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Plant Photobiology in the Last Half-Century[1]

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The absorption of light energy not only nourishes the plant through photosynthetic phosphorylation and concomitant carbon dioxide fixation, but it also determines the nature and the direction of plant growth. This review will summarize the major advances in the physiology of both of the latter processes over the last half-century. Because of severe limitations of space, only a few key concepts can be mentioned, let alone emphasized, and some fields, like algal phototaxis, must be omitted entirely.

Although there was certainly provocative earlier work by Klebs and others (100), our present understanding of the control of form by light, or photomorphogenesis, began with the clear enunciation of the principles of photoperiodism by Garner and Allard (55, 56) a little more than 50 years ago. Photomorphogenetic transformations are mediated mainly by the longer wavelengths of the visible spectrum (105), and are these days attributed mainly or exclusively to absorption by phytochrome. The development of the phytochrome concept by Hendricks and Borthwick (74), and the isolation, partial identification, localization, and study of the mode of action of this pigment is certainly one of the most brilliant accomplishments of modern plant physiology. The history and current status of phytochrome research are well summarized in a recent symposial volume (96), but I shall attempt to summarize a few key concepts and provide a general updating. The second process, phototropism, is initiated entirely by the shorter wavelengths of the visible spectrum (84). For about 25 years, there has been a sporadically lively debate as to whether carotenoids or flavins are the most likely photoreceptors for phototropism and related processes (49, 144). Although a definite conclusion cannot yet be made, the bulk of recent evidence favors the flavin hypothesis. I shall try to summarize this evidence and certain other aspects of the physiology of phototropism as well. Although experimentation in phototropism has been relatively quiescent for about 20 years, recent developments provide encouragement for new insights into this process.

Some methodological problems are unique to this kind of biology. Decisions about photoreceptor pigments depend on correlations between action spectra for the process and absorption spectra for the pigment (10, 160). Light, to be effective, must first be absorbed, and in energy ranges where photon absorption limits the rate or extent of a process, there should be rough correspondence between regions of the spectrum that are most active in eliciting a process and regions of high light absorption by the pigment. One cannot push these coincidences too far, however, because other pigments can act as light screens, differential refraction of different wavelengths inside the tissue can produce distortion in relative actinic effectiveness, attachment of pigments to different proteins can alter their absorption properties in different ways, resonance transfer of energy between pigments or unequal quantum efficiencies at different wavelengths can alter curves, and dichroic orientation of a pigment in a lamella may produce aberrant or unrepresentative values. Despite these caveats, action spectra remain the major method for inferring the nature of the photoreceptor (130, 131).

Action spectra are prepared by doing dose-response energy experiments at each of the wavelengths employed. From such data, provided that the reciprocity law holds, one can calculate the energy required to produce a standard effect, *e.g.* 50% promotion of seed germination. When corrected for the different energy of quanta in the different portions of the spectrum and transformed to reciprocal values, these data tell us the relative effectiveness of quanta in producing the observed physiological effect. A plot of this parameter against wavelength gives the standard action spectrum.

Deducing the nature of the photoreceptor pigment is only the first step along the difficult road of understanding the physiology of the process itself. For example, unlike photosynthesis, in which light energy participates stoichiometrically with quantum yields of less than 1.0, both photomorphogenesis and phototropism are actuated by low irradiances, so that the final quantum yield, in any chemical or physical sense, is far greater than unity (49, 144, 160). Investigators in this field have to face the problem of defining an amplification mechanism that follows photon absorption. Among the well known invoked cellular amplifiers have been (*a*) gene repression and derepression, (*b*) enzyme activation or inactivation, (*c*) control of the binding, release, or metabolism of oligodynamic substances such as hormones, which then control metabolic pathways in various ways, and (*d*) control of membrane properties. At the moment, the membrane is considered a likely locale for the action of both photomorphogenic and phototropic pigments. This may represent either a historical confluence of insights into the inner workings of cells, or merely a current fad, destined to be replaced by another generalization in the years ahead. I believe it is too early to tell.

PHOTOMORPHOGENESIS

The discovery of photoperiodism just over 50 years ago not only immediately subsumed much information within the confines of a single generalization, but also provided an experimental basis for further explorations of physiology. If daylength indeed determines form and reproductive habit, it becomes appropriate to inquire whether the length of the light or the dark period is most important in the regulatory process. The answer came easily through the use of artificial daylengths and controlled chambers (65), certainly one of the first productive uses of this technique in plant physiology. From such experiments it became clear that although a certain minimum length of daylight was essential to provide enough photosynthate to nourish the plant, the dark period was most impor-

[1] Dedicated to the memory of Harry A. Borthwick, a wise and compassionate man. Generous support from the National Science Foundation has made possible the author's active involvement in this field for more than 20 years.

tant in the measurement of time. It was discovered that the effect of the dark period, whether promotive or inhibitory to flowering, could be annulled by low irradiances of light, especially in the middle of the dark period (103). This made it feasible to probe the action spectrum for the promotion and inhibition of flowering by relatively low irradiances of monochromatic light (105) instead of studying more complex interactions arising from prolonged growth of plants in high intensities of selected wavelengths of light (154).

Experiments with localized application of effective photoperiods revealed that although the bud responds to photoperiodic stimuli, it is the leaf that perceives the photoperiodic stimulus (65). This led to the postulate of a mobile, floral-inducing hormone, or florigen, moving from leaf to bud (30). The florigen theory was fortified by the demonstration of the graft transmissibility of the floral stimulus (31), even by single leaves (73), as long as tissue union occurred (162). Despite the availability of adequate bioassays and the induction and inhibition of flowering by selected chemical probes, florigen has thus far eluded extraction and identification. The discovery of photoperiodic induction (6) and the effectiveness of a single inductive dark period in some plants (65, 80) has led to conjectures about self-perpetuating metabolic alterations triggered by the inducing stimulus (76).

The concept of a single pigment controlling a wide variety of developmental processes arose from similarities in action spectra prepared for photoperiodic inhibition and promotion of flowering in long day and short day plants (104, 105), deetiolation in various seedlings (8, 106), photocontrol of seed germination (9) and chloroplast development (161), and photocontrol of flavonoid pigment formation (98, 133, 134). For all these processes, the major action peak is in the red region of the spectrum, near 650 nm; a lesser peak, in the blue, varies in exact position between 400 and 450 nm. A search of available absorption spectra led to an early postulate of a phycobilin photoreceptor, resembling allophycocyanin (7). Yet, a comprehensive search for such a pigment in higher plants led nowhere. The impasse was resolved partly by perusal of old literature dealing with both promotion and inhibition of lettuce seed germination by light (44). The recognition that the promotive action of R[2] upon germination might be negated by the inhibitory action of FR (and vice-versa) was the intuitive leap which made possible not only the theory of two mutually interconvertible pigment forms, but also the design of an appropriate photometer for detecting small changes in red- and far red-absorbancies against a large background absorption (26). Armed with an assay, biochemists could now turn to the isolation of the pigment. This followed after several years (99, 135), but not without the expenditure of considerable sweat along with, I suppose, some blood and tears. So active has the field now become that a large symposial volume (96) was recently required to review the work.

Phytochrome is a protein with a mol wt of about 120,000 attached to a bilitriene chromophore (17). The mol wt is also sometimes reported as 60,000 or 240,000 or even higher, leading naturally to the postulate that the various mol wt categories may result from aggregation and disaggregation of subunits (36). Attempts to demonstrate this *in vitro* have so far been without success, although a protease has been shown to degrade phytochrome from high (240,000) to lower mol wt without loss of photoreversibility (107). Photoreversibility is the hallmark of phytochrome, and R-FR mutual reversibility is generally taken as evidence for the participation of phytochrome in the reaction.

[2] Abbreviations: R: red light; FR: far red light.

Pr is thought to be the more stable form of the pigment. Pfr is more sensitive to denaturation by urea and −SH complexing agents like *p*-chlorimercuribenzoate (27); however Pr is more sensitive to aldehydes commonly used in fixation, such as formaldehyde and glutaraldehyde (118). Flash photolysis experiments have demonstrated that the Pr → Pfr transition has several intermediates (91); the reverse transformation has intermediates as well. Although it has been proposed that these short lived intermediates may represent the active form of the molecule (16), most people regard Pfr as the active form, Pr merely serving as a reservoir from which Pfr is formed at appropriate times. Both forms are stable in dehydrated tissue such as dormant seeds, and the temperature sensitivity and requirement of a seed for light reflect the status of phytochome at the time the seed was dehydrated on the mother plant (148).

By the use of the"Ratiospect" two-wavelength spectrometer, phytochrome has been detected and relative quantities measured along the main axis of various plants and within the various organs (18). In general, the highest titers of phytochrome are found in the most rapidly growing areas, although there are some exceptions to this (110). Through the use of antibodies coupled to peroxidase, it has been shown that phytochrome is not distributed uniformly in the cell; there seems to be some association with organelles, and apparently there is a changed distribution upon irradiation, such that Pfr associates with a dense cytoplasmic body, while Pr is distributed more generally (93). Microspectrophotometric evidence indicated the possible localization of phytochrome on discrete portions of the nuclear envelope (50), but the studies of Haupt with the alga *Mougeotia* (69) indicate a plasma membrane localization. In this alga, phytochrome controls the orientation of the chloroplast, but the chloroplast is not itself the photoreceptor for this effect. Moreover, the plane of polarization of R or FR given to the cell greatly affects the efficiency of the light in causing chloroplast rotation. A total scan of the cell with polarized microbeams revealed the presence of phytochrome in an oriented layer of the same shape and size as the cell, *i.e.* the plasmalemma. Because the plane of polarization of the reversing FR is optimal when 90° offset from the plane of polarization of the activating R, Haupt concluded that both Pr and Pfr are oriented in the membrane, but in different planes (70).

The attachment of phytochrome to some cellular particulate, presumably membranous, was first demonstrated by Rubinstein *et al.* (120). This system has recently been studied in great detail and the association of Pfr with membranous vesicles, both *in vivo* and *in vitro,* has been demonstrated experimentally (5, 94). But, in the light of Haupt's finding that both Pr and Pfr are attached in an oriented fashion, it is difficult to give complete physiological credence to the findings that only Pfr attaches, and that Pr comes off the membrane. Because, in these experiments, precise conditions of pH and Mg^{2+} were found to govern this specific attachment of Pfr to the membranes, it is reasonable to hypothesize that both forms are really attached *in vivo,* and that some additional bond, not suitably restored *in vitro,* is ruptured during the homogenization.

The demonstrated attachment of phytochrome to some cellular receptor has helped to resolve or at least to set aside several "phytochrome paradoxes." Briggs and Chon (15) had found that pretreatment with R, *i.e.* Pfr, sensitizes maize coleoptiles to subsequent phototropically active blue light. But when dose-response curves were constructed, it was found that the physiological result (increased curvature) was energy-saturated when no photometrically detectable phytochrome conversion had occurred. Only when a thousand-fold greater

energy input had occurred did phytochrome photoconversion become apparent. Hillman (77) similarly found that pea sections without detectable Pfr were still sensitive to FR. One way out of these paradoxes is the hypothesis that only a small part of the cell's total phytochrome, presumably the physiologically active part, is found in a discrete location, separated from the bulk (inactive) phytochrome of the cell (78). Thus, conversion of the localized, active phytochrome from Pr to Pfr could saturate the physiological process without being reflected in any way in the spectrophotometric phytochrome determination. But this theory also implies that the localized, active phytochrome in the Briggs experiment is preferentially photoconverted; otherwise, its approach to the photosaturation plateau should parallel that of all the phytochrome molecules. Similarly, Pfr at the active center in Hillman's experiment must dark-revert to Pr more slowly than bulk phytochrome.

The major question of concern to physiologists is, of course, the mechanism whereby phytochrome exerts its varied effects. Is it possible to construct a single mechanism of action which will include, *e.g.*, control of flowering, opposite control of stem and leaf growth in de-etiolation, opening of the plumular hook, promotion of seed germination, and sensitization to phototropic stimuli? Because the phytochrome concept matured in the age of the double helix, it is not surprising that attempts were made to explain all these phenomena in terms of the control of gene activity and of the synthesis of specific proteins required for the processes to occur (97). The inhibitory effects of such compounds as cycloheximide and actinomycin D on phytochrome-induced phenomena were taken as evidence for this view (28, 127); the photocontrol of the synthesis of phenylalanine ammonia-lyase and other enzymes involved in flavonoid (136) and other syntheses (128) also implies derepression of specific genes (40).

Difficulties soon arose in accepting this as a universal mode of action of phytochrome. The control by phytochrome of chloroplast rotation in *Mougeotia* is insensitive to inhibitors of RNA and protein synthesis (69); furthermore, the effect follows so soon after phytochrome conversion as to make it unlikely that gene activation, followed by mRNA synthesis and all the steps involved in the synthesis and ultimate action of a specific protein could be involved. The gene activation hypothesis as a general mode of phytochrome action was further weakened by the discovery of a whole host of still more rapid responses to phytochrome conversion. The nyctinastic movements of leguminous leaflets (45) and the potassium fluxes underlying them (123) were shown to occur within about 10 min after irradiation, and to be insensitive to inhibition of protein and RNA synthesis. The surface charge of plant tissues (141) and bioelectric potentials in various plant organs (82) are altered following phytochrome phototransformation; such changes occur within several sec and are also insensitive to inhibitors of protein and nucleic acid synthesis. These rapid phenomena could be ascribed more logically to changes in the membrane, and it was thus inferred that phytochrome acts through control of membrane properties (45), much as rhodopsin is known to do (59). Such a mechanism of action could also account for control of gene activity, either through localization of phytochrome in the nuclear envelope (50) or through ion activation of genetic events (89). Some workers have preferred to subscribe to a theory of dual phytochrome action, one involved in rapid, nonmorphogenetic processes such as leaf movements and chloroplast rotation, the other involved in processes involving growth, such as de-etiolation, flowering, and germination control (98). Aside from violating Occam's razor, this dual theory is not objectionable.

Although one can explain the longer range effects of phytochrome conversion, such as flower induction and de-etiolation, as secondary manifestations of a more rapidly induced change, such as membrane permeability, there are other dichotomies which are more difficult to rationalize under a single mechanism of action. Some effects of phytochrome conversion, like the induction of anthocyanin synthesis (40), are strictly localized to those cells in which the Pr→ Pfr conversion has occurred, but others, like control of stem elongation (92) and floral induction (65), are transmitted from cell to cell. Furthermore, some effects, like the promotion of seed germination, are independent of time of day, while others, like photoperiodic induction and control of leaf movement, are time-dependent. The latter phenomenon gives us, I believe, an important lead.

In the 1920's, shortly after investigations into photoperiodism became fashionable, Erwin Bünning began his now classical investigations into biological rhythms. Faced at first with widespread skepticism, Bünning demonstrated that such daily oscillations as the sleep movements of leaves are truly endogenous, and once set into motion, will persist in the absence of further environmental perturbations (24). The oscillations of the biological clock responsible for the overtly manifested rhythms in leaf movement furnish a changing backdrop against which the effect of any defined stimulus, such as a light flash, must be measured. Thus, a flash of light given at one point in the cycle might be expected to have an effect different from that of the same flash given at another point in time. When Bünning was able to show, in a comparative study of 10 varieties of soybeans (22), that those varieties with pronounced leaf movements are obligate short day plants, while those with only slight movements are day neutral, he made it likely that the observed requirement for a specific photoperiod for floral induction is the result of a necessary time linkage between a light stimulus and oscillations of the biological clock. Further, Bünning's model of a tension-relaxation oscillator has been useful in explaining other phytochrome-controlled processes (24).

Consider the sleep movements of leaves (23). Normally, leaves are open during daylight hours and closed during the night. Close observation reveals, however, that opening usually anticipates the dawn, while closing may anticipate the dark period. If such nyctinastic leaves are placed in total darkness, they will continue to open and close with a circadian frequency; clearly, the biological clock is controlling such movements. Phytochrome also plays a role, however, for if leaves are transfered from light to darkness after several hours of illumination, they will close promptly, unless the phytochrome of the pulvinus is converted to the Pr form by a flash of FR (45). This effect is repeatedly R-FR reversible. Both the rhythm and phytochrome can be shown to control potassium flux in key pulvinar motor cells (122, 122a), but in different ways. Rhythmic closure and K^+ efflux occur during a phase in which diffusive K^+ leakage predominates over any pump activity; the operation of an inwardly directed pump is shown by the accelerating effect of low temperature upon closure (124). Contrariwise, nyctinastic closure is inhibited by anaerobiosis, azide, and uncouplers of oxidative phosphorylation (123). Because phytochrome can be maximally effective only when channels are closed, *i.e.* when pump action predominates, phytochrome presumably acts on pump activity. Yet, a phytochrome conversion before the rhythmic channel opening may prevent such leakage during that circadian cycle. Electrophysiological measurements show that the observed electronegativity of the membrane increases during K^+ uptake, while a massive drop in resistance precedes observed ion leakage (113).

The discovery of phytochrome-rhythm interaction in the control of membrane properties furnishes the basis for a general theory of the phytochrome control of all morphogenetic

processes which it affects. It may even help us understand the mysterious florigen phenomenon; that long sought hormone may turn out to be nothing more than a mobile stimulus which progressively depolarizes (or prevents the depolarization of) membranes between the leaf and the flower bud. Jaffe (83) has in fact proposed that phytochome controls morphogenetic phenomena by the control of acetylcholine titer. Evidence both for (68, 85, 125) and against (87, 121, 142, 158) this proposal has been presented; the only facts that are clear at present are that acetylcholine is present in plants, that its titer can be affected by phytochrome conversion in some but not all instances, and that it is photomimetic in some systems, but not in others. Some evidence has also been presented for the involvement of IAA, GA, ethylene, cytokinins, and ABA in the phytochrome reaction (4), but there is no hard evidence that any of these hormone-phytochrome interactions are primary. Recent evidence that IAA activates a membrane-localized proton pump (34, 114) makes it an interesting candidate for participation in phytochrome-initiated events, especially because it potentiates phytochrome action in controlling potassium uptake into key motor cells of leguminous pulvini (124). This raises the interesting possibility that a proton pump is involved in K^+ uptake, as previously demonstrated for other systems (109) and that both phytochrome and auxin affect proton pump activity.

The postulate that phytochrome works in membranes has also been supported by direct demonstration of phytochrome incorporation into "black lipid" model membranes containing oxidized cholesterol (119). The resistance of such membranes change markedly with alternating R and FR, indicating that conformational changes of the phytochrome alters membrane structure. Direct evidence for changed phytochrome orientation and conformation during phototransformation comes from experiments with polarized light (70), titration of aldehyde-sensitive lysine residues in the two forms (117), circular dichroism studies (149), absorption spectra (79), and differential sensitivity of the two forms to denaturation (27).

PHOTOTROPISM

Fifty years ago, our knowledge of the biophysics of phototropism was already quite advanced, but our understanding of the internal events controlling the curvature response was hampered by the fact that the auxin concept had not yet fully emerged. The unambiguous demonstration of the existence of auxin did not come until late in the 1920's (156, 157) despite provocative experiments almost 2 decades earlier (12).

Let us recall the basic facts about phototropism. Cylindrical plant organs placed in a gradient of visible light energy generally react by curving toward or away from the brighter light. In general, stems and other aerial organs are positively phototropic, bending toward unilateral illumination, whereas roots and other subterranean organs are negatively phototropic, bending away from light. This generalization is frequently violated, however; some tendrils are negatively phototropic, some roots are nonphototropic, and some organs change from negative to positive behavior as they age. Furthermore, the energy dose-response curve for such organs as the *Avena* coleoptile are very complex, showing several maxima and intervening minima, usually designated as first, second, and third positive curvatures and first and second negative curvatures (39). This complexity may arise from separate "tip" and "base" reactions, as well as low irradiance and high irradiance reactions. Only the first-positive, tip-mediated photoreaction has been investigated extensively.

Emphasis on grass coleoptiles in tropistic studies resulted from the early work of Charles Darwin (37). Darwin had shown many years previously that the tip of the coleoptile is the main if not the only locus of perception, whereas the zone of curvature is several mm down the organ. He postulated a chemical connection between the region of perception and the region of response. This suggestion led Boysen-Jensen (12) to try to transmit the phototropic stimulus across a wound gap, an experiment which was successfully completed a little more than 60 years ago. Aided by Páal's subsequent demonstration (102) that curvature could also be induced in unilluminated, decapitated coleoptiles by asymmetrical replacement of the tip, physiologists made the logical move to the theory that the tip of the coleoptile is the locus of production and export of a diffusible, growth-promoting substance. This theory was proved by the well known simple but elegant experiments of Went (157), in which auxin, diffused from coleoptile tips into agar, was able to elicit straight growth when replaced symmetrically on decapitated coleoptiles, and curvature when replaced asymmetrically. This led to the postulate of Cholodny, exactly 50 years ago (32), that geotropic curvature could be caused by an asymmetrical distribution of growth hormones produced by the tip. Later (33), phototropism was included in the theory, which was then confirmed beautifully by the data of Went (155), showing a roughly 2:1 distribution of diffusible auxin between shaded and illuminated sides of a unilaterally illuminated coleoptile.

On the biophysical side, the experiments of Blaauw (2) more than 60 years ago had established that in both grass coleoptiles and *Phycomyces* sporangiophores, the reciprocity law holds over an extraordinary range of intensity and time, covering 8 orders of magnitude. This fact made the investigation of action spectra straightforward, and by the early 1930's (84), it was clear that phototropism resulted from the absorption of blue light. There were obvious peaks of activity at about 440 and 480 nm and a long wavelength cut-off point at about 510 nm. Shortly thereafter, carotenoid pigments with roughly that absorption spectrum were shown to exist in the *Avena* coleoptile (153), and to be concentrated toward (21), but not at (14) the tip. It was generally accepted, on these grounds, that carotenoid photoreception is responsible for phototropic curvature; this theory was not to be challenged for more than a decade. Thus, fully 40 years ago, phototropism was explained on the basis that light absorption by carotenoids somehow led to asymmetrical distribution of auxin, which in turn led to inequality of growth rates on the two sides of the coleoptile and the subsequent curvature.

In the remainder of this review, I shall attempt to analyze the following questions in terms of successive historical developments: (*a*) what is the most likely photoreceptor for phototropism; (*b*) what accounts for the unequal auxin distribution on the two sides of the coleoptile; and (*c*) what kind of amplification mechanism is involved in translating absorption of a few light quanta into large and lasting effects on growth?

In our previous discussion of photomorphogenesis, we noted that many processes besides flowering are controlled by phytochrome, and that the nature of these processes influenced the kinds of theories advanced to explain the mechanism of phytochrome action. In the same vein, other light-controlled processes in plants, described below, have action spectra sufficiently similar to that for the phototropic curvature of grass coleoptiles as to make it logical to ascribe them to the same photoreceptor. This fact has also had profound consequences for the construction of a general theory of the physiology of phototropism.

We have already mentioned that the sporangiophores of fungi such as *Phycomyces* and *Pilobolus* bend toward or away

from light; in the energy ranges producing curvature toward the light, the action spectrum is strikingly similar to that for higher plant phototropism (38, 143). Although such fungi contain auxin, their growth seems not to be affected by auxin (63), and in any event, there is no evidence that auxin is redistributed across the sporangiophore under the influence of unilateral light. This means that auxin cannot be involved critically in the basic reaction involving the photoreceptor, although it could certainly reflect such changes by its later distribution.

When leaves of *Elodea* are exposed to light after a period of darkness, the viscosity of their cytoplasm changes; when placed into a horizontal centrifuge microscope, the contents of light-exposed cells are more easily displaced than the contents of darkened cells (139). Similar effects have been noted in the moss *Mnium* and in the alga *Spirogyra* (150). The effects of irradiation are limited to the cell which receives the light energy, and the action spectrum for the process resembles very closely the action spectrum for higher plant phototropism (151). Auxin has been reported to produce similar effects on protoplasmic viscosity (139) and on heat coagulability (54) of cytoplasm; this ties the two processes even more closely to phototropism, because obviously unilateral auxin application can mimic unilateral light in causing tropistic curvature. One major difference is, however, that light effects are transmitted from cell to cell in phototropism, but not in the light effect on viscosity.

The cytoplasm of healthy *Avena* coleoptile cells streams vigorously; when the cell is darkened for a long time, such streaming comes to a virtual halt. The action spectrum for the resumption of vigorous streaming closely resembles that for phototropism (11), and the energies required resemble those for the elicitation of phototropic curvature. Like phototropism and the light effect on viscosity, this phenomenon is also affected by auxin. Auxin-deprived coleoptiles stream very slowly or not at all, and auxin in physiological concentrations restores active streaming within several min (145, 146). There is another resemblance, in that low intensity light seems to depress streaming, whereas high intensity light speeds it (11).

In both grass coleoptiles and *Phycomyces* sporangiophores, blue light administered symmetrically results in transient changes in the growth rate. This has been called the "light-growth reaction." In coleoptiles, there is at first (3–20 min) a depression, then (25–45 min) an elevation, and then a return to the control rate at about 60 min (3, 155). In *Phycomyces*, there is at first an elevation, then a depression, and a return to the normal rate (2). The over-all effect is thus completely gone within several hours, but the phenomenon is of significance, because the action spectrum for the changed growth rate matches the phototropic action spectrum for the particular organism. In fact, Blaauw (3) concluded that the problem of phototropism is entirely a problem of the light-growth reaction. In the *Avena* coleoptile, where blue light initially depresses growth, a unilateral irradiation would set up the kind of growth asymmetry required for initial curvature. It is a little harder to understand what would happen after the first 30 min, when the transitory growth inhibition is converted to a transitory growth increase, but perhaps the time relationships are altered under conditions of unilateral irradiation.

In the light-growth reaction of the *Phycomyces* sporangiophore, growth is accelerated initially. How then, can one account for curvature toward, instead of away from unilateral light? The answer is to be found in the path of light refraction within the sporangiophore. This body acts as a cylindrical lens, focusing light on the "shaded" side, causing a greater photochemical reaction and thus a greater growth acceleration on that side of the organ (29). This explanation is strengthened greatly by the finding that immersion of the sporangiophore under paraffin oil transforms a positive to a negative curvature (20). Since paraffin oil has a high index of refraction, light entering the sporangiophore would be dispersed rather than focused on the far side of the organ; thus the light-growth reaction would occur primarily on the illuminated side of the organ, and the resulting light-growth reaction would cause curvature away from the light. This explanation is so satisfying that it comes as something of a shock to find that the *Avena* coleoptile shows a similar reversal of phototropic response when immersed under paraffin oil (164). No satisfactory explanation for this phenomenon has yet been advanced.

It seems a reasonable supposition that the light-growth reaction, the effect of light on protoplasmic viscosity and streaming, and phototropism are all manifestations of the same basic reaction, initiated by photoactivation of some blue light-absorbing pigment. Blue light is also known to induce changes in cell permeability (90) and ion absorption (35), electrical properties (129) and plasticity of the cell wall (57), and fungal sporulation (163) but because the action spectrum for the induction of these effects is not well known, it is premature to include them in the phototropism package.

Nature of the Photoreceptor Pigment. Carotenoids are certainly strong and logical contenders for the role of photoreceptor for these various blue light activated processes, on the grounds of comparative biochemistry (152), their presence in the light-sensitive organs (153), and the general coincidence of their absorption spectra with the visible part of the action spectra for these processes (143). The suggestion in 1949 that riboflavin might be the photoreceptor (47) was therefore greeted with something less than enthusiasm (143). The suggestion arose from the observation that riboflavin added to the growth medium surrounding excised stem segments of etiolated peas caused a marked growth inhibition, but only in the light (51); in the dark, there was either no effect on plant tissue (95) or a slight promotion (51). This "photodynamic action" was shown to be the result of a riboflavin-sensitized photo-oxidation of IAA present in the medium (47). Such a reaction can easily be carried out *in vitro;* its action spectrum closely resembles that of phototropism, it has a quantum yield of a little less than 1, it is first order with respect to IAA, and is obligately aerobic. Anaerobically, the reaction proceeds until all the riboflavin is converted to colorless leucoflavin; upon the admission of oxygen, the reaction proceeds to completion. When IAA is added to a cell-free homogenate of etiolated pea epicotyls, the action spectrum for its photodestruction also resembles that for the riboflavin-IAA reaction, and because this occurs in well dialyzed preparations presumably free of low mol wt forms of riboflavin, it was suggested that a flavoprotein enzyme had been light activated (53).

IAA is not the only material whose photo-oxidation is sensitized by riboflavin; other indole compounds, including tryptophan, are good substrates (48). So are various purines (81), methionine, tyrosine, and peptides containing any of the sensitive amino acids (48), ascorbate (66), 3,4-dihydroxyphenylalanine (81), 2,4-dichlorophenylacetic acid (1, 67), α-naphthaleneacetic acid (60), tryptophan-containing enzymes such as α-amylase (52) but not tryptophan-free enzymes such as lysozyme (132), bacteriophage T6r (48), and even intact erythrocytes (71). Furthermore, there is nothing special about riboflavin, since other fluorescent pigments, like eosin, fluorescein, and quinine will sensitize similar photo-oxidations (43). When riboflavin is complexed with metals it can sensitize photoreductions, instead of photo-oxidations (101); electrons from the oxidizable metal pass through riboflavin, forming a transient semi-

quinone, and then go on to reduce such materials as tetrazolium salts.

All these flavin-sensitized photoreactions seemed to provide an embarrassment of riches and to be too nonspecific to be important. How meaningful could they be in the organism? As early as 1934 (42), high quantities of riboflavin were found in the pigmented epithelium of the retina of certain deep sea fishes, but not in rods and cones, and in 1939, Karrer (86) considered the possible role of riboflavin in light perception in animals in general. Heiman (72) cites evidence for diminished acuity of vision and photophobia under conditions of riboflavin deficiency; these abnormal symptoms are cured by administration of flavin. In plants, Brauner (13) and later, Reinert (116) were able to simulate phototropically sensitive organs with riboflavin-IAA mixtures, and to account for the action spectra on the basis of carotenoid shielding of the active flavin photoreceptor.

Yet, such evidence is circumstantial and inconclusive, and the dilemma could not be resolved by action spectra in the visible region alone (25). Both flavins and carotenoids could be rationalized with the action spectrum, especially because many flavins acquire fine structure in the visible region when dissolved in nonpolar solvents (147). It became clear that new action spectra including the near UV would have to be constructed, because the absorption by carotenoids continues to decrease with decreasing wavelength from their peak at 420 nm, while flavins have a major absorption peak near 370 nm. When several such spectra were produced (38, 143) and showed peaks at 370 nm, the argument for flavin photoreception was strengthened considerably. But, it was argued that certain *cis*-carotenoids would be expected to have significant peaks near 370 nm (144), and the later demonstration that carotenoids dissolved in polar solvents develop considerable absorbance near 360 nm (64) once again strengthened the possibility that a carotenoid could be involved. Nature seemed to have provided an experimental impasse to the resolution of the photoreceptor problem.

Clearly, other techniques must be employed to make the distinction between the two possible photoreceptors, and two major ones have been employed. One depends on the use of mutants, lacking entirely or almost entirely the carotenoid pigments; unfortunately, the reciprocal experiment, involving flavinless mutants, seems to be impossible because such mutations are lethal. In both higher plants and fungi, there is clear evidence of phototropic sensitivity, sometimes undiminished with respect to the wild type, in mutants lacking substantial fractions of their carotenoids (references in 49).

Another approach has been the use of chemical agents that interfere with the synthesis of one or another of the putative photoreceptors. Once again, inhibitors of carotenoid biosynthesis have been satisfactorily employed, especially with *Phycomyces;* there has been little success in lowering the titer of riboflavin by such manipulation. If *Phycomyces* is grown on lactate, or in the presence of diphenylamine, the sporangiophores have less than 1% of the normal content of carotenoid, yet the phototropic sensitivity is unaltered (115). Although these experiments with both mutants and inhibitors have strengthened the case for riboflavin they have not provided unambiguous evidence that permits a clear decision to be made; the fact remains that the small residual quantities of carotenoids could be fully functional in phototropism if they were concentrated at some active photochemical center.

The presence of phytochrome in natural membranes and its photochemical effectiveness when added to both natural and synthetic membranes constituted strong evidence for phytochrome's membrane localization and action on permeability or transport. Similar evidence is available for both carotenoids and flavins. Carotenoids are found in both photosynthetic membranes of chloroplasts and visual membranes of retinal rods and cones. Plant carotenoids can receive and transmit radiant energy to the photosynthetically active center (41). Mutants lacking carotenoids show rapid bleaching of the Chl in the light (140); one infers that their natural function involves protection of Chl against such an effect. Rhodopsin added to isolated membranes attaches and upon irradiation changes its conformation and ion flux through the membranes (112).

Recent evidence indicates that membrane fractions from both corn coleoptiles and *Phycomyces* hyphae contain both a flavoprotein and a Cyt fraction. The absorption of the flavoprotein, in both instances, strongly resembles the action spectrum for phototropism (Briggs, Jesaitis, and Reau, personal communication). When the preparation is illuminated, the Cyt becomes reduced, and the action spectrum for this Cyt reduction is that of a flavin (108). The inference is that the photoactivated flavoprotein transfers electrons to the Cyt. The membranes containing this system seem to include both a plasmalemma fraction, identified by the binding of labeled naphthylphthalamic acid and other auxins, and an endoplasmic reticulum fraction, containing an NADH-reducible Cyt. Song *et al.* (137, 138) have also adduced *in vitro* photochemical evidence, based on fluorescence properties, luminescence, and phototautomerism for favoring flavins over carotenoids as probable photoreceptors in such systems.

Klein (88) found that bean hook opening, normally relatively insensitive to blue light, became sensitized to blue after infiltration of flavin mononucleotide. This reaction was independent of added IAA, indicating photoeffects on a nonauxin system. Riboflavin has also been incorporated into model membranes, and when photo-activated, produced altered membrane properties (46).

The original suggestion that flavins could be photoreceptors in phototropism and related processes fell into disfavor partly because it was closely linked to the sensitized photodestruction of auxin, and further evidence (19, see below) reaffirmed that transverse auxin migration, rather than destruction, is involved in the phototropic response. But, the question of photoreceptor should be dissociated from the question of the nature of the subsequent physiological reactions. The recent evidence, cited above, certainly indicates that flavoproteins must be taken very seriously as candidates for the role of photoreceptor in phototropism.

What causes unequal auxin distribution on the two sides of the coleoptile? An unirradiated coleoptile releases equal quantities of diffusible auxin from the two halves of its longitudinally split tip, but unilateral irradiation with phototropically active light causes an alteration to an asymmetrical situation in which twice as much auxin is released from the dark as from the light side (156).

What causes this asymmetry? The first thing to note is that comparisons between calculations of quanta absorbed by the photoreceptor and numbers of molecules of auxin displaced reveal a disparity of at least 3 orders of magnitude; *i.e.* each absorbed quantum results in the displacement of at least 1000 auxin molecules (49, 144). Because there is virtually no net decrease in the amount of auxin released from unilaterally irradiated coleoptiles compared with dark controls (156), one cannot invoke photodestruction alone. Destruction might, of course, occur, but would have to be followed by increased synthesis, if some homeostatic mechanism for auxin level were involved. Lateral migration is made very likely by an experiment, originally performed by Boysen-Jensen (12) and later by Briggs *et al.* (19), showing that insertion of a mica or glass bar-

rier in the tip perpendicular to the plane of the unilateral light prevents curvature, whereas a similar barrier parallel to the plane of the unilateral light does not interrupt phototropic curvature. This shows that tissue continuity between light and "dark" halves of the coleoptile tip is required for curvature to occur. By inserting progressively higher barriers from below, Briggs *et al.* were able to show that only a small amount of tissue continuity at the tip suffices to redistribute the auxin. The logical inference is that transfer of auxin from light to dark sides occurs in the solid tip in the region of tissue continuity. Such a mechanism is also supported by the unambiguous transverse migration of unilaterally applied ^{14}C-IAA under the influence of a gravitational stimulus (58); there are data which indicate a similar transverse migration of ^{14}C-IAA during phototropism (106a) but the unambiguous technique of unilateral auxin application on the side opposite ultimate auxin accumulation has not yet been applied to this problem.

If it is assumed that transverse migration under the influence of unilateral light occurs, to what can it be attributed? One attractive possibility, emphasized during the 1930's and 1940's, was the hypothesized electrophoretic migration of auxin across a transversely polarized coleoptile. Unilateral illumination does set up a transverse electrical potential of about 100 mv, with the dark side positive to the illuminated side (129); electrophoretic migration of the indoleacetate is thus logical. But more recent investigations with the vibrating reed electrometer technique (61, 62) show that decapitated, auxin-starved coleoptiles do not develop such a transverse potential unless auxin is administered. From comparisons with careful kinetic studies of the movement of labeled IAA, it appears that lateral displacement of auxin occurs before the potential appears; thus, the potential is a result rather than a cause of auxin asymmetry. In a sense, the initial polarization does not depend on auxin, because light given in the absence of auxin so polarizes the tissue that subsequently administered auxin is asymmetrically distributed, and a measurable transverse potential is established. There is obviously much to be learned from further experiments with light-generated transverse potentials, in view of probable common membrane localization of auxin (75), auxin-activated proton pump (34, 114), and the phototropic pigment (Briggs, Jesaitis, and Reau, personal communication).

THE HIGH IRRADIANCE REACTION

Some reactions controlled by phytochrome also depend on prior exposure to high irradiances of visible light (98, 133, 134). Action spectra for such processes frequently indicate peaks in the blue and the far red regions (98); other photomorphogenic phenomena are potentiated more by light at roughly 720 nm than any other part of the spectrum (98).

What is the photoreceptor for such reactions? Several theories have been advanced, each with supporting evidence, but none entirely convincing. Among such theories are (*a*) photoreception by Pfr (68), a special form of Pfr, or by intermediates between Pr and Pfr (98), (*b*) photoreception by Chl active in cyclic photophosphorylation (126), and (*c*) photoreception by flavin pigments, interacting with phytochrome (see above). The first of these theories has been rejected by its original proponents, at least in the original form, but now derives some support from the demonstration of separate populations of phytochrome molecules, each having its own reaction kinetics and response to physical and chemical stimuli (111). The theory of Chl photoreception is supported by experiments in which inhibition of cyclic photophosphorylation inhibits anthocyanin formation, while inhibition of noncyclic photophosphorylation actually favors anthocyanin formation, probably by indirect promotion of cyclic photophosphorylation (126). The theory of cooperativity between flavin and phytochrome molecules is supported by those experiments showing their coexistence in isolated membrane fragments (Briggs, Jesaitis, and Reau, personal communication). Absorption by either pigment might contribute, possibly in opposite directions, to a common result, thus accounting for blue and far red action peaks. Resonance transfer of energy between contiguous pigments in an oriented lamella (159) might also account for the interaction.

EPILOGUE

Even a solution of the molecular mode of action of phytochrome will leave unanswered many crucial questions in the area of photomorphogenesis, and a solution of the troublesome photoreceptor question will not end phototropic investigations. In the hope that a list of provocative questions may help future inquiries, I offer the following: (*a*) Why are some plants short day and some long day? Is this a result of a difference in basic phytochrome mechanism, interaction with rhythm, or some other aspect of physiology? (*b*) Why are day neutral plants largely insensitive to photoperiod? It is known that such plants contain phytochrome, for their seedlings show typical phytochrome-mediated de-etiolation phenomena. Does the phytochrome mechanism cease to function in the mature plant? Or is there an altered rhythmic mechanism, as Bünning suggests? (*c*) During de-etiolation, why is leaf growth promoted and stem growth inhibited by conversion of phytochrome to Pfr? Is this a competitive phenomenon between similar growth systems, or are different growth regulators involved? (*d*) Although R inhibits stem elongation in etiolated plants, unilateral R does not cause curvature. Why? (*e*) In view of the above, why does blue light cause curvature? Why does R given as a pretreatment affect subsequent response to blue? (*f*) Both R and blue light cause changes in electrical potential of plant tissues. Do they act through the same mechanism? Why does R not cause the appearance of a transverse potential under conditions of unilateral irradiation? (*g*) What is the role of polar auxin transport in phototropism? Is the photo-induced lateral transport mediated through alteration of polar transport? (*h*) What is the role of auxin in the generation of the observed transverse electrical potentials following unilateral irradiation with blue light? (*i*) How does phototropism in green plants in intense light differ from the much studied phenomenon in etiolated seedlings? (*j*) What regulates the plagiophototropism of leaf blades?

From these questions and many others that could be formulated, it is clear that students of plant photobiology have many fascinating things to do in the years ahead.

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