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Correlation of Blue Light-Induced Phosphorylation to Phototropism in *Zea mays* L.[1]

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The physiology of light-induced phototropic curvature has been studied extensively in coleoptiles of grasses, particularly in *Avena* and *Zea mays* L. In *Z. mays* L., we have found that, in addition to curvature, blue light also induces rapid phosphorylation of a 114-kD protein in the tips of coleoptiles, and, in a previous report, we reported several characteristics of the phosphorylated substrate protein and kinase (J.M. Palmer, T.W. Short, S. Gallagher, W.R. Briggs [1993] Plant Physiol 102: 1211–1218). Here, we compare the phosphorylation response to several known aspects of phototropism physiology. Blue light-induced phosphorylation occurs only in the upper portion of the coleoptile and is absent from the node and mesocotyl. The specific activity of phosphorylation is highest in the extreme apical portion of the tip, which is also the site of maximal sensitivity to phototropic stimuli (A. W. Galston [1959] *In* Physiology of Movements, Encyclopedia of Plant Physiology, Springer, Berlin). Fluence-response determinations indicate that light dosage levels that stimulate curvature also stimulate phosphorylation. However, the threshold for inducing detectable phosphorylation in maize cannot be matched to the threshold for curvature induction. The recovery of sensitivity to phototropic stimuli after exposure to high fluences of light occurs with kinetics that are very similar to those for recovery of the phosphorylation response after a previous high-fluence light exposure. In addition, wavelengths of light in the blue and near-ultraviolet regions of the spectrum that maximally stimulate phototropic curvature also maximally stimulate in vitro phosphorylation in maize. The pattern of stimulation matches the absorption spectra of flavoproteins, which have been proposed as candidates for blue light photoreceptors.

Phototropism, the bending of plants or plant organs toward a directional light source, is primarily a response to blue and near-UV light, although red light may alter the sensitivity and magnitude of the response (Iino, 1990). Phototropism has been studied extensively in dark-grown seedlings of grass coleoptiles because these plant organs are anatomically simple and free of potentially interfering photosynthetic pigments. These studies have revealed a surprising complexity in the physiological bending response to blue light, which can be divided into at least two phases defined by light dosage (fluence) and time requirements. First-positive curvature is generally described as bending of the apical tip of the coleoptile toward a unilateral light delivered in brief pulses at very low fluences. Second-positive curvature occurs with prolonged irradiation in a time-dependent fashion (for review, see Iino, 1990). In the case of first-positive phototropic curvature, the major site of light perception is in the extreme apical tip of the coleoptile (Sierp and Seybold, 1926; Lange, 1927; Galston, 1959). Presumably, this region contains a high density of blue light photoreceptors capable of transducing an inductive light pulse into the biochemical and physiological changes that result in curvature.

The molecular nature of the blue light photoreceptor for phototropism has not yet been elucidated. A number of candidate molecules have been proposed as the chromophore moiety of the photoreceptor based on comparisons between the absorption spectra of these molecules and the action spectra of light wavelengths that most efficiently induce curvature. Several lines of evidence implicate flavins as the putative chromophore for phototropism in higher plants. The absorption spectra of flavoproteins (Ghisla et al., 1975) are nearly identical with the phototropism action spectra measured for alfalfa (Baskin and Iino, 1987) and *Avena* (Shropshire and Withrow, 1958). Several inhibitors that inactivate flavins also inhibit phototropism in maize (*Zea mays* L.) coleoptiles (Schmidt et al., 1977) and in *Avena*; acifluorfen both alters the activity of a Cyt-flavin complex and increases the sensitivity of phototropism (Leong and Briggs, 1982). However, direct confirmation of the identity of the chromophore awaits purification and characterization of the blue light photoreceptor.

Recently, rapid blue light-stimulated phosphorylation of a high mol wt protein has been described in a variety of higher plants (Short and Briggs, 1990; Reymond et al., 1992a). This phosphorylation event appears to be an early step in the signal transduction chain for phototropism based on the location of the response in phototropically sensitive tissues, the fluence levels of blue light required to initiate the response (Short et al., 1992), and the altered kinetic efficiency of phosphorylation in an *Arabidopsis thaliana* mutant that shows greatly reduced light sensitivity for first-positive phototropic curvature (Reymond et al., 1992b). In etiolated maize, blue light stimulates phosphorylation of a 114-kD protein in vivo in excised coleoptiles and in vitro in purified plasma membranes (Palmer et al., 1993). The response is rapid enough to precede the development of phototropic curvature, and the phosphorylated protein itself has an apparent ATP-binding site, which suggests that it may be capable of kinase activity.

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In the following report, we further characterize the nature of blue light-induced phosphorylation in maize and compare the results to some of the known physiological properties of maize phototropism.

MATERIALS AND METHODS

Plant Material and Membrane Preparation

Zea mays L. (Northrup King Hybrid pX9540) seedlings were grown in vermiculite with one-fourth-strength Hoagland medium for 5 d in total darkness. Harvests of coleoptile tissue samples were performed under dim red safelights. For preparation of 1-mm-long tissue samples of the apex, a custom-built cutting device was used to harvest the desired length of tips. For in vivo irradiations, excised tip samples were floated on deionized distilled water during exposure to blue light. Microsomal membranes were prepared by grinding the tissue on ice with a mortar and pestle, followed by differential centrifugation as described by Gallagher et al. (1988), except for the homogenization and resuspension buffers, which were prepared according to the method of Short et al. (1992). Membrane samples were assayed for total protein as described by Short et al. (1992), divided into small aliquots, and stored in the dark at −80°C until thawed for in vitro phosphorylation reactions.

Light Sources

The red and blue light sources used in these experiments were described by Short et al. (1992) and Palmer et al. (1993). Irradiations for action spectroscopy were performed in a fluorimeter (Photon Technology Intl.) equipped with a xenon lamp and a 5-nm half-bandwidth. Fluence levels were determined for each wavelength by measuring light intensity at the position of the sample cuvette with a thermopile (model 2M; Dexter Research Inc.) and converting the measurement to μmol m^{-2} s^{-1}. A correction factor was included to account for light absorption by the polystyrene cuvettes used in each assay.

Phosphorylation Assays and Gel Electrophoresis

Phosphorylation in vivo was assayed with samples of 100 coleoptile tips 5 to 8 mm long, excised from seedlings grown for 5 d in total darkness. The tips were incubated for 1 h on 5 mL of 10 mM Tris (pH 7.3) with 1 mM sodium phosphate and 0.5 mCi of ^{32}Pi (100 μL, 8500 Ci/mmol; New England Nuclear). Following the incubation, different samples were irradiated at different fluence levels of blue light, obtained with 1-min irradiation times and the appropriate neutral density filter. Microsomal membranes were prepared as described above, and 50 μg of total protein from each sample were electrophoresed at 5.5 mA (constant current) for 14 h at room temperature on 5 to 20% gradient polyacrylamide gels containing SDS (Gallagher et al., 1988). The gels were dried and exposed to Kodak XAR-5 film for autoradiography.

Phosphorylation assays in vitro contained 200 μg of total protein from each microsomal membrane sample in a Tris-Mes buffer supplemented with magnesium and 0.5% Triton X-100 as described by Short et al. (1992). Individual frozen aliquots of membranes were thawed and immediately added to the assay buffer, warmed to 30°C for 30 s, irradiated or mock irradiated, and incubated with 13.3 μCi of [γ-^{32}P]ATP and 10 nmol of ATP for 2 min at 30°C. Reactions were stopped by the addition of an SDS-containing gel-loading buffer as described by Short et al. (1992), then electrophoresed, and autoradiographed as described above. Phosphorylation levels of individual protein bands in dried gels were quantitated on a beta scanner (PhosphorImager; Molecular Dynamics, Sunnyvale, CA).

RESULTS

Tissue Distribution of Phosphorylation in Maize Coleoptiles

Blue light-induced phosphorylation of a 114-kD protein in plasma membrane fractions from the tips of *Zea mays* L. coleoptiles has been described (Palmer et al., 1993). To determine the distribution of the phosphorylation reaction, tissue sections from 5-d-old etiolated seedlings were analyzed for their response to blue light. Sections of 5 to 7 mm in length were removed under dim red light from the apical tip and basal region of the coleoptile, the node, and the mesocotyl. Microsomal membranes were prepared, and each sample was phosphorylated in vitro in standard reactions and electrophoresed through denaturing gradient acrylamide gels. Figure 1 is an autoradiograph of phosphorylation of the 114-kD protein in each of the tissue samples from etiolated maize seedlings. The strongest phosphorylation response occurred in the apical tip of the coleoptile; more basal regions also showed substantial phosphorylation. Blue light-induced phosphorylation was not detected in the node or mesocotyl or in any other proteins in the microsomal membranes of

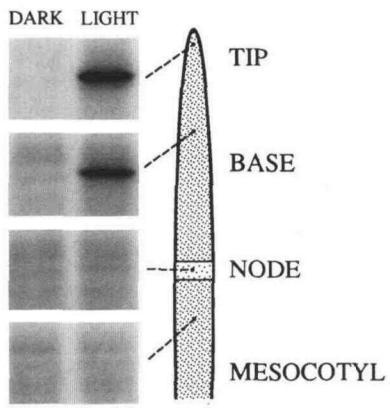

Figure 1. Distribution of the phosphorylation response in maize seedlings. Etiolated maize coleoptiles grown for 5 d in complete darkness were cut into 5-mm-long sections under dim red light. Microsomal membranes were prepared from sections of the extreme tip, coleoptile base, node, and mesocotyl. Each membrane sample was phosphorylated in vitro with [γ-^{32}P]ATP after either mock irradiation or exposure to blue light, then electrophoresed on a denaturing acrylamide gel, and exposed to film for autoradiography. The autoradiograph panel from each tissue sample shows phosphorylation at the level of the 114-kD protein in mock-irradiated (DARK) or blue light-exposed (LIGHT) samples.

these tissues. The primary leaves, which are ensheathed by the coleoptile, showed strong phosphorylation of many proteins, but none was induced by exposure to blue light (data not shown).

The localization of phosphorylation to the coleoptile tip was investigated further by comparing the specific activity of phosphorylation in 1- versus 5-mm-long apical tissue samples. The results are shown in Figure 2. In the equal protein amounts loaded into each gel lane, blue light-induced phosphorylation is increased by slightly more than 50% in the sample prepared from 1 mm of apical tissue. A comparable increase in phosphorylation is also detected in mock-irradiated (dark) samples in the 1-mm tip preparation. These results suggest there may be a higher concentration of the 114-kD protein in the most apical portions of the coleoptile tip.

Sensitivity of the Phosphorylation Response to Different Light Fluences

The fluence of blue light required to induce phosphorylation in vivo was determined by perfusing excised coleoptile tips, 5 to 8 mm long, with 0.5 mCi of ^{32}Pi for 1 h in the dark at room temperature. Six samples of 100 tips each then were either mock irradiated or irradiated with a different fluence of blue light, ranging from 10^{-1} to 10^{3} μmol m^{-2}. Microsomal membranes were prepared from each sample and electrophoresed on denaturing gradient polyacrylamide gels. The autoradiograph is shown in Figure 3A. Blue light-induced phosphorylation in the 114-kD protein is first detected in vivo at 10^{1} μmol m^{-2}, indicating that the threshold for induction is between 1 and 10 μmol m^{-2}. Phosphorylation of the 114-kD band increases steadily up through 10^{3} μmol m^{-2}. Pretreatment with red light for 1 h before harvesting the tips did not alter the results (data not shown).

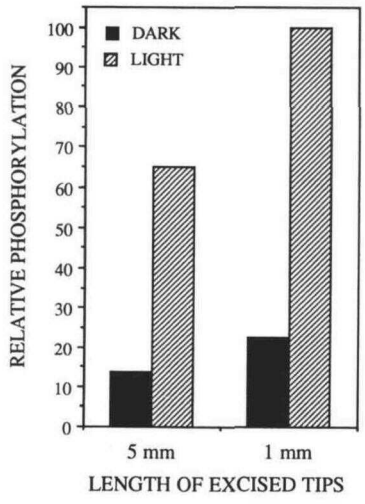

Figure 2. Specific activity of phosphorylation in 1- versus 5-mm-long coleoptile tip sections. Crude membranes were prepared from coleoptiles as in Figure 1, except that one sample was derived entirely from sections that included only 1 mm of tissue at the coleoptile tip. The second sample consisted of 5-mm-long apical sections. After phosphorylation in vitro of equal protein amounts from each preparation and separation on a denaturing acrylamide gel as in Figure 1, the level of phosphorylation in the 114-kD protein band was quantitated on a beta scanner.

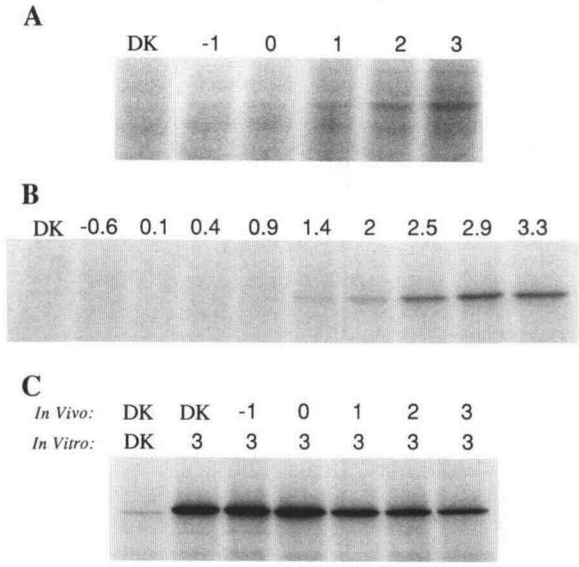

Figure 3. Fluence levels of blue light that stimulate phosphorylation of the maize 114-kD protein. Autoradiographs of phosphorylation at different doses of blue light: the numbers above each lane are the log of the fluence used to irradiate the sample. All irradiations were for 1 min; fluence levels were varied with neutral density filters. Following the phosphorylation assays, proteins in the samples were electrophoresed on denaturing acrylamide gels, dried, and exposed to film for autoradiography. A, In vivo phosphorylation. Coleoptile tips from etiolated maize seedlings were perfused with ^{32}Pi for 1 h in total darkness. Individual samples, each including 100 perfused tips, were either mock irradiated (DK) or irradiated with blue light at the indicated fluence level before extracting microsomal membranes. B, In vitro phosphorylation. Etiolated coleoptile tip sections were excised as in A, and the microsomal membrane fractions were extracted, divided into equal protein aliquots, and phosphorylated in vitro in standard assays at the indicated fluences of blue light. C, In vivo irradiation followed by in vitro phosphorylation. Coleoptile tips were excised and exposed to different fluences of blue light as in A. Microsomal membranes were prepared from each sample and were phosphorylated in vitro in the standard assay after mock irradiation (DK) or exposure to 10^{3} μmol m^{-2} of blue light.

Phosphorylation in vitro is first detected at $10^{0.9}$ μmol m^{-2} and increases steadily up through $10^{3.3}$ μmol m^{-2} (Fig. 3B). For this experiment, standard in vitro phosphorylation reactions were prepared with microsomal membranes of etiolated seedlings. The exposure time for all samples was 1 min; fluences were adjusted with neutral density filters. The threshold for induction in vitro appears to fall between $10^{0.4}$ and $10^{0.9}$ μmol m^{-2}, similar to the light level required for in vivo phosphorylation.

Fluence requirements were also investigated in an experiment combining in vivo irradiation with in vitro phosphorylation (Fig. 3C). Coleoptile tip samples were exposed to different levels of blue light in vivo, and microsomal membranes were prepared from each sample immediately following irradiation and then assayed for phosphorylation in vitro in the standard reaction. In this experiment, prior irradiation in vivo,

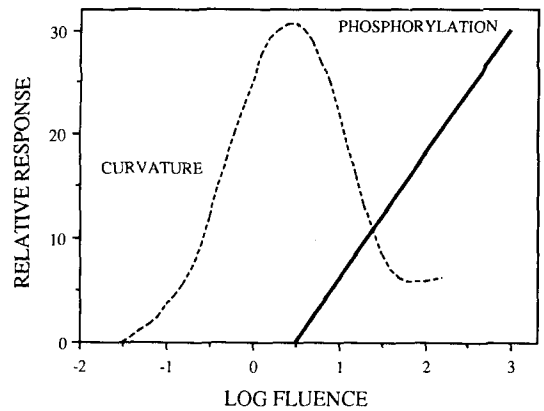

Figure 4. Comparison of blue light fluence levels that stimulate first-positive phototropic curvature with those that stimulate detectable phosphorylation. The curvature data are redrawn from Baskin (1986) and indicate, for various fluences, the relative amount of curvature elicited in maize coleoptiles pretreated with red light (dotted line). Relative phosphorylation levels, at these same fluences, are represented by the solid line.

in the absence of labeled phosphate, reduces the level of phosphorylation in a subsequent in vitro reaction with radioactive ATP, presumably because many potential phosphorylation sites were filled in vivo with endogenous phosphate. As shown in Figure 3C, maximum in vitro phosphorylation levels are obtained in samples that received no blue light in vivo (dark) or either 10^{-1} or 10^{0} μmol m^{-2}. Reduced phosphorylation levels are first apparent in samples receiving 10^{1} μmol m^{-2} blue light in vivo, and phosphorylation steadily decreases up through in vivo exposure to 10^{3} μmol m^{-2}. The results of this subtraction experiment set a similar level to the in vivo and in vitro assays for a threshold of phosphorylation induction between 10^{0} and 10^{1} μmol m^{-2}.

Figure 4 is a graph comparing the fluences of blue light that stimulate phosphorylation of the 114-kD protein in maize membranes with those that stimulate curvature in maize coleoptiles pretreated with red light. By the above gel analysis, the threshold for induction of phosphorylation in vivo falls somewhere between 10^{0} and 10^{1} μmol m^{-2} (Fig. 3A), and phosphorylation levels increase in a more or less linear fashion to a maximum value at or above 10^{3} μmol m^{-2} . In contrast, induction of first-positive phototropism in red light-grown maize follows a bell-shaped curve with a threshold near $10^{-1.5}$ μmol m^{-2} and peak induction at $10^{0.5}$ μmol m^{-2}. Fluences between $10^{0.5}$ and 10^{3} are increasingly inefficient at stimulating curvature (redrawn from Baskin, 1986).

Dark Recovery of Phosphorylation and Phototropism in Maize

Etiolated maize seedlings exposed to supersaturating doses of white light do not exhibit light-induced curvature and, for a period of time, are unable to respond to lower levels of light that are normally inductive (Briggs, 1960). If the exposed seedlings are subsequently incubated in the dark, they will gradually recover the ability to respond to an inductive light pulse. The kinetics of dark recovery were investigated for blue light-induced phosphorylation of the 114-kD protein in maize. Coleoptile tips, 5 to 8 mm in length, were excised from etiolated maize seedlings and divided into different samples. Each sample was exposed to 10^{3} μmol m^{-2} of blue light. This fluence level fails to induce curvature in maize when delivered as a brief pulse but is near saturation for inducing phosphorylation (Fig. 4). The samples were then incubated for varying times in the dark at room temperature. Nonirradiated control samples were incubated for either 0 or 40 min. Following the incubations, microsomal membranes were prepared from each sample, and the membranes were assayed for phosphorylation in vitro in the standard reaction. The results are shown in Figure 5. The filled symbols on the graph represent the data points for recovery of the phosphorylation response, and the solid line shows the average of these data points. The level of in vitro phosphorylation is barely above the level of the dark control for samples incubated only 2.5 min in the dark following in vivo light exposure. Recovery of sensitivity occurs steadily and reaches a maximum after 20 min of dark incubation. Further incubation for 40 min causes a slight decline in phosphorylation, comparable to that of the light control, also incubated for 40 min.

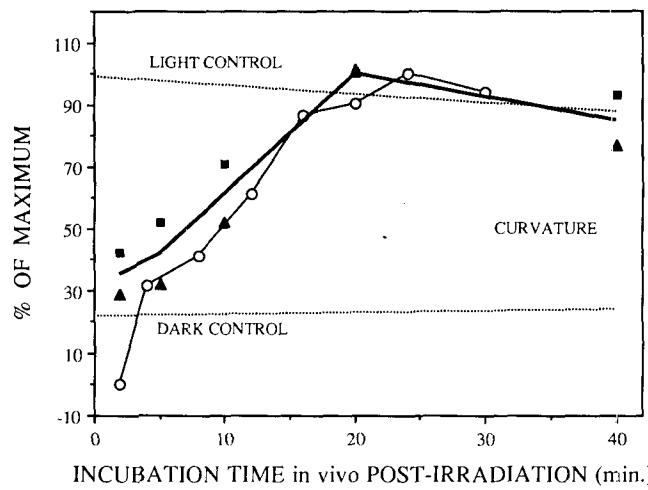

Figure 5. Dark recovery kinetics for phosphorylation and phototropism in maize. The curvature assay results (line with open symbols) are redrawn from Briggs (1960). Etiolated maize seedlings were exposed to fluences of white light that suppress first-positive curvature. Following incubation in darkness for the indicated times, the seedlings were irradiated unilaterally with fluences of light that normally induce first-positive curvature. For the phosphorylation assay (filled symbols; the solid line represents the average values), etiolated maize coleoptile tips were excised and exposed to the same fluences of blue light that suppress curvature in seedlings. The irradiated samples were incubated in the dark for the indicated times, and then microsomal membranes were prepared from each. The extracted membrane samples were irradiated in vitro in the standard phosphorylation assay. The DARK CONTROL and LIGHT CONTROL lines indicate the difference in phosphorylation levels between membrane samples that were not irradiated in vivo but were incubated for either 0 or 40 min before membrane preparation and then either mock irradiated or exposed to light in vitro in the standard phosphorylation assay.

These results are superimposed on the data points for recovery of curvature (open symbols), redrawn from Briggs (1960). After exposure to high-fluence light, maize seedlings recover the ability to respond to a subsequent inductive light pulse with phototropic curvature at a rate that is very similar to the recovery of phosphorylation.

Phosphorylation at Different Wavelengths of Blue Light

The effectiveness of different wavelengths of blue light in inducing phosphorylation was examined in standard in vitro phosphorylation assays with maize microsomal membranes. The samples were irradiated in polystyrene cuvettes in a fluorimeter in which wavelengths could be selected with 5-nm half-bandwidths. Irradiations were for 10 s or less and were adjusted to the same subsaturating total fluence at each wavelength. The results are shown in Figure 6. The dotted lines without symbols represent the relative effectiveness of each wavelength at inducing phototropic curvature in *Avena* or alfalfa (redrawn from Baskin and Iino, 1987). The solid line in the large graph indicates the intensity of phosphorylation in the 114-kD protein band at each of the indicated wavelengths of light. This line is an average of the data points from four separate experiments, shown in the smaller graphs along the border. The effectiveness of different wavelengths at stimulating either phosphorylation in maize or curvature in *Avena* or alfalfa is very similar. Major peaks occur at 375 and 445 nm, with decreased effectiveness at 400 nm. A fluence-response curve of phosphorylation was performed at 375, 400, and 445 nm, with fluence varied between 0 and 10^4 μmol m^{-2} at each of the selected wavelengths.

Phosphorylation increases at each wavelength in an essentially linear fashion between 10 and 10,000 μmol m^{-2} blue light, but the slope of the increase is much steeper at 445 nm than at either 400 or 375 nm (data not shown).

DISCUSSION

Blue light-induced phosphorylation in maize is localized to the upper region of the coleoptile and increases in specific activity in the most apical portions of the tip (Figs. 1 and 2). The location of the phosphorylation response correlates well with the region in grass coleoptiles known to be sensitive to phototropic stimuli (Galston, 1959; Iino and Briggs, 1984; Vogelmann and Haupt, 1985). The apical 0.5 mm of the coleoptile tip is also the major site of lateral auxin translocation for both first-positive and time-dependent curvature in maize (Briggs, 1963). According to the Cholodny-Went theory, lateral translocation of auxin is induced by photoperception of the phototropic stimulus and is a prerequisite for development of the differential cell elongation that results in curvature.

The fluence levels of blue light that stimulate phosphorylation in maize coleoptiles are similar in vivo and in vitro (Fig. 3). The apparent threshold for phosphorylation induction is between 10^0 and 10^1 μmol m^{-2}, with maximum phosphorylation occurring at or above 10^3 μmol m^{-2}. In the Alaska pea, the threshold for in vitro phosphorylation is similar to that in maize, but the reaction is detectable in vivo with 10-fold less light than is required in vitro, which suggests that extraction of plasma membranes alters the components of the reaction in a way that reduces overall efficiency (Short et al., 1992). In maize, the inability to detect greater sensitivity of the reaction in vivo may relate to the technical difficulty of perfusing cone-shaped coleoptile tips with ^{32}Pi so that labeled phosphate is at high concentration in the distal portion of the tip. Red light pretreatment of maize seedlings did not alter the fluence sensitivity of the phosphorylation response, as it does for phototropic curvature (Chon and Briggs, 1966; Iino, 1988). However, the effects of red light on sensitivity occur at fluences of blue light below the threshold detected for inducing phosphorylation (Fig. 4).

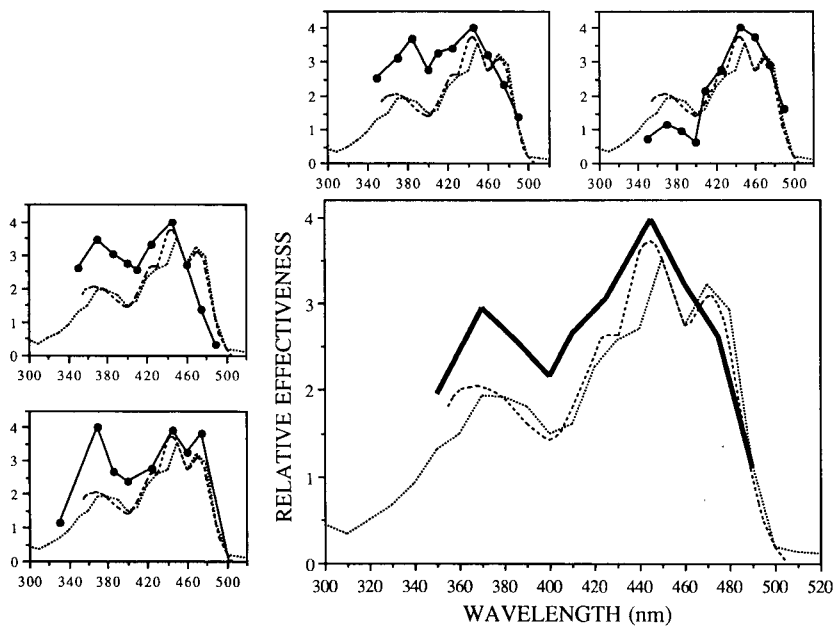

Figure 6. Effect of different wavelengths of blue light on stimulating phototropic curvature and phosphorylation in maize. The small graphs show the data points from individual experiments; the large graph shows the average derived from the four data sets (solid heavy line). The data for curvature induction in *Avena* and alfalfa (dotted lines) are redrawn from Baskin and Iino (1987). The phosphorylation assays of maize microsomal membrane samples were performed by the standard method, except that a fluorimeter was used for irradiations at different wavelengths of light. Fluence levels were adjusted to 500 μmol m^{-2} for each sample (below saturation), and irradiation times were 10 s or less. After the samples were incubated in [γ-^{32}P]ATP and were electrophoresed, phosphorylation levels in the 114-kD protein were quantitated on a beta scanner.

Phosphorylation in maize is triggered by levels of blue light that maximally stimulate phototropic curvature (Iino and Briggs, 1984; Iino, 1987); yet the threshold for induction of curvature is at least an order of magnitude more sensitive than the fluence levels required to detect phosphorylation (Fig. 4). There are a number of possible explanations for this discrepancy: (a) Determination of the true threshold may be complicated by the difficulty of extracting only the most phototropically sensitive tissue for use in the assays. In *Avena*, phototropic sensitivity declines 60-fold at a distance of 0.5 mm from the tip and nearly 1000-fold at a distance of 1.5 mm from the tip (Sierp and Seybold, 1926; Lange, 1927; Galston, 1959). In pea, in which the phototropic sensitivity is more evenly distributed in the growing region of the upper internode, the threshold for phosphorylation in vivo is detected at fluences similar to the threshold for curvature induction (Baskin, 1986; Short et al., 1992). (b) Phosphorylation and curvature induction may occur via separate photoreceptors. The involvement of multiple photoreceptors in blue light-induced signal transduction is suggested by the physiological responses of two different sets of *Arabidopsis* mutants: for phototropism, Konjevic et al. (1989); for hypocotyl growth inhibition, Young et al. (1992). (c) Phosphorylation may not be the primary event in initiating phototropic curvature but may, instead, be a mechanism for returning the system to an initial ground state following induction or may be a signaling event required for a pathway related to the development of curvature. The functional involvement of phosphorylation as a key element at some step in the phototropism signal transduction chain is strongly suggested by mutants of *Arabidopsis* that have reduced sensitivity to blue light induction of both first-positive curvature and phosphorylation (Reymond et al., 1992b). Further experimentation will be required to determine the exact relationship between phosphorylation and phototropism in maize.

Brief pulses of blue light delivered at fluences beyond those that induce first-positive phototropism fail to elicit curvature in maize coleoptiles (Briggs, 1960). First-positive curvature is described by a bell-shaped curve over several orders of magnitude in fluence, with the descending arm of the curve identifying those fluences that are increasingly inefficient at inducing a detectable physiological response (Fig. 4). Following exposure to high fluences that fail to elicit curvature, maize seedlings incubated in the dark will gradually recover sensitivity to a normally inductive blue light pulse during a 20-min period. Similarly, if excised coleoptile tips are pulsed with light levels that fail to induce curvature in seedlings and then are incubated in the dark for varying times before membrane preparation, very little detectable phosphorylation is evident in a subsequent standard in vitro irradiation/phosphorylation assay in those samples incubated in the dark for only 2.5 min (Fig. 5). Samples given longer dark incubations in vivo show steady recovery of the phosphorylation response to normal levels during a 20-min period. Thus, the recovery kinetics for sensitivity to phototropic stimuli closely match those for recovery of the phosphorylation response. It must be noted that fluence levels of blue light that are high enough to inhibit first-positive curvature in both pea and maize (Iino and Briggs, 1984; Baskin, 1986; Iino, 1987, 1988; Parker et al., 1989) stimulate maximum phosphorylation in vivo of the 117-kD protein in pea (Short et al., 1992) and the 114-kD protein in maize (Figs. 3 and 4). Recovery of the phosphorylation response in maize during dark incubation in vivo, therefore, may reflect the time needed either to dephosphorylate the substrate protein and return it to an initial ground state, to reassemble, or to synthesize new components in the photoreceptor/kinase/substrate induction chain.

In photobiology, action spectra are used as a means of identifying the probable photoreceptor for a biological response by comparing the wavelengths of light that induce the response with the absorption spectra of potential chromophore molecules. Detailed action spectra have been measured for phototropism in alfalfa (Baskin and Iino, 1987) and in *Avena* coleoptiles (Shropshire and Withrow, 1958). Curvature is most efficiently induced by wavelengths of light at or near 445 nm with additional peaks at 475 and 375 nm. Phosphorylation of the 114-kD protein in maize follows a similar pattern of induction (Fig. 5), indicating that the same wavelengths of blue light that maximally stimulate curvature also maximally stimulate phosphorylation. The pattern of induction of both curvature and phosphorylation matches the absorption pattern of flavoproteins (Ghisla et al., 1975), which suggests that some form of flavin may be the chromophore in photoperception for phototropism and for phosphorylation.

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LITERATURE CITED

Baskin TI (1986) Redistribution of growth during phototropism and nutation in the pea epicotyl. Planta **169:** 406–414

Baskin TI (1986) Phototropism: light and growth. PhD thesis. Stanford University, Stanford, CA

Baskin TI, Iino M (1987) An action spectrum in the blue and ultraviolet for phototropism in alfalfa. Photochem Photobiol **46:** 127–136

Briggs WR (1960) Light dosage and phototropic responses of corn and oat coleoptiles. Plant Physiol **35:** 951–962

Briggs WR (1963) Mediation of phototropic responses of corn coleoptiles by lateral transport of auxin. Plant Physiol **38:** 237–247

Chon HP, Briggs WR (1966) Effect of red light on the phototropic sensitivity of corn coleoptiles. Plant Physiol **41:** 1715–1724

Gallagher S, Short TW, Ray PM, Pratt LH, Briggs WR (1988) Light-mediated changes in two proteins found associated with plasma membrane fractions from pea stem sections. Proc Natl Acad Sci USA **85:** 8003–8007

Galston AW (1959) Phototropism of stems, roots and coleoptiles. *In* W Ruhland, ed, Physiology of Movements, Encyclopedia of Plant Physiology, Vol 17. Springer, Berlin, pp 493–529

Ghisla S, Massey V, Lhoste J-M, Mayhew S (1975) Fluorescence characteristics of reduced flavins and flavoproteins. *In* K Yagi, ed, Reactivity of Flavins. University Park Press, Baltimore, MD, pp 15–24

Iino M (1987) Kinetic modelling of phototropism in maize coleoptiles. Planta **171:** 110–126

Iino M (1988) Desensitization by red and blue light of phototropism in maize coleoptiles. Planta **176:** 183–188

Iino M (1990) Phototropism: mechanisms and ecological implications. Plant Cell Environ **13:** 633–650

Iino M, Briggs WR (1984) Growth distribution during first positive phototropic curvature of maize coleoptiles. Plant Cell Environ **7:** 97–104

Konjevic R, Steinitz B, Poff KL (1989) Dependence of the phototropic response of *Arabidopsis thaliana* on fluence rate and wavelength. Proc Natl Acad Sci USA **86:** 9876–9880

Lange S (1927) Die Verteilung der Lichtempfindlichkeit in der Spitze der Haferkoleoptile. Jb Wiss Bot **67:** 1–5

Leong TY, Briggs WR (1982) Evidence from studies with acifluorfen for participation of a flavin-cytochrome complex in blue light photoreception for phototropism of oat coleoptiles. Plant Physiol **70:** 875–881

Palmer JM, Short TW, Gallagher S, Briggs WR (1993) Blue light-induced phosphorylation of a plasma membrane-associated protein in *Zea mays* L. Plant Physiol **102:** 1211–1218

Parker K, Baskin TI, Briggs WR (1989) Evidence for a phytochrome-mediated phototropism in etiolated maize seedlings. Plant Physiol **89:** 493–497

Reymond P, Short TW, Briggs WR (1992a) Blue light activates a specific protein kinase in higher plants. Plant Physiol **100:** 655–661

Reymond P, Short TW, Briggs WR, Poff KL (1992b) Light-induced phosphorylation of a membrane protein plays an early role in signal transduction for phototropism in *Arabidopsis thaliana*. Proc Natl Acad Sci USA **89:** 4718–4721

Schmidt W, Hart J, Filner P, Poff KL (1977) Specific inhibition of phototropism in corn seedlings. Plant Physiol **60:** 736–738

Short TW, Briggs WR (1990) Characterization of a rapid, blue light-mediated change in detectable phosphorylation of a plasma membrane protein from etiolated pea (*Pisum sativum* L.) seedlings. Plant Physiol **92:** 179–185

Short TW, Porst M, Briggs WR (1992) A photoreceptor system regulating in vivo and in vitro phosphorylation of a pea plasma membrane protein. Photochem Photobiol **55:** 773–781

Shropshire W Jr, Withrow RB (1958) Action spectrum of phototropic tip-curvature of *Avena*. Plant Physiol **33:** 360–365

Sierp H, Seybold UA (1926) Untersuchungen uber Lichtempfindlichkeit der Spitze und des Stumpfes in der Koleoptile von *Avena sativa*. Jb Wiss Bot **65:** 592–610

Vogelmann TC, Haupt W (1985) The blue light gradient in unilaterally irradiated maize coleoptiles: measurement with a fiber optic probe. Photochem Photobiol **41:** 569–576

Young JC, Liscum E, Hangarter RP (1992) Spectral-dependence of light-inhibited hypocotyl elongation in photomorphogenic mutants of *Arabidopsis*: evidence for a UV photosensor. Planta **188:** 106–114