. the level of photoconversion intermediates;
(*e*) should the responses be quantitatively correlated to phytochrome parameters determined at the time of light treatment or at the time at which the responses are measured?

With such serious conditions placed upon the experimental approach it is quite surprising that any correlations at all have been observed; on the other hand the existence of so many conditional factors allows wide latitude in interpretation of conflicting data! Perhaps the most important point about correlative evidence, however, is that it can never be anything more than circumstantial. No matter how many instances of positive correlations are accumulated, it only takes one single definitive and authenticated negative correlation to disprove the working hypothesis.

This article is concerned principally with data from light-grown seedlings but some coverage of the correlations observed with dark-grown seedlings is necessary to set the scene.

(*a*) *Dark-grown seedlings*

For dark-grown seedlings given brief light treatments essentially three types of positive correlations have been observed. These are: (1) the response is related to the logarithm of P_{fr} concentration (i.e. $\log[P_{fr}]$); (2) the response shows a biphasic linear relation to $[P_{fr}]$; (3) the response is related to $[P_{fr}]$ on a threshold basis.

Early observations by Hillman and others (Hillman 1965, 1966; Loercher 1966) showed that response to brief treatments of red light or to mixed red and far-red light became saturated at relatively low levels of P_{fr}/P_{total} and that the relation below saturation approximated to a logarithmic curve. Subsequently, Mandoli & Briggs (1981) were able to pull together much of the early data and show that the relations between response and $[P_{fr}]$ could be plotted together on a log–linear basis. Mohr and colleagues (Drumm & Mohr 1974; Steinitz *et al.* 1979), however, studying anthocyanin synthesis in mustard seedlings described the relation between $[P_{fr}]$ and response as biphasic–linear. This is expressed as a much greater degree of sensitivity to changes in $[P_{fr}]$ at low $[P_{fr}]$ than at higher $[P_{fr}]$. The third type of relation was shown by Oelze-Karow & Mohr (1970, 1973) studying lipoxygenase in mustard seedlings, where they

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

found that the increase in activity of lipoxygenase was switched on at a $[P_{fr}]$ below a certain critical, threshold value; there was no graded response at all in this case.

If we assume from these correlations that P_{fr} is the only active component the question arises as to whether P_{fr} acts differently in the three types of relation. A logarithmic relation between the concentration of an effector and the response to that effector is not common in biology, although other examples can be found, e.g. the relation between the concentration of certain plant growth substances and response is in many cases logarithmic. Depending on the reliability of the data, however, it is not always easy to distinguish between a logarithmic relation and a hyperbolic relation, which might be more understandable in terms of classical biochemistry (i.e. Michaelean analysis). The biphasic–linear relation of Drumm & Mohr (1974) may also be a manifestation of a hyperbolic function although the linear fits are impressive. The authors interpret their data in terms of a biphasic cooperativity between P_{fr} molecules associated with a presumed membrane matrix; the problems of understanding this biphasic relation are not trivial. The threshold relation is perhaps the easiest to understand biologically (e.g. some form of cascade control sequence), but it seems to have limited importance judging from the rarity of confirmatory reports.

Even though it might be difficult to reconcile the three different types of relation between $[P_{fr}]$ and response, nevertheless on the evidence quoted above it is not necessary to postulate activity associated with any component other than, or additional to, P_{fr}. There are, however, some quite celebrated examples of a lack of correlation between $[P_{fr}]$ and response. These have generally been termed 'paradoxes' and the most serious is the so-called *Zea* paradox in which red light induces a response that saturates at fluences well below those required for detectable photoconversion of P_r to P_{fr}, and which is reversed by fluences of far-red that establish higher P_{fr}/P_{total} than does the red light fluence required to saturate the response (Chon & Briggs 1966; Briggs & Chon 1966). I know of no acceptable argument for ignoring these data, but they seem generally to be discounted in considerations of whether or not P_{fr} is the only active form of phytochrome. Similar extreme sensitivity to very low levels of red light has been reported more recently in a number of other responses observed with totally dark-grown seedlings (Raven & Shropshire 1975; Small *et al.* 1979; Mandoli & Briggs 1981) and dark-imbibed seeds (VanDerWoude & Toole 1980). In these cases the organisms show substantial responses to $[P_{fr}]$ well below those that can be detected by spectrophotometry *in vivo*. Such responses – which have been termed 'very low fluence responses' by Mandoli & Briggs (1981) – may be quite general with dark-grown plants, having been missed previously by investigators who used green 'safe lights' even for their dark controls; exposure of the material to any light whatsoever is sufficient to induce the very low fluence response. The earlier observations of Hillman and others did, however, show significant physiological response to light treatments that established a $[P_{fr}]$ not detectable by spectrophotometry (see Hillman 1967, 1972 for reviews). Sensitivity to low $[P_{fr}]$ may also be markedly affected by a pretreatment with light. This has been shown particularly with anthocyanin synthesis in mustard cotyledons, where so-called 'sensitivity amplification' is induced by pretreatment for several hours with light, itself apparently operating through phytochrome (Mohr *et al.* 1979; Johnson 1980). Since the long-term light pretreatment leads to partial de-etiolation, this topic is dealt with in more detail below.

When considering the data for dark-imbibed seeds and dark-grown seedlings, therefore, it seems useful to draw attention to the extremely high sensitivity to changes in $[P_{fr}]$ at low levels of $[P_{fr}]$, and to the variety of different relations exhibited between $[P_{fr}]$ and response.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*b*) *High-irradiance responses*

High-irradiance responses are responses of dark-grown seedlings to continuous light, and many different action spectra have been produced, most showing peaks of action in the blue and in the red or far-red (or both) wavelength bands. The action in the red and far-red band was shown by Hartmann (1966, 1967*a*), using the now classical bichromatic irradiation techniques, to be mediated by phytochrome. In some of the best-studied examples – e.g. lettuce hypocotyl growth (Hartmann 1967*b*) and mustard hypocotyl growth (Beggs *et al.* 1980) – the response is maximal in the far-red, where very low [P_{fr}] is established. The action spectra in general are dependent upon the length of irradiation treatment and the pretreatment conditions and also on the level of response chosen as being a standard response (see Beggs *et al.* 1980; Holmes & Schäfer 1981). During the period of the irradiation the concentration of total phytochrome is constantly changing and thus any possible relation between [P_{fr}] and response must be quite complex. Although it is possible that the responses are related to the integral of [P_{fr}] over the time period of the irradiation treatment, a direct relation between response and [P_{fr}] is not obvious.

On the other hand, even on simple models, under continuous irradiation [P_{fr}] becomes independent of wavelength and strongly dependent on fluence rate (Schäfer & Mohr 1974; Gammerman & Fukshansky 1974). Heim & Schäfer (1982) have shown that both [P_{fr}] and P_{fr}/P_{total} show strong fluence-rate dependence under continuous red light and repeated brief red light treatments, and that these effects on phytochrome parameters at least partly parallel the effects of different fluence rates of red light on hypocotyl growth. Recently, the same authors (Heim & Schäfer 1983) have tackled the closely related question of whether or not the fluence-rate dependence of mustard hypocotyl growth under continuous far-red (Beggs *et al.* 1980; Holmes & Schäfer 1981) is paralleled by a fluence-rate dependence of [P_{fr}]. Their data show a lack of correlation between the measured phytochrome parameters and the observed responses, and they conclude that P_{fr} cannot be solely responsible for the action of phytochrome in the high-irradiance response, although they wisely do not speculate upon which other component is likely also to be involved.

Others have also concluded that P_{fr} cannot be the only active form of phytochrome in the high-irradiance response. Johnson & Tasker (1979) concluded that P_{fr} interacted with a cycling-driven process (see below for detailed treatment). Recently, Bartley & Frankland (1982) have investigated a high-irradiance response in seed germination, in which the response operates antagonistically to that elicited by brief red light; here again it was proposed that phytochrome cycling was responsible for the inhibitory action of continuous light.

(*c*) *Light-grown plants*

In recent years, studies of the photomorphogenetic reactions of light-grown plants have become fashionable. The transition from etiolation growth to growth under light takes a substantial time and during the transition period plants appear to exhibit responses that are characteristically different from either those shown by dark-grown plants or those shown by mature, fully de-etiolated plants. It is therefore necessary to establish criteria for the responses that appear to be characteristic of mature, de-etiolated, light-grown plants. For complete de-etiolation it seems necessary to have quite a long period (24–28 h at least) of relatively high fluence rate (more than *ca.* 50 μmol m^{-2} s^{-1}) of light, which establishes a relatively high P_{fr}/P_{total}.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

During the transition period the extension growth rate of the seedling drops from the high rate characteristic of etiolation growth to the very much lower rate characteristic of light-grown seedlings. In mustard, for example, elongation growth in the dark can be of the order of 20–40 μm min^{-1}, whereas in a fully de-etiolated young seedling the growth rate is commonly 1–2 μm min^{-1}; as the seedlings mature in the light, growth rate usually increases again, reaching 5–8 μm min^{-1} after 3–4 weeks. Mustard seedlings growing at the low rate in the light initially retain the same growth rate when transferred to darkness, although within 1 or 2 hours the growth rate begins to decline even further (Child & Smith, unpublished data). A reasonable criterion of de-etiolation would therefore be the establishment of a minimum growth rate. Such seedlings growing under fluorescent white light, when given additional far-red light – which depresses P_{fr}/P_{total} – exhibit a substantial increase in extension growth, and it is this far-red mediated increase in extension growth that is the true characteristic of the photomorphogenetic responses of the fully de-etiolated plant (Holmes & Smith 1975, 1977; Morgan & Smith 1976, 1978). As a rule of thumb, it seems that seedlings that still have extending hypocotyls react in a transitional way to light, and the far-red-induced growth increases do not become fully apparent until hypocotyl elongation has ceased and elongation growth of the seedling is solely due to the growth of true internodes.

If we concentrate first on seedlings given short, partly de-etiolating light treatments, it is now recognized that subsequent phytochrome-mediated phenomena show unusual relations between $[P_{fr}]$ and response. As mentioned briefly above, the partial de-etiolation treatment can lead to a large increase in the subsequent sensitivity of responses to brief red light treatments; this is described as 'sensitivity amplification' and interpreted as an increased response to low $[P_{fr}]$ (Mohr *et al.* 1979; Johnson 1980). Sensitivity amplification is a relatively short-lived phenomenon, with the increase in response disappearing over about 6 h (Schmidt & Mohr 1982). Schmidt & Mohr (1983) have recently concluded that the mechanism of sensitivity amplification does not reside in an acceleration of the transduction process between phytochrome and response (anthocyanin synthesis), whereas Mohr & Schäfer (this symposium) discuss the possible ways in which amplification of the 'P_{fr} signal' may be achieved. It seems unlikely, however, that an increased level of 'receptor sites' for P_{fr} could explain the observed amplification (Oelmüller & Mohr 1983). Whitelam & Johnson (1981), investigating sensitivity amplification of the phytochrome control of nitrate reductase activity in mustard cotyledons, showed that amplification was a function of the fluence rate of the light pretreatment. They concluded that the process occurring during the pretreatment causes sensitivity amplification through the time-dependent and fluence-rate-dependent (i.e. cycling-dependent) synthesis or transport of a component that interacts with P_{fr}. Thus P_{fr} is considered to be active, but its activity is considered to be increased through interaction with another component whose level is dependent on phytochrome action, operating in a manner *independent* of $[P_{fr}]$.

Fully de-etiolated seedlings exhibit a type of phytochrome-controlled growth response that seems, from the phenomenological point of view, different from both the red–far-red inducible responses and the high-irradiance responses shown by dark-grown plants. Stem extension in a light-grown plant, when measured over a period of days, is linearly related to the P_{fr}/P_{total} calculated from the spectral distribution of the actinic radiation (Morgan & Smith 1976, 1978). There are problems with estimations of P_{fr}/P_{total}, because it is clearly impossible to measure phytochrome spectrophotometrically in green plant tissues, and P_{fr}/P_{total} is calculated from the spectral photon distribution of the incident radiation. Others, however, have reported

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

similar linear relations in light-grown plants (Vince-Prue 1977; Holmes, this symposium). The linear relation between extension growth and P_{fr}/P_{total} has now been seen in a wide range of species, and may be regarded as a characteristic of light-grown plants (Morgan & Smith 1979; Smith 1982).

The responses of light-grown plants to a change in the red:far-red ratio are very rapid. Transducer measurements of growth rate in mustard seedlings have shown a 10–15 min lag between the onset of additional far-red and the first detectable increase in growth rate (Morgan *et al.* 1980). After the 10–15 min lag the response becomes maximal in approximately 20–30 min. The display is red–far-red reversible; this means that after the new higher growth rate has been established by additional far-red the growth rate can be reduced by additional red. Apart from satisfying the operational criterion of red–far-red reversibility, this result indicates that the growth responses are closely related to whatever is the primary action of phytochrome. The responses are best seen as a continuous and reversible modulation of growth rate by a red:far-red ratio operating somehow through phytochrome.

The transducer methodology allows growth rate to be related to $[P_{fr}]$ because, during the short period between light treatment and measurement of the response, it is unlikely that there is a substantial change in the total amount of phytochrome present. If the increases in growth rate caused by added far-red are plotted against P_{fr}/P_{total} measured *in vitro* by using phytochrome samples exposed to the light sources in exactly the same geometry as the irradiated plant tissues, the relation shows extreme sensitivity to a small decrease in P_{fr}/P_{total}. If different wavelengths of additional far-red are used (i.e. 700, 719, 739 nm) the increases in growth rate can be seen to lie on the same curve when plotted against P_{fr}/P_{total} (Morgan *et al.* 1980, 1981). These three additional far-red wavelengths should increase phytochrome cycling to different degrees, and yet they establish the same relation between growth rate and P_{fr}/P_{total}. In another experiment to assess the possible influence of phytochrome cycling, different fluence rates of background white light were used; again the increments in growth rate caused by the far-red could be plotted on the same curve against P_{fr}/P_{total}. In all experiments we have done using the transducer the extreme sensitivity to a small decrease in P_{fr}/P_{total} was observed.

To account for the responses of light-grown plants it is necessary to explain:

(*a*) the extreme sensitivity to a small decrease in P_{fr}/P_{total} at high values of P_{fr}/P_{total} observed when the growth effects are measured continuously;

(*b*) the apparent lack of this extreme sensitivity when the growth effects are measured over a long time;

(*c*) the linear relation between elongation and P_{fr}/P_{total} observed with long-term measurements of growth.

On points (*a*) and (*b*) it is possible that changes in the amounts of P_{total} over the long times used might account for the apparent lack of extreme sensitivity to far-red seen on a long-term basis. We have been unable in our laboratory to measure the phytochrome content of light-grown mustard seedlings *in vivo*, even when treated with the bleaching herbicide Norflurazon; however, we have done similar experiments with maize seedlings and the level of total phytochrome has been shown to increase in seedlings exposed over several days to white light containing a relatively high proportion of far-red (i.e. low red:far-red ratio), whereas seedlings exposed to white light with a high red:far-red ratio showed a small decrease in P_{total} (Smith 1981). In these experiments the growth of the maize seedlings was shown to be linearly related

to P_{fr}/P_{total} even after 48–72 h of exposure to light, at which point $[P_{fr}]$ was equal in all four treatments given; in this example therefore there was no obvious relation between $[P_{fr}]$ and growth,

The most difficult point to reconcile between the responses of the light-grown plant and those of the dark-grown plant is the form of the relation between response and P_{fr}/P_{total} observed when the growth effects are measured by transducer, i.e. under conditions in which P_{total} should not change. Formally, the relations observed in dark-grown plants (described earlier) could not account for the shape of this curve, with a high sensitivity to a small change in P_{fr}/P_{total} at high P_{fr}/P_{total} levels. Indeed on the argument advanced above in relation to the very early work on phytochrome, one would be justified in concluding that the production of a small amount of P_r leads to an amplified effect on growth, and that in light-grown plants it is P_r rather than P_{fr}, that is active! It does seem, however, that a useful generalization may be that dark-grown plants are exceptionally sensitive to a small change in P_{fr}/P_{total} at low P_{fr}/P_{total} levels, whereas light-grown plants are exceptionally sensitive to a small change in P_{fr}/P_{total} at high P_{fr}/P_{total} levels.

Models

There is no shortage of models for phytochrome action but it seems that none of the current models is capable of accounting for all the responses outlined in this article. Strangely enough, none of the currently more fashionable models considers P_{fr} to be the *only* active form. The model of Schäfer (1975) adheres most closely to the central concept but even here it was found necessary to postulate two different forms of P_{fr}, one responsible for the red–far-red inducible responses and the other for the high-irradiance responses. It is difficult to incorporate into Schäfer's model the extreme sensitivity to small amounts of far-red exhibited by the light-grown plant. The other model most actively discussed at present is that of Johnson & Tasker (1979). In this model, action of phytochrome in the red–far-red reversible induction responses is considered to be a direct function of P_{fr}, whereas action in the high-irradiance response is due to an interaction between P_{fr} and a substance X, of which the level, or availability, is determined by some cycling-driven process. Again it is difficult to build into this model a very high sensitivity to small changes in P_{fr}/P_{total} at high values of P_{fr}/P_{total}. Very recently, VanDerWoude (1982, 1983) has developed a model of phytochrome action based upon the proposal that phytochrome exists *in vivo* as a dimer in which each monomer has a single chromophore. The model was developed to account for the extreme sensitivity of lettuce seeds, previously given a high-temperature treatment before germination, to very small amounts of red light. The model proposes that the extreme sensitivity to red light is due to the conversion of one of the pair of chromophores in the dimer to P_{fr}; at levels of red light that convert both chromophores to P_{fr} the sensitivity becomes reduced. Even in this model, however, activity is not considered to be strictly a function of $[P_{fr}]$, with P_r–P_{fr} considered to have different activity from that of P_{fr}–P_{fr}. It may be possible to build into the VanDerWoude model a high sensitivity to small amounts of far-red, if this would convert P_{fr}–P_{fr} into P_{fr}–P_r, but it would be necessary to assume that a small amount of P_{fr}–P_r would exert a marked effect in the presence of relatively very large amounts of P_{fr}–P_{fr}.

One way of circumventing the awkward fact that spectrophotometrically measurable $[P_{fr}]$ does not always correlate with response is to postulate the existence of (at least) two populations of P_{fr}, one present in small amounts and responsible for the growth effects, and the other

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

present in much larger amounts and therefore measurable spectrophotometrically, but physiologically inactive. This idea has been repeatedly adduced in attempts to resolve the paradoxes between response and [P_{fr}] (see Hillman (1967, 1972) for reviews) and is currently popular once more. Evidence is accumulating that two populations of phytochrome exist that differ in their susceptibility to degradation, such that most of the phytochrome in the dark-grown plant is of the unstable variety, whereas that in light-grown plants is relatively very stable (Brockmann & Schäfer 1982; see also the papers by Furuya, Mohr & Schäfer, and Quail *et al.* in this symposium). Indeed, the view is developing that the unstable phytochrome present in large amounts in etiolated plants serves an 'antennae' function, enabling the seedling under the soil to detect the soil surface with great sensitivity (see Quail *et al.* this symposium), whereas the stable phytochrome of the mature plant operates essentially as a detector of red:far-red ratio (see Smith 1982). Although the second part of this attractive concept is quite soundly based, much research will be needed to establish the antenna function proposal.

Blaauw-Jansen (1983) has recently suggested that P_{fr} in dark-grown seedlings is stable until a critical concentration is reached (*ca.* 2 % P_{total} in *Avena*), above which P_{fr} degradation occurs as a first-order reaction. Such behaviour is formally equivalent to two populations, one stable and one unstable and, it is claimed, can account for the very high sensitivity of etiolated seedlings to small amounts of red light.

Although the concept of two populations of phytochrome may possibly reconcile the paradoxes between the physiology and the spectrophotometry, it should be borne in mind that taking refuge in such a concept also renders highly questionable those cases in which measured [P_{fr}] appears to correlate positively with response (cf. Smith 1975, p.107). If there are two populations of P_{fr} in the one case, why should there not also be two populations in the other?

On the face of it none of the current models, nor any of the relations observed in the dark-grown plant, appear to account for the relations observed in the light-grown plant. Obviously much more information will be necessary before we can integrate all the various relations between response and phytochrome parameters, and this is particularly true for the light-grown plant where as yet very few intensive investigations have been mounted. It does seem, however, as if it will be necessary to construct a model for phytochrome action that allows for extreme sensitivity to low [P_{fr}] in the dark-grown plant and extreme sensitivity to small changes in [P_{fr}] at high [P_{fr}] in the light-grown plant. It clearly would be very difficult to do this if one adheres to the limiting concept that P_{fr} is the *only* active component of phytochrome.

Conclusions

The concept that P_{fr} is the only active form of phytochrome is certainly an attractive hypothesis, but equally certainly it is not based on a critical and unbiased view of the available evidence. This evidence is basically correlative in nature and there are sufficient examples of lack of correlation between [P_{fr}] and response to indicate to the cautious mind that scepticism should be in order. Lack of correlation can be seen in the responses of dark-grown plants, in the high-irradiance responses, and in the responses of light-grown plants. Furthermore, even in cases where [P_{fr}] appears to be correlated with response the relations are not uniform and indeed some of the relations are difficult to interpret. Particular emphasis should be placed upon the observations of extreme sensitivity to low levels of [P_{fr}] in the dark-grown plant and dark-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

imbibed seeds, and the equally extreme sensitivity to small amounts of added far-red light in plants growing in white light establishing a high P_{fr}/P_{total}. It can only be concluded that the present models for phytochrome action are inadequate to account for all the various relations that appear to exist, and one is forced to the conclusion that P_{fr} is unlikely to be the *only* active form of phytochrome.

References

Bartley, M. R. & Frankland, B. 1982 Analysis of the dual role of phytochrome in the photoinhibition of seed germination. *Nature, Lond.* **300**, 750–752.

Beggs, C. J., Holmes, M. G., Jabben, M. & Schäfer, E. 1980 Action spectra for the inhibition of hypocotyl growth by continuous irradiation in light- and dark-grown *Sinapis alba* L. seedlings. *Pl. Physiol.* **66**, 615–618.

Blaauw-Jansen, G. 1983 Thoughts on the possible role of phytochrome destruction in phytochrome-controlled responses. *Pl. Cell Envir.* **6**, 173–179.

Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H. & Toole, V. K. 1952 A reversible reaction controlling seed germination. *Proc. natn. Acad. Sci. U.S.A.* **38**, 662–666.

Borthwick, H. A., Hendricks, S. B., Toole, E. H. & Toole, V. K. 1954 Action of light on lettuce-seed germination. *Bot. Gaz.* **115**, 205–225.

Briggs, W. R. & Chon, H. P. 1966 The physiological versus the spectrophotometric status of phytochrome in corn coleoptiles. *Pl. Physiol.* **41**, 1159–1166.

Brockmann, J. & Schäfer, E. 1982 Analysis of P_{fr} destruction in *Amaranthus caudatus* L. – evidence for two pools of phytochrome. *Photochem. Photobiol.* **35**, 555–558.

Chon, H. P. & Briggs, W. R. 1966 Effects of red light on the phototropic sensitivity of corn coleoptiles. *Pl. Physiol.* **41**, 1715–1724.

Drumm, H. & Mohr, H. 1974 The dose response curve in phytochrome-mediated anthocyanin synthesis in the mustard seedling. *Photochem. Photobiol.* **20**, 151–157.

Gammerman, A. Ya. & Fukshansky, L. 1974 A mathematical model of phytochrome – the receptor of photomorphogenetic processes in plants. *Ontogenez.* **5**, 122–129.

Hartmann, K. M. 1966 A general hypothesis to interpret 'high energy phenomena' of photomorphogenesis on the basis of phytochrome. *Photochem. Photobiol.* **5**, 349–366.

Hartmann, K. M. 1967*a* Phytochrome 730 (P_{fr}), the effector of the 'high energy photomorphogenic reaction' in the far-red region. *Naturwissenschaften* **54**, 544.

Hartmann, K. M. 1967*b* Ein Wirkungsspektrum der Photomorphogenese unter Hochenergiebedingungen und seine interpretation auf der Basis des Phytochroms (Hypokotylwachstumshemmung bei *Lactuca sativa* L.). *Z. Naturf.* **22***b*, 1172–1175.

Heim, B. & Schäfer, E. 1982 Light-controlled inhibition of hypocotyl growth in *Sinapis alba* L. seedlings. Fluence-rate dependence of hourly light pulses and continuous irradiation. *Planta* **154**, 150–155.

Heim, B. & Schäfer, E. 1983 Hypocotyl growth in dark-grown *Sinapis alba* L.: fluence-rate dependence of continuous and hourly pulsed irradiation with far-red. *Pl. Cell Envir.* **6**. (In the press.)

Hillman, W. S. 1965 Phytochrome conversion by brief illumination and the subsequent elongation of isolated *Pisum* stem segments. *Physiologia Pl.* **18**, 346–358.

Hillman, W. S. 1966 Responses of *Avena* and *Pisum* tissues to phytochrome conversion by red light. *Pl. Physiol.* **41**, 907–908.

Hillman, W. S. 1967 The physiology of phytochrome. *A. Rev. Pl. Physiol.* **18**, 301–324.

Hillman, W. S. 1972 On the physiological significance of *in vivo* phytochrome assays. In *Phytochrome* (ed. K. Mitrakos & W. Shropshire Jr), pp. 573–584. New York and London: Academic Press.

Holmes, M. G. & Schäfer, E. 1981 Action spectra for changes in the 'high irradiance reaction' in hypocotyls of *Sinapis alba* L. *Planta* **153**, 267–272.

Holmes, M. G. & Smith, H. 1975 The function of phytochrome in plants growing in the natural environment. *Nature, Lond.* **254**, 512–514.

Holmes, M. G. & Smith, H. 1977 The function of phytochrome in the natural environment. IV. Light quality and plant development. *Photochem. Photobiol.* **25**, 551–557.

Johnson, C. B. 1980 The effect of red light in the high irradiance reaction of phytochrome: evidence for an interaction between P_{fr} and a phytochrome cycling-driven process. *Pl. Cell. Envir.* **3**, 45–51.

Johnson, C. B. & Tasker, R. 1979 A scheme to account quantitatively for the action of phytochrome in etiolated and light-grown plants. *Pl. Cell Envir.* **2**, 259–265.

Loercher, L. 1966 Phytochrome changes correlated to mesocotyl inhibition in etiolated *Avena* seedlings. *Pl. Physiol.* **41**, 932–936.

Mandoli, D. F. & Briggs, W. R. 1981 Phytochrome control of two low-irradiance responses in etiolated oat seedlings. *Pl. Physiol.* **67**, 733–739.

Mohr, H. 1972 *Lectures on photomorphogenesis*. Berlin, Heidelberg and New York: Springer-Verlag.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Mohr, H., Drumm, H., Schmidt, R. & Steinitz, B. 1979 The effect of light pretreatments on phytochrome-mediated induction of anthocyanin and phenylalanine ammonia-lyase. *Planta* **146**, 369–376.
Morgan, D. C., Child, R. & Smith, H. 1981 Absence of fluence rate dependency of phytochrome modulation of stem extension in light-grown *Sinapis alba* L. *Planta* **353**, 497–498.
Morgan, D. C., O'Brien, T. & Smith, H. 1980 Rapid photomodulation of stem extension in light-grown *Sinapis alba* L. *Planta* **150**, 95–101.
Morgan, D. C. & Smith, H. 1976 Linear relationship between phytochrome photoequilibrium and growth in plants under simulated natural radiation. *Nature, Lond.* **262**, 210–212.
Morgan, D. C. & Smith, H. 1978 The relationship between phytochrome photoequilibrium and development in light-grown *Chenopodium album* L. *Planta* **142**, 187–193.
Morgan, D. C. & Smith, H. 1979 A systematic relationship between phytochrome-controlled development and species habitat, for plants grown in simulated natural radiation. *Planta* **145**, 253–258.
Oelmüller, R. & Mohr, H. 1983 Responsivity amplification by light in phytochrome-mediated induction of chloroplast glyceraldehyde-3-phosphate dehydrogenase (NADP-dependent, EC 1.2.1.13) in the shoot of milo (*Sorghum vulgare* Pers.). *Pl. Cell Environ.* (In the press.)
Oelze-Karow, H. & Mohr, H. 1970 Phytochrome-mediated repression of enzyme synthesis (lipoxygenase): a threshold phenomenon. *Proc. natn. Acad. Sci. U.S.A.* **65**, 51–57.
Oelze-Karow, H. & Mohr, H. 1973 Quantitative correlation between spectrophotometric phytochrome assay and physiological response. *Photochem. Photobiol.* **18**, 319–330.
Parker, M. W., Hendricks, S. B., Borthwick, H. A. & Scully, N. J. 1946 Action spectrum for the photoperiodic control of floral initiation of short-day plants. *Bot. Gaz.* **108**, 1–26.
Parker, M. W., Hendricks, S. B., Borthwick, H. A. & Went, F. W. 1949 Spectral sensitivities for leaf and stem growth of etiolated pea seedlings and their similarity to action spectra for photoperiodism. *Am. J. Bot.* **36**, 194–204.
Raven, C. W. & Shropshire, W. Jr 1975 Photoregulation of logarithmic fluence-response curves for phytochrome control of chlorophyll formation in *Pisum sativum* L. *Photochem. Photobiol.* **21**, 423–429.
Schäfer, E. 1975 A new approach to explain the 'high irradiance response' of photomorphogenesis on the basis of phytochrome. *J. math. Biol.* **2**, 41–56.
Schäfer, E. & Mohr, H. 1974 Irradiance dependency of the phytochrome system in cotyledons of mustard (*Sinapis alba* L.). *J. math. Biol.* **1**, 9–15.
Schmidt, R. & Mohr, H. 1981 Time-dependent changes in the responsiveness to light of phytochrome-mediated anthocyanin synthesis. *Pl. Cell Envir.* **4**, 433–437.
Schmidt, R. & Mohr, H. 1982 Evidence that a mustard seedling responds to the amount of P_{fr} and not to the $P_{fr}:P_{total}$ ratio. *Pl. Cell Envir.* **5**, 495–499.
Schmidt, R. & Mohr, H. 1983 Time course of signal transduction in phytochrome-mediated anthocyanin synthesis in mustard cotyledons. *Pl. Cell Envir.* **6**, 235–238.
Small, J. G. C., Spruit, C. J. P., Blaauw-Jansen, G. & Blaauw, O. H. 1979 Action spectra for light-induced germination in dormant lettuce seeds. *Planta* **144**, 125–131.
Smith, H. 1975 *Phytochrome and photomorphogenesis*. London: McGraw-Hill.
Smith, H. 1981 Evidence that P_{fr} is not the active form of phytochrome in light-grown maize. *Nature, Lond.* **293**, 163–165.
Smith, H. 1982 Light quality, photoperception, and plant strategy. *A. Rev. Pl. Physiol.* **33**, 481–518.
Steinitz, B., Schäfer, E., Drumm, H. & Mohr, H. 1979 Correlation between far-red absorbing phytochrome and response in phytochrome-mediated anthocyanin synthesis. *Pl. Cell Envir.* **2**, 159–163.
VanDerWoude, W. J. 1982 Mechanism of photothermal interactions in phytochrome control of seed germination. In *Strategies of plant reproduction* (BARC Symposium no. 6) (ed. W. J. Meudt), pp. 135–143). New Jersey: Allanheld, Osmun.
VanDerWoude, W. J. 1983 A dichromophoric model for the action of phytochrome: evidence from photothermal interactions in lettuce seed germination. *Proc. natn. Acad. Sci. U.S.A.* (In the press.)
VanDerWoude, W. J. & Toole, V. K. 1980 Studies of the mechanism of enhancement of phytochrome-dependent lettuce seed germination by prechilling. *Pl. Physiol.* **66**, 220–224.
Vince-Prue, D. 1977 Photocontrol of stem elongation in light-grown plants of *Fuchsia hybrida*. *Planta* **133**, 149–156.
Whitelam, G. C. & Johnson, C. B. 1981 Temporal separation of two components of phytochrome action. *Pl. Cell Envir.* **4**, 53–57.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES