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INDUCTION OF RAPID CHLOROPHYLL ACCUMULATION IN DARK GROWN SEEDLINGS. III. TRANSPORT MODEL FOR PHYTOCHROME ACTION

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SUMMARY

An explanation is proposed for the differences between completely dark grown and de-etiolated seedlings of some higher plants in their sensitivity to far red inductive light and red-far red photoreversibility. It is based upon the idea that the morphogenic pigment phytochrome is transported during the process of de-etiolation to certain receptor sites of restricted capacity within the plant cell which sites then become activated to initiate the physiological photoresponse.

1. INTRODUCTION

Attempts to correlate spectrophotometric phytochrome assays with the physiological reaction controlled by this pigment generally have had negative results. In a number of cases, data about the concentration and state of the pigment derived from these two types of measurement were greatly contradictory ("phytochrome paradoxes"). The situation has been reviewed by HILLMAN (1967, 1972).

A similar discrepancy seems to exist with respect to the far red photoreversibility of red-induced rapid chlorophyll (Chl) accumulation in pea and bean. We have reported previously (RAVEN & SPRUIT 1972b) that in completely dark grown seedlings far red displayed a relatively high inductive capacity as compared with red. Far red also proved hardly or not at all antagonistic to red in the standard photoreversibility experiments, undoubtedly as a consequence of its own inductive action. However, this inductive capacity of far red became lost upon progressive de-etiolation of the tissue. Concomitantly, red-far red photoreversibility became much more pronounced.

The action spectrum for induction of Chl accumulation in pea (RAVEN & SPRUIT 1972a) pointed to phytochrome as the only pigment involved. The fact that upon very prolonged dark incubations photoreversibility became more pronounced in completely dark grown leaves, too, strengthens this conclusion (RAVEN & SPRUIT 1972b). On the other hand, BLAAUW et al. (1968) and BOTTOMLEY (1970) have tried to explain similar findings by assuming the presence of two photoreceptor pigments, a far red reversible and a far red irreversible one, both mediating the low-energy effects of red light.

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In the present paper we attempt to attribute the difference in photoreversibility between completely dark grown and de-etiolated tissue as described above to an extremely low P_{fr} requirement of the induction reaction in addition to transport of phytochrome during the process of de-etiolation.

Migration of phytochrome might also explain some of the other phytochrome paradoxes (SPRUIT et al., in the press).

2. TRANSPORT MODEL FOR PHYTOCHROME ACTION IN RAPID CHLOROPHYLL ACCUMULATION

The model to be discussed below is based upon some assumptions. First, we suppose that in seedlings grown in complete darkness phytochrome is present in a comparatively large fraction of the cell volume. This assumption is closely connected with the problem of the intracellular localization of phytochrome within a completely dark grown seedling. PRATT & COLEMAN (1971), using an immunocytochemical assay, found it associated with both nuclei and plastids, in addition to the cytoplasm. MARMÉ & SCHÄFER (1972) demonstrated its presence in the plasmalemma. WELLBURN & WELLBURN (1973) reported that the ultrastructural development of isolated etioplasts was still under phytochrome control. An obvious conclusion from these observations could be that, at least in completely dark grown tissue, phytochrome is not restricted to a particular cell organelle only.

On the other hand, SPRUIT & SPRUIT (1972) concluded from the distortion of phytochrome difference spectra by the presence of chlorophyllous pigments in leaves (SPRUIT 1967) as well as in stem tissue (GRILL 1972) of irradiated pea seedlings that this pigment is distributed in a non-homogeneous way.

This leads us to the next assumption, viz. that in plant cells a number of reaction centres of relatively small volume exist. Upon irradiation of completely dark grown tissue, the far red-absorbing form of phytochrome (P_{fr}) is assumed to migrate to these reaction centres which then become activated.

At the moment we need not speculate about the site and nature of these reaction centres except that they are associated with specific physiological reactions. We have tried to illustrate this with the aid of *fig. 1*. The seven squares in this figure indicate the part of the plant cell accessible to phytochrome. Initially, in the completely dark grown state (*fig. 1*, a), phytochrome is assumed to be present only in the compartment marked 'R', in the red-absorbing form P_r. The rectangular compartment at the right side of the cell represents the reaction centre associated with induction of rapid Chl accumulation that can be activated by P_{fr}. The rectangle at the bottom of the cell represents the other places where phytochrome may become bound. The relatively small quantities of active phytochrome inside the Chl reaction centre (p_r and p_{fr}) are indicated by small letters, r and f, respectively. The larger amounts of inactive phytochrome outside the centre (P_r and P_{fr}) have been denoted by capitals R and F. We have indicated the relative amounts of phytochrome involved by different sizes of these letters.

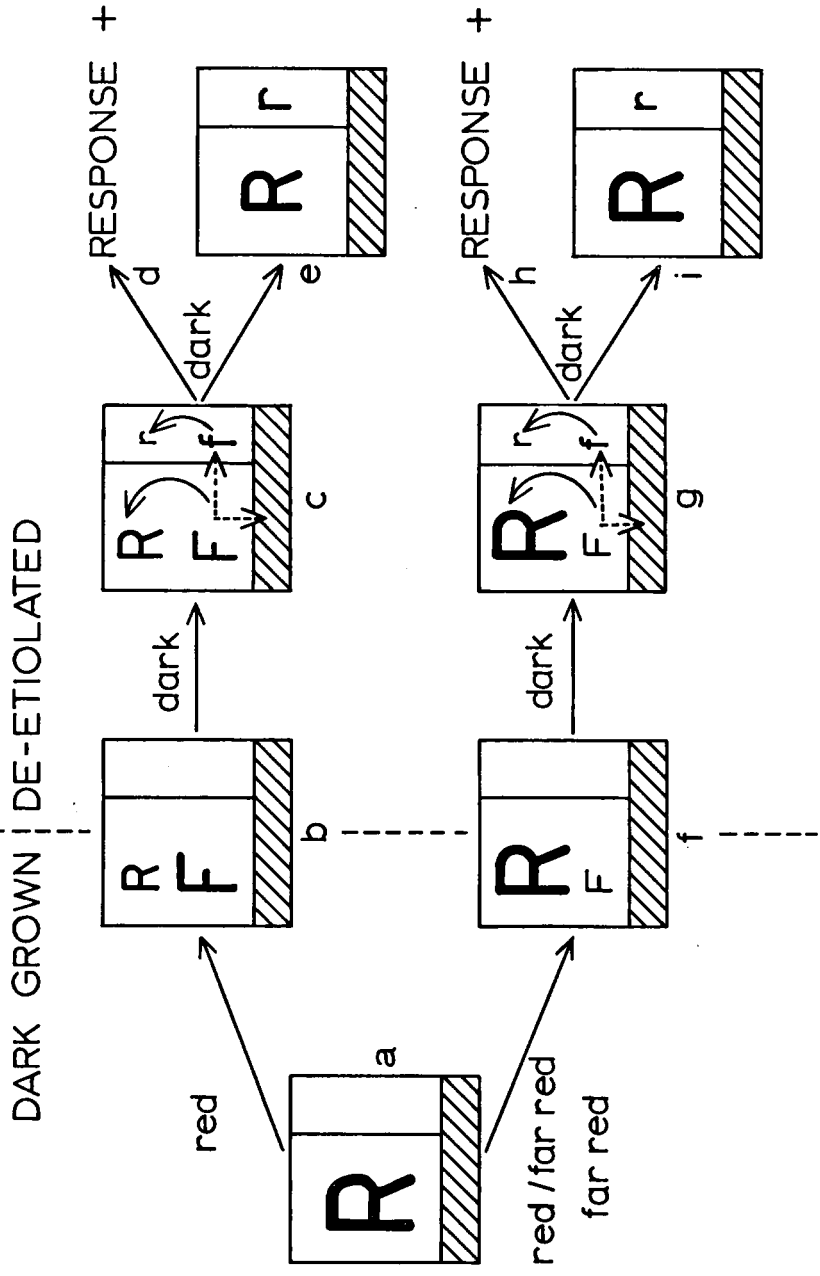

Fig. 1. Model illustrating processes during the establishment of the de-etiolated state by various light treatments. The squares represent the fraction of the cell volume accessible to phytochrome. Inactive phytochrome (P_r and P_{fr}) is indicated by R and F, respectively; active phytochrome (p_r and p_{fr}) is indicated by r and f. Migration of P_{fr} is represented by dashed arrows, dark reversion by curved arrows. Other receptor sites than the one involved in induction of rapid Chl accumulation are indicated by the hatched rectangle forming the lower part of each square. For further explanation see text.

Starting with completely dark grown tissue (*fig. 1*, a), a brief red exposure gives rise to a certain percentage P_{fr} (*fig. 1*, b). In darkness this is followed by migration of P_{fr} to the reaction centre for Chl induction as well as to other reaction centres and by reversion of P_{fr} to P_r (*fig. 1*, c). As soon as the concentration of p_{fr} inside the reaction centre rises above a certain threshold level for a sufficient period (MOHR 1970), the physiological induction process is assumed to start. Light regimes that experimentally resulted in a stimulation of the Chl-*a* accumulation rate above either the dark control or those already pretreated with light have been indicated by a plus sign (*fig. 1*, d), in agreement with the results of our earlier experiments on Chl-*a* induction and its reversion (RAVEN & SPRUIT 1972b).

Phytochrome inside the reaction centre (p_{fr}) is assumed to undergo dark reversion to p_r. As soon as this reaction is completed, we have reached the de-etiolated state which differs in our model from the initial dark grown state in that there is now p_r in the reaction centre (*fig. 1*, e). As indicated before, we call phytochrome inside the reaction centre "active phytochrome". The remaining phytochrome outside the centre is called "inactive phytochrome".

Since it appears reasonable to assume that active phytochrome in the form p_{fr} is bound in some way, the kinetics of its dark reversion may well be different from those for the dark reversion of inactive phytochrome (KENDRICK & SPRUIT, in prep.). We have found that in bean seedlings a renewed red irradiation, given after a dark interval of about 2 hours, enhances the inductive action of the first (RAVEN & SPRUIT 1972b). A possible explanation for this observation may be that inside the reaction centre dark reversion is completed within this period. Alternatively, it may indicate that after that time the p_{fr} concentration is about to return to the threshold level or is already lower, while the system itself is still sensitive to further induction. We suppose, therefore, that the maintenance of the inductive capacity for Chl-*a* formation over a period of at least 48 hours (RAVEN & SPRUIT 1972a, 1972b) does not reflect the continuous presence of p_{fr} at concentrations above the threshold level. On the contrary, we assume that the inductive capacity of a certain light dose as well as the duration of the period over which this induction is maintained are determined by the extent to which the initial p_{fr} concentration exceeds the threshold level. The fact that the induction of Chl formation is maintained over such long dark periods may then be explained by the irreversible nature of the development of the etioplasts occurring simultaneously. The latter process may be the basic feature of the Chl-*a* induction phenomenon (RAVEN & SPRUIT 1972b).

When red inductive irradiation of completely dark grown tissue is immediately followed by far red, nevertheless a small amount of P_{fr} will remain as a result of the overlap of the absorption bands of the two forms of phytochrome (*fig. 1*, f). This P_{fr} may or may not revert partly to P_r in subsequent darkness (*fig. 1*, g). At any rate, part of it can be transported in the meantime to and become concentrated in the empty reaction centre (*fig. 1*, g). There it could still elicit a certain level of induction if its concentration rises above the thresh-

old (*fig. 1*, h). Such a mechanism could explain the absence a of red-far red antagonism.

This model does not, of course, exclude the possibility of existence of completely photoreversible reactions in dark grown seedlings, since this may depend on both the relative capacity of the reaction centre and the height of the threshold. In other words, photoreversibility depends upon the "p_{fr}-capacity" of the reaction centre and "p_{fr}- requirement" of the respective physiological response.

A similar explanation can be given for the inductive capacity of far red not preceded by red, when administered to completely dark grown tissue (*fig. 1*, f–i).

In fact, all light treatments we have applied so far were found to induce an increased Chl-*a* accumulation rate in completely dark grown leaves in a number of cultivars of pea, bean, and maize (RAVEN & SPRUIT 1972b). In our model this indicates that in such cases p_{fr} indeed surpassed the threshold level for induction as expressed by the plus signs in *fig. 1*.

An inductive treatment resulting in the de-etiolated state does not necessarily lead to a positive physiological response, as might be concluded from *fig. 1*: Green safelight can act like red with respect to the increase in photoreversibility, without, however, inducing any directly measurable physiological response (RAVEN & SPRUIT 1972b). We can ascribe this to the circumstance that P_{fr} after being produced in small amounts by the green light, is slowly transported to the reaction centre where simultaneous dark reversion prevents the p_{fr} concentration from rising above the threshold (SPRUIT et al., in the press). Nevertheless, the slow accumulation of active phytochrome in the form p_r establishes a more or less completely de-etiolated state, without, however, triggering the induction of Chl formation. A similar experiment should be possible with light of very low intensities in wavelength regions other than green.

With tissues already de-etiolated by previous exposure to light the responsivity towards a second irradiation becomes quite different (*fig. 2*). In this case p_r is already present inside the reaction centre (*fig. 2*, a). A (second) red dose will therefore transform both active and inactive phytochrome (*fig. 2*, b). It is the immediate reappearance of p_{fr} inside the reaction centre (*fig. 2*, b–c) that, possibly, gives a new impulse to a further increase in physiological response (*fig. 2*, d). We suppose that the reaction centre, if already completely occupied by active phytochrome (as is the case upon saturating de-etiolation) cannot accomodate more p_{fr}. Molecules newly formed outside the reaction centre may be transported to reaction centres associated with other physiological responses (*fig. 2*, c and g). One may also consider the possibility that certain phytochrome sinks exist, where P_{fr} remains inactive or, finally, may be used as substrate in some other biosynthetic chain (KENDRICK & HILLMAN 1972).

These assumptions may explain the difference in far red sensitivity and photoreversibility between dark grown and de-etiolated tissues. Far red following red as well as far red alone give rise to a small amount of P_{fr} outside the reaction centre, as illustrated in *fig. 2*, f. Also inside the centre some p_{fr} is formed. The ratios P_{fr}/P_r (and p_{fr}/p_r) established by this irradiation will, of

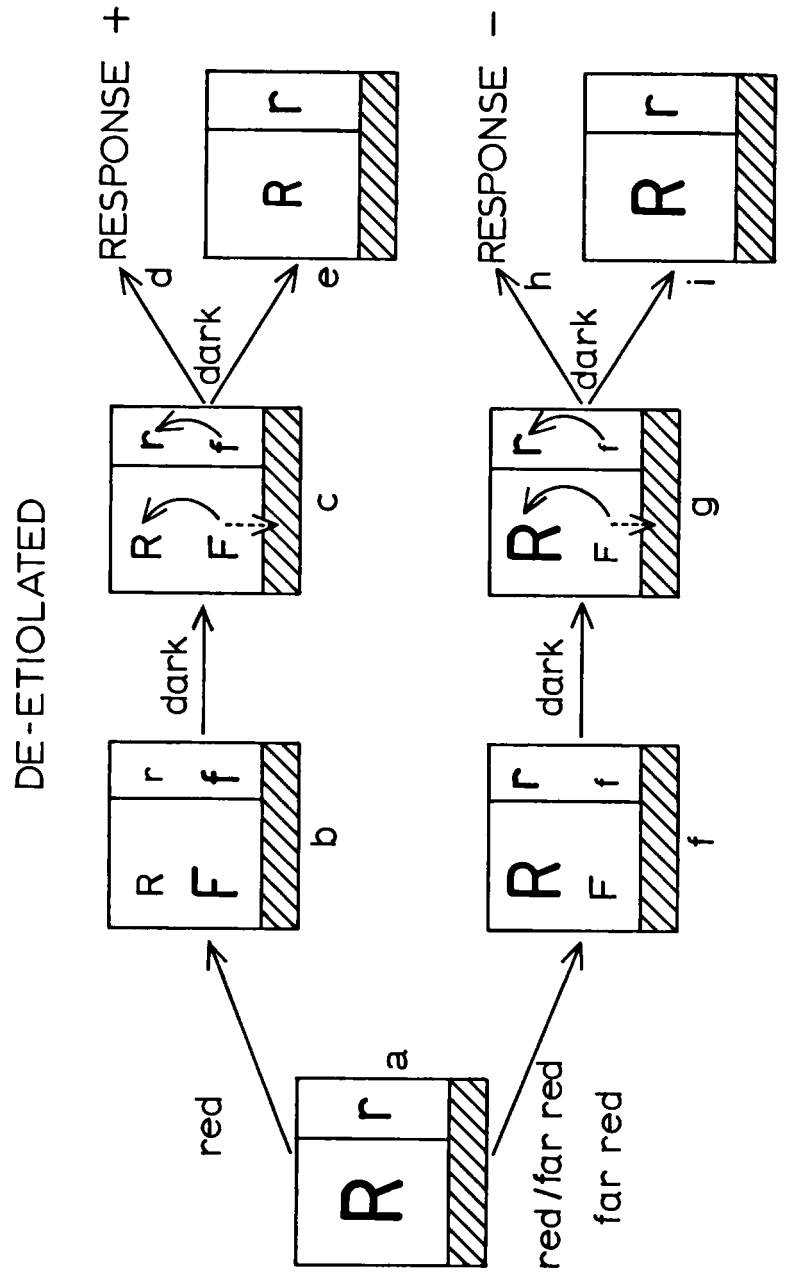

Fig. 2. Model illustrating processes that occur upon various light treatments in tissue de-etiolated by previous exposure to red. For details see *fig. 1* and text.

course, be the same as in completely dark grown material. Since, however, the completely filled reaction centre should not allow the penetration of any new P_{fr}-molecules, the absolute concentration of its p_{fr} will remain low and should not surpass the physiological threshold (*fig. 2*, g–h). Obviously, this condition should also lead to complete photoreversals in de-etiolated tissue.

3. DISCUSSION

In the previous section we have tried to explain the increase in far red reversibility of red-induced rapid Chl-*a* accumulation (RAVEN & SPRUIT 1972b) with a model attempting to describe the process of de-etiolation. The basic features of this model are the intracellular translocation of P_{fr} and its concentration into receptor sites of restricted capacity. Predictions from this model were shown to give possible explanations for some paradoxical observations from the literature (SPRUIT et al., in the press). We will discuss here only those phytochrome data, that are immediately related to our findings on the induction of rapid Chl-*a* accumulation. Among these the observations of FOX & HILLMAN (1968a, 1968b) are of great interest. They found that the inductive effect of red light, causing inhibition of growth of stem segments of pea, escaped far red reversibility much earlier in dark grown tissue than in de-etiolated plant material. It also appeared that dark grown tissue showed greater reactivity to the lower photostationary states used. This implies either that P_{fr} acts more rapidly in dark grown seedlings than in de-etiolated ones, or that the sensitivity of the system towards P_{fr} changes upon de-etiolation (HILLMAN 1972). Especially the latter possibility comes very close to what we observed for the inductive capacity of far red only. Fox & Hillman assumed differences between dark grown and de-etiolated tissue in the concentration of a substrate on which P_{fr} action might depend. Their condition for de-etiolation implied a 16-hour pretreatment during which period a number of substrate-requiring formative reactions might occur. However, we think that this hypothesis is not valid for our case for two reasons: Firstly, the inductive action of far red only upon Chl formation is less pronounced in seedlings pretreated with green safelight, although the light dose applied did not evoke any measurable physiological response by itself. Secondly, de-etiolation, as measured by increased red-far red photoreversibility, could be demonstrated already within 2 hours after a short red exposure.

A more or less similar time scale for de-etiolation has recently been reported by KENDRICK & HILLMAN (1972). The high degree of similarity between the data of Fox & Hillman and ours makes it very tempting to explain also their observations with the transport model for phytochrome. Before discussing this further, it should be stressed that the photostationary state levels as mentioned by Fox & Hillman are derived from spectrophotometric measurements. However, according to our model, mainly the inactive forms of phytochrome can be estimated in this way. Furthermore, one has to assume that the size of the reaction centre(s) are relatively small. Obviously, the lower photostationary

state levels of phytochrome required for the inhibition of growth of dark grown stem segments may then be ascribed to the rapid saturation with active phytochrome of the empty reaction centres as a result of phytochrome transport. The threshold level for induction is rapidly reached, and the small doses of light required explain the low photostationary state levels necessary for dark grown material.

In de-etiolated pea stem segments, however, higher doses of light or higher photostationary state levels are needed for re-establishing a p_{fr} concentration above the threshold level, since the centre is already saturated and the concentration effect resulting from P_{fr} migration is no longer possible.

To make this very clear let us assume, e.g., that we start with a completely dark grown tissue. Irradiation with a particular far red source establishes a photostationary state of, say, 3%. If this amount is adequate for saturating the centre, the latter is fully occupied with phytochrome, all as p_{fr}, after completion of P_{fr} migration. After dark reversion the centre is still fully occupied, but with p_r. A repeated irradiation with the same light source now establishes a photostationary state in the centre of 3% and the p_{fr} concentration now levels off at 0.03 times the first, which is below the activation threshold. The original light source is now no longer able to activate the centre and a light source giving a much higher photostationary state would be required.

Confirmation of this explanation would require the estimation of phytochrome concentrations in situ inside the plant cell or the study of phytochrome-controlled photoreactions in cell organelles, isolated either from dark grown or from de-etiolated tissues.

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