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Blue-Light-Absorbing Photoreceptors in Plants [and Discussion]

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Blue-light-absorbing photoreceptors in plants

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Evidence is presented that more than one blue-light photoreceptor plays a role in morphogenesis, and that there are at least three, distinguishable on the basis both of action spectra and other criteria, which may be found both in green plants and fungi. One of these has been tentatively identified as a flavoprotein–cytochrome complex, most probably located in the plasma membrane. Studies with oat seedlings suggest that it may be involved in photoreception for phototropism, at least for the first positive curvature response. Both photoreduction of the cytochrome, via excitation of the flavin, and phototropic sensitivity in the first positive curvature range are similarly affected by diphenylether herbicides. The second class of photoreceptors can be distinguished from the first in *Neurospora* by both genetic and physiological evidence, as well as by the action spectrum. It could be either flavin or carotenoid, although a different moiety is not excluded. The third class, distinguished only by action spectroscopy, shows a single sharp action peak near 475 nm, and seems unlikely to be either a flavin or a carotenoid, though they are not rigorously excluded. The first positive phototropic curvature response in maize shows a redistribution of growth consonant with the Cholodny–Went hypothesis for tropic responses, with an increase in the growth rate of the shaded side over dark controls, a concomitant decrease on the illuminated side, and no net change in overall growth rate.

Introduction

To understand any photosensory system, it is necessary to characterize the entire sequence of events from the primary photoact through to the final consequence of photoexcitation. The effects of blue light on higher plants and fungi are no exception. Light must first be absorbed by a photoreceptor moiety, leading to some primary photochemistry. The photochemistry, be it a photoreduction or oxidation, an isomerization, or some other type of reaction, then leads through a series of dark reactions to transduction of the light signal into a measurable physiological response. In only a very few systems (e.g. vision), do we have anything approaching complete information from identity of the photoreceptor, knowledge of its pertinent photochemistry, and knowledge of the transduction steps leading to the final response. Indeed in the blue light responses to be discussed below, we do not yet have definitive identification of the photoreceptor in any case, though in some cases the photoreceptor can be assigned to a particular class of pigments. Not surprisingly, in the absence of knowledge of the chemical nature of the photoreceptor it is difficult to unravel the transduction steps leading to the response.

In blue-light-sensitive systems, efforts have been expended either at the photoreceptor end of the chain (action spectroscopy, inhibitor studies, pigment isolation attempts, etc.) or the response end (e.g. the relation between auxin distribution and tropic responses), with few studies in between. This paper will deal primarily with the question of photoreceptor

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characterization in several systems, emphasizing three systems that may be shared between green plants and fungi. It will then consider briefly the response mechanism in one particular case. It is not our purpose here to be comprehensive. For more detailed coverage, the reader is referred to the proceedings of a recent symposium (Senger 1980) and several recent reviews. Senger (1982) and Senger & Briggs (1981) provide general treatments, Tan (1978) treats the filamentous fungi in detail, and Lenci & Columbetti (1978) deal with the photoresponses of a broad range of microorganisms.

Responses in which a flavoprotein is implicated as photoreceptor

Action spectroscopy

A number of blue-light photoresponses have been described that have an action spectrum highly suggestive of the absorption spectrum of a flavoprotein. These action spectra all show maximum activity between 400 and 500 nm, with fine structure revealing at least three bands, the largest of which is between 450 and 460 nm. They also show a single somewhat smaller

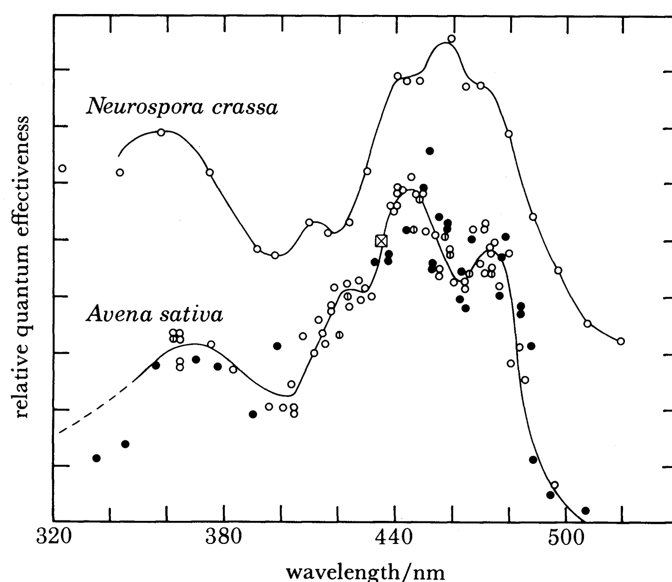

Figure 1. Action spectra for first positive phototropic curvature of *Avena* coleoptiles (lower curve, redrawn after Thimann & Curry (1960)), and for photoreduction *in vivo* of a *b*-type cytochrome in *Neurospora* mycelium (upper curve, redrawn after Muñoz & Butler (1965)).

broad band of activity in the near ultraviolet, centred near 360 nm. Among such photoresponses are phototropic curvature of etiolated *Avena sativa* coleoptiles (Shropshire & Withrow 1958; Thimann & Curry 1960) (figure 1, lower curve); phototropic curvature of *Phycomyces* sporangiophores (Curry & Gruen 1959); light-induced carotenogenesis in *Fusarium aquaeductuum* (Rau 1967); and suppression of a circadian rhythm of conidiation in a mutant strain of *Neurospora crassa* (Sargent & Briggs 1966). There are a number of other such responses, several of them treated in detail by Tan (1978). All of these action spectra resemble fairly closely the absorption spectrum of a typical flavoprotein, e.g. NADPH–cytochrome *c* oxidoreductase (Kamin *et al.* 1966), leading to the hypothesis that the flavin moiety of a flavoprotein is the

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photoreceptor. Because the absorption spectra of most flavoproteins are extremely similar, however, the action spectra cited above are of little assistance in indicating which flavoprotein might be the best candidate.

Light-induced flavin-mediated cytochrome reduction

About 10 years ago, Butler and his colleagues first detected blue-light-induced absorbance changes *in vivo* in several fungi (see Senger & Briggs (1981) for review). The careful studies by Muñoz & Butler (1975) showed that the light-induced absorbance change in *Neurospora crassa* mycelium involved the photoreduction of a *b*-type cytochrome, with the simultaneous reduction of a flavoprotein. Both constituents became reoxidized in the dark. The action spectrum for the process (figure 1, upper curve) was very much like that for the various processes described in the previous section. Brain *et al.* (1977*a*) subsequently found the photoactive cytochrome–flavin to be located in the plasma membrane of *Neurospora* and showed similar photoactivity in a membrane fraction from corn coleoptiles. Goldsmith *et al.* (1980) improved the techniques for measuring the photoreaction so that it could be induced consistently in corn membrane fractions, and showed that the flavin moiety was firmly bound. They also presented spectral evidence that the photoreaction was specific only for a *b*-type cytochrome with a room temperature α band near 560 nm. This specificity was obtained, despite the presence of both cytochrome b_5 from the endoplasmic reticulum and significant mitochondrial contamination. The specificity was also obtained whether the photosensitizer was the endogenous flavin moiety or externally added flavins or methylene blue. Exogenous photosensitizers could be readily washed away, but the endogenous photoreceptor for the photoreduction was firmly membrane-bound. Leong & Briggs (1981) used sucrose and renografin gradient centrifugation to separate the membrane fraction containing the photoactivity from mitochondria, endoplasmic reticulum, and Golgi, and showed that it cosedimented with glucan synthetase II activity. It seems likely that as with *Neurospora* (Brain *et al.* 1977*a*), this fraction is also the plasma membrane (see Quail (1979) for an evaluation of the evidence that glucan synthetase II is a reliable marker for plasma membrane in higher plants). Leong & Briggs (1981) also reported that the complex could be separated from the bulk of the membrane fraction by mild detergent treatment with little loss of photoactivity. Hence it was probably associated with a peripheral membrane protein rather than an intrinsic one. Finally, Leong *et al.* (1981) obtained reduced-minus-oxidized spectra for the cytochrome at liquid nitrogen temperature. The single sharp α band at 555 nm clearly distinguishes this *b*-type cytochrome both from the cytochrome b_5 of the endoplasmic reticulum, with a split α band showing peaks at 550 and 556 nm, and the several mitochondrial cytochromes. Leong *et al.* (1981) also determined that the redox potential of the cytochrome at 50% reduction was −65 mV.

There are several lines of evidence that the photoreceptor in the complex is actually a flavin. First, as mentioned above, the action spectrum of the cytochrome photoreduction, at least in *Neurospora*, resembles the absorption spectrum of a flavoprotein (Muñoz & Butler 1975). Second, the purified photoactive membrane fraction clearly contains a flavin: the fluorescence excitation spectrum for emission at 525 nm is clearly that of a flavoprotein (Leong *et al.* 1981). Third, phenylacetic acid, which reacts with flavins in their triplet state to form a covalent linkage with them (Hemmerich *et al.* 1967), and iodide and azide, both effective in depopulating the excited states of flavins (Heelis *et al.* 1978), are effective inhibitors of the blue-light-induced photoreduction (Caubergs *et al.* 1979).

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Relevance of flavin-mediated cytochrome photoreduction to photoresponses in vivo

None of the evidence so far cited provides any convincing argument that this photosensitive flavoprotein–cytochrome complex is indeed the biologically active photoreceptor for any of the responses cited. There is, however, accumulating evidence, both for coleoptile phototropism and the conidiation response in *Neurospora*, that this complex may indeed be the actual photoreceptor. First, the same three inhibitors that were effective in blocking the photoreduction *in vivo* (phenylacetic acid, iodide and azide (see Caubergs *et al.* 1979)) preferentially inhibited the phototropic over the geotropic response of corn coleoptiles (Schmidt *et al.* 1977). Second, a mutant of *Neurospora*, *poky*, which is deficient in all of its *b*-type cytochrome, has only about 1% of the wild-type photoreducible cytochrome activity, and is less than 1% as sensitive as wild-type to photosuppression of the circadian conidiation pattern (Brain *et al.* 1977*b*). Third, methylene blue, which photosensitizes reduction of the *b*-type cytochrome to red light (Britz *et al.* 1979), also sensitizes photosuppression of the conidiation banding in *Neurospora* and phase-shifting of the banding (probably simply a special case of suppression) in response to red light (see Briggs 1980). Finally, Leong & Briggs (1982) showed that the diphenyl ether herbicide acifluorfen both increased the level of photoreduction of the *b*-type cytochrome in membrane fractions from *Avena* coleoptiles and significantly sensitized the first positive phototropic curvature reaction. The herbicide was entirely without effect on the geotropic reaction. While not one of these lines of evidence is more than circumstantial, and each case requires further careful analysis, they, together with the action spectra mentioned above, make the hypothesis that this light-sensitive plasma-membrane-associated flavin–cytochrome complex is the actual photoreceptor for phototropism in coleoptiles and for photosuppression of conidial banding in *Neurospora* an attractive one.

Responses in which either a flavin or carotenoid might be the photoreceptor

Carotenogenesis in Neurospora crassa

Many years ago, Zalokar (1955) first published an action spectrum between 400 and 500 nm for carotenogenesis in *Neurospora*. Although frequently included in collections of action spectra thought to share a common photoreceptor (Muñoz & Butler 1975), in particular those discussed above that are hypothesized to have a flavoprotein as photoreceptor, there are features of this action spectrum that are not consistent with those mentioned above. First, the wavelength for maximum photoactivity is much nearer 480 nm than 450–460 nm; and second, the relative activity at 400 nm with respect to the maximum is considerably lower than in the spectra mentioned above. A more recent detailed action spectrum for this process (De Fabo *et al.* 1976) confirms and extends these conclusions (figure 2, upper curve). There are two sharp peaks, one at 450 nm and the other at 480 nm, with the latter being the highest. Relative activity is indeed low, though not absent, between 300 and 400 nm.

There are currently three lines of evidence suggesting that the photoreceptor for carotenogenesis in *Neurospora* is not identical with that for the suppression of conidial banding. The first of these involves study of the *poky* mutant. E. Schrott (unpublished) found that although, as mentioned above, the deficiency in *b*-type cytochromes brought about a reduction to less than one-hundredth in light sensitivity for the suppression of conidial banding, it was entirely without

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effect on carotenogenesis: the dose–response curve for carotenogenesis in the *poky* mutation was indistinguishable from that of the wild-type. The second line of evidence involved studies with an external photosensitizer. Schrott (unpublished) showed that although methylene blue would sensitize both the suppression of conidial banding and phase-shifting of the circadian rhythm of conidiation to red light (see above), red light administered in the presence of methylene blue failed to have any effect whatever on carotenogenesis either in *poky* or the wild-type. The third line of evidence comes from studies by Paietta & Sargent (1981) with two riboflavin mutants,

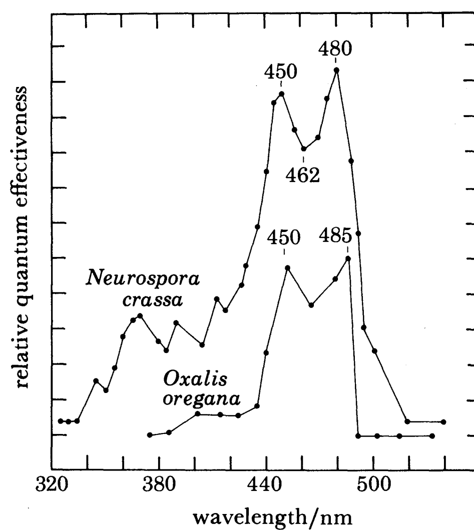

FIGURE 2. Action spectra for carotenogenesis in *Neurospora* (upper curve, redrawn after De Fabo *et al.* (1976)), and light-induced leaflet closure in *Oxalis oregana* (lower curve, redrawn after Björkman & Powles (1981)).

rib-1 and *rib-2*, of *Neurospora*. Flavin deficiency reduced light sensitivity for suppression of conidial banding to just over one-hundredth, whereas it reduced light sensitivity for carotenogenesis to only about one-quarter.

These results, taken together, strongly suggest that there are two different blue-light photoreceptors in *Neurospora*, one probably mediated by a light-sensitive flavin–cytochrome complex localized in the plasma membrane, and the other clearly not. The second photoreceptor could be a flavin, a carotenoid, or something else. There is at present no evidence other than action spectroscopy bearing on its chemical nature, and that evidence is ambiguous.

Blue-light-induced leaflet closure in Oxalis oregana

Björkman & Powles (1981) have recently described a rapid light-induced closure of the leaflets of *Oxalis oregana*, a ground cover species commonly found deep in California's redwood forests. These plants normally flourish in deep shade, and are very well adapted to these shade conditions. Exposure to light fluence rates equivalent to full sunlight leads within minutes to significant damage to the photosynthetic apparatus, and prolonged exposures lead to damage that requires hours of recovery, or that indeed may be permanent. Even in the deepest shade of the forest floor, however, there are occasional sunflecks in which the light fluence rate approaches or equals that of unobstructed sunlight. The fluence rate may increase as much as 200-fold within 1 min or less. In Nature, the plants respond by rapid folding of the leaflets

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so that only their edges are exposed to the full sunlight. With a very much reduced area of light interception for the same amount of chlorophyll, photoinhibition is completely avoided. The reaction begins within seconds of the arrival of a sunfleck as the Earth rotates, and goes almost to completion within as little as 3 min. On departure of the sunfleck, the leaflets then require approximately ½ h to return to their normal extended orientation.

The action spectrum for this response (Björkman & Powles 1981) is shown in figure 2 (lower curve), along with that for carotenogenesis in *Neurospora* (figure 2, upper curve). Like the *Neurospora* spectrum, that for the *Oxalis* response shows two distinct peaks in the visible, one near 450 nm and the other near 480 nm (at a slightly longer wavelength for *Oxalis* than for *Neurospora*), and low activity in the near ultraviolet (lower in *Oxalis* than in *Neurospora*). Finally, both spectra share a minimum at about 460 nm. Though a flavin is not excluded in either case, the low activity in the near ultraviolet makes a flavin a somewhat unlikely candidate, and a carotenoid more likely. Even if the photoreceptors were flavins, it is highly unlikely that the flavins would be the same as those involved in the other responses so far discussed.

Responses where neither a flavin nor a carotenoid is a promising candidate for the photoreceptor

Responses in fungi

In addition to the blue light responses discussed above, there are several that have action spectra showing only a single sharp action peak near 475 nm, and no other significant fine structure. Among these is that for the inhibition of conidiation in the fungus *Stemphylium botryosum* (Leach 1968), and that for promotion of sporulation in *Penicillium isariiforme* (Benninck 1971). That for *Stemphylium* is shown in figure 3 (lower curve). In comparison with the two

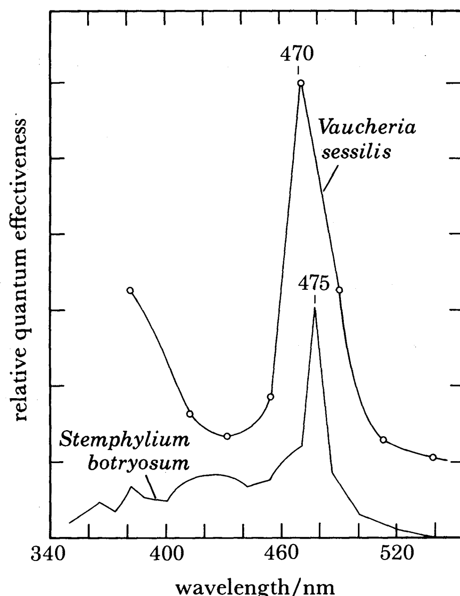

Figure 3. Action spectra for inhibition of conidiation in the fungus *Stemphylium botryosum* (lower curve, redrawn after Leach (1968)), and for light-induced chloroplast aggregation in the alga *Vaucheria sessilis* (upper curve, redrawn after Blatt (1980)).

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responses discussed in the preceding section, a flavin as photoreceptor in these cases seems even more unlikely. It is also hard to invoke a carotenoid when the action peak is as narrow as these are.

Chloroplast aggregation in Vaucheria sessilis

The final process to be discussed is the light-induced aggregation of chloroplasts in the alga *Vaucheria sessilis*. Blatt (1980) measured the rates of chloroplast aggregation as a function of fluence rate for a series of wavelengths between 385 and 528 nm. He had previously shown that there was no effect of red light on this system. The action spectrum for the relative quantum efficiency as a function of wavelength is shown in figure 3 (upper curve). As with the two fungal responses just mentioned, the action spectrum shows only a single peak near 475 nm. *Vaucheria* does show somewhat more sensitivity in the near ultraviolet than either of the fungal responses. The requirement for using fibre optics both for excitation and measuring beams precluded measurement of the response below 385 nm, because adequate quantum fluences could not be obtained.

On the multiplicity of blue-light photoreceptors

It should be clear from the above discussion that there must be more than one blue-light photoreceptor both in fungi and in green plants. This idea is hardly original, as Tan (1978) stresses it for the filamentous fungi, and both Shropshire (1980) and De Fabo (1980) and others have addressed this possibility. Even the notion that there may be two different blue-light photoreceptors in the same organism, as may occur in *Neurospora*, is not new. Mikolajaczyk & Diehn (1975) proposed that more than one pigment is involved in the photophobic responses of *Euglena* to blue light. One of these photoreceptors showed strong inhibition in the presence of potassium iodide, and hence could have been a flavin; the other was iodide-insensitive. In addition, Jayaram *et al.* (1979) present arguments favouring two different photoreceptors for carotenogenesis in *Phycomyces*. In this connection, it is of some interest that the action spectrum for photoinduction of carotenoid synthesis for the water mould *Fusarium aquaeductuum* (Rau 1967) is similar to that for flavin-mediated photoreduction of the membrane-associated *b*-type cytochrome in *Neurospora* (Muñoz & Butler 1975); and both the *Fusarium* response (Lang-Feulner & Rau 1975) and the cytochrome photoreduction (Britz *et al.* 1979) can be potentiated by red light if methylene blue is present. By contrast, the action spectrum for carotenogenesis in *Neurospora* itself (De Fabo *et al.* 1976) does *not* resemble that for photoreduction of the *Neurospora* cytochrome, and the process is *not* potentiated by red light in the presence of methylene blue. Hence not only may *Neurospora* contain two different photoreceptors, but a single process in the filamentous fungi, namely carotenogenesis, may be potentiated by different photoreceptor molecules in different organisms.

In summary, a review of a number of different action spectra suggests that the fungi may share at least three classes of photoreceptors with their photosynthetic cousins. One of these classes is clearly a flavin, and at least in the phototropism of corn and oat coleoptiles and the photosuppression of conidial banding in *Neurospora* it may involve a plasma membrane-associated flavin–cytochrome complex as the photoreceptor. A second class, that associated with carotenogenesis in *Neurospora* and leaflet closure in *Oxalis*, could be a flavin, a carotenoid, or some other compound. If this class is a flavin, it is unlikely to be the same one as involved in the first class, because, at least in *Neurospora*, a *b*-type cytochrome is evidently not involved. The third class, found in some filamentous fungi and, at least in *Vaucheria*, among green plants,

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shows just a single sharp action peak in the blue. It seems unlikely that it could be either a flavin or a carotenoid, though neither can be completely ruled out.

Action spectra do not, of course, necessarily reflect the absorption spectrum of the photoreceptor pigment. They may be altered by screening pigments or may be a consequence of photochromicity, reflecting the absorption spectrum of neither member of the photochromic pair of molecular species directly (see Shropshire 1980). Because the two photosystems in *Neurospora* can be distinguished on evidence other than action spectroscopy, it is immaterial to the hypothesis that there are two different photoreceptors whether the action spectra precisely resemble the photoreceptor absorption spectra or not. Furthermore, as Shrophire (1980) points out, the participation of a screening pigment in the carotenogenesis response is unlikely because the irradiations were done through a single mycelial pad, and what little absorbance was found was insufficient to modify the action spectrum in any significant way. A role of screening is likewise unlikely for suppression of conidial banding (Sargent & Briggs 1966) for similar reasons. Screening is also most unlikely to be the cause of the single sharp action peak near 475 nm in *Vaucheria*: the photoreceptor is located in the plasma membrane or outermost cortical cytoplasm (Fischer-Arnold 1963), with only the cell wall intervening between incident light and photoreceptor. Photochromicity cannot at present be ruled out, though if cycling of a photochromic pigment is required for action, a mechanism that *could* produce the sharp peak, this system must differ from the others described, where a mechanism requiring such cycling for action cannot be readily invoked

The transduction step in coleoptile phototropism

The Went–Cholodny hypothesis for tropisms states that the tropisms are the consequence of a light-induced or gravity-induced lateral redistribution of auxin, leading to a redistribution in growth, causing the directed growth response (see Went & Thimann 1937). All of the early studies were based on experiments with grass coleoptiles, and later studies adding support to the hypothesis for gravitropism (Gillespie & Briggs 1961; Gillespie & Thimann 1963) and phototropism (Briggs *et al.* 1957; Briggs 1963; Pickard & Thimann 1964) also involved auxin studies with coleoptiles. These more recent studies clearly demonstrated that gravitational or light stimuli leading to tropic curvature in coleoptiles were indeed accompanied by lateral transport of auxin, and that neither photoinactivation of auxin nor inhibition of auxin synthesis by light could account for the auxin differential.

Firn & Digby (1980) have raised some provocative questions concerning the Went–Cholodny hypothesis. They point out first that if the hypothesis is really valid, one would expect an increase in the growth rate of the shaded (positive phototropism) or lower (gravitropism) side of the coleoptile, with respect to an unstimulated control, and a corresponding decrease in growth on the opposite side. They also point out that for the auxin differential to be responsible for the growth curvature, its formation would have to *precede* the actual changes in growth rate. Recently Franssen *et al.* (1981, 1982) have reported that continuous blue light administered under conditions of fluence rate and duration sufficient to yield second positive curvature (see Zimmerman & Briggs 1963) caused a complete cessation of growth on the illuminated side of *Avena* coleoptiles, with little change in the growth rate of the shaded side, a result clearly not consistent with the Went–Cholodny hypothesis.

There is accumulating evidence that there are phytochrome responses in corn and oat

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seedlings that are so sensitive that even short exposures (minutes or even seconds) of dim green or blue light can cause substantial growth changes (Blaauw *et al.* 1968; Briggs & Chon 1966; Chon & Briggs 1966; Iino 1982*a*, *b*; Iino & Carr 1981; Mandoli & Briggs 1981). It therefore seems possible that either the blue light used for phototropic induction or the green safelight used for handling the plants could bring about growth responses that were unrelated to the tropic responses, but which could easily obscure the growth differential which was the actual basis for the tropic response. Indeed, Pratt & Briggs (1966) showed spectrophotometrically that both blue and green light could bring about significant P_r–P_{fr} phototransformation *in vivo* in corn [maize] coleoptiles.

We therefore set out to measure growth changes brought about by blue light dosages leading to first positive curvature under conditions under which all phytochrome reactions would be

Table 1. Growth redistribution during first positive curvature of corn coleoptile

(Curvature was developed for 100 min after the onset of illumination.)

$\log_{10}\{\text{fluence}/(\mu\text{mol m}^{-2})\}$	curvature/deg	growth change (% of control) illuminated side	growth change (% of control) shaded side
−1.0	2	−5	5
−0.2	12	−30	30
0.7	24	−45	55
1.6	12	−15	40
2.2	8	0	35

saturated. In brief, corn seedlings were raised under continuous red light (0.15 μmol m^{-2} s^{-1}) at 24–25 °C for 3 days from the time of sowing. Phototropic induction with blue light and subsequent curvature development also took place under red light. In this way, we hoped to minimize any effects of phytochrome phototransformation by the blue light itself. The reasoning was that the very low fluences of blue light used for phototropic induction would have a negligible effect on the phytochrome photoequilibrium, which was sustained by the high fluence-rate red light. The phototropic fluence–response curve so obtained yielded the same threshold and saturation values as those obtained in 1966 by Chon & Briggs with dark-grown corn seedlings given only a brief exposure to red light 2 h before phototropic induction.

For growth measurements, each coleoptile was carefully marked just before phototropic induction exactly 1.5 cm below the coleoptile apex. An image of a set of plants was obtained by photocopying, and the photocopies used to measure the starting lengths of the sides that would be illuminated or shaded in the experimental plants. After curvature had developed for 100 min after the onset of blue-light treatment, the experimental plants were also photocopied for subsequent analysis. Length measurements were obtained with a digitizer directly on line with a computer. Growth increments were obtained for both shaded and illuminated sides, and the results expressed as percentage of those of control plants, which had received no phototropic induction (table 1).

The results obtained over the entire first positive curvature range showed an increase in growth on the shaded side of the coleoptile, and a compensatory decrease on the illuminated side: precisely what one would expect if the Went–Cholodny hypothesis were valid. The relative size of the increase or decrease is directly related to the amount of curvature obtained, with maximal curvature being accompanied by the maximal differential. At the higher fluences, there was some evidence for an overall stimulation of growth, but this tendency was eliminated

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when just the coleoptile tips were irradiated with blue light (data not shown). The kinetics of the differential growth response were also obtained. For these measurements, enlargements of photographic images of the coleoptiles, obtained with red-sensitive film, were used to increase resolution. The results obtained for maximal first positive curvature showed a clear stimulation of the growth rate of the shaded side and a decrease in the growth rate on the illuminated side. Net growth was hardly changed. These results will be presented in detail elsewhere.

We hope to begin studies on the kinetics of auxin redistribution soon, in order to address the second point raised by Firn & Digby (1980). We also hope to obtain measurements in the second positive curvature range to determine whether the mechanism in that range is really different, as suggested by the experiments of Frannsen *et al.* (1981, 1982), or whether the difference found can be attributed to light reactions unrelated to the direct physiological consequence of photostimulation of the blue-light photoreceptor.

Concluding remarks

It is clear that there are complexities involved at both the photoreceptor and response ends of the blue-light-excited sensory transduction chain. At the photoreceptor end, hypotheses based on the expectation of one kind of photoreceptor may be invalid because quite another photoreceptor is involved. At the response end, the interaction of photoreceptors may obscure a response mechanism, or different types of responses (e.g. first as against second positive curvature in coleoptiles or coleoptile tropisms compared with those of hypocotyls) may involve different mechanisms. Further experimentation is needed to resolve these problems, and is certainly required before any intermediate steps in photosensory transduction in response to blue light can be characterized in any of these systems.

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Discussion

A. W. Galston (*Plant Breeding Institute, Trumpington, U.K.*). Dr Briggs has proposed, on the basis of slightly different action spectra, that different photoreceptors exist for several of the blue-light-mediated reactions in plants. Because it is well known that the same chromophore attached to different proteins can yield slightly different absorption spectra, would Dr Briggs consider that this type of situation could satisfactorily explain the slightly differing flavin-like action spectra? In other words, could these be the 'different' photoreceptors that he calls for?

W. R. Briggs. There is no reason why the 'different' photoreceptors I am suggesting could not each be flavins in different environments: on different proteins, in different membranes, etc.

A. W. Galston. Is anything known about the mechanism of the light-fleck-induced leaf movements in *Oxalis*? Is there, for example, a trans-pulvinar shuttle of ions, as in *Albizzia* and *Samanea*?

W. R. Briggs. To my knowledge, there has been no work at all on the mechanism of *Oxalis* leaf movement, though the elegant work that Dr Galston and Ruth Satter and colleagues have done on *Albizzia* and *Samanea* certainly suggests things to investigate. Indeed, the pulvini are large, the plants available in large numbers (and easy to grow in the greenhouse), and *Oxalis oregana* might be first-rate for studying some aspects of leaf movement.

J. Digby and R. D. Firn (*Department of Biology, University of York, U.K.*). In contrast to the results that Dr Briggs has presented, Mr K. MacLeod in our laboratory has recently found that in dark-grown *Avena* coleoptiles, first positive phototropic curvature is brought about almost entirely by a growth retardation on the illuminated side, and the growth rate of the shaded side is virtually unchanged. It is possible, as in the phototropism of some hypocotyls, that the contribution to curvature of changes in growth rate on the shaded side may vary, depending on the growth conditions. This suggests that simple curvature requirements are really inadequate as a means of describing phototropism, yet it is just such simple curvature measurements that we seem to rely on for phototropic action spectra.

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W. R. Briggs. There is certainly no question that under different conditions the relative contribution of growth rate changes on the two sides may vary widely. However, simply because both sides of a unilaterally irradiated coleoptile slow down, one more than the other (for example) does not in itself eliminate a lateral auxin differential in the Went–Cholodny sense from being the reason for curvature. If there are two separate photoreactions, one simply a general inhibition of growth, and the other an induction of lateral transport of auxin, it would still be the lateral transport of auxin that brought about the curvature response. The questioners are quite correct in that if this is so (multiple photosystems and multiple light responses), then action spectroscopy is not as helpful as one might hope. In the present limited experiments, we have simply tried to saturate any red-induced effects on growth, as a way of isolating blue-light effects on phototropism. The results *in this case* are clearly consistent with the Went–Cholodny hypothesis. Until similar kinds of experiments with other curvature responses are demonstrated, we do not extrapolate beyond this one system, first positive curvature in corn, grown under these specific conditions.

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