Exposure of Oat Seedlings to Blue Light Results in Amplified Phosphorylation of the Putative Photoreceptor for Phototropism and in Higher Sensitivity of the Plants to Phototropic Stimulation

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Phototropism has been studied in a variety of dicotyledonous and monocotyledonous plants over the last century (for reviews, see Firn and Digby, 1980; Briggs and Baskin, 1988; Iino, 1990; Firn, 1994). The most extensive studies on phototropic-curve reactions were done on the coleoptile of etiolated oat (Avena sativa L.) seedlings, a classic object of phototropism research. From the fluence-response curves obtained by unilateral irradiation of oat coleoptiles with blue or white light at least three different types of bending reactions have been distinguished with respect to tissue distribution, fluence-response characteristics, and dark-recovery kinetics (for review, see Short and Briggs, 1994). In addition, we recently demonstrated the asymmetric distribution of phosphorylation of the protein within the coleoptile after unilateral irradiation (Salomon et al., 1997a, 1997b). The above correlations were further supported by detailed biochemical and genetic analysis of an

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Abbreviations: FNPC, first-negative phototropic curvature; FPPC, first-positive phototropic curvature; SPPC, second-positive phototropic curvature.

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Arabidopsis mutant lacking all phototropic responses (Liscum and Briggs, 1995). It turned out that the deficiency in phototropism of this mutant was due to a mutation within a single gene locus (nph1), and there is now excellent evidence that this gene probably encodes the photoreceptor responsible for all phototropic responses. Furthermore, the concomitant loss of the 120-kD protein and of any blue light-dependent phosphorylation gave rise to the hypothesis that phototropism may be regulated by a photoreceptor that becomes phosphorylated upon irradiation with blue light (Liscum and Briggs, 1995), and earlier experiments suggested that the protein itself was the kinase (Reymond et al., 1992).

However, most of our knowledge about the physiology and photobiology of phototropism is still based on the studies of the different bending reactions after unilateral light treatment. It has been pointed out that the Bunsen-Roscoe reciprocity law does not hold for SPPC. The main characteristic of this response can be summarized as follows: Increasing the time of irradiation results in a continual shift of the reaction toward lower total fluences, and SPPC, therefore, must be considered as a time-dependent rather than a fluence-dependent response. Although different physiological approaches have been used to investigate this phenomenon (Zimmermann and Briggs, 1963; Everett and Thimann, 1968; Steinitz et al., 1988), nothing is known about the possible biochemical and molecular processes accounting for the observed time dependence of SPPC.

In the present work we used a recently described microscope system for protein phosphorylation (Salomon et al., 1997a) to study time-dependent changes in light-induced protein phosphorylation in vitro after preirradiation of etiolated oat coleoptiles in vivo. This approach proved to be appropriate for unravelling the molecular mechanism underlying the invalidity of the Bunsen-Roscoe reciprocity law with regard to SPPC.

**MATERIALS AND METHODS**

**Plant Material and Conditions of Growth**

Oat (Avena sativa L. cv Pirol) (Ackermann, Irlbach, Germany) seedlings used for the biochemical studies of protein phosphorylation were grown on vermiculite at 25°C for 72 h in the dark and were then exposed for 16 h to a dim-red light source (TL 40 W/15, Philips; 1 μmol m⁻² s⁻¹). Plants used for measurements of phototropic curvature were grown from seeds sown in covered plexiglass boxes laid out with tap water-soaked filter paper. The resulting tissue samples were collected in microtubes for cell extract preparation. For that purpose single samples were removed and homogenized for 30 s in 40 μL of kinase buffer (50 mM Hepes/KOH, pH 8.0, and 5 mM MgSO₄) using a conical tissue grinder for 1.5-mL microfuge tubes. The samples were briefly centrifuged for 10 s at 12,000g. Membrane vesicles and the plasma membrane-associated 116-kD protein remained in the supernatant that was immediately used for phosphorylation.

**In Vitro Phosphorylations**

In our experiments we used a light source with glass fiber optics and an IR filter (KL 1500, electronic, Schott, Mainz, Germany). Blue light was supplied by a broad-band (380–450 nm) blue glass filter (filter set for KL 1500 electronic, Schott). Light intensities were measured with a Li-189 quantum photometer (Lambda Instruments Corp., Lincoln, NE).

To determine the levels of in vitro phosphorylation in preirradiated plants the coleoptiles were cut just below the node and placed into small vessels filled with tap water. Unless stated otherwise, preirradiations were carried out under saturating conditions by exposing each side of the coleoptile for 30 s to a fluence rate of 1000 μmol m⁻² s⁻¹. Subsequent dark incubations of the plants were carried out under red light at 22°C. In those studies in which we investigated the distribution of phosphorylation within the coleoptile after a unilateral light exposure, we used the same experimental protocol as recently described in detail (Salomon et al., 1997a).

For phototropic stimulation and measurements of curvature the intact plants were positioned in a rack and placed in a wooden box just behind a 15 × 2-cm window cut out from the front side of the box. To avoid light reflection from the backside a mirror was fixed inside of the box in such a way that the incoming light was deflected upward. The blue light source was placed in front of the window at an appropriate distance, ensuring virtually identical light conditions along the entire length of the window. Five plants were irradiated at one time, with the entire coleoptile being illuminated. After phototropic stimulation curvature was permitted to develop on a clinostat for 120 min. The degrees of curvature were determined by taking shadowgraphs under red light and comparing them to those taken prior to unilateral irradiation.

**Preparation of Crude Cell Extracts**

For the preparation of cell extracts the coleoptiles were separated from the primary leaf. Either a single 5-mm cross-section or two 5-mm longitudinal half-sections from the coleoptile zone to be investigated were obtained by cutting the coleoptiles with a razor blade on a glass plate with graph paper. The resulting tissue samples were collected in microcentrifuge tubes and stored in liquid nitrogen until needed for cell extract preparation. For that purpose single samples were removed and homogenized for 30 s in 40 μL of kinase buffer (50 mM Hepes/KOH, pH 8.0, and 5 mM MgSO₄) using a conical tissue grinder for 1.5-mL microfuge tubes. The samples were briefly centrifuged for 10 s at 12,000g. Membrane vesicles and the plasma membrane-associated 116-kD protein remained in the supernatant that was immediately used for phosphorylation.
ylation reactions were performed at 22°C and were stopped after 60 s with 10 μL of SDS-sample buffer (110 mm Tris-HCl, pH 6.8, 16% [w/v] glycerin, 4% [w/v] SDS, 2% [v/v] 2-mercaptoethanol, and 0.01% [w/v] bromphenol blue). Equal volumes of each sample were then separated on 8% polyacrylamide gels followed by Coomassie blue-staining of the proteins. In general, the protein content of the different samples was very uniform. For quantification of the radiolabeled protein bands we only used those lanes with uniform protein-staining patterns and intensities. Because of the small sample size and the low total protein concentration this method turned out to be more precise than protein determination prior to gel analysis. Quantification of \(^{32}\)P-incorporation was performed on a beta-scanner phosphor imager (BAS-1500, Fuji, Tokyo) using the software programs BAS-Reader and TINA, both from Raytest (Isotopenmeßgeräte GmbH, Straubenhardt, Germany).

RESULTS

Increase in Phosphorylation of the 116-kD Protein in the Coleoptile Base after Preirradiation with Blue Light

For maize (Zea mays L.) it has been reported that blue light-dependent protein phosphorylation is not restricted to the coleoptile tip, but is also detectable in the more basal parts of the coleoptile (Palmer et al., 1993a). Precise quantitation of the tissue distribution of protein phosphorylation in oat showed that, although the response declined exponentially from the tip to the node, it was detectable along the entire coleoptile (Salomon et al., 1997b).

We previously reported that phototropic stimulation results in asymmetric distribution of blue light-dependent protein phosphorylation across the oat coleoptile tip (Salomon et al., 1997a). More recent experiments showed that for a fluence of 120 μmol m\(^{-2}\) a corresponding phosphorylation gradient in the upper coleoptile base (5–10 mm below the tip) could be found with irradiation times of 5 and 10 min, but not with a 30-s blue light pulse (Salomon et al., 1997b). Since the number of photons required to activate the photoreceptor molecules was the same in all cases, one would expect to find a constant percentage of proteins phosphorylated following light treatment for each duration of irradiation. Therefore, to account for the observed differences in asymmetric protein phosphorylation, one must hypothesize that other time-dependent biochemical processes are simultaneously taking place, with their effect becoming more apparent with longer irradiation times. Recovery of the initial ground state of the photoreceptor can be regarded as a process possibly acting in this way.

For the determination of dark-recovery kinetics we preirradiated both sides of oat coleoptiles for 30 s with 1000 μmol m\(^{-2}\) s\(^{-1}\) of blue light. Five-millimeter-long cross-sections of the upper-basal region (5–10 mm from the tip) were then prepared and frozen in liquid nitrogen at 1, 5, 20, 60, 90, 120, and 180 min after the end of in vivo irradiation. In vitro phosphorylations of cell extracts from these tissue sections gave the results depicted in Figure 1.

Immediately upon irradiation there is a rapid decline in the amount of incorporated radiolabeled phosphate, indicating that the applied light dosage resulted in almost quantitative in vivo phosphorylation of the 116-kD protein. The in vitro phosphorylation values recovered in the dark rapidly and reached the level of the nonirradiated control plants within 20 min. A further dramatic increase in phosphorylation was found for preirradiated plants that were incubated in the dark for up to 90 min prior to harvest. At this time we observed the maximum value of \(^{32}\)P-incorporation exceeding the initial level by a factor of about 3.5, reaching a level comparable to that found in the coleoptile tip. After 120 min the level of the blue light-stimulated increase in phosphorylation was somewhat lower (about 2.5-fold) but did not drop further by 180 min. The phosphorylation levels of the dark controls remained constant throughout the entire period of time (Fig. 1, O). Since all of the plants were kept under red light during the experiment, the observed amplified phosphorylation can be regarded as a genuine blue light effect.

Figure 2 shows the recently published (Salomon et al., 1997b) basipetal distribution of blue light-dependent protein phosphorylation in a 2-mm apical section and in 5-mm basal sections of the oat coleoptile (shaded columns). We carried out the same experiment with plants that were preirradiated as described above. After 90 min in the dark, where the maximum increase in phosphorylation was found for the upper base, the phosphorylation levels of each of the residual base segments also turned out to be 3- to 3.5-fold over the dark values of the respective tissue section (Fig. 2, white columns). In contrast, only a 15% higher response was observed for the uppermost 2 mm of...
fluences were carried out at a fluence of 30, 150, 600, 1500, or 6000 μmol m\(^{-2}\) and a constant irradiation time of 30 s, followed by a dark incubation of the plants for 90 min. The total capacity of phosphorylation of the 116-kD protein was then determined by \(^{32}\)P-labeling of cell extracts prepared from upper-base segments (5–10 mm below the tip). Figure 3A clearly demonstrates that the magnitude of the increase in phosphorylation depends on the blue light dosage administered in vivo. The threshold of the reaction was somewhat below 30 μmol m\(^{-2}\) and saturation was reached between 1500 and 6000 μmol m\(^{-2}\). At fluences ranging from 30 to 1500 μmol m\(^{-2}\) there is an almost linear increase of the response when the logarithms of the fluences are plotted against the increment of phosphorylation (Fig. 3B). This finding suggests a direct correlation between the phosphorylation state of the 116-kD protein and the magnitude of the response.

Comparison between Dark-Recovery Kinetics and Blue Light-Stimulated Increase in Phosphorylation

From published data it is known that following in vivo blue light irradiation, \(^{32}\)P-phosphorylation disappeared at

first in vitro but slowly recovered when the plants were incubated in the dark. In maize complete recovery in the coleoptile tip was observed within 20 min after irradiation (Palmer et al., 1993a). In those experiments the phosphorylation values were almost comparable to, but never higher than, those of nonirradiated plants. We also found only slightly elevated levels of protein phosphorylation in the tip (Figs. 2 and 4, ○), indicating that almost no amplification of phosphorylation occurs within this tissue and that the observed time-dependent reappearance of in vitro phosphorylation is mainly due to dark recovery, i.e. return of the photoreceptor and the kinase to the initial ground state. However, in contrast to maize, about 90 min were necessary to achieve full recovery in oat.

However, recovery of in vitro phosphorylation at the base proceeded much faster, and the initial value was attained after 20 min of dark incubation (Fig. 4, □). Assuming identical dark-recovery kinetics for the tip and the base, the real values accounting for the blue light-stimulated
increase in phosphorylation can be calculated by subtracting the tip-derived curve from that determined for the base. The resulting curve (Fig. 4, A) clearly indicates that the response is initiated immediately upon blue light stimulation.

Blue Light-Stimulated Increase in Phosphorylation after Unilateral Irradiation

Coleoptiles were unilaterally irradiated for 5 min at a fluence rate of 2 μmol m⁻² s⁻¹. The resulting fluence of 600 μmol m⁻² was known to result in phosphorylation values that are 2.5-fold that of the dark controls at the maximum (see Fig. 3A). Longitudinal half-sections of the upper-basal segment were prepared from the irradiated and the shaded sides of these coleoptiles, either immediately after illumination or after subsequent incubation for 20 or 60 min in the dark. In vitro phosphorylation of these tissue sections showed that immediately after the end of the in vivo light treatment, asymmetric phosphorylation could be found, as expected, with lower rates of ³²P-incorporation, and hence higher levels of in vivo phosphorylation in the irradiated side (Fig. 5). This initial distribution rapidly changed during dark incubation. After 20 min the phosphorylation values for both sides of the coleoptile were almost identical to those of nonirradiated plants. The effect became even more apparent when dark incubation was carried out for another 40 min. In this case we observed a net increase in ³²P-incorporation of 88% for the light-exposed side and of 32% for the opposite side, suggesting that the light gradient across the coleoptile results in different rates of increase in the concentration of the 116-kD protein on both sides of the coleoptile.

Phototropic-Curvature Responses of Preirradiated Plants to Usually Ineffective Blue Light Dosages

The lack of either specific gene probes or antibodies raised against the 116-kD protein did not allow direct measurements of transcript and protein levels in preirradiated plants, which could test whether the observed light-triggered amplification of phosphorylation was indeed caused by up-regulation of the concentration of the 116-kD protein. Therefore, we elected to use an alternative strategy. Assuming that the amount of the 116-kD protein directly correlates to the photoreceptor concentration, an elevated concentration of this protein might be expected to lead to increased photosensitivity of the coleoptile base to blue light. Such a change should concomitantly result in altered fluence-response characteristics for phototropic curvature.

In our bending studies we used conditions of irradiation that have been described to be located in the valley between FPPC and SPPC on the dose-response curves for phototropism in oat coleoptiles, and thus were ineffective in eliciting either of the responses (Everett and Thimann, 1968). From the published data those conditions were best met with a fluence rate of 0.2 μmol m⁻² s⁻¹ and an irradiation time of 110 s. This light regime was used for phototropic stimulation of nonpreirradiated control plants, as well as seedlings that were preirradiated under saturating conditions, followed by incubation for varying times in the dark. Almost no positive curvature (about 3% of the total plants) was observed for the controls, as expected. Most of the plants did not respond at all and some showed slight negative bending (Fig. 6A, shaded columns). In contrast, we found significant SPPC when plants were preirradiated prior to phototropic stimulation. The best results were obtained after a subsequent dark period of 30 min, where we found a marked, positive bending reaction of the entire coleoptile base in about 66% of the investigated plants and average degrees of curvature of 20°. No curvature was observed for the remaining plants (Fig. 6A, white columns).
phosphorylation assay carried out immediately after phototropism values determined 30 min after preirradiation (white columns) sides of the upper coleoptile base immediately after unilateral irradiation (1 s at a fluence rate of 0.2 μmol m⁻² s⁻¹). The plants were phototropically stimulated by unilateral irradiation and shaded sides by the complementary in vitro blue light-induced phosphorylation relative to those plants showing positive phototropism was determined 30 min after preirradiation, and curvature was permitted to develop for 120 min for 110 s at a fluence rate of 0.2 μmol m⁻² s⁻¹). Phosphorylation revealed a strong correlation with our curvature data. There was no phosphorylation gradient at the upper coleoptile base immediately after unilateral irradiation (1 s at a fluence rate of 0.2 μmol m⁻² s⁻¹). Phosphorylation values determined 30 min after preirradiation (white columns) and those determined for nonpreirradiated plants (shaded columns) are shown. The average degree of curvature of those plants showing positive phototropism was 20°. The distribution of in vitro blue light-induced phosphorylation relative to nonirradiated dark controls found in the irradiated and the shaded sides of the upper coleoptile base immediately after unilateral irradiation (110 s at a fluence rate of 0.2 μmol m⁻² s⁻¹). Phosphorylation values determined 30 min after preirradiation (white columns) and those determined for nonpreirradiated plants (shaded columns) are shown (means ± st, n = 8).

Measurements of the in vivo phosphorylation state in the irradiated and shaded sides by the complementary in vitro phosphorylation assay carried out immediately after phototropic stimulation under the same conditions of irradiation revealed a strong correlation with our curvature data. There was no phosphorylation gradient at the upper coleoptile base of the control plants (Fig. 6B, shaded columns), whereas a well-pronounced asymmetry of phosphorylation was detectable when unilateral irradiation was performed 30 min after the blue light pretreatment (Fig. 6B, white columns).

**DISCUSSION**

Based on the different fluence-response characteristics that can be described for FPPC, FNPC, and SPPC, some authors concluded that phototropism might be regulated by at least two distinct photoreceptors (Zimmermann and Briggs, 1963; Konjevic et al., 1989). However, genetic and biochemical analysis of an Arabidopsis null-mutant for phototropism that also lacks blue light-dependent protein phosphorylation (Liscum and Briggs, 1995) prompted the authors to hypothesize that this phosphoprotein probably represents the sole photoreceptor responsible for all phototropic responses in Arabidopsis. If this is true, the question arises of how a single photoreceptor can be responsible for the simultaneous stimulation of both FPPC, a fluence-dependent process, and SPPC, a time-dependent reaction. Based on the results presented here, we will address this question and propose preliminary answers to this up-to-now unsolved problem.

Dark recovery of in vitro blue light-mediated protein phosphorylation after high-irradiance blue light treatment in vivo showed substantially different kinetics for the uppermost region of the tip and the coleoptile base. The capacity of ³²P-incorporation at the tip recovered slowly within 90 min in the dark to approximately the same level found prior to in vivo irradiation. Similar dark-recovery kinetics were also reported by other investigators (Short and Briggs, 1990; Palmer et al., 1993a) and have been discussed as the time necessary to completely restore the initial ground state of all components of the photoreceptor/kinase system.

In contrast, within the same period of time, dramatically increased phosphorylation of the 116-kD protein was measured for the entire coleoptile base of preirradiated plants. For each coleoptile section investigated the values found were about 3-fold those of the corresponding dark controls, i.e. nonpreirradiated plants. The kinetics of this blue light-stimulated, increased phosphorylation were significantly faster. The response started immediately upon irradiation without any lag phase and progressed linearly until reaching its maximum after 90 min of dark exposure (Figs. 1 and 4). It could not be induced by red light (not shown). Under the assumption that the capacity of phosphorylation is an appropriate measure of the total number of phosphorylated 116-kD proteins the observed higher phosphorylation values can be interpreted in terms of a corresponding rise of the concentration of this protein, possibly caused by blue light-induced gene expression. Since the response is very rapid, the assumed de novo protein synthesis therefore must start immediately upon light activation. Such fast inductions of gene expression are not uncommon and have been also reported for other blue light- and red light-regulated nuclear genes in higher plants (for review, see Thompson and White, 1991), e.g. the rates of transcription of Cab genes in pea increased within a few minutes after blue light stimulation in a linear way, very similar to the light-induced increase in phosphorylation described in the present work (Marrs and Kaufman, 1991).

However, we also have to consider alternative explanations that could account for the observed amplified phosphorylation of the 116-kD protein, such as sensor-adaptation, e.g. by light-induced increase in kinase activity. Assuming such a mechanism, the velocity of the phosphorylation reaction at a constant concentration of the 116-kD protein would be expected to become higher following blue light exposure, but saturating phosphorylation should...
be the same in all cases provided that the reaction time chosen is sufficiently long. From Palmer et al. (1993b) and M. Salomon, and M. Zacherl (unpublished data) we know that the kinetics of the in vitro phosphorylation reaction is very fast, reaching saturation within 1 (oat) and 2 (maize) min when membrane preparations of nonpreirradiated plants were used. Since the duration of in vitro phosphorylation was always 60 s in this work, the observed dramatic changes of the phosphorylation levels of preirradiated plants under these saturating conditions of in vitro phosphorylation are difficult to understand by the simple assumption of an increased kinase activity.

Another possibility would be a blue light-triggered activation of additional phosphorylation sites or removal of inhibitory components from already present photoreceptor molecules. Considering the linearity of the increase in phosphorylation over 90 min (Figs. 1 and 4), it must be postulated that the transmission of the light energy to the target molecules and the subsequent inter- and/or intermolecular conformational changes leading to the activation must occur at a constantly slow rate.

Even though we cannot completely rule out these alternative possibilities, an increase in the concentration of the 116-kD protein seems to be the most probable explanation, and, therefore, the results of this work will be discussed under this assumption.

The results presented here further demonstrate that the extent of the amplification of phosphorylation depends on two additional factors, namely the tissue-specific, total capacity of blue light-inducible protein phosphorylation found prior to preirradiation, which we hypothesize to be directly related to the corresponding concentration of the photoreceptor (Fig. 2), and the fluence applied during preirradiation (Fig. 3). Together, these findings clearly suggest that the rate of increase in phosphorylation depends strictly on the portion of the 116-kD protein becoming phosphorylated during preirradiation, and that the phosphorylated protein may act as the primary signal for the induction of its own synthesis.

Unfortunately, we cannot definitively test this conclusion, because direct measurements of steady-state transcript levels and photoreceptor concentrations were not possible in this work. However, our studies on phototropic curvature performed with preirradiated oat seedlings convincingly showed that some plants exhibited a significantly higher photosensitivity to blue light (Fig. 6A) when compared with nonpreirradiated plants. About 70% of the investigated plants showed average degrees of curvature of 20° when phototropic stimulation was performed with blue light dosages known to induce neither FPPC nor SPPC (Everett and Thimann, 1968) in control plants. The observed bending reaction is obviously not the result of desensitization, i.e. a shift of the fluence-response curve for FPPC toward higher fluence after high irradiance preirradiation (Iino, 1988b), since desensitization is a special feature of FPPC but not of SPPC. Further, the average degree of curvature that we measured under comparable experimental conditions (a blue light fluence of 25 μmol m⁻² s⁻¹ administered 32 min after preirradiation) was only about 5°, whereas we found a 20° curvature 30 min after preirradiation at a fluence of 22 μmol m⁻². Therefore, we conclude that preirradiation results in a higher photosensitivity of the coleoptile base.

This is exactly what would be expected if the light-mediated increased phosphorylation corresponds to a rise in the amount of activatable photoreceptors. Further, the rise of photosensitivity is reflected by a concomitant asymmetric distribution of phosphorylation after unilateral irradiation (Fig. 6B) not found in nonpreirradiated plants. We therefore hypothesize that the magnitude of the phosphorylation gradient across the coleoptile is an appropriate measure of the actual photoreceptor concentration.

Together, the results presented here provide evidence that the oat coleoptile apparently possesses the ability to adjust the tissue-specific photoreceptor concentration according to the prevalent conditions of illumination, resulting in altered photochemical properties of the coleoptile. With those conditions it is evident why SPPC is a time-dependent rather than a fluence-dependent phenomenon and why the Bunsen-Roscoe reciprocity law does not hold for SPPC. This law is only valid for an optical system that exhibits well-defined and invariable physical properties with respect to light absorption and light transmission, and that is limited by first-order photochemistry. The above studies conclusively demonstrate that the oat coleoptile cannot be regarded as such a system.

The key toward understanding the molecular events occurring during long-term unilateral irradiation came from our finding that preirradiation at higher fluences of blue light also resulted in a corresponding higher increase in phosphorylation of the 116-kD protein. Unilateral irradiation generates a directional gradient of light across the coleoptile (Vogelmann and Haupt, 1985), and, hence, the fluence at the irradiated side will be higher than that at the shaded side. According to the fluence dependence of the blue light-stimulated increase in phosphorylation, more 116-kD proteins will become synthesized in the light-exposed side than in the opposite side of the coleoptile, resulting in a concentration gradient of the 116-kD protein that should be more pronounced the longer the reaction is permitted to proceed. In agreement with that, 60 min after unilateral in vivo irradiation (5 min × 2 μmol m⁻² s⁻¹) we found net increases of phosphorylation of 88% for the irradiated and of only 32% for the shaded side (Fig. 5).

Assuming that initiation of differential growth and phototropic curvature predominantly depends on the relative difference in phosphorylation between the irradiated and the shaded sides of the coleoptile rather than on the total amount of phosphorylated proteins on both sides, the creation of the required gradient will be a function of the irradiation time rather than of the applied fluence rate. The above interpretations of our results are in agreement with the fluence-response curves determined for SPPC under various conditions by several workers (Zimmermann and Briggs, 1963; Everett and Thimann, 1968; Pickard et al., 1969), who all characterized SPPC to be a time-dependent rather than a fluence-dependent response.

Another special case of SPPC can also be explained by our model. Steinitz et al. (1988) found that a low-fluence blue light pulse resulted in FPPC when given as a single
pulse, but resulted in a significantly more pronounced bending reaction, typically ascribed for SPPC, when the same total fluence was administered in five pulses at 20 min-intervals. They further found that within a limited time range prolonged dark exposure between the single pulses also increased the degrees of curvature. This situation best reflects the conditions of our own experiment shown in Figure 5, where we demonstrated the direct relationship between the duration of dark incubation and the differences in the blue light-stimulated increase in phosphorylation found at the irradiated and the shaded sides of the coleoptile. Once the system is activated by blue light the subsequent adjustment of the concentration of the 116-kD protein obviously occurs independent of light. Thus, continual unilateral irradiation is not necessarily needed to obtain second-positive curvature. Thus, pulse-induced SPPC can be explained as follows: Prior to the first pulse there will be a uniform distribution of the 116-kD protein across the entire coleoptile. But in the same way as the photosensitivity of the irradiated and the shaded sides will rise rapidly during the intervals of dark incubation, the more effective each of the subsequent light pulses will be in the capacity to induce a well-pronounced gradient of protein phosphorylation.

In summary, the data from our experiments are consistent with the hypothesis that part of the signal transduction chain for phototropism functions via a fluence-dependent induction of the expression of the 116-kD protein permitting the plant to adjust photosensitivity according to the varying daylight conditions. Whether this induction is regulated at the transcriptional or at the posttranscriptional level remains to be elucidated in future work.

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