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*Communication*

# Rapid Changes in Protein Phosphorylation Associated with Light-Induced Gravity Perception in Corn Roots[1]

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## ABSTRACT

**The effect of light and calcium depletion on *in vivo* protein phosphorylation was tested using dark-grown roots of Merit corn. Light caused rapid and specific promotion of phosphorylation of three polypeptides. Pretreatment of roots with ethylene glycol bis *N,N,N',N'* tetraacetic acid and A23187 prevented light-induced changes in protein phosphorylation. We postulate that these changes in protein phosphorylation are involved in the light-induced gravity response.**

Significant advances have been made in our understanding of gravity signal perception and transduction. Recent advances include high resolution analysis of differential growth kinetics (11) and characterization of light-induced changes in protein synthesis and identification of mRNAs in dark-grown Merit corn (4, 5). Understanding the sequence of events in the transduction of environmental signals such as gravity remains a subject of fundamental interest to many researchers.

Elucidation of the pathway of signal transduction in plants depends on identification of the rapid responses to a stimulus. Some of the rapid responses to gravity that have been identified to date include amyloplast movement, asymmetries in auxin distribution and electrical potential, and rapid mRNA and protein synthesis (3–5, 13, 14, 16). In this paper, we report light-induced rapid changes in protein phosphorylation, and suggest that these changes participate in the development of the gravitropic response.

In animal systems, it is well known that protein phosphorylation plays a key role in signal transduction (2). Signal reception can trigger changes in protein kinases such as protein kinase C (15, 17, 19). Furthermore, receptors for a variety of growth factors are themselves protein-tyrosine kinases (22). In addition, the responsiveness of receptors to a stimulus can be regulated by phosphorylation. In many cases, signal transduction is also mediated by calcium- and calmodulin-dependent protein kinases (16, 22).

Evidence for stimulus transduction pathways in plants similar to those known in animal systems is accumulating. Calcium- and calmodulin-dependent protein phosphorylation has been reported in plants (7, 18, 23, 24), and attempts have been made to isolate plant protein kinases and determine their substrates (6, 7, 19, 20). Previous work from this laboratory has provided evidence for calcium- and calmodulin-dependent protein phosphorylation in corn roots (18). The data presented here extends that work by showing rapid and specific calcium-dependent changes in protein phosphorylation induced by light treatment of dark-grown roots of Merit corn.

## MATERIALS AND METHODS

**Plant Material.** Corn seeds (*Zea mays* L., cv Merit) were imbibed for 12 h, then planted on plastic trays in paper towels under dim green light. Seedlings were grown for 2 d in darkness, then 1 cm apical segments (100 per treatment) were harvested under dim green light, using a Safelight Kodak filter (Wratten Series 3) and a 7 W bulb.

***In Vivo* Protein Phosphorylation.** One hundred segments per treatment were preloaded with $^{32}P$ in the dark by incubating in 1 ml of buffer (25 mM MES, pH 6.2, 5 mM $MgSO_4$, 250 mM sucrose) with 0.5 mCi of carrier free $[^{32}P]H_3PO_4$ for 1 h. Then excess label was removed with two washes of 2 ml each of buffer. Preloading was done to prevent the possibility that treatments could affect uptake of label and alter the cytoplasmic phosphate pool. After removal of label, 1 ml of buffer was added, and root segments were treated in one of three ways: (a) incubation for 15 min in darkness, then frozen in liquid $N_2$; (b) incubation for 8 min in darkness, then 7 min in light, and frozen in liquid $N_2$; (c) incubation for 8 min in darkness in the presence of 1 μM A23187 and 5 mM EGTA,[2] then 7 min in light, and frozen in liquid $N_2$. During preliminary experiments, light-induced changes in phosphorylation were measured after 1, 7, and 15 min. Significant increases in phosphorylation were observed after 7 min. Hence, further experiments were carried out after 7 min of light exposure. Experiments were repeated four times.

**Separation of Phosphoproteins.** After freezing, roots were homogenized, and labeled proteins were separated by two-dimensional gel electrophoresis as described previously (18). Frozen root tips were homogenized in a mortar and pestle with 4 ml of homogenization buffer (50 mM MES-NaOH, pH 7.0, 10 mM $KH_2PO_4$, 1 mM EDTA, 10 mM NaF, 0.5 mM PMSF, and 1 mM DTT) containing 5 μg/ml RNAase I. The homogenate was centrifuged at 10,000*g* for 10 min. The supernatant was made up to 5 ml with homogenization buffer. DNAase I was added and incubated for 20 min at 4° C. The protein pellet was obtained by precipitation with an equal volume of 20% TCA, then cen-

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[2] Abbreviations: EGTA, ethylene glycol bis *N,N,N',N'* tetraacetic acid; PMSF, phenylmethylsulfonyl fluoride.

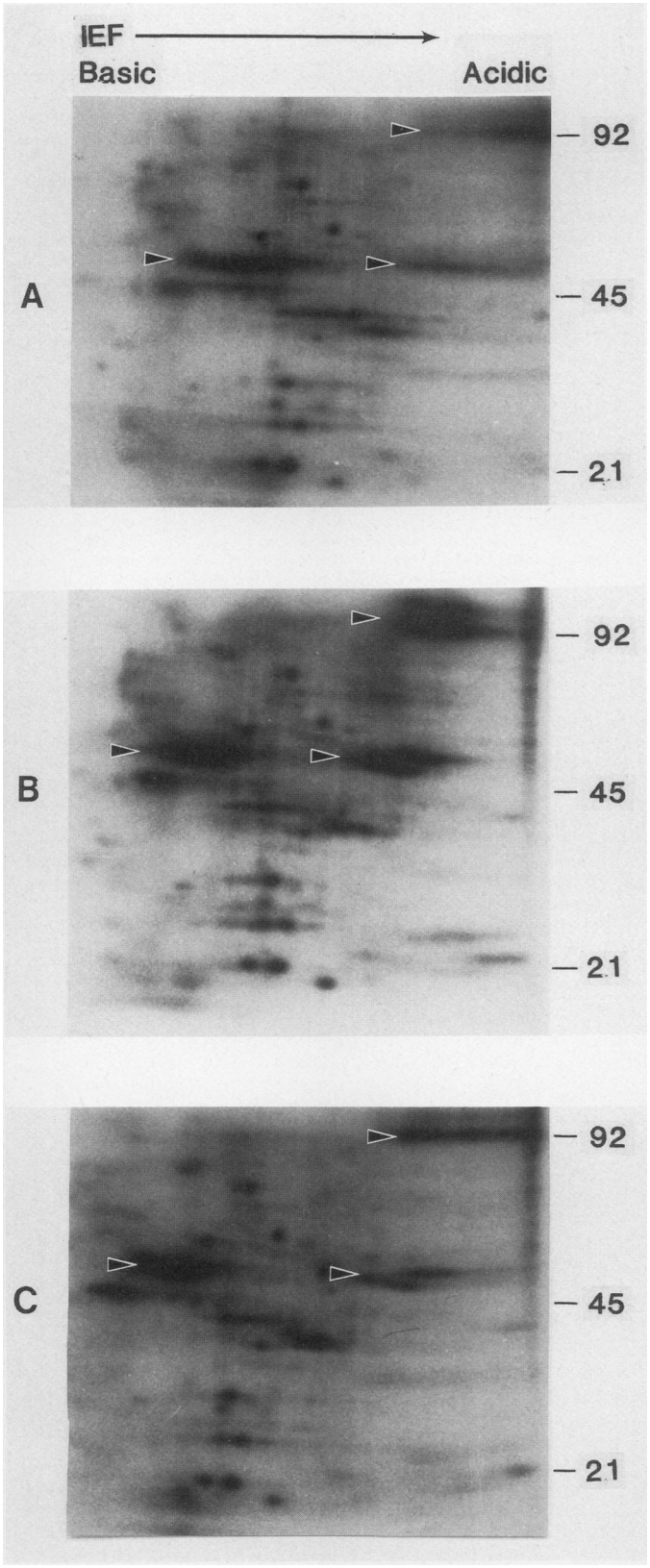


FIG. 1. Light-induced changes in *in vivo* protein phosphorylation in corn roots and the effect of calcium depletion. Apical segments of dark-grown corn roots were preloaded with $^{32}P$ for 1 h, then label was removed and samples were given one of the three treatments. One set of roots (A) was incubated for 15 min in dark, then frozen in liquid $N_2$. A second set (B) was incubated for 8 min in dark, then 7 min light, and frozen. The third set (C) was incubated for 15 min with 5 mM EGTA and 1 μM A23187 with the same light treatment as in B. See text for details of methods.

trifugation at 27,000*g* for 20 min. Pellets were washed twice in 80% acetone with 2% sucrose followed by 100% acetone. The samples were freeze-dried and dissolved in 150 μl of IEF sample buffer. Protein estimation was performed as described by Bensadoun and Weinstein (1).

Phosphoproteins were separated on first dimension using 1.5 mm i.d. tube gels following the method of O'Farrell (12) with modifications. NP-40 was replaced by Triton X-100 in the gel mixture. Equal radioactivity (about 20,000 CPM) was loaded on the gels and IEF was carried out at 400 V constant voltage for 16 h followed by 1 h at 1000 V. SDS-gel electrophoresis was performed as described earlier (23). The dried gels were exposed to Kodak X-Omat AR films for autoradiography in the presence of intensifying screens.

## RESULTS

Light treatment of dark-grown seedling roots had a significant effect on protein phosphorylation. Comparison of sample pairs showed that light consistently promoted total protein phosphorylation. The average increase in protein phosphorylation was found to be 60.9% and was determined to be significantly different at the 0.025 level (Student's *t* test).

In Figure 1, the effect of light on phosphorylation of specific polypeptides is shown. Comparison of A and B on Figure 1 shows that phosphorylation of polypeptides of $M_r$ 48,000, $M_r$ 92,000, and $M_r$ 94,000 was promoted by light. Distinct changes in phosphorylation occurred within the 7 min of light treatment in these experiments. In addition, Figure 1C shows that depletion of calcium by addition of EGTA and A23187 prior to light treatment significantly decreased light-induced promotion of the phosphorylation of the acidic polypeptides. The decrease in the light-induced phosphorylation of the basic polypeptide was less distinct as compared to the acidic polypeptides. Most other proteins in the gel show little or no change in phosphorylation with these treatments.

## DISCUSSION

It is becoming clear that calcium plays a key role in linking gravity stimulation to the gravitropic growth response in roots (8, 13, 16). Lee *et al.* (8) have shown that application of calcium chelators to the root cap can prevent gravitropism, and lateral placement of chelators or calcium can cause curvature in vertically oriented roots. In addition, gravitropic stimulation of corn roots results in polar transport of calcium towards the lower side of the root cap (9). Miyazaki *et al.* (10) also observed redistribution of calcium in corn roots during light-induced gravitropism. Some of the changes associated with root gravitropism have recently been reviewed by Poovaiah *et al.* (16).

The light-induced gravitropic response of dark-grown roots of Merit corn has been useful in understanding early events of the gravitropic response (3–5). It has been recently shown in our laboratory (5) that calcium depletion in dark-grown Merit corn roots can inhibit gravitropic response. Thus, the rapid changes in protein phosphorylation shown in Figure 1, are correlated with the responsiveness of roots to gravity.

The calcium-dependent and light-induced increases in protein phosphorylation (Fig. 1C) suggests the involvement of calcium in the gravitropic response. Many key regulatory proteins are controlled by calcium-dependent protein phosphorylation (15), and there are several reports of calcium regulation of protein kinase activity in plants (15, 19). Since the gravitropic response

itself is calcium-dependent, demonstration of calcium-dependent changes in protein phosphorylation suggests these changes could be involved in the gravitropic response. Further experiments are in progress to show whether the observed changes in protein phosphorylation occur in the root cap, elongation zone, or both.

A significant feature of the increases in phosphorylation described in this report is the speed of the response. Published reports of changes in phosphorylation to date have focused on changes in phosphorylation that occur over a longer time frame (15, 19), while the changes described in this paper were observed within 7 min. Thus, the changes in protein phosphorylation described here are among the most rapid responses observed to date, and are consistent with the idea that protein phosphorylation can play a role in the rapid events of signal transduction.

In addition, the rapid response is significant because it places phosphorylation within the time frame of other rapid responses to gravitropic stimulation. As reported recently by Pickard (13, 14), rapid responses are those which occur within 10 min (21). Changes which include asymmetrical distributions of calcium, electrical potential, and mRNA synthesis in dark-grown Merit corn roots are known to occur rapidly during gravitropic bending (4, 13, 14). The light stimulation of protein phosphorylation associated with gravitropic responsiveness reported in this paper suggests that these changes are among the earliest responses to gravitropic stimulation.

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