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Perception of a Unilateral Light Stimulus [and Discussion]

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Perception of a unilateral light stimulus

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An organism can detect light direction given a gradient in light intensity within the organism. This gradient, which may be measured temporally or spatially, can be produced by screening or by refraction. The ramifications of the method of producing the gradient are potentially great, with possible effects on the shape of dose–response curves and action spectra. Two biological systems, amoebal phototaxis in *Dictyostelium* and phototropism by monocot seedlings, illustrate some potential problems. In the former system, no obvious mechanism exists for producing a substantial internal gradient in light intensity. This indicates our lack of knowledge concerning the amount of gradient necessary for an organism to measure light direction. In the latter system, it is evident that a gradient in light intensity is established by screening for second positive phototropism. However, screening may not be the method used for first positive phototropism. The implications of refraction as the mechanism involved in first positive phototropism are sufficiently great to warrant a thorough examination of the role of screening and refraction in first positive phototropism.

Introduction

Many examples are known in which an organism perceives not only light but also the direction in which the light is propagated. Typically, this results in a movement or growth response related in some way to the directional stimulus. (This discussion will for ease of expression use the term 'light direction' to refer to the direction in which light is propagated.) For an organism to detect light direction, there must be some difference or gradient in light intensity within the organism, which can be translated into a gradient in light absorption. The gradient in light intensity can be measured in space (a spatial measurement) or in time (a temporal measurement), but it is the gradient which permits the detection of light direction regardless of the basis for its measurement (whether spatial or temporal).

This discussion will present the known methods whereby an internal gradient in light intensity can be produced and some of the ramifications that derive from these methods. Finally, two biological examples will be described to illustrate some of the more intriguing problems.

Screening

The mechanisms available for establishing an internal light gradient are screening and refraction (figure 1). (A dichroic receptor pigment may be used to measure the plane in which the light is propagated, but in the absence of screening or refraction, a dichroic receptor pigment cannot be used to measure light direction.) Screening decreases the light intensity beyond the screen relative to that before the screen, and may occur as a result of scattering or absorption. Scattering must be assumed to occur in all organisms, although the extent of the scattering

may vary. Thus, no biological system should exist in which screening is solely by absorption. However, by definition, a photoresponsive system must contain a photoreceptor pigment, which must contribute some absorption to the tissue. It is therefore not theoretically possible to have an organism in which the screening is solely by scattering, and scattering and absorption both must contribute to screening, although either may be relatively insignificant.

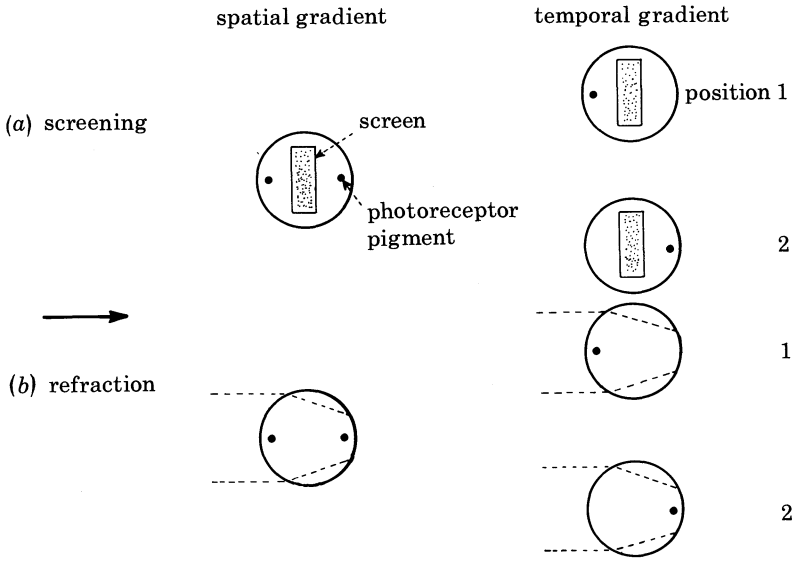

FIGURE 1. Schematic drawing of the mechanisms by which a gradient in light intensity can be established across an organism illuminated from the left. The drawings on the left depict a spatial perception of the gradient and the drawings on the right depict a temporal perception of the gradient. For a spatial perception, with a gradient established either by screening or refraction, a minimum of two detections must occur at two places in the gradient. For a temporal perception, two detections must also occur but separated in time rather than space. This separation in time is represented by the organism's moving from position 1 to position 2. (Adapted from Feinleib (1980).)

If the screening is largely by absorption, then it can be reasoned that the response is dependent upon absorption of light by the photoreceptor pigment and, in addition, is dependent upon absorption of light by the screening pigment (Thimann & Curry 1961). It follows directly from this argument that one of the major constraints for action spectroscopy is difficult to meet with a system in which the light intensity gradient is established by absorption. Namely, the response or action is not dependent only on the photoreceptor pigment but is dependent on two pigments. An action spectrum measured for such a system will indicate a complex product of the absorption spectrum of the photoreceptor pigment and the absorption spectrum of the screening pigment. Similarly, if the photoreceptor pigment itself serves as the screening pigment, the action spectrum will represent a complex product of the absorption spectrum of the photoreceptor pigment multiplied by itself.

REFRACTION

Refraction of light at a curved air–organism interface can focus the light within the organism. For an organism with a circular cross section and a relatively low internal absorbance, this results in a higher light intensity and a longer pathlength over which light can be absorbed

on the distal side than on the proximal side (figure 2). Given the assumptions that the photoreceptor pigment is evenly distributed throughout the organism and that the initial reaction products do not readily diffuse throughout the organism, a greater number of pigment molecules will be 'excited' on the distal side than on the proximal side and thus the light direction will be detected. In a positive phototaxis or phototropism, movement or growth would be away from the side with the greater number of 'excited' pigment molecules.

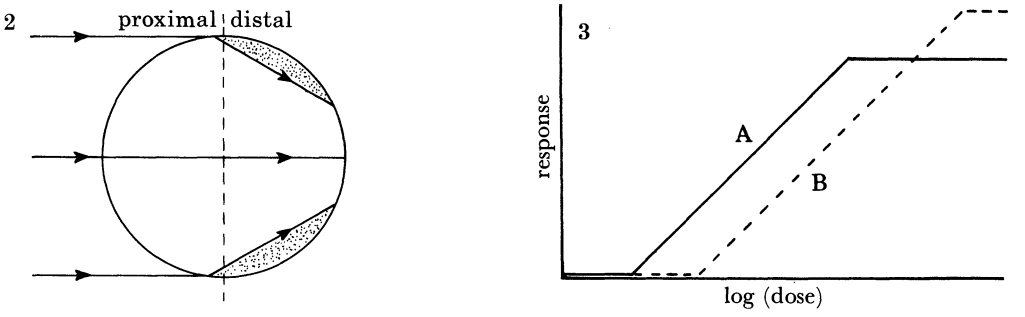

FIGURE 2. Schematic drawing of the cross section of an organism exposed to unilateral light. The lines represent light rays from a light source to the left of the organism. Because of refraction at the air–organism interface, light is focused onto the distal side of the organism. Note the darkened areas on the distal side of the organism.

FIGURE 3. Hypothetical dose–response curves for an organism that detects light direction by using a refraction-generated light gradient. Curve A represents the photoproduct formation per unit volume on the distal side; curve B represents photoproduct formation per unit volume on the proximal side. It is assumed that the photoreceptor pigment is evenly distributed and that the primary photoproducts are not readily diffusible throughout the organism.

One might expect a hypothetical stimulus response curve like figure 3*a*, with response increasing with the logarithm of the stimulus to saturation for the distal side of the organism. This saturation can result from any rate-limiting reaction in the stimulus–response sequence. Because the receptor pigment stimulation is greater on the distal side, saturation would occur first on that side. At a still higher fluence rate, saturation also would occur on the proximal side (figure 3*b*). However, because of the lens effect, only a portion of the distal side will be illuminated whereas almost the entire proximal side will be illuminated. This would result in a greater number of pigment molecules being stimulated on the proximal side than on the distal side. Thus, if one considers the number of photoproducts found on the two sides, saturation on the proximal side will occur not only at a higher fluence rate but also at a higher 'response' level than on the distal side. At these higher fluence rates, if growth or movement were still away from the side with the greater number of 'excited' pigment molecules, then the organism would grow or move away from the light.

Saturation might be expected at lower doses with a high fluence rate than with a low fluence rate. In such a case, the extent of a 'negative' response would be fluence-dependent.

BIOLOGICAL EXAMPLES

This discussion will not attempt to review the many biological systems where evidence is available concerning the mechanism whereby a light gradient is established and light direction detected. Rather, two biological examples will be discussed to illustrate some of the areas of uncertainty.

Phototaxis by amoebae of Dictyostelium discoideum

Amoebae of the cellular slime mould *Dictyostelium discoideum* move towards or away from unilateral light, depending on the light intensity (Häder & Poff 1979*a*, *b*, *c*; Hong, *et al.* 1981). Because of the small size of the organism and the relatively long wavelength to which it is sensitive, this organism presents a particular challenge for understanding the mechanism whereby light direction is detected.

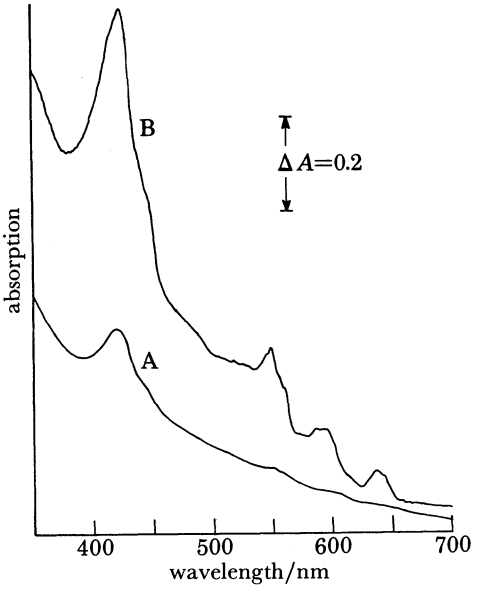

FIGURE 4. Absorption spectra of *Dictyostelium* amoebae at 23 °C (A) and 77 K (B). The sample consisted of 4×10^7 cells in 0.4 ml suspension buffer. The pathlength was approximately 0.3 cm. The spectra were measured by using a single-beam spectrophotometer on line with a small computer. Note the distinct absorption maximum at 640 nm in cells at 77 K, and the relatively low absorbance of cells at 23 °C.

Action spectra for both positive and negative amoebal phototaxis show a major peak at 405 nm, with secondary maxima at 440–520, 580 and 640 nm, Of these, the action maximum at 640 nm is of particular interest. This action peak has been associated with an absorption maximum *in vivo* at 640 nm, which may be easily seen in cells at 77 K (figure 4*a*). It is not readily evident that any of the known mechanisms are sufficient to establish any substantial gradient of 640 nm light in *Dictyostelium* amoebae.

1. The ability of a lens to focus light decreases rapidly as the diameter of the lens approaches the wavelength of light. The diameter of an amoeba is approximately 10 μm but is highly irregular, whereas the diameter of the more regular pseudopodium is about 1 μm. Clearly, 640 nm (0.64 μm) light is perceived by the amoeba. However, at this wavelength the amoeba should be relatively ineffective as a lens.

2. Scattering is, in general, inversely proportional to some power of the wavelength. Scattering could be quite significant in establishing a light gradient in the blue, but would be much less effective at 640 nm.

3. Establishing any significant gradient of light intensity by absorbance screening is unlikely, given the very low absorbance at 640 nm for cells at 22 °C (figure 4), and the very short path length (10 μm) through an amoeba.

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Both scattering and absorbance screening may in fact operate to produce a significant gradient in light intensity in *Dictyostelium* amoebae. It should be noted, however, that any lens effect would operate to diminish the gradient established by scattering and absorbance screening.

The fact that none of the mechanisms for producing a light gradient is substantial in *Dictyostelium* amoebae exposed to unilateral 640 nm light raises a major question concerning the extent of the gradient required. How large must the ΔI be between the proximal and distal sides for an organism to measure the difference? Surely a ΔI of 50% would be sufficient, but would a ΔI of 10^{-6}% suffice? It should be possible to calculate, for a given number of excitations, the difference on the distal and proximal sides necessary for statistical significance. Note that such a calculation would be valid only for a particular number of excitations or for a particular fluence rate. Unfortunately, this calculation presupposes a knowledge of the 'noise' level for the particular reaction or pathway modulated by light.

In summary, none of the known mechanisms is obviously sufficient to establish a significant gradient of 640 nm light in *Dictyostelium* amoebae.

Phototropism in monocot seedlings

For many monocots, the dose–response curve for phototropism is very complex, typically showing at least three separable components, which have been termed first positive phototropism, first negative phototropism, and second positive phototropism (figure 5). Considerable evidence has been accumulated that separable mechanisms are involved in these responses (Zimmerman & Briggs 1963), although the nature of the mechanisms and their difference are largely unknown. These differences could be based on different photoreceptor pigments, different response mechanisms, or different mechanisms for detecting light direction in the first and second positive phototropic responses.

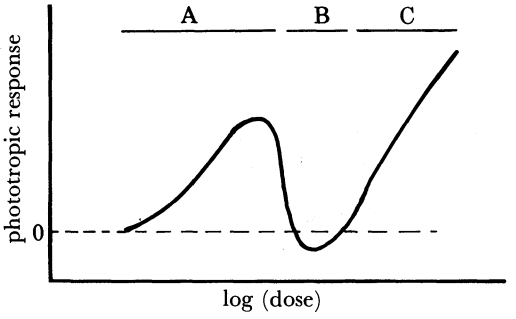

FIGURE 5. Idealized dose–response curve for phototropism by the shoot of a monocot seedling. A, B and C represent first positive phototropism, first negative phototropism and second positive phototropism, respectively.

In spite of the evidence that the first and second positive phototropic responses differ considerably, it has frequently been tempting to extrapolate from one to the other, equating the two responses. Such an approach may in fact delay an understanding of the phenomenon of phototropism. Perhaps the best example of this is the evaluation of the role of screening in phototropism.

It has clearly been established that screening is involved in second positive phototropism, i.e. that the high absorption in the primary leaf within the coleoptile shades the distal side of the coleoptile such that an intensity gradient is established between the proximal and distal

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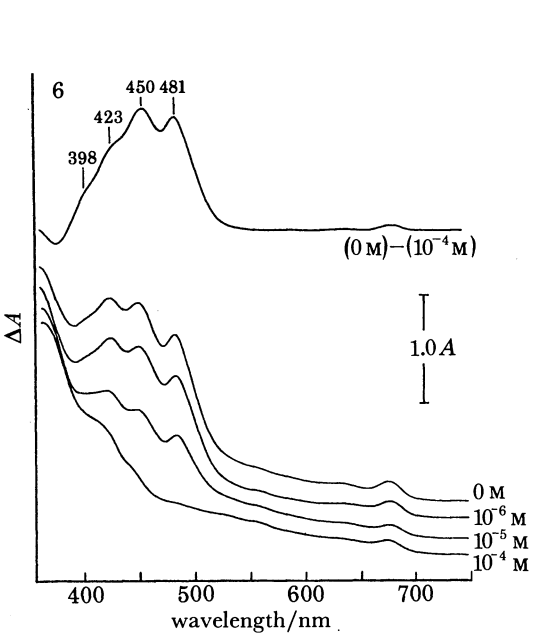
sides. This has been demonstrated through a number of experiments: (1) if the primary leaf is removed, the screening is dramatically reduced and phototropism decreased; (2) if the primary leaf is replaced by an absorbing dye, the screen and phototropism are regenerated (Brauner 1955; Bunning *et al.* 1953); (3) if one treats seedlings with SAN 9789, an inhibitor of carotenoid biosynthesis, both the screen in the coleoptile and primary leaf and phototropic sensitivity are substantially reduced (figures 6 and 7). Thus one may conclude that light direction is detected in second positive phototropism through the mechanism of screening.



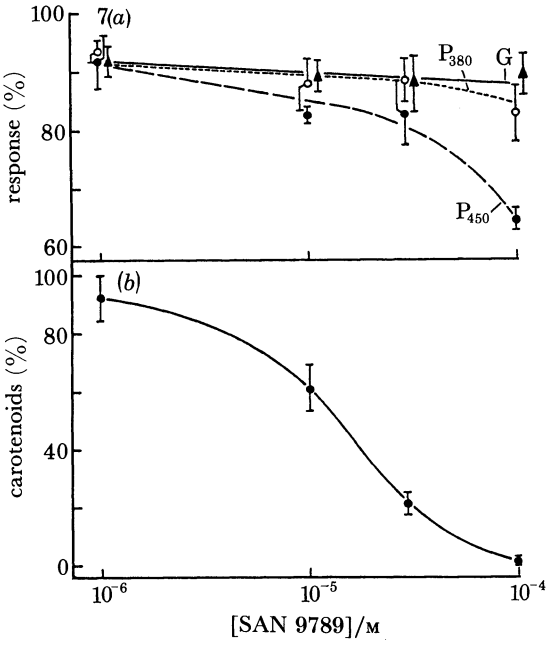


FIGURE 6. Absorption spectra of corn [maize] seedlings germinated with water or different concentrations SAN 9789. The sample consisted of 0.5 g of seedling tips including the primary leaf homogenized in 0.5 ml distilled water. Spectra were measured by using a single-beam spectrophotometer on line with a small computer. (Taken from Vierstra & Poff (1981).)

FIGURE 7. The effect of SAN 9789 on carotenoid accumulation, phototropism and geotropism in corn seedlings. (*a*) The effect of SAN 9789 on phototropism and geotropism of corn seedlings. Geotropic bending (G; ▲) relative to control seedlings germinated in distilled water was measured after 3 h geotropic stimulus. Phototropic bending to 380 nm (○) and 450 nm (●) light, relative to control seedlings germinated in distilled water, was measured after a 3 h phototropic stimulus. The vertical bars represent ±1 standard error. Each point represents from four to six independent experiments comparing ten seedlings treated with SAN 9789 with ten control seedlings.

(*b*) The effect of SAN 9789 on carotenoid accumulation in corn seedlings. The carotenoid content of SAN 9789 treated seedlings was determined from the absorbance at 481 nm of 0.5 g homogenized seedling tips and compared with that of control seedlings. The vertical bars represent ± one standard deviation.

It is not equally clear that screening is involved in first positive phototropism. Moreover, it should be noted that a relatively high-absorbance screen is available in the coleoptile only in the primary leaf (figure 8). In contrast, the absorbance of the tip of the coleoptile above the primary leaf is low and may not be consistent with screening as a mechanism for the detection of light direction but may be consistent with a refraction mechanism.

Considerable attention has been given to the tip and base responses of *Avena* and to the correlation of the 'tip response' with first positive phototropism (Thimann & Curry 1961;

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Curry 1969). Dennison (1979), however, argues that the distinction between the 'tip response' and 'base response' is morphological and spurious. However, these arguments seem only to be directed toward the bending response and do not negate the observations that only the tip is sensitive at low light doses.

If the tip of the coleoptile is indeed responsible for the first positive phototropism, then the possible role of the refraction mechanism for the detection of light direction should be carefully examined given a low transverse absorbance in the coleoptile tip. If refraction is the mechanism for the detection of light direction in the tip, and if the photoproducts are not easily diffusible,

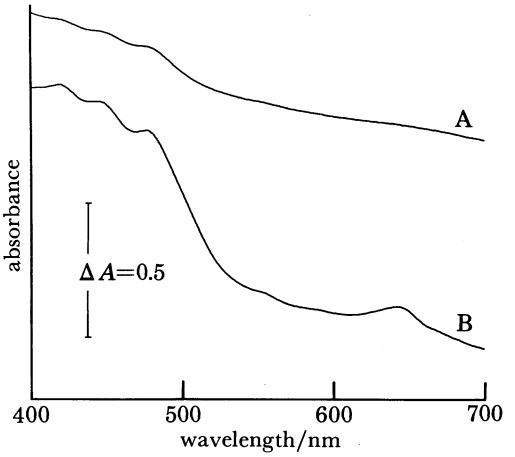

FIGURE 8. Absorption spectra measured across the shoot of a corn seedling. Spectrum A was measured through the tip of the coleoptile above the primary leaf. Spectrum B was measured 0.5 cm below the tip of the coleoptile, where the primary leaf is present. The seedlings were grown for 5 days in darkness with 1 h red light each day. Spectra were measured by using a single-beam spectrophotometer on line with a small computer.

then one would expect a 'negative' response after the first positive response. This would occur after the photoreceptor-response mechanism is saturated on the distal side and before saturation on the proximal side. Thus the extent and perhaps the existence itself of the 'negative' response should be fluence-dependent and would be expected to be more extreme at higher fluence rates where saturation would be more severe. That such a fluence-dependent first negative phototropism is indeed observed may be purely chance and should not be accepted as evidence that refraction is involved in first positive phototropism. However, this should be sufficient to stimulate a closer examination of the mechanism whereby light direction is measured in first positive phototropism.

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Discussion

S. Obrenović (*Institute for Biological Research 'Siniša Stanković', Belgrade, Yugoslavia*). Concerning the effect of norfluorazone on carotenoids as screening pigments and on the phototropic response, I wish to ask whether Dr Poff has measured its effect on the presumed photoreceptor for blue light, the flavin–cytochrome complex. We have measured the light-induced absorbance change in the 50000 *g* pellet fraction from corn coleoptiles and found that norfluorazone does affect it. The effect of norfluorazone on the phototropic reaction was established by Dr Konjevic only in light-grown plants (*Phaseolus aureus* Roxb.) and not in etiolated ones. A comparable effect was obtained on the light-induced absorbance change *in vivo*. So it seems that norfluorazone can affect directly the presumed photoreceptor for blue light, and thus its effect on the phototropic reaction can not be unequivocally ascribed to the lack of carotenoids.

K. L. Poff. No, we have not measured the effect of norfluorazone on the blue-light-induced absorbance changes. The results just described are interesting and may suggest an effect, whether direct or indirect, on the photoreceptor pigment itself. In our experience, the specificity of inhibitors is dependent upon the concentration used so one should be cautious in extrapolating results from one experiment to another.

Several lines of evidence support the conclusion that the carotenoids function primarily as a screening pigment. (1) Fluence response curves with and without norfluorazone extrapolate to zero response at the same fluence. This is probably not compatible with an effect of the inhibitor directly on the photoreceptor pigment. (2) Experiments where the primary leaf is removed from the coleoptile result in a decreased phototropism. The addition of a dye in place of the primary leaf regenerates the phototropic response (Bunning 1953; Brauner 1955).

I agree that one should be cautious in the use of inhibitors remembering that few if any are specific at all concentrations. However, in this case, I believe that the data support the conclusion that the carotenoids function as a screening pigment in corn coleoptiles and that norfluorazone inhibits phototropism through the decrease of that screen.

S. Obrenović. What are Dr Poff's views on the involvement of phytochrome in the phototropic reaction?

K. L. Poff. Although it is clear that phytochrome is related in some way to the phototropic response, perhaps potentiating the response, I would be extremely hesitant to propose a specific role of phytochrome. No attempt has been made in this paper to include phytochrome because

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I am aware of no evidence suggesting that phytochrome directly establishes the light gradient in etiolated corn shoots. However, we must understand the role of phytochrome in phototropism before we truly understand the mechanism of phototropism.

W. Haupt (*Institut für Botanik und Pharmazeutische Biologie, Erlangen, F.R.G.*). I have a comment on the expected composite action spectrum, containing characteristics of photoreceptor and screening pigments. In the temporal gradient, due to periodic screening (e.g. in *Euglena*), I would not expect any major contribution of the photoreceptor's absorption spectrum to the action spectrum. The photoreceptor is adapted to the steady-state intensity and the response depends on the proportional step-down signal, irrespective of the steady-state level. The size of this step-down signal is solely a function of the absorption in the screening pigment (given the fact that the photoreceptor can absorb at all). This comment does not concern the situation with spatial gradients.

K. L. Poff. That is a very interesting suggestion. It would appear that in such a system, given absorption by the photoreceptor pigment, adaptation to the 'unscreened' light, and a response proportional to the step-down signal, the contribution by the photoreceptor pigment to the action spectrum would be minimal. Thus the major contribution of the photoreceptor pigment to the action spectrum would be to set the wavelength limits for the response.

R. D. Firn (*Department of Biology, University of York, U.K.*). I wonder whether Dr Poff might not be underestimating the contribution of diffusion and light scattering to the creation of a light gradient across a coleoptile. Some studies we have recently made on light gradients in hypocotyls suggests that these factors are important and it is evident from Dr Poff's data that coleoptiles lacking the carotenoid screening pigments still show 60 % of the normal phototropic response.

K. L. Poff. As I indicated, screening may be accomplished through scattering or absorption either by the photoreceptor pigment itself or by a second pigment. Of these, the least complicated to manipulate experimentally is absorptive screening by a second pigment. One must not forget that scattering and absorption must both be present inherently. A quantitative study of the relative importance of each of these factors has not yet been made in any system but would be a significant contribution.

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