THE FUNCTION OF PHYTOCHROME IN REGULATION OF PLANT GROWTH

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Communicated October 2, 1967

Many aspects of growth and development of seed plants, ferns, and mosses are affected by the change in form of the blue chromoprotein phytochrome.[1] These include flower initiation and development, germination of seeds and spores, and enlargement of such structures as fronds, leaves, and stems. This diversity of final display has invited speculation about the course of phytochrome action. The light-induced molecular change in phytochrome, which is the first step in action, is known, as also are the thermal steps through intermediates to a final form. Immediate consequences of establishing the final molecular form (P_{fr}) within the cell, however, have remained unknown—in part because of the long times required for the physiological displays. Recent finding of several rapid displays and re-evaluation of some previous results indicate that an early consequence of phytochrome action is a change in permeability of the cells involved and possibly of intracellular components. Our purpose is to collate the information leading to this conclusion and to place some aspects of previous work in a new perspective.

Physiological studies show that phytochrome (P) exists in two forms, P_r and P_{fr}, of considerable persistence, which are photochemically intraconvertible.[2] Absorption maxima are at 660 nm (P_r) and 730 nm (P_{fr}). The course of intraconversions is now known from flash excitation of the isolated pigment.[3, 4] The first-order rate constant at 0°C for the slow step in the appearance of P_{fr} upon irradiation of P_r is 0.3 corresponding to a half-conversion time of 2.3 seconds. The reverse reactions from P_{fr} to P_r are rapid with rate constants of 1010, 710, and 170 sec^{-1} for the three intermediate steps. Studies *in vivo*, both physiologically[5, 6] and by bichromatic spectrometry,[7] show a dark reversion of P_{fr} to P_r. Half times for reversion in several plants range from an hour or less to several days, depending on the influence of factors associated with the action.

The physiologically active form of P is P_{fr}. This follows from the rapid change in response with change in P_{fr} when P_{fr} is a small part of total P, as contrasted with the slow change in the reverse direction when P_{fr} is predominant. An example is the approximately linear dependence of enlargement of the dark-grown pea leaf on the logarithm of the incident irradiance at 660 nm effective in changing the initially very predominant P_r to P_{fr} (Fig. 1).[8] Half-saturation of this response requires conversion of only about 2 per cent of the P_r to P_{fr}. This conclusion is sometimes accepted because of viable persistence of some nongerminating buried seed for as much as 1,700 years[9] with P in the inactive P_r form. Such seeds are brought to quick germination upon exposure to light, as occurs through disturbing the soil, which converts P_r to P_{fr} in imbibed seed.

Developing knowledge of phytochrome action has depended almost solely on the photoreversible change of the two forms. An action can be initiated by

quickly (in seconds) establishing P_{fr}, through irradiation, in a plant previously having predominant P_r. An initiated action can be terminated when desired by the photoconversion of P_{fr} back into P_r, in which direction, $P_{fr} \rightarrow P_r$, absorbancies of the two forms (at 730 nm) do not seriously overlap. Displays are known which depend on the presence of P_{fr} only for seconds, which also indicates that P_{fr} is the active form of P. These are crucial ones in the present context. Many displays, however, require P_{fr} to be present for hours.

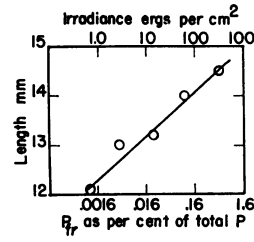

FIG. 1.—Length of the 1st + 2nd leaf of pea seedlings plotted as a function of the daily irradiation at 650 nm. The corresponding per cent conversion of P_r to P_{fr} in a solution of the isolated pigment is also shown as abscissa.

There is predilection to mistrust deductions about initial steps in slowly expressed displays requiring days or seasons, such as flowering. A search accordingly is made both for responses requiring the presence of P_{fr} for only brief periods and for those quickly expressed—within minutes, if possible.

A moderately rapid response, which we have long appreciated, is *absence* of induced bending of etiolated pea and barley seedlings upon unilateral exposure to red light (>600 nm). The light reduces stem elongation and increases stem stiffness. The amplification afforded by stem curvature would make differences in elongation of cells on opposite sides of a stem evident within less than 30 minutes, as is seen when wavelengths are <500 nm, which leads to the phototropic bending activated by auxin. Absence of curvature in red light is evidence of synchrony of elongation induced radially about the stem by P_{fr} even though the light absorption is asymmetric—being greatest where the light falls. This result is evidence of effective transport of a material controlling stem elongation or some other compensatory action. Deduction is blunted, however, both by the negative nature of the display—that is, lack of curvature—and a possible unknown interaction with auxin, which also controls stem elongation.

Very rapid distribution of an effect of radiation on cell enlargement is evident in the unrolling of the first leaf of wheat, barley, and several other etiolated grasses. The dark-grown leaf remains rolled because of restricted enlargement of mesophyll cells near the upper surface of the leaf. Virgin[10] found the expected features for phytochrome control of unrolling of the first wheat seedling leaf. The detailed action spectrum has a maximum at 660 nm, with saturation of response at low irradiances of 1.0×10^{-8} einsteins/cm^2. The potentiated response, moreover, is repeatedly reversible by far-red radiation but only to within about 40 per cent of the increase in width of the red-irradiated leaves over the dark controls. Wagné[11] finds that if one-fourth to one-half of a leaf is irradiated and then removed, the unirradiated part unrolls in 24 hours to within about 40 per cent of the increased width shown by the irradiated part. The minimum irradiation and manipulation time in severing the irradiated portion was about 20 seconds.

Rapid response to the presence of P_{fr} is also shown in control of flowering of several plants. Nakayama *et al.*[12] found that flowering of the Japanese morning-glory (*Pharbitis nil* Chois.) (*Ipomoea nil* (L) Roth) induced by 16-hour nights can be prevented by partial conversion of P_r to P_{fr} after 8 hours of darkness.

The potentiated response, which requires about ten days to develop, is not reversed by changing P_{fr} back to P_r in the course of five minutes. Fredericq,[13] however, found that the potentiated response is partially reversed if the total irradiation time, red + far red, at the middle of the 16-hour night is 60 seconds (Table 1). He obtained similar results over a period of about five minutes with potential suppression of flowering of *Kalanchoe blossfeldiana*.[14]

TABLE 1

LOSS OF PHOTOREVERSIBLE SUPPRESSION OF FLOWERING OF *Pharbitis nil* WITH TIME FOLLOWING EXPOSURE TO RED LIGHT AT THE EIGHTH HOUR OF EACH OF THREE INDUCTIVE 16-HOUR NIGHTS*

Treatment	Flowering after 10 days development (buds per plant)	Estimated P_{fr} after treatment (per cent of P)
Dark control	6.6	—
30-sec R	0.7	>70
30-sec R + 30-sec FR	3.7	<10
30-sec R + 180-sec dark + 30-sec FR	0.4	<10

* After Fredericq (ref. 13).

In seed germination, for which lettuce (*Lactuca sativa* (L) var. Grand Rapids) is representative, promotion of germination by P_{fr} can be partially reversed after periods as long as 12 hours.[15] The light requirement for germination is eliminated by gibberellic acid (10^{-4} M GA_3). Recently, Bewley *et al.*[16] found considerable enhancement of germination (18% dark controls to 36% lighted) induced by the presence of P_{fr} for five minutes when suboptimal amounts of GA_3 were supplied.

The several potentiated responses of P_{fr} action terminated by photoreversion clearly show a response requiring the presence of P_{fr} only for seconds. This time is no more than an order of magnitude slower than the half time (2.3 sec) for establishing P_{fr} if radiant energy is nonlimiting. In each of the experiments considered, the amount of P_{fr} was limited by radiation intensity and the half time for fully establishing P_{fr} was about 20 seconds. The actions are accordingly potentiated about as rapidly as P_{fr} appears. Success in reversal of the potentiated responses in periods shorter than 60 seconds shows that action is not a consequence of once having established P_{fr} irrespective of its later reversal.

Rapid actual display of P_{fr} action, to be compared with potentiation of a much slower display, as in the cases just discussed, is shown by the sleep movements of some leaves. Fondeville *et al.*[17] studied such movements of leaves of the sensitive plant (*Mimosa pudica* (L)). Open leaflets on plants in normal sunlight begin to fold together about the tertiary pulvini when darkened. If the P_{fr} is transformed to P_r at the beginning of darkness, however, the leaflets remain open. Closing is again induced by reestablishing P_{fr}. The closing movement induced by the presence of P_{fr} is readily detectable after 5 minutes and is half complete in about 20 minutes at 25°. A similar rate of display has been found with leaflets of the silk tree *Albizzia julibrissin* and several other legumes.[18, 19]

The leaf movement responses of *M. pudica* and *A. julibrissin* operate through turgor control of special cells, in pulvini. In the case of *M. pudica*, leaves of which respond to touch, the turgor control has been an object of study throughout the past 100 years. Excitation of movement by touch is known both to give action

potentials[20] and to be accompanied by salt efflux into tissue spaces.[21] Jaffe and Galston[19] found for *A. julibrissin*, which is apparently insensitive to touch, that photoexcitation leading to P_{fr} causes salt release to surrounding water.

We conclude from the actions studied that P_{fr} mediates cell permeability in the several grasses and legumes. Some materials released by the stimulated cells are transported within seconds to other cells at distances as great as 1 cm.

Another display of P_{fr} action, which is about as rapid (detectable in 5 min) as leaf movement, was found by Haupt[22] for plastid orientation at low light intensities in the alga *Mougeotia*. Moreover, by studying the response to polarized light, Haupt showed that the phytochrome is oriented in the cytoplasm of a cell with respect to the single plastid. This indicates P_{fr} control over a body within a cell and a degree of associated action at a membrane.

The direction of development of protonemata of spores of the fern *Dryopteris filix mas* (L) Schott is influenced by light. Etzold[23] found that the action spectrum for the response has maxima near 460 and 660 nm, with the former much the more effective of the two. He concluded that two pigments are involved in the action. One of these seems to be phytochrome, although, as with the quickly potentiated leaf-unrolling effects, photoreversibility is only partially shown in the effect of adaptation to red light. Results obtained with polarized light show that both pigments are dichroic and are located close to or in the cell wall with the axes of maximum absorption parallel to the cell wall. Etzold concludes that the axis of maximum absorption of phytochrome at the cell wall turns through 90° in the transition from P_r to P_{fr}. He also concluded that bending of the protonemata is on the side of maximum absorption of polarized radiation by P_r.

Isolation procedures for phytochrome,[24] based on the bichromatic spectrometry assay, are straightforward, as might be expected for a nonparticulate protein. Procedures, though, require cell breakage, which is drastic in a sense and involves shifts of pH towards higher values than in the unbroken cells. Recoveries are far from quantitative and possibilities of only a part of the total P_{fr} being "active" are currently speculated about. The isolation does not preclude membrane association of phytochrome, nor do the actions affecting permeability require the presence of P in a membrane as contrasted to action of P_{fr} on a membrane. Similar questions of membrane association arise in action of insulin on cell permeability for glucose in man.

An effect of P_{fr} on cell permeability could well lead to a variety of eventual displays. Steps remote from the primary control might relate back to the primary permissive step affected by P_{fr}. Mohr and his co-workers,[25, 26] who have adduced evidence that P_{fr} results in derepression of gene action, are chiefly concerned with these remote steps. The prevention of several potentiated P_{fr} responses by puromycin or actinomycin D, as suppressors of protein synthesis[27] —for example, suppression of phenylalanine deaminase formation in mustard seedlings—is in accord

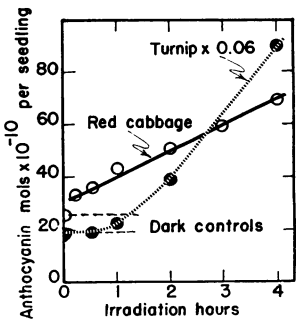

FIG. 2.—Variation in anthocyanin synthesis in red cabbage and turnip seedlings with irradiation at 0.6 mw per cm² at 700 nm. The seedlings were extracted for analysis 24 hr after zero time (after Siegelman and Hendricks, ref. 28).

with some displays eventually involving gene action. Mohr, in recognizing extremes of displays as showing long or short induction periods for potentiation, perhaps became involved in early steps of P_{fr} action in the latter. He considered the responses with short induction periods, however, to involve gene derepression. These are illustrated by P_{fr} action on photochemically enhanced formation of anthocyanin in red cabbage and turnip seedlings (Fig. 2).[28] An induction period is absent or very short in red cabbage, which responds to the presence of P_{fr} within five minutes even though only about 1 per cent of P is in the P_{fr} form. An induction period is present in turnip seedlings, which do not respond in this regard to P_{fr}. The observed response in red cabbage is considered by us as possibly arising from a P_{fr}-determined cell permeability, which could allow more rapid entry of substrates required in anthocyanin synthesis.

Phytochrome action in plants is somewhat similar to three phenomena in animals; namely, excitation of vision, control of photoperiodism by light, and several hormonal actions. The photochemically induced molecular changes in phytochrome[3, 4] markedly resemble those of rhodopsin[29, 30] in involving a sequence of rapidly appearing intermediate forms of a chromophore despite the respective chromophores of the chromoproteins being a phycobilin and retinal. One of these steps is photoreversible in both cases. In vision, the first response following the initial photoaction is the appearance of an early receptor potential, which is displayed in less than a millisecond after excitation.[31] This is followed by a late receptor potential, which rises to a maximum in about 10 msec. There is evidence that the early receptor potential involves a rapid molecular change of an early intermediate, while the late receptor potential arises from a membrane depolarization induced by the over-all molecular change. The phytochrome actions in *M. pudica* and *A. julibrissin* are induced by the over-all molecular change. Depolarization across a membrane is known to take place in *M. pudica*.[20]

The photoperiodic control referred to in animals is a stimulation of cells in the insect[32] or avian[33] brain by light as a first step. This is followed by neural transport of hormonally active material leading finally to a circadian rhythmic display. The first step likely involves a change in cell permeability, although we do not know of critical information on this point. Later steps in the animal responses involve a multiplicity of hormone actions, some of which mediate gene derepression as illustrated by eventual involvement of the insect hormone, ecdysone.

The course of photoperiodic control of flowering, although largely hypothetical, possibly has a similar course of action as involved in insect metamorphosis. The initial photostimulating step, the one affecting permeability, is postulated as being followed at some stage by release of a "hormone," the hypothetical "florigen."[34] "Florigen" acts either to enhance or to suppress flowering, depending upon the type of plant. It is transported from a leaf, where P_{fr} acts, to a distant developing meristem, such as the terminus of the stem axis, where the eventual flowering display occurs. The final differentiation in flowering surely involves the genic apparatus. The transport of the stimulus from a leaf to the meristem was early found to require tissue continuity throughout the transport system.[35] The movement is slow—the order of 10 cm/day, as shown by effects on flowering of removing the irradiated leaf after various intervals. Both observations indicate

that the flower-controlling material released by P_{fr} action has a high molecular weight or includes such a component.

Summary.—A number of plant responses indicate that phytochrome acts on cell permeability as a first or early step in its control of plant growth and development.

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