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Asymmetric, Blue Light-Dependent Phosphorylation of a 116-Kilodalton Plasma Membrane Protein Can Be Correlated with the First- and Second-Positive Phototropic Curvature of Oat Coleoptiles[1]

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The possible correlation between blue light-dependent phosphorylation of a 116-kD protein and phototropic responses of etiolated oat (*Avena sativa* L.) seedlings was tested by a micromethod for protein phosphorylation. Quantitation of the basipetal distribution of this protein showed that the in vitro ^{32}P phosphorylation values declined exponentially from tip to node, with more than 50% of the total label being found in the uppermost 5 mm. Nonsaturating preirradiation of the coleoptiles in vivo resulted in partial phosphorylation with endogenous ATP. Subsequent in vitro phosphorylation under saturating irradiation allowed the determination of the degree of in vivo phosphorylation. Unilateral preirradiation resulted in higher in vivo phosphorylation on the irradiated than on the shaded side of the coleoptile. The fluence-response curve for the difference in phosphorylation between both sides of the coleoptile resembles the fluence-response curve for first-positive phototropic curvature, although it is shifted by two orders of magnitude to higher fluences. Possible reasons for this shift are discussed. In the coleoptile base the phosphorylation gradient across the coleoptile becomes larger with increasing time of irradiation at a constant fluence. Thus, phosphorylation of the 116-kD protein, in accordance with second-positive phototropic curvature, does not obey the Bunsen-Roscoe reciprocity law.

Bending of plants toward light (phototropism) is a well-known phenomenon that has been studied by numerous workers for more than 150 years (Firn and Digby, 1980; Briggs and Baskin, 1988; Iino, 1990; Firn, 1994). Phototropism includes among other things two processes of "fundamental importance" (Firn, 1994), namely blue light perception and differential cell elongation. The classic experimental approach uses unilateral irradiation of etiolated dicot seedlings or monocot coleoptiles. The resulting fluence-response curves for phototropism in this seemingly simple system have revealed a surprisingly high degree of complexity (Zimmermann and Briggs, 1963; Everett and Thimann, 1968; Meyer, 1969a, 1969b; Iino, 1988; Janoudi and Poff, 1990, 1991) and can be summarized as follows: short pulses of low-fluence light lead to the FPPC observable close to the coleoptile tip of grass seedlings. This curvature obeys the Bunsen-Roscoe reciprocity law. The fluence-response curve is bell shaped. Increasing fluences result at first in increasing bending of the coleoptile tip to a maximum value. Further increase of the fluence causes a gradual decrease in bending to zero. Even higher fluences lead to the SPPC that is observed at the coleoptile base. The SPPC strongly depends on the time of irradiation and does not obey the Bunsen-Roscoe reciprocity law.

It has been shown that the light gradient across the coleoptile found upon unilateral irradiation with blue light is created by a combination of light absorption and light scattering, resulting in lower fluences of blue light on the shaded than on the irradiated side of the coleoptile (Parsons et al., 1984; Vogelmann and Haupt, 1985). The postulate that the light gradient should create some kind of molecular difference between irradiated and shaded cells has not yet been substantiated. An unequal distribution of auxin, which has been predicted and discussed for a long time, was not found with a sensitive micromethod (Feyerabend and Weiler, 1988).

A new approach to molecular events related to phototropism started with the finding of blue light-dependent phosphorylation of a plasma membrane protein (Gallagher et al., 1988). Light-dependent phosphorylation of this protein, sized 114 to 130 kD in various plants, seems to be ubiquitous in higher plants (Reymond et al., 1992a). The photobiological correlation between this phosphorylation and the FPPC has been summarized by Short and Briggs (1994). In oat (*Avena sativa* L.) coleoptiles the protein is tightly bound to the plasma membrane only in young (3-d-old) etiolated seedlings and becomes loosely associated later in 5-d-old etiolated seedlings (Salomon et al., 1996).

When Arabidopsis mutants that lack phototropic responses were investigated in detail it turned out that mutation of one gene locus (nph1) resulted in the disappearance of the 120-kD protein and/or its phosphorylation (Liscum and Briggs, 1995). It is remarkable that, although the mutants were isolated by screening for the SPPC, all phototropic responses including the FPPC were impaired. It was concluded that the protein encoded by the nph1

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

gene is the apoprotein of a dual- or multichromophoric photoreceptor that is responsible for all of the phototropic responses of Arabidopsis.

We have recently developed a micromethod for investigation of the blue light-dependent phosphorylation of the 116-kD plasma membrane protein of oat (Salomon et al., 1997). This enabled us to investigate this reaction in small sections of the oat coleoptile. Here we describe the asymmetry of phosphorylation after unilateral irradiation of oat coleoptiles, the fluence-response characteristics, and the relationship between this reaction and FPPC and SPPC.

MATERIALS AND METHODS

Oat (*Avena sativa* L. cv Pirol) (Ackermann, Irlbach, Germany) seedlings were grown on vermiculite at 25°C for 72 h in the dark and exposed for a further 16 h to a dim-red light source (TL 40 W/15, Philips [Eindhoven, The Netherlands]; 1 μmol m^{-2} s^{-1}) to create conditions comparable to those used in most studies of phototropic bending reactions under elimination of phytochrome-mediated side effects. All experimental manipulations were also carried out under this red light source.

Light Sources and Unilateral Irradiation of Coleoptiles

The experimental protocol used for unilateral irradiation of coleoptiles was the same as previously described (Salomon et al., 1997). For unilateral irradiation we used a light source with glass fiber optics and an IR filter (KL 1500, electronic, Schott, Mainz, Germany). Blue light was generated by a blue glass filter (380–450 nm, filter set for KL 1500, electronic, Schott) and light dosages were measured with a Li-189 quantum photometer (Lambda Instruments Corp., Lincoln, NE).

The coleoptiles were cut just below the node and were irradiated for 30 s at a fluence rate of 0.033, 0.166, 1, 4, 40, or 200 μmol m^{-2} s^{-1}, resulting in blue light fluences of 1, 5, 30, 120, 1200, and 6000 μmol m^{-2}, respectively. For basal segments of the coleoptiles we varied the duration of irradiation at a constant fluence of 120 μmol m^{-2} using additional irradiation times of 5 and 10 min. Individual irradiations were carried out with four coleoptiles.

Preparation of Crude Cell Extracts

Tissue distribution of blue light-dependent protein phosphorylation was investigated by cutting coleoptiles of uniform tip-to-node length (2.5 cm) after removal of the primary leaf into either 5- or 2.5-mm cross-sections using a razor blade. One 5-mm and two 2.5-mm sections of each zone of the coleoptile were collected in separate microcentrifuge tubes and were immediately frozen in liquid nitrogen. From the unilaterally irradiated plants we prepared longitudinal half-sections of the irradiated and the shaded sides either from the uppermost 5 mm of the tips or from basal segments (5–10 mm from the tip). The tissue sections were frozen as described above. Each probe contained two 5-mm-long coleoptile halves corresponding to a fresh weight of 5 mg. For preparation of crude cell extracts a single tube was removed from the liquid nitrogen. The frozen plant material was ground and further homogenized with a conical tissue grinder after the addition of 40 μL of kinase buffer (50 mM Hepes/KOH, pH 8.0, 5 mM $MgSO_4$). After a brief centrifugation step (10 s at 12,000*g*) the supernatant was immediately used for phosphorylation. Only trace amounts of residual kinase activity were found when the cell debris were phosphorylated after resuspension in kinase buffer. In all of our experiments the time for preparing this crude homogenate was kept constant at exactly 2.5 min.

In Vitro Phosphorylations

For each phosphorylation reaction we used 16 μL of the crude cell extract. After the addition of 2 μL of 5% (w/v) Triton X-100, the probes were irradiated at a high fluence of blue light (15 s × 1500 μmol m^{-2} s^{-1}) to ensure quantitative activation of the photoreceptor molecules within a few seconds. The radiolabeled ATP (0.5 μL of carrier-free [γ-^{32}P]ATP [110 TBq $mmol^{-1}$]) was added at the moment the blue light was turned on to prevent phosphorylation by the endogenous ATP present in the cell extracts. After 1 min the reactions were stopped 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 sample volumes were separated on 8% polyacrylamide gels. Due to the limited number of available probes, protein determinations carried out before gel analysis proved to be unreliable. For quantitation we used only those lanes that showed equal intensities of the protein bands after Coomassie blue staining. Quantitation of the labeled protein bands was carried out using a beta-scanner phosphor imager (BAS-1500, Fujifilm, Tokyo, Japan) and the software programs BAS-Reader and TINA, both from Raytest (Isotopenmeßgeräte GmbH, Straubenhardt, Germany). All manipulations were carried out at 22°C.

RESULTS

Tissue Distribution of Blue Light-Dependent Protein Phosphorylation along the Coleoptile

Previous investigations (Salomon et al., 1996) had shown that the blue light-dependent phosphorylation of a 116-kD protein was restricted to the plasmalemma fraction. We later found (Salomon et al., 1997) that there was no interfering phosphorylation of any other protein above 100 kD in the crude extract when only the coleoptile tissue was used and the leaf tissue was removed before homogenization. With the newly developed micromethod for protein phosphorylation, we determined blue light-dependent phosphorylation of the 116-kD protein in small segments of the oat coleoptile. At first, tissue distribution of this reaction within the coleoptile was investigated. For this purpose, tissue segments of etiolated oat seedlings were harvested and extracted in the dark. The phosphorylation reaction was performed under saturating irradiation. Under these conditions the number of phosphate groups per

protein should always be the same. Therefore, the amount of radioactivity incorporated into the 116-kD protein should reflect the amount of this protein in the respective section.

In accordance with previous results with maize (Hager and Brich, 1993; Palmer et al., 1993) the amount of phosphorylation decreases from the tip to the base of the coleoptile. Hager and Brich (1993) found light-dependent phosphorylation only in the tip, but not in the base, of maize coleoptiles. Palmer et al. (1993) had determined a higher specific activity of phosphorylation in 1- versus 5-mm-long coleoptile tip sections. Since our experimental approach is more sensitive than earlier procedures, we could detect the blue light-dependent phosphorylation in 5 or 10 sections along the whole coleoptile from the tip to the node (Fig. 1). Although the number and the size of the cells

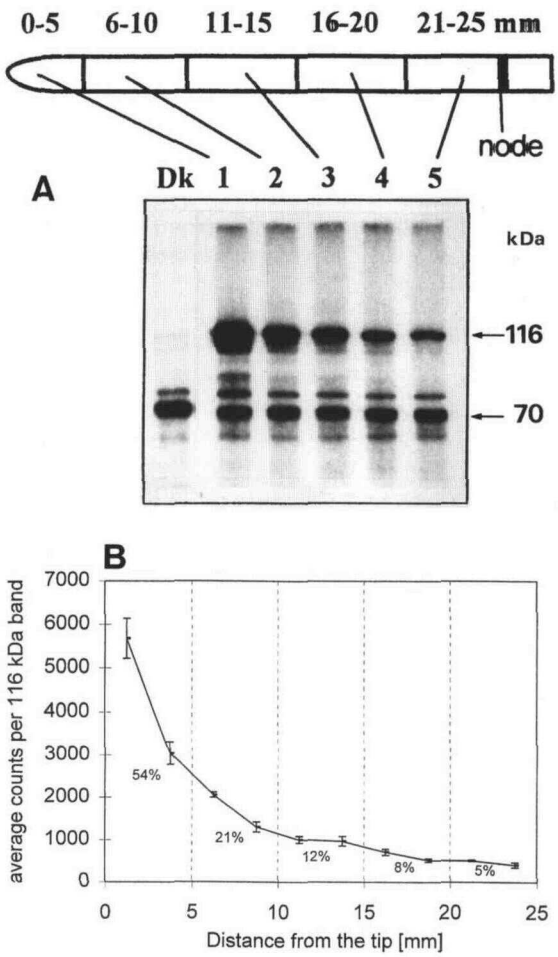

Figure 1. Tissue distribution of blue light-dependent protein phosphorylation in the oat coleoptile. In vitro phosphorylations of cell extracts prepared from 5-mm (A) or 2.5-mm cross-sections (B) of 2.5-cm-long coleoptiles. The position of the blue light-dependent phosphorylated 116-kD protein and of the 70-kD protein are marked by arrows in A. B shows the exponential, basipetal decline in phosphorylation of the 116-kD protein and the percentage of the total phosphorylation capacity of the protein found in each 5-mm section. Each data point in the diagram represents the means ± SD of six individual experiments.

along the coleoptile differ greatly during the early development of the organ, those differences disappear almost completely when the coleoptiles reach a length of 14 mm (Avery et al., 1945). In agreement with that, we found uniform protein composition and protein-staining patterns in our samples of the different coleoptile sections, indicating that the total protein content did not vary significantly down the coleoptile (not shown). This is further supported by the patterns of the phosphorylated proteins shown in Figure 1A. Only the blue light-dependent phosphorylation of the 116-kD protein decreases from the tip to the base, but ^{32}P incorporation into other proteins, e.g. the 70-kD protein, remains about the same throughout the length of the coleoptile (Fig. 1A, lanes 1–5) and does not depend on irradiation (Fig. 1A, lane Dk).

We consider this to be an important internal control, indicating that both the concentration of the 70-kD protein and the ratio of [^{32}P]ATP to endogenous ATP in the tissue sections did not vary significantly. Therefore, the exponential decrease in phosphorylation of the 116-kD protein from the tip to the node found in our experiments (Fig. 1B) can be regarded as a corresponding decrease in the concentration of this protein. Figure 1B further demonstrates that more than 50% of the radioactivity, and hence of the phosphorylated protein, were found within the uppermost 5 mm of the coleoptile tip.

Unilateral Pulse Irradiations and Asymmetric Phosphorylation within the Tip and the Base of the Coleoptile

Another application of the micromethod for protein phosphorylation is the investigation of longitudinal sections after unilateral preirradiation of the seedlings. The principle of in vivo preirradiation has been demonstrated before as follows (Reymond et al., 1992a; Palmer et al., 1993; Salomon et al., 1997): preirradiation of seedlings results in phosphorylation of the 116-kD protein in vivo with endogenous ATP. The phosphorylation sites of the protein are then occupied with unlabeled phosphate so that these sites cannot be labeled upon subsequent irradiation and incubation with [^{32}P]ATP in vitro. Nonsaturating preirradiation leads only to partial kinase activation and partial in vivo phosphorylation. The extent of in vivo phosphorylation can indirectly be determined by the extent of subsequent in vitro phosphorylation with knowledge of the minimum and maximum values of in vitro phosphorylation for saturating preirradiated and for nonirradiated seedlings, respectively.

Nonsaturating unilateral preirradiation had already indicated a higher degree of phosphorylation at the irradiated, rather than at the shaded side of the coleoptile tip (Salomon et al., 1997). In Figure 2 we present the results of a detailed investigation of this phenomenon. As expected, increasing fluences of preirradiation lead to decreasing in vitro phosphorylation (and hence increasing in vivo phosphorylation) in the coleoptile tip and base. The percentage of in vitro phosphorylation relative to the dark controls is higher in the basal segments than in the tips at a given fluence. This effect is further evaluated below (see Fig. 3).

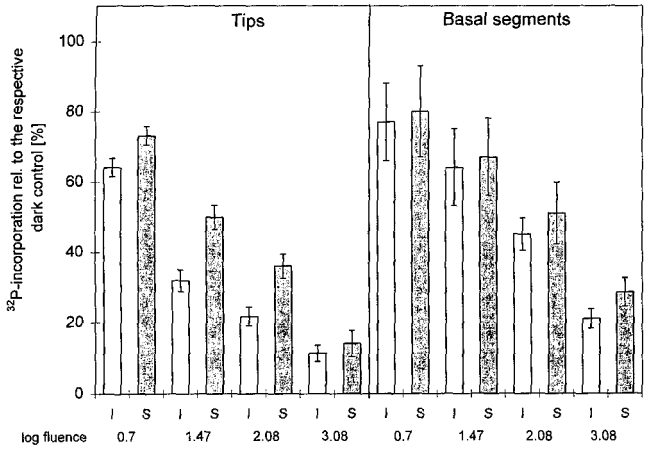

Figure 2. Fluence dependence of the distribution of light-induced phosphorylation across the tip and the base of oat coleoptiles after unilateral blue light irradiation in vivo. The coleoptiles were unilaterally irradiated at 5, 30, 120, or 1200 μmol m^{-2} (shown as the logarithm of the fluence). In each case the time of irradiation was 30 s. Coleoptile halves of the irradiated (I) and the shaded (S) sides were prepared either from 5-mm-long tip sections or from a basal segment of an equal length obtained after removing the uppermost 5 mm of the coleoptile tip. The diagram shows the means ± SD of 8 and 10 individual in vitro phosphorylations for each of the tip and basal sections, respectively, relative to those of corresponding tissue sections of nonpreirradiated plants.

For the coleoptile tips, there are significant differences in phosphorylation between the preirradiated and the shaded sides, especially at blue light fluences of 30 and 120 μmol m^{-2} (log fluence 1.47 and 2.08, respectively, in Fig. 2). In comparison, the basal region shows significant asymmetric in vitro phosphorylation only at the highest applied fluence of 1200 μmol m^{-2} (log fluence 3.08). The difference between the preirradiated and shaded sides is further discussed below (see Figs. 4 and 5).

The data in Figure 2 allow the calculation of the degree of in vivo phosphorylation at various fluences. The result of this calculation is shown in Figure 3 for the irradiated side of coleoptile tips and basal segments. It can be deduced from the resulting curves that there are different thresholds for the reaction in the base and the tip. At 1 μmol m^{-2} (values not shown in Fig. 2) no phosphorylation could be detected in the basal region, but about 27% phosphorylation was found in the tips. Furthermore, the fluence necessary to phosphorylate 50% of the 116-kD protein present in the corresponding tissue is about 10 times higher for the base when compared with the tip. These data indicate that the vertical decline of the amount of the 116-kD protein in the coleoptile (see Fig. 1) results in a concomitant loss of sensitivity to blue light. The same conclusion can be drawn from a corresponding, very similar diagram using the shaded side of coleoptile tips and basal segments (not shown).

The difference between the irradiated and shaded sides of the coleoptile tips is plotted in Figure 4 as a function of the blue light fluence of preirradiation. We found the largest difference with a preirradiation of 30 μmol m^{-2} blue light. No difference between the irradiated and shaded sides was detected at very low (0.5 μmol m^{-2}) or very high (6000 μmol m^{-2}) fluences. The bell shape of the curve for fluence-dependent asymmetric phosphorylation is similar to that described for the FPPC (Iino, 1988). However, the curve for phosphorylation is shifted about 100-fold toward higher fluences compared with the curve for phototropism.

The Effect of Irradiation Time at a Constant Fluence on Asymmetric Phosphorylation in the Coleoptile Base

The distribution of the phosphorylated protein along the coleoptile (Fig. 1) prompted us to investigate the question of asymmetry of the light-potentiated phosphorylation in the base in more detail. The coleoptile base is the location of the SPPC. With short light pulses, the SPPC appears only at high fluences. In accordance with this property, asymmetry of phosphorylation was observed only at the highest fluence (1200 μmol m^{-2}), when a 30-s blue light pulse was applied (Fig. 2). However, the SPPC occurs typically in a time-dependent fashion with irradiation of longer duration. It does not obey the Bunsen-Roscoe reciprocity law (see Firn, 1994). Therefore, we investigated the phosphorylation of the 116-kD protein at the irradiated and the shaded sides of the coleoptile base with different fluence rates of preirradiation and different periods of time, but with constant total fluence (Fig. 5). The fluence (120 μmol m^{-2}) was chosen such that no significant asymmetry was detectable after a pulse of 30 s. However, significant asymmetry was obtained with the same fluence after 300 s or longer periods of irradiation and corresponding lower fluence rates. There was nearly no change in the phosphorylation at the irradiated side down to 0.2 μmol m^{-2} s^{-1} of blue light. The shaded side exhibited more phosphorylation in vitro (and hence less phosphorylation in vivo), when the applied fluence rate was reduced from 4 to 0.4

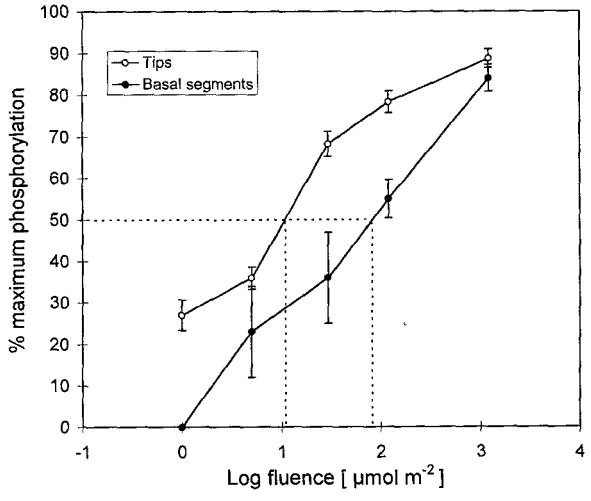

Figure 3. Degrees of in vivo phosphorylation after unilateral irradiation at the irradiated side of the tip and the base. The in vivo phosphorylation values were calculated from the data shown in Figure 2 as follows: phosphorylation in vivo = 100 − relative ^{32}P incorporation in vitro. The obtained values are plotted as a function of the logarithm of blue light fluence. The dashed lines indicate the fluences needed for 50% in vivo phosphorylation.

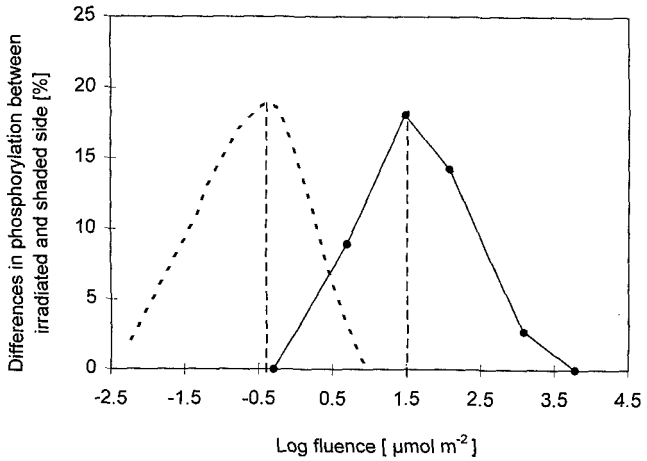

Figure 4. Correlation between the extent of asymmetric phosphorylation across the coleoptile tip and FPPC. The differences in phosphorylation between the shaded and the irradiated sides found at blue light fluences of 0.5, 5, 30, 120, 1200, and 6000 µmol m^{-2} were compared with the fluence-response curve for FPPC (dashed line) of red light-grown oat coleoptiles, according to Iino (1988). The shape of the curve resulting from the fluence-dependent degrees of asymmetric phosphorylation is similar to that found for the bending reaction. However, the curve is shifted about two orders of magnitude toward higher fluences.

µmol m^{-2} s^{-1} and the time period increased from 30 to 300 s.

DISCUSSION

Phototrophic responses of higher plants have been correlated with blue light-dependent phosphorylation of a plasma membrane protein on the basis of photobiological (Short and Briggs, 1994) and genetic evidence (Reymond et al., 1992b; Liscum and Briggs, 1995). Genetic evidence indicated the possibility that the phosphorylated protein could be the photoreceptor responsible for all phototropic reactions. The present study of the blue light-dependent phosphorylation in small sections of oat coleoptiles supports this view and reveals several remarkable properties of this system.

The distribution of blue light-dependent phosphorylation along the coleoptile corresponds to the sensitivity of phototropism. The highest concentration of the 116-kD protein was found in the coleoptile tip, the most photosensitive tissue. There is a steep decrease toward the base, but the phosphorylated protein is detectable in all parts of the coleoptile, in agreement with the phototropic sensitivity of the entire organ (MacLeod et al., 1984). The lower concentration at the base could explain the lower sensitivity of this region. The lower sensitivity is connected with a higher threshold for phosphorylation (Fig. 3) and for the phototropic reaction of the coleoptile base (Zimmermann and Briggs, 1963).

An important finding of the present work came from investigation of sections from the irradiated and the shaded sides of the coleoptile after unilateral irradiation with blue light. In vivo phosphorylation of the shaded side, quantified via the complementary phosphorylation in vitro, was either lower than or equal to that of the irradiated side. It was never higher than that of the irradiated side. On the basis of the fluence-response characteristics of the system (Fig. 3, this paper; and Salomon et al., 1997), the direction of the light gradient can be derived. It declines from the irradiated to the shaded side in agreement with direct measurements of light intensities across the coleoptile (Parsons et al., 1984; Vogelmann and Haupt, 1985).

The fluence-response curve for the difference of phosphorylation between the irradiated and shaded sides is an optimum curve. This can be explained as follows: with increasing fluence, the difference must at first become larger until saturation of the phosphorylation is reached at the irradiated side. Any further increase in fluence does not change the phosphorylation at the irradiated side but still induces an increase of phosphorylation at the shaded side. Thus, the difference becomes smaller until saturation is reached at both sides, i.e. a difference of zero.

The fluence-response curve for the differences of phosphorylation is shifted by two orders of magnitude toward higher fluences compared with the corresponding curve for FPPC. Palmer et al. (1993), when investigating the fluence-dependent protein phosphorylation in maize coleoptiles, found a 10- to 100-fold shift of the threshold values for protein phosphorylation compared with the threshold of blue light fluence that stimulates the FPPC. The authors offered several explanations that should also be considered for our results with oat coleoptiles: (a) The possibility of different photoreceptors for protein phosphorylation and phototropism can be ruled out given the convincing correlation deduced from results with several

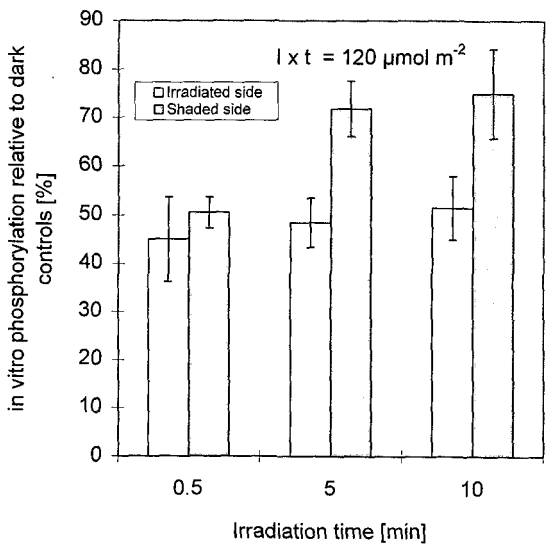

Figure 5. The effect of duration of unilateral irradiation on asymmetric distribution of in vitro phosphorylation across the coleoptile base. ^{32}P phosphorylation values for the irradiated and the shaded sides of the coleoptile base were determined for different times of irradiation at a constant fluence of 120 µmol m^{-2}. The fluence rate was changed from 4 to 0.4 and 0.2 µmol m^{-2} s^{-1}, and the exposure time varied between 30, 300, and 600 s. The evaluated values are the means ± SD of 10 independent experiments.

phototropic mutants (Liscum and Briggs, 1995). (b) Since it was not possible to extract only the most phototropically sensitive tissue, the average sensitivity in the investigated tissue might be lower than that responsible for bending. Palmer et al. (1993) summarized previous data on phototropic responses, indicating a decline in sensitivity in oat coleoptiles by a factor of 60 within 0.5 mm and by a factor of 1000 within 1.5 mm from the tip. The factor of 100 determined in this paper for 5-mm sections from the tip is within this range. (c) It has been reported that blue light-dependent phosphorylation of the 120-kD pea protein occurs at multiple phosphorylation sites (Short et al., 1994). One possible explanation could be a different reactivity of these sites. Indeed, some sites seem to be phosphorylated at low fluences of blue light, whereas higher fluences are needed to phosphorylate the residual sites (Briggs, 1996). If we assume that phosphorylation at the rapidly reacting residues already causes phototropic curvature and that the additional phosphorylation sites are associated with destruction of the protein (Briggs, 1996) or with other processes, e.g. adaptation, then this could account for the observed shift.

Surprisingly, a correlation between the blue light phosphorylation of the 116-kD protein and phototropism holds also for the SPPC that can be observed at the coleoptile base. Like the phototropic response, the difference in phosphorylation between the irradiated and shaded sides does not obey the Bunsen-Roscoe reciprocity law. The difference becomes larger when the same fluence is given instead within 30 s during a longer period of time, e.g. 300 to 600 s. The molecular basis for this effect is not clear. With the assumption that the phosphorylated protein is the blue light photoreceptor for all phototropic responses (Liscum and Briggs, 1995), a complex transduction chain resulting in different phosphorylation of this protein can be excluded. The effect could be understood under the assumption of a change in the concentration of the 116-kD protein following long-term unilateral irradiation resulting in an altered *trans*-coleoptile distribution of the protein. Such a change must be very rapid so that the resulting effect, a decrease in phosphorylation at the shaded side of the coleoptile, can be observed 300 s after onset of irradiation. If such a rapid change is considered unlikely, one has to postulate a direct interaction of an independent factor with the 116-kD protein. The simplest assumption is that of a phosphatase acting in vivo with limited capacity independently from light. Assuming the same low-enzyme capacity (V_{max}) in irradiated and shaded sides of the coleoptile, the same number of phosphate groups would become cleaved off on both sides. However, since the degree of phosphorylation is lower at the shaded than at the irradiated side, a higher percentage of phosphate groups gets lost at the shaded side. This is what we indirectly measure by phosphorylation in vitro. A slow dephosphorylation in the dark after irradiation of etiolated seedlings has been correlated with recovery of phototropic sensitivity in the dark after irradiation (Short and Briggs, 1990; Hager and Brich, 1993). We cannot exclude more complex models, however, e.g. those including turnover of the photoreceptor that can only be determined with antibodies raised against this protein.

Our results clearly suggest that the establishment of a pronounced gradient of protein phosphorylation might be a prerequisite for differential growth resulting in bending of the plant toward a light source. From the fluence-response curve depicted in Figure 4 it can be hypothesized that the degree of asymmetric phosphorylation determines the degree of curvature. At least for the basal part of the coleoptile, the results presented here suggest that the extent of asymmetric distribution of protein phosphorylation is regulated by time-dependent processes, e.g. adaptation mechanisms, occurring during irradiation rather than by the magnitude of the light gradient, i.e. the total number of photons available for the stimulation of phosphorylation on the irradiated and the shaded sides of the coleoptile.

In summary, we have shown here a biochemical process that is asymmetric in oat coleoptiles after unilateral irradiation. This process, namely blue light-dependent phosphorylation of a plasma membrane protein that is probably the photoreceptor for phototropism, can be correlated with FPPC and SPPC of oat coleoptiles. More detailed studies of this process will be possible after isolation and cloning of the gene encoding this photoreceptor.

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