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Calcium and Gravitropism

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I. INTRODUCTION

Environmental signals such as light and gravity control many aspects of plant growth and development. In higher plants, the directional growth of an organ in response to stimuli such as gravity and light is considered a tropic movement. Such movement could be either positive or negative with respect to a specific stimulus. In general, stems show a positive response to light and negative response to gravity. In contrast, most roots show a positive response to gravity and a negative response to light.

Investigations on plant tropism date back a century when Darwin studied the phototropic responses of maize seedlings (Darwin, 1880). Although the precise mechanism of signal perception and transduction in roots is not understood, Darwin recognized over 100 years ago that the root cap is the probable site of signal perception. He discovered that the removal of the root cap eliminates the ability of roots to respond to gravity. Other investigators have since confirmed Darwin's observation (Konings, 1968; Evans et al., 1986). In recent years, especially with the advent of the U.S. Space Program, there has been a renewed interest in understanding how plants respond to extracellular signals such as gravity (Halstead and Dutcher, 1987). Studies on the mechanisms involved in perception and transduction of gravity signal by roots would ultimately help us to better understand gravitropism and also to grow plants under microgravity conditions as in space.

In this chapter, we restrict ourselves to the role of calcium in transduction of the gravity signal. In doing so, emphasis is given to the role of calcium-modulated proteins and their role in signal transduction in gravitropism. Detailed reviews on various other aspects of gravitropism (Scott, 1972; Torrey, 1976; Pilet 1979, 1983; Wilkins, 1979; Firm and Digby, 1980; Feldman, 1985; Pickard, 1985a, 1985b; Moore and Evans, 1986; Halstead and Dutcher, 1987; Poovaiah et al., 1987) and on the role of calcium as a messenger in signal transduction in general have been published (Helper and Wayne, 1985; Poovaiah and Reddy, 1987, 1993; Roberts and Harmon, 1992; Bowler and Chua, 1994; Gilroy and Trewavas, 1994).

Plant roots have been widely used to study the transduction of gravity and light signals (Poovaiah et al., 1987a; Roux and Serlin, 1987). Most roots show positive gravitropic response in either dark or light. However, roots of some varieties of plants (e.g., *Zea mays* L., cv Merit, and *Zea mays* L., cv Golden Cross Bantam 70) show positive gravitropic response only in light (Feldman, 1983;

Miyazaki et al., 1986). Investigations from various laboratories indicate that calcium acts as a messenger in transducing gravity and light signals in plant roots (Pickard, 1985a, 1985b; Evans et al., 1986; Poovaiah et al., 1987a).

II. MESSENGER ROLE OF CALCIUM IN GRAVITY SIGNAL PERCEPTION AND TRANSDUCTION

Free calcium is the most common signal transduction element in both plant and animal cells (Poovaiah and Reddy 1993; Clapham, 1995). Calcium is essential for survival, yet prolonged high intracellular calcium levels can kill the cell. Hence, cells stringently control intracellular calcium levels through numerous binding proteins. Several reviews have appeared on this topic (Poovaiah and Reddy, 1993; Gilroy and Trewavas, 1994; Bowler and Chua, 1994; Reddy, 1994). The realization that changes in cytoplasmic calcium could mediate diverse plant responses, coupled with the development of fluorescent and luminescent indicators to monitor changes in free calcium in living cells, has led calcium to become one of the best-characterized second messengers in plants (Poovaiah and Reddy, 1993; Gilroy and Trewavas, 1994). A number of studies in recent years have demonstrated that many environmental and hormonal signals cause an elevation of cytosolic calcium concentration. The increase in cytosolic calcium is believed to initiate a cascade of biochemical events that are discussed below. Several studies have shown that calcium is important in gravity signal perception and transduction.

The gravitropic response is separated into three phases: signal perception, transduction, and response. The primary event that takes place in roots subjected to gravity is the initial perception of the signal. This initial perception takes place in the root cap. The root cap is composed of short-lived parenchymalike cells, and new cells are continuously being added to it. Hence, the root cap persists throughout the growing life of the root. The events that take place between stimulus perception and final growth response are grouped under the term *transduction*. The third phase is the final response of altered growth pattern in the elongation zone of the roots, which leads to curvature.

Evidence for the importance of calcium in gravitropism is obtained by simple but elegant experiments using calcium chelators (e.g., ethylene glycol bis[β -aminoethylether] N, N'-tetraacetic acid [EGTA]) and calcium ionophores (e.g., A23187). Manipulation of calcium gradients in the root cap can change the gravitropic response (Lee et al., 1983b). Depletion of calcium in the root cap, using calcium chelators, results in the loss of gravisensitivity (Fig. 1A1), and subsequent replenishment of calcium to depleted roots restores gravisensitivity (Fig. 1A2). Furthermore, root curvature can also be induced by creating calcium gradients across the root cap using calcium or calcium chelators such as EGTA (Fig. 1A3 and 1A4). Studies have shown that the root tip contains four times more calmodulin than the root base (Poovaiah et al., 1987). Amyloplasts in the columella cells of the root cap are known to contain a high concentration of calcium (Chandra et al., 1982) and large quantities of calmodulin (Dauwalder et al., 1986). Calmodulin inhibitors inhibit gravitropism without inhibiting the growth rate of the roots (Biro et al., 1982). Inhibitory effects of KN-93, an inhibitor of calcium calmodulin-dependent protein kinase II on light-regulated maize root gravitropism have also been observed (Lu and Feldman, 1993). Moreover, calmodulin antagonists inhibit polar transport of calcium in roots (Stinemetz and Evans, 1986), suggesting that establishment of the calcium gradient, which is essential for gravitropism, is a calmodulin-dependent process. Gehring et al. (1990) demonstrated that gravity induces a rapid increase in cytosolic calcium in the elongating cells on the growing side of gravistimulated maize coleoptiles.

Primary roots of a mutant maize (cv Merit) do not exhibit gravitropism in the dark but become gravisensitive only after brief exposure to light (Fig. 1B). Roots of maize (cv Merit) that were depleted of calcium by EGTA and A23187 (calcium ionophore) prior to light treatment did not show gravitropic curvature (Reddy et al., 1987b). However, replenishment of calcium to depleted roots restored sensitivity to gravity. Perdue and Leopold (1988) reported that treatments such as heat shock and cold shock that cause an influx of calcium can substitute for light, causing positive gravitropism in the dark. Furthermore, the calcium channel blocker, verapamil, was found to inhibit the light response. Calmodulin antagonists such as calmidazolium and compound 48/80 were shown to inhibit

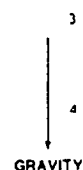


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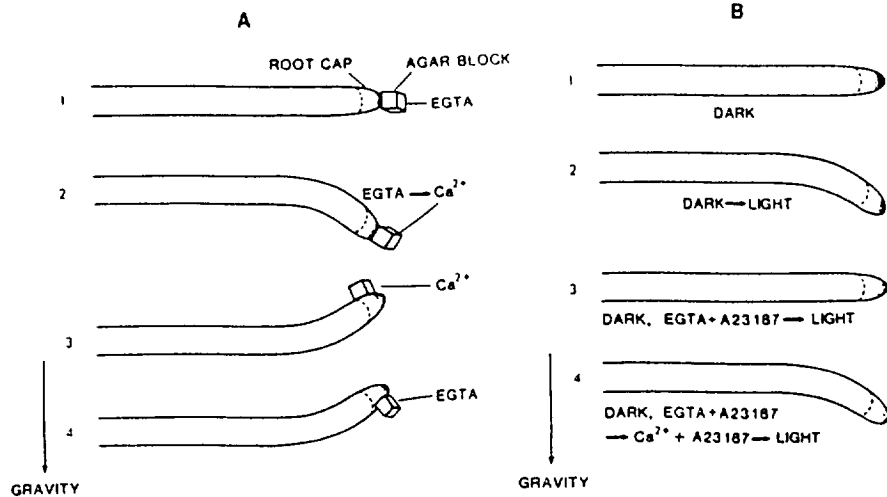


Figure 1 Diagrammatic representation of the effect of calcium on gravitropic response in maize roots. (A) Effect of calcium or EGTA applied in agar blocks on gravitropism: (1) EGTA applied to the root cap prevents gravitropic response; (2) Calcium application following EGTA pretreatment restores gravitropic response; (3) Calcium application to one side of the cap causes bending towards the calcium; (4) EGTA application to one side of the cap causes bending away from EGTA. (Adapted from Lee et al., 1983b.) (B) Effect of calcium manipulation on light-induced gravitropic response in maize roots (cv Merit): (1) dark-grown roots nonresponsive to gravity; (2) dark-grown roots exposed to light; (3) dark-grown roots treated with EGTA + A23187 (calcium ionophore) prior to light exposure; (4) dark-grown roots treated with EGTA + A23187 and transferred to calcium + A23187 prior to light exposure. (From Poovaiah et al., 1987a.)

light-induced gravitropism in maize (cv Merit) roots (Bjorkman and Leopold, 1987). Recent reports have also indicated an increase in the level of calmodulin and its activity in maize (cv Merit) roots exposed to light (Stinemetz et al., 1987). These studies suggest that the effects of calcium could be mediated through calmodulin. The discovery of various components of the calcium messenger system in roots such as calmodulin and calcium-regulated protein kinases, and the observation of an increase in cytosolic calcium in response to gravity, has led researchers to propose a messenger role for calcium in gravitropic bending.

A. Calcium-Modulated Proteins

Elevation of cytosolic calcium initiates a cascade of events resulting in a physiological response. The changes in cytosolic calcium are sensed by a group of calcium-modulated proteins that are believed to be involved in cellular regulation. These binding proteins have a structural feature called the EF-hand which is present in multiple copies and binds calcium with high affinity (Heizmann and Hunziker, 1991). Many calcium-binding proteins have been identified and characterized in animals and several in plants (Moncrief et al., 1990; Heizmann and Hunziker, 1991, Roberts and Harmon, 1992). Calcium-binding proteins are inactive in the absence of bound calcium, but when the concentration of cytosolic calcium increases, the calcium-binding proteins such as calmodulin (CaM) and calcium-dependent protein kinases bind to calcium and become active. Once bound to calcium, these proteins become active and interact with other proteins in the cell and alter their activity. Fig. 2 shows a schematic diagram illustrating how elevated levels of cytosolic calcium affect various enzymes and proteins in the cell. Hence, in this chain of events, calcium acts as a simple on-off switch that conveys the signal from cell surface to the metabolic machinery, eventually resulting in a physiological response. So far, two calcium-modulated proteins, CaM and calcium-dependent protein kinase, have

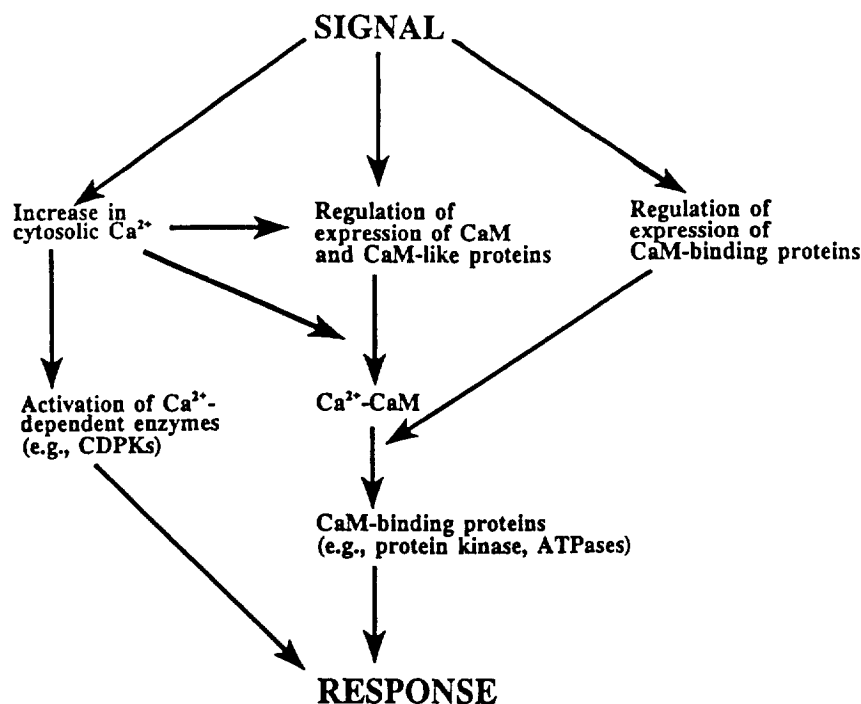


Figure 2 Schematic illustration of the proposed events involving calcium, CaM, and CBP in signal transduction. Signals induce changes in cytosolic calcium and these changes in cytosolic calcium are transmitted to the metabolic machinery through CBP. (From Poovaiah and Reddy, 1993.)

been well characterized in plants. In addition, a chimeric calcium/CaM-dependent protein kinase has recently been cloned from plants (Patil, et al., 1995). There are indications for the presence of CaM-like proteins and additional calcium-binding proteins that could be involved in sensing cytosolic calcium changes and mediating calcium action in plants (Krause, et al., 1989; Braam and Davis, 1990; Zielinski, 1990; Clark et al., 1992; Poovaiah and Reddy, 1993). In addition, calcium-dependent proteinase activity was also detected recently in *Arabidopsis* root cultures (Reddy et al., 1994).

1. Calmodulin

CaM is a highly conserved protein and is considered to be a multifunctional protein because of its ability to interact and regulate the activity of a number of other proteins (Roberts et al., 1986; Poovaiah and Reddy, 1987). The properties of plant CaM are very similar to animal CaM. In recent years, there has been considerable progress in studying CaM gene expression and the organization of CaM genes in plants.

cDNAs or genomic clones that code for CaM were isolated from a number of plant systems (Roberts and Harmon, 1992; Poovaiah and Reddy, 1993). Analysis of cDNA and genomic clones suggest the presence of multiple calmodulin genes in plants (Ling et al., 1991; Perera and Zielinski, 1992; Takezawa et al., 1995). Recent studies have shown that CaM and CaM-related genes are highly responsive to signals. Various physical and chemical signals have been shown to induce mRNA corresponding to CaM and CaM-related genes. Exposure of dark-grown Merit corn root tips to light increased the CaM mRNA level (Jena et al., 1989). In *Arabidopsis*, Braam and Davis (1990) have shown rapid (10–30 min) induction of mRNAs corresponding to four cDNAs (TCH 1, TCH 2, TCH 3, and TCH 4) in response to a variety of stimuli such as touch, wind, rain, and wounding. Of these four genes, TCH 1 is identified as CaM and TCH 2 and TCH 3 are identified as CaM-related genes.

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Other studies have confirmed the induction of calmodulin genes by touch stimuli (Perera and Zielinski, 1992; Watillon et al., 1992). These studies suggest that physical and chemical signals induce the expression of CaM. By manipulating cytosolic calcium the expression of some of the touch genes is found to be regulated by calcium (Braam, 1992). Studies have shown that touch and wind signals elevate cytosolic calcium (Knight et al., 1991, 1992). Hence, the probable sequence of events in touch signal transduction is elevation of cytosolic calcium, which in turn regulates the expression of the specific genes, including those that code for its own receptor. The availability of CaM genomic clones from *Arabidopsis* (Perera and Zielinski, 1992), rice (Y. J. Choi, B. W. Poovaiah, and G. An, unpublished results), apple (Watillon et al., 1992), and potato (Takezawa et al., 1995) will help in identifying regulatory elements in CaM promoters. In addition to CaM, there are reports of CaM-related genes in plants (Braam and Davis, 1990; Zielinski et al., 1990). In *Arabidopsis*, a cDNA for a CaM-like protein (p21) that shares 65% amino acid similarity with the higher plant CaM sequences has been isolated (Zielinski et al., 1990; Ling and Zielinski, 1993). From the same system, Braam and Davis (1990) isolated two partial cDNAs, TCH 2 and TCH 3, that code for CaM-related proteins. These showed 44% and 70% amino acid identities, respectively, with CaM. p21 has several unique structural features including a 45-amino acid carboxyterminal extension with no homology to any known proteins. Another CaM-related cDNA was isolated from petunia which contained an extra domain of 35 amino acids at the carboxyterminal end. Forty percent of the amino acid residues in the extra domain of petunia CaM-like protein are positively charged (H. Fromm, E. Carlenor, and N. H. Chua, data obtained from Genbank). The significance and function of these CaM-like proteins are not known at this time.

2. Calcium- and Calcium/Calmodulin-Regulated Enzymes

Calcium either directly or through calmodulin regulates the activity of a number of enzymes and the function of structural proteins that play a key role in cellular regulation (Klee, 1991; Meader et al., 1992). A number of proteins that interact with calcium/CaM have been isolated, characterized, and identified in animals (Bachs and Carafoli, 1987; Bachs et al., 1990; Colbran and Soderling, 1990; Klee, 1991). These include protein kinases, protein phosphatase (calcineurin), nitric oxide synthase, calcium ATPase, IP3 kinase, and several structural proteins (Colbran and Soderling, 1990; Klee, 1991). Identification of these proteins has greatly increased our understanding of how calcium and CaM regulate various biochemical and molecular processes that eventually lead to a physiological response in animal cells. Very little is known about the number, localization, and identity of CaM-binding proteins in plants, although enzymes such as NAD kinase, calcium ATPase, nuclear NTPases, and protein kinases are known to be activated by CaM (Poovaiah and Reddy, 1987; Roberts and Harmon, 1992). The lack of information on calmodulin-binding proteins and their identities has been a major limitation in elucidating the calcium-mediated signal transduction mechanisms in plants.

a. **Protein Kinases.** Protein phosphorylation, which is catalyzed by protein kinases, is one of the major mechanisms of signal integration in eukaryotic cells. Protein kinases play a pivotal role in the majority of the signal transduction pathways (Ranjewa and Boudet, 1987; Nishizuka, 1988; Cohen, 1990, 1992; Poovaiah and Reddy, 1990; Asaoka et al., 1992). Extracellular signals, either directly or through second messengers, regulate the activity of protein kinases, which in turn regulate the activity of their substrates by phosphorylation. The diverse actions of various signals and amplification of signals are largely achieved through protein kinases (Cohen, 1992). Extensive studies in animal systems indicate that CaM-dependent protein kinases are central to calcium-mediated signal transduction pathways (Colbran and Soderling, 1990).

Calcium-Dependent and Calmodulin-Independent Protein Kinases. Calcium-dependent protein kinases are one of the best characterized and widely distributed protein kinases in plants. Since the discovery of a calcium-dependent and calmodulin-independent protein kinase (CDPK) in soybean, such protein kinases have been purified and characterized from a number of plant systems (Harmon et al., 1987; Putnam-Evans et al., 1990; Roberts and Harmon, 1992). CDPK is activated by micromolar concentration of calcium and is not dependent on CaM for its activity. The CDPK showed calcium-dependent mobility shift as well as calcium-binding.

Harper et al. (1991) isolated a cDNA (SK5) from soybean that codes for a CDPK. The deduced amino acid sequence of soybean CDPK contains a catalytic domain and a CaM-like region with four calcium-binding domains at the carboxyterminal end. The presence of these calcium-binding domains explains direct calcium activation of CDPK. The kinase domain showed highest homology (39%) with the catalytic domain of the α -subunit of CaM KII. So far, this new type of protein kinase, where the kinase domain is fused to a CaM-like region, has been found only in plants. However, in the case of calpain, a calcium-activated protease, the catalytic domain is fused to CaM-like regulatory domain (Suzuki and Ohno, 1990). Isolation of a partial cDNA (SK2) which codes for a protein that shows 70% identity with CDPK and the observation that SK5 hybridizes to multiple fragments on Southern blots indicate the presence of multiple CDPK isoforms in soybean. Using a variety of approaches, the presence of CDPK-like enzymes have been shown in a number of plants, indicating the ubiquitous nature of these enzymes in plants (Harmon and McCurdy, 1990; Polya and Chandra, 1990; Roux et al., 1990; Li et al., 1991; Roberts and Harmon, 1992). Partial cDNAs that code for CDPK have been isolated from carrot (Choi and Suen, 1991) and corn root tip (A. Bhatia, S. Patil, and B. W. Poovaiah, unpublished results). Alignment of the deduced amino acid sequences of the CDPKs of soybean, carrot, and corn root tip is shown in Fig. 3.

Calcium-Regulated Protein Phosphorylation in Roots. *In vivo* protein phosphorylation studies have shown calcium-dependent protein phosphorylation in roots (Raghothama et al., 1987). To study the role of calcium-dependent protein phosphorylation in light-dependent gravitropism in corn root tips, we have performed *in vivo* protein phosphorylation studies in dark-grown and light-treated roots by manipulating tissue calcium levels (McFadden and Poovaiah, 1988). Exposure of dark-grown roots to 7 min of light resulted in the promotion of phosphorylation of specific polypeptides corresponding to 94,000, 92,000, and 48,000 D (Fig. 4). In later studies, we were able to detect the light-dependent changes in protein phosphorylation within 1 min. Interestingly, the light-dependent changes in protein phosphorylation were observed only in the root tips, which are considered to be the site of light and gravity signal perception. No effect of light on the phosphorprotein pattern was observed in the root base, suggesting the specificity of light-dependent changes and the physiological significance of these changes in light-induced gravitropism. Depletion of calcium by addition of EGTA and the calcium ionophore A23187 prior to light treatment decreased light-induced promotion of the phosphorylation of these polypeptides. Replenishment of calcium to depleted root tips restored the light effect on protein phosphorylation. These results strongly suggest that light induces rapid and specific changes in protein phosphorylation and that these changes are mediated by calcium.

Calcium/calmodulin-dependent protein kinases. Five types of calcium/CaM-dependent protein kinases (CaM kinase I, CaM kinase II, CaM kinase III, phosphorylase kinase, and myosin light chain kinase) have been well characterized in mammalian systems, although there are other CaM-dependent kinases in animals (Colbran and Soderling, 1990; Fujisawa, 1990; Klee, 1991). All of these CaM-dependent protein kinases, except CaM KII, have limited substrate specificity. CaM KII phosphorylates a wide range of substrates, and it is therefore considered to be a multifunctional protein kinase. CaM KII is present in different species of vertebrates, invertebrates, yeast, and other fungi (Colbran and Soderling, 1990; Pausch et al., 1991). cDNAs that code for five different polypeptides of CaM KII have been isolated and characterized (Tobimatsu and Fujisawa, 1989). Because of the important role played by CaM kinases in animals, plant scientists have attempted to identify CaM-dependent protein kinases. Several reports have indicated the presence of CaM-dependent protein kinases (reviewed in Roberts et al., 1986; Poovaiah and Reddy, 1987). A majority of these studies have been performed with crude protein preparations and conclusions drawn are based on inhibition of calcium-stimulated protein phosphorylation by CaM inhibitors or stimulation of phosphorylation by exogenous CaM. Many of these studies are considered inconclusive because of: (1) the nonspecific effects of CaM inhibitors which are known to inhibit CaM-independent enzymes, and (2) the high concentration of CaM required to stimulate phosphorylation. Although purification of CaM-dependent protein kinase is needed to establish unequivocally the presence of CaM-dependent enzymes, some of the recent results coupled with earlier reports (Veluthambi and Poovaiah, 1984a, 1984b; Blowers et al., 1985; Echevarria et al., 1988; Blowers and Trewavas, 1989; Trewavas and Blowers, 1990) suggest the presence of CaM-dependent protein kinases in plants.

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relation studies (1987). To study the effect of light on corn root growth in corn root tip-treated roots and dark-grown roots corresponding to the light-dependent changes in protein synthesis of light and dark-grown roots and the role of these changes in the calcium-dependent phosphorylation effect on specific changes

dependent protein kinase myosin light chain (MLC) and other CaM-dependent kinases. All of these kinases are phosphorylated by CaM KII. Multifunctional protein kinase, and other five different kinases (Sawa, 1989). The authors attempted to identify the role of CaM-dependent kinases drawn from corn root tip stimulation in response to light because of the CaM-independent nature of the response. Although the presence of calcium-binding sites in the protein was demonstrated in uthambi and wavas, 1989; in plants.

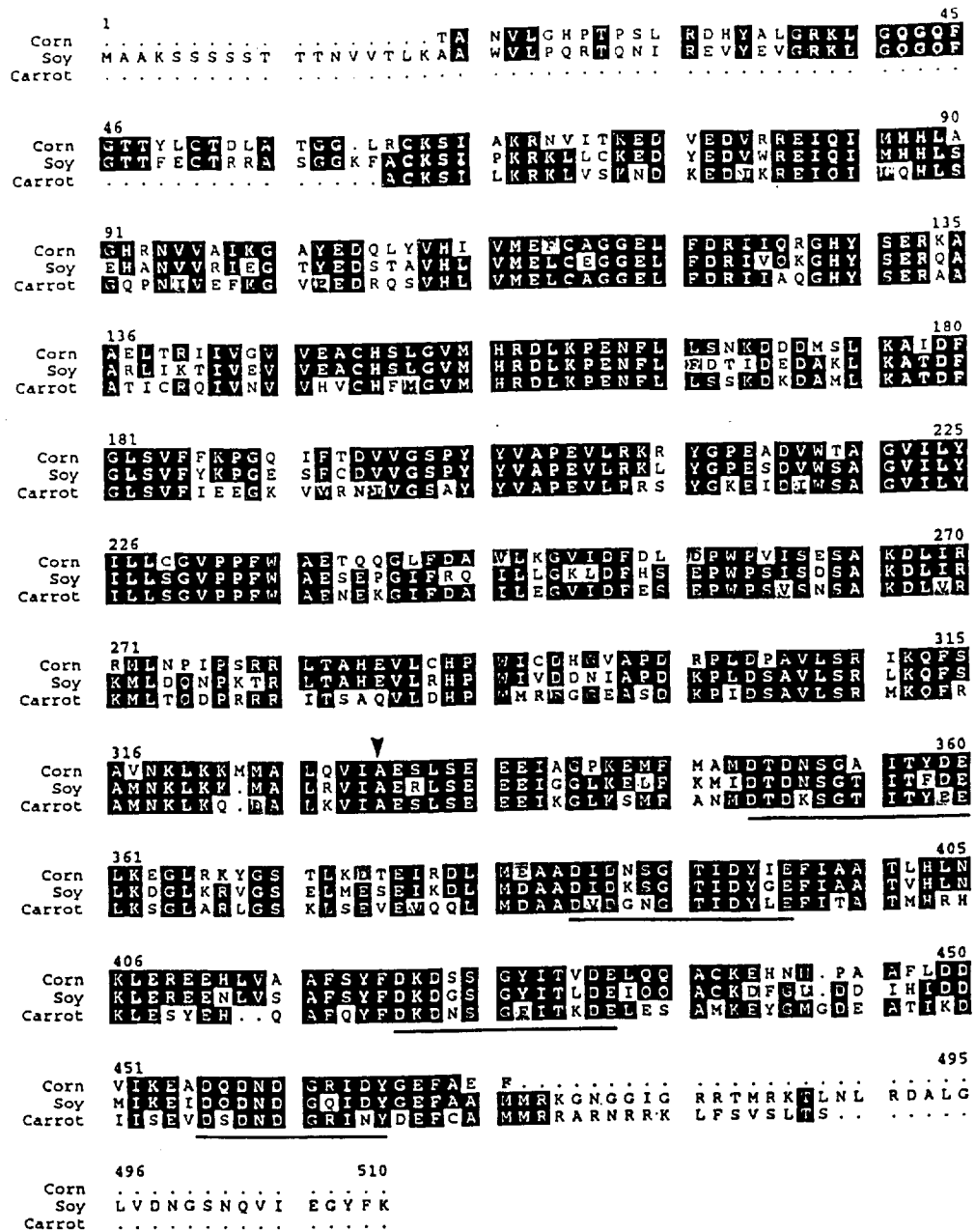


Figure 3 Alignment of amino acid sequence of calcium-dependent and CaM-independent protein kinase from soybean, carrot, and corn root tip. Arrow indicates the beginning of calmodulin-like domain; calcium-binding sites are underlined. (From Poovaiah and Reddy, 1993.)

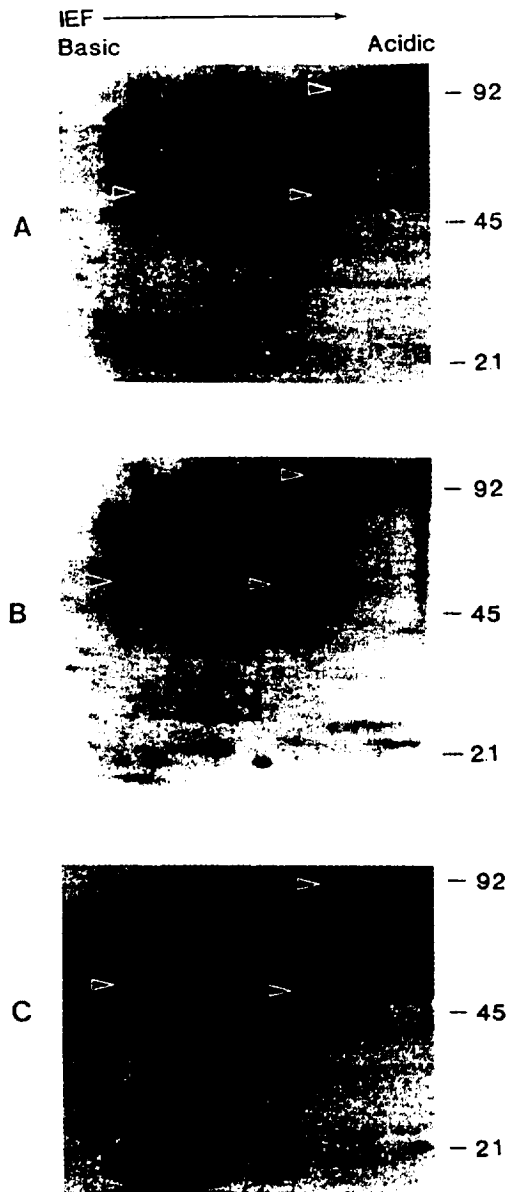


Figure 4 Rapid changes in protein phosphorylation associated with gravity perception in roots of maize (cv Merit). Apical segments of dark-grown roots were preloaded with ^{32}P for 1 h and, then washed in buffer. (A) Roots were left in buffer for 15 min in the dark (control). (B) Roots were exposed to light for 7 min after 8 min of dark incubation. (C) Light treatment was the same as in (B) but EGTA + A23187 were present for 15 min. Proteins were extracted and separated by two-dimensional gel electrophoresis as described earlier (Raghothama et al., 1987). Arrows indicate the phosphoproteins that are affected by light. (From McFadden and Poovaiah, 1988.)

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Antipeptide antibodies produced against the α -subunit of rat brain CaM KII were found to detect one or two bands (54–56 kD) in soluble proteins (Reddy et al., 1991; Poovaiah et al., 1992). The molecular weight of the cross-reacting proteins was similar to the mammalian CaM KII, indicating that plants may have a homologue of CaM KII. Watillon et al. (1992a) isolated a cDNA (CB1) clone by screening an expression library with ^{125}I -labeled CaM. The deduced amino acid sequence of this CaM-binding protein showed sequence similarities with rat brain CaM KII isoforms. The similarities include a CaM-binding domain and domain XI of protein kinase, suggesting that the plant CaM-binding protein could be CaM kinase. The missing 3' and 5' ends of the CB1 were isolated by polymerase chain reaction and sequenced. The deduced amino acid sequence of the full-length CB1 has a CaM-binding domain with all the conserved domain of protein kinase and showed homology with mammalian CaM KII (Watillon et al., 1993). These results provide further evidence indicating the existence of CaM-dependent protein kinases in plants. Northern analysis indicated that CB1 represents a rare mRNA, which is probably one of the reasons why it was not represented in the pool of various protein kinases isolated by using oligoprobes made to the conserved domain of protein kinases. If the mRNA level is the indication of protein level, one would expect very low levels of this CaM-kinase which may have contributed to problems in purifying this enzyme. Southern analysis of genomic DNA from *Arabidopsis*, chicory, and tomato with the CB1 insert (which mostly contains the CaM-binding region) under low-stringency conditions showed a small number of major hybridizing bands suggesting the presence of similar genes in other plants. More recently, a chimeric calcium/calmodulin-dependent protein kinase (CCaMK) with a catalytic domain, calmodulin-binding domain and a visininlike calcium-binding domain was cloned and characterized from lily anther (Patil et al., 1995). Plants, unlike animal systems, seem to have both calcium-dependent protein kinases (CDPK) and calcium/CaM-dependent protein kinases. Having these two different types of protein kinases would explain how calcium might regulate diverse physiological processes in plants.

3. Other Calmodulin Target Proteins

Gel overlay assay was used to detect the number and distribution of CaM-binding proteins in plants. In *Fucus*, Brawley and Roberts (1989) have demonstrated changes in CaM-binding proteins during development. Oh et al. (1992) detected several CaM-binding proteins in the carrot embryo extract. Some of these CaM-binding proteins were found to change during carrot embryogenesis and germination. A 54-kD CaM-binding protein markedly increased during embryo germination. In different tissues of *Vicia faba* and guard cell protoplasts, CaM-binding proteins were analyzed by gel overlay assay (Ling and Assmann, 1992). Several CaM-binding proteins that are specific to metabolically active plant parts have been detected (Ling and Assmann, 1992). These studies indicate that there are several CaM-binding proteins in plants and some of them are specific to a particular tissue or cell type. To better understand the mode of calcium/CaM action, it is essential to identify and characterize all CaM-binding proteins in plants. Characterization of various CaM-binding proteins from animal systems has revealed a CaM-binding domain containing a basic amphiphilic alpha helix (O'Neil and DeGrado, 1990). However, there is no amino acid sequence conservation in the CaM-binding domain among different CaM-binding proteins. Hence, it has not been possible to design oligonucleotide probes to clone CaM-binding proteins. Sikela and Hahn (1987) developed a method to isolate CaM-binding proteins from a cDNA expression library using ^{125}I -labeled CaM. Using this method, Watillon et al. (1992a) isolated a cDNA for a CaM-binding protein which was identified as a CaM-dependent protein kinase based on sequence similarity. In recent years, this method has been improved by using ^{35}S -labeled CaM (Asselin et al., 1989; Widada et al., 1989; Fromm and Chua, 1992). Nonradioactive methods to isolate calmodulin-target proteins have also been described (Fordham-Skelton et al., 1994; Stirling et al., 1994). Two cDNAs (CBP-1 and CBP-5) that code for CaM-binding proteins have been isolated from a corn tip cDNA library using ^{35}S -labeled calmodulin (Reddy et al., 1993). Comparison of the deduced amino acid sequence of CBP-1 and CBP-5 clones showed an overall 50% identity. However, 100% conservation of the 34-amino acid stretch at their carboxyterminal end was observed in other regions of amino acid sequence. Hence, this conserved region could be a potential CaM-binding domain. The highly conserved 34-amino acid stretch

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contained putative CaM-binding domain, a basic amphiphilic alpha helix. The putative CaM-binding domain has a cluster of basic residues (hydrophilic region) facing hydrophobic amino acids. The fact that the 34-amino acid stretch is highly conserved between two different CBP clones and forms an amphiphilic alpha helix strongly suggest that it is a CaM-binding domain in CBP-1 and CBP-5. However, further studies to confirm the CaM-binding domain by deletion analysis or by competition experiments using synthetic peptides are needed. A computer search of nucleotide and protein databases with both nucleotide and deduced amino acid sequences of CBP-1 and CBP-5 has not revealed any significant homology between CBP sequences and known nucleic acid and protein sequences.

Different CaM-binding proteins isolated from animals indicate that there is no amino acid sequence conservation. However, CBPs that belong to a particular class, for instance, different isozymes of CaM-dependent protein kinases, have the same amino acid sequence in the CaM-binding domain (Tobimatsu and Fujisawa, 1989). Hence, the highly conserved amino acid region in the putative CaM-binding region of CBP-1 and CBP-5 suggests that these two proteins may be related or perform similar functions. The genes corresponding to CBP-1 and CBP-5 are expressed in all the parts tested, although there was a difference in the extent of expression. CBP-5 is expressed almost equally in all the parts tested, whereas CBP-1 mRNA was found to be differentially expressed in different parts with very low levels in the root elongation zone. The fact that the CBP-1 and CBP-5 are expressed in all the parts tested indicates a broader role for these proteins. Southern analysis indicates that CBP-1 and CBP-5 are coded most likely by one or two genes.

a. Calcium Asymmetry and Differential Growth. Bode (1959) showed that the gravistimulation of sunflower hypocotyls leads to asymmetrical redistribution of calcium, in these tissues. Since then, calcium asymmetry in gravistimulated roots has been reported by several investigators using different systems (Goswami and Audus, 1976; Roux and Serlin, 1987). In gravistimulated roots, calcium was shown to accumulate preferentially on the lower side, which is slower growing. Studies have shown that calcium redistribution occurs prior to gravicurvature, suggesting that asymmetrical distribution of calcium could participate in the development of differential growth (Roux and Serlin, 1987). Using ^{45}Ca , it has been shown that calcium moves from the upper to the lower side of horizontally oriented maize roots (Lee et al., 1983a). Measurements of calcium levels in gravistimulated and light-stimulated maize roots, using proton-induced x-ray emission, showed higher levels of calcium in the lower half as compared with the upper half both in root caps and in the elongation zone (Miyazaki et al., 1986). In both stems and roots, curvature is toward the side with higher calcium levels. Exogenous calcium application can reduce the growth rate rapidly (Cleland and Rayle, 1977), and cell wall extension is greatly affected by apoplastic calcium (Cleland and Rayle, 1977; Slocum and Roux, 1983). Furthermore, Sakai-Wada and Yagi (1993) have observed changes in the calcium localization in the dividing cells of the maize root tip.

The sequence of biochemical events that could occur in gravitropism are illustrated in a schematic diagram (Fig. 5). According to this model, the initial event in gravity perception is the localized increase in cytosolic calcium in root cap cells. Although the mechanism by which gravity induces the

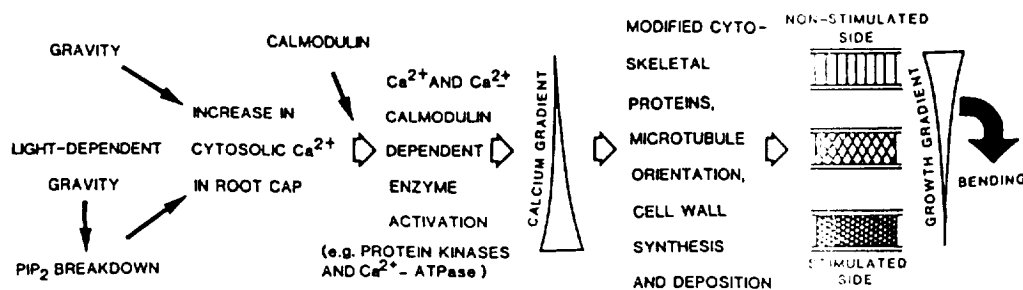


Figure 5 A schematic diagram illustrating the involvement of calcium in gravitropism in roots. (Poovaiah et al., 1987a.)

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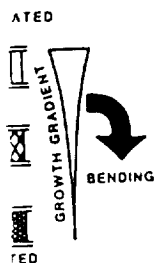
REFERENCES

- Asaoka, Y., N.
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CaM-binding proteins. The fact that CaM forms an 1:1 complex with 3P-5. How this complex is regulated in all the different databases revealed any differences. The amino acid sequence, different CaM-binding regions in the protein may be related to the function in all the different databases. The most expressed almost all the proteins are expressed in the CaM and CBP-5 domain analysis.

Stimulation of growth. Since then, using different calcium levels, we have shown that the distribution of calcium is different (Miyazaki and Reddy, 1977), and that the localized calcium induces the growth.

In a schematic diagram, the localized calcium induces the growth.



Calcium in roots.

increase in cytosolic calcium is unclear, it is likely that phosphoinositide hydrolysis could be involved in light-induced gravity response. Increases in cytosolic calcium activate calmodulin, leading to stimulation of calcium-dependent and calcium/CaM-dependent enzymes such as Ca-ATPase and protein kinases, ultimately leading to creation of both intra- and extracellular calcium gradients. This asymmetrical calcium distribution could differentially modify cytoskeletal proteins, microtubule orientation, and cell wall synthesis and deposition. As a result, growth gradient is created with more growth on the nonstimulated side resulting in bending.

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REFERENCES

- Asaoka, Y., Nakamura, S., Yoshida, K., and Nishizuka, Y. (1992). Protein kinase C, calcium and phospholipid degradation. *Trends Biochem. Sci.* 17: 414-417.
- Asselin, J., Phaneuf, S., Watterson, D. M., and Haiech, J. (1989). Metabolically ³⁵S-labeled recombinant calmodulin as a ligand for the detection of calmodulin-binding proteins. *Anal. Biochem.* 178: 141-147.
- Audus, L. J. (1979). Plant geosensors. *J. Exp. Bot.* 8:235-249.
- Bachs, O., and Carafoli, E. (1987). Calmodulin and Calmodulin-binding proteins in liver cell nuclei. *J. Biol. Chem.* 262: 10786-10790.
- Bachs, O., Lanini, I., Serratos, J., et al. (1990). Calmodulin-binding proteins in the nuclei of quiescent and proliferatively activated rat liver cells. *J. Biol. Chem.* 265: 18595-18600.
- Berridge, M. J., and Irvine, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312: 315-321.
- Biro, R. L., Hale, C. C., II, Wiegand, O. F., and Roux, S. J. (1982). Effects of chlorpromazine on gravitropism in *Avena* coleoptiles. *Ann. Bot.* 50: 737-745.
- Bjorkman, T., and Leopold, A. C. (1987). Effect of inhibitors of auxin transport and of calmodulin on a gravisensing-dependent current in maize roots. *Plant Physiol.* 84: 847-850.
- Blowers, D. P., and Trewavas, A. J. (1989). Rapid cycling of autophosphorylation of Ca²⁺-calmodulin regulated plasma membrane located protein kinase from pea. *Plant Physiol.* 90: 1279-1285.
- Blowers, D. P., Hetherington, A., and Trewavas, A. J. (1985). Isolation of plasma membrane bound calcium/calmodulin dependent protein kinase from pea and modification of catalytic activity by autophosphorylation. *Biochem. Biophys. Res. Commun.* 143: 691-696.
- Bode, H. R. (1959). Über den Einfluss der Goeinduktion auf die Kationenverteilung im Hypotyl von *Helianthus annuus*. *Planta* 54: 15-33.
- Boss, W. F., and Massel, M. O. (1985). Polyphosphoinositides are present in plant tissue culture cells. *Biochem. Biophys. Res. Commun.* 132: 1018-1023.
- Bowler, C., and Chua N.-H. (1994). Emerging themes of plant signal transduction. *Plant Cell.* 6: 1529-1541.
- Braam, J., and Davis, R. W. (1990). Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in *Arabidopsis*. *Cell* 60: 357-364.
- Brawley, S. H., and Roberts, D. M. (1989). Calmodulin-binding proteins are developmentally regulated in gametes and embryos of fucoid algae. *Dev. Biol.* 131: 313-320.
- Casey, P. J., and Gilman, A. G. (1988). G protein involvement in receptor-effector coupling. *J. Biol. Chem.* 263: 2577-2580.
- Caspar, T., Somerville, C., and Pickard, B. G. (1985). Geotropic roots and shoots of a starch-free mutant of *Arabidopsis*. *Plant Physiol.* 77(Suppl.): 105.
- Chandra, S., Chabot, J. F., Morrison, G. H., and Leopold, A. C. (1982). Localization of calcium in amyloplasts of root-cap cells using ion microscopy. *Science* 216: 1221-1223.
- Choi, J. H., and Suen, K.-L. (1991). Isolation and sequence analysis of a cDNA for a carrot calcium-dependent protein kinase: Homology to calcium/calmodulin-dependent protein kinases and calmodulin. *Plant Mol. Biol.* 17: 581-590.
- Clark, G. B., Dauwalder, M., and Roux, S. J. (1992). Purification and immunolocalization of an annexin-like protein in pea seedlings. *Plant* 187: 1-9.

- Cleland, R. E., and Rayle, D. L. (1977). Reevaluation of the effect of calcium ion on auxin-induced elongation. *Plant Physiol.* 60: 709-712.
- Cohen, P. (1982). The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature* 296: 613-619.
- Cohen, P. (1985). The role of protein phosphorylation in the hormonal control of enzyme activity. *Eur. J. Biochem.* 151: 439-448.
- Cohen, P. (1990). The structure and regulation of protein phosphatases. In *The Biology and Medicine of Signal Transduction* (Y. Nishizuka, Ed.). Raven Press, New York, p. 230.
- Cohen, P. (1992). Signal integration at the level of protein kinases, protein phosphatases and their substrates. *Trends Biochem. Sci.* 17: 408-413.
- Colbran, R. J., and Soderling, T. R. (1990). Calcium/calmodulin-dependent protein kinase II. *Curr. Top. Cell Regul.* 31: 181-221.
- Darwin, C. (1880). *The Power of Movement in Plants*. John Murray, London.
- Dauwalder, M., Roux, S. J., and Hardison, L. (1986). Distribution of calmodulin in pea seedlings: Immunocytochemical localization in plumules and root apices. *Planta* 168: 461-470.
- Dillenschneider, M., Hetherington, A., Graziana, A., et al. (1986). The formation of inositol phosphate derivatives by isolated membranes from *Acer pseudoplatanus* is stimulated by guanine nucleotides. *FEBS Lett.* 208: 413-417.
- Drobak, B. K., and Ferguson, I. B. (1985). Release of Ca^{2+} from plant hypocotyl microsomes by inositol-1,4,5-trisphosphate. *Biochem. Biophys. Res. Commun.* 130:1241-1246.
- Drobak, B. K., Allan, E. F., Comerford, J. G., et al. (1988). Presence of guanine nucleotide-binding proteins in a plant hypocotyl microsomal fraction. *Biochem. Biophys. Res. Commun.* 150: 899-903.
- Echevarria, C., Vidal, J., Le Marechal, P., et al. (1988). The phosphorylation of Sorghum leaf phosphoenolpyruvate carboxylase is a Ca^{++} -calmodulin dependent process. *Biochem. Biophys. Res. Commun.* 155: 835-840.
- Elliott, D. C., and Kokke, Y. S. (1987a). Cross-reaction of a plant protein kinase with antiserum raised against a sequence from bovine brain protein kinase C regulatory sub-unit. *Biochem. Biophys. Res. Commun.* 145: 1043-1047.
- Elliott, D. C., and Kokke, Y. S. (1987b). Partial purification and properties of a protein kinase C type enzyme from plants. *Phytochemistry* 26:2929-2935.
- Ertlinger, C., and Lehle, L. (1988). Auxin induces rapid changes in phosphatidylinositol metabolites. *Nature* 331: 176-178.
- Evans, M. L., Moore, R., and Hasenstein, K.-H. (1986). How roots respond to gravity. *Sci. Am.* 255: 112-119.
- Feldman, L. J. (1983). Light-enhanced protein synthesis in gravitropically stimulated root caps of corn. *Plant Physiol.* 72: 833-836.
- Feldman, L. J. (1985). Root gravitropism. *Physiol. Plant.* 65: 341-344.
- Firm, R. D., and Digby, J. (1980). The establishment of tropic curvatures in plants. *Ann. Rev. Plant Physiol.* 31: 131-148.
- Fordham-Skelton, A. P., Safadi, F., Golovkin M., and Reddy, A. S. N. (1994). A non-radioactive method for isolating complementary DNAs encoding calmodulin-binding proteins. *Plant Mol. Biol. Rep.* 12: 355-363.
- Fromm, H., and Chua, N.-H. (1992). Cloning of plant cDNAs encoding calmodulin-binding proteins using ^{35}S -labeled recombinant calmodulin as a probe. *Plant Mol. Biol. Rep.* 12: 199-206.
- Fujisawa, H. (1990). Calmodulin-dependent protein kinase II. *BioEssays* 12: 27-29.
- Gehring, C. A., Williams, D. A., Cody, S. H., and Parish, R. W. (1990). Phototropism and geotropism in maize coleoptiles are spatially correlated with increases in cytosolic free calcium. *Nature* 345: 528-530.
- Gilroy, S., and Trewavas, T. (1994). A decade of plant signals. *BioEssays* 16: 677-682.
- Gilroy, S., Hughes, W. A., and Trewavas, A. J. (1986). The measurement of intracellular calcium levels in protoplasts from higher plant cells. *FEBS Lett.* 199: 217-221.
- Goswami, K. K. A., and Audus, L. J. (1976). Distribution of calcium, potassium and phosphorus in *Helianthus annuus* hypocotyls and *Zea mays* coleoptiles in relation to tropic stimuli and curvatures. *Ann. Bot.* 40: 49-64.
- Halstead, T. W., and Dutcher, F. R. (1987). Plants in space. *Ann. Rev. Plant Physiol.* 38: 317-345.
- Harmon, A. C., Putnam-Evans, C., and Cormier, M. J. (1987). A calcium-dependent but calmodulin-independent protein kinase from soybean. *Plant Physiol.* 83: 830-837.
- Harper, J. F., Sussman, M. R., Schaller, G. E., et al. (1991). A calcium-dependent protein kinase with a regulatory domain similar to calmodulin. *Science* 252: 951-954.
- Hasunuma, K., Furukawa, K., Funadera, K., et al. (1987a). Partial characterization and light-induced regulation of GTP-binding proteins in Lemna paucicostata. *Photochem. Photobiol.* 46: 531-535.
- Hasunuma, K., Furukawa, K., Tomita, K., et al. (1987b). GTP-binding proteins in etiolated epicotyls of *Pisum sativum* (Alaska) seedlings. *Biochem. Biophys. Res. Commun.* 148: 133-139.
- Heim, S., and Wagner, K. G. (1986). Evidence of phosphorylated phosphatidylinositols in the growth cycle of suspension cultured plant cells. *Biochem. Biophys. Res. Commun.* 134: 1175-1181.

Heim, S., B
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- Heim, S., Bauleke, A., Wylegalla, C., and Wagner, K. G. (1987). Evidence of phosphatidylinositol and diacylglycerol kinases in suspension cultured plant cells. *Plant Sci.* 49: 159-165.
- Hepler, P. K., and Wayne, R. O. (1985). Calcium and plant development. *Ann. Rev. Plant Physiol.* 36:397-439.
- Hespler, J., DeGroot, P., Linskens, H. F., and Jackson, H. F. (1986). Phosphatidylinositol phospholipase C activity in pollen of *Lilium longiflorum*. *Phytochemistry.* 25:2053-2055.
- Hetherington, A. M., and Trewavas, A. (1984). Activation of a pea membrane protein kinase by calcium ions. *Planta* 161: 409-417.
- Konings, H. (1968). The significance of the root cap for geotropism. *Acta Bot. Neerl.* 17: 203-211.
- Lapetina, E. G., and Reep, B. R. (1987). Specific binding of [γ - 32 P]GTP to cytosolic and membrane-bound proteins of human platelets correlates with the activation of phospholipase C. *Proc. Natl. Acad. Sci. USA* 84: 2261-2265.
- Lee, J. S., Mulkey, T. J., and Evans, M. L. (1983a). Gravity-induced polar transport of calcium across root tips of maize. *Plant Physiol.* 73: 874-876.
- Lee, J. S., Mulkey, T. J., and Evans, M. L. (1983b). Reversible loss of gravitropic sensitivity in maize roots after tip application of calcium chelators. *Science* 220: 1375-1376.
- Ling, V. and Assmann, S. (1992). Cellular distribution of calmodulin and calmodulin-binding proteins in *Vicia faba* L. *Plant Physiol.* 100: 970-978.
- Litosch, C., Wallis, J. N., and Fain, J. N. (1985). 5-Hydroxytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary glands: Evidence for a role of GTP in coupling receptor activation to phosphoinositide breakdown. *J. Biol. Chem.* 260: 5464-5471.
- Lu, Y. T., and Feldman, L. J. (1993). Inhibitor effects of KN-93, and inhibitor of Ca^{2+} calmodulin-dependent protein kinase II, on light-regulated root gravitropism in maize. *Plant Physiol. Biochem.* 31: 857-862.
- McFadden, J. J., and Poovaiah, B. W. (1988). Rapid changes in protein phosphorylation associated with light-induced gravity perception in corn roots. *Plant Physiol.* 86: 332-334.
- McMurray, W. C., and Irvine, R. F. (1988). Phosphatidylinositol 4,5-bisphosphate phosphodiesterase in higher plants. *Biochemistry. J.* 249: 877-881.
- Melin, P.-M., Sommarin, M., Sandelius, A. S., and Jergil, B. (1987). Identification of calcium-stimulated polyphosphoinositide phospholipase C in isolated plant plasma membranes. *FEBS Lett.* 223: 87-91.
- Miyazaki, A., Kobayashi, K., Ishizaka, S., and Fujii, T. (1986). Redistribution of phosphorus, sulfur, potassium and calcium in relation to light-induced gravitropic curvature in *Zea* roots. *Plant Cell. Physiol.* 27: 693-700.
- Moore, R. (1985). Dimensions of root caps and columella tissues of primary roots of *Ricinus communis* characterized by differing degrees of gravi-responsiveness. *Ann. Bot.* 55: 375-380.
- Moore, R., and Evans, M. L. (1986). How roots perceive and respond to gravity. *Am. J. Bot.* 73: 574-587.
- Morre, D. J., Gripshover, B., Monroe, A., and Morre, J. T. (1984). Phosphatidylinositol turnover in isolated soybean membranes stimulated by the synthetic growth hormone 2,4-dichlorophenoxyacetic acid. *J. Biol. Chem.* 259: 15364-15368.
- Morse, M. J., Crain, R. C., and Satter, R. L. (1987a). Light-stimulated inositolphospholipid turnover in *Samanea saman* leaf pulvini. *Proc. Natl. Acad. Sci. USA* 84: 7075-7078.
- Morse, M. J., Crain, R. C., and Satter, R. L. (1987b). Phosphatidylinositol cycle metabolites in *Samanea saman* pulvini. *Plant Physiol.* 83: 640-646.
- Morse, M. J., Cote, G. G., Crain, R. C., and Satter, R. L. (1988). Light-modulated phosphatidylinositol turnover in *Samanea saman*. *Plant Physiol.* 86(Suppl.): 93.
- Muto, S., and Hirose, T. (1987). Inhibition of adventitious root growth in *Tradescantia* by calmodulin antagonists and calcium inhibitors. *Plant Cell Physiol.* 28: 1569-1574.
- Nishizuka, Y. (1986). Studies and perspectives of protein kinase C. *Science* 233: 305-312.
- Nishizuka, Y. (1988). The molecular heterogeneity of a protein kinase C and its implication for cellular regulation. *Nature* 334: 661-665.
- Oh, S-H, Steiner, H-Y, Douglall, D. K., and Roberts, D. M. (1992). Modulation of calmodulin levels, calmodulin methylation, and calmodulin-binding proteins during carrot cell growth and embryogenesis. *Arch. biochem. Biophys.* 297: 28-34.
- Olah, Z., and Kiss, Z. (1986). Occurrence of lipid and phorbol ester activated protein kinase in wheat cells. *FEBS Lett.* 195: 33-37.
- O'Neil, K. T., Wolfe, H. R., Jr., Erickson-Vitanen, S., and De Grado, W. F. (1987). Fluorescence properties of calmodulin-binding peptides reflect alpha-helical periodicity. *Science* 236: 1454-1456.
- Patil, S., Takezawa, D., and Poovaiah, B. W. (1995). Chimeric plant calcium/calmodulin-dependent protein kinase gene with a neural visinin-like calcium-binding domain. *Proc. Natl. Acad. Sci.* 92: 4897-4901.
- Perdue, D. O., and Leopold, A. C. (1988). Light regulation of gravitropism: Evidence for G-protein regulation of inositol trisphosphate release. *Plant Physiol.* 86(Suppl.): 68.
- Perdue, D. O., La Farve, A. K., and Leopold, A. C. (1988). Calcium in the regulation of gravitropism by light. *Plant Physiol.* 86: 1276-1280.
- Pfaffmann, H., Hartmann, E., Brightman, A. O., and Morre, D. J. (1987). Phosphatidylinositol specific phos-

- pholipase C of plant stems. Membrane associated activity concentrated in plasma membranes. *Plant Physiol.* 85: 1151-1155.
- Pickard, B. G. (1985a). Roles of hormones, protons, and calcium in geotropism. In *Encyclopedia of Plant Physiology: Hormonal regulation of Development III. Role of Environmental Factors, New Series, Vol II* (R. P. Pharis and D. M. Reid, Eds.), Springer-Verlag, Berlin, pp. 193-281.
- Pickard, B. G. (1985b). Early events in geotropism of seedling shoots. *Ann. Rev. Plant Physiol.* 36: 55-75.
- Pilet, P. -E. (1979). Growth inhibitors in growing and geo-stimulated maize roots. In *Plant Growth Regulation* (P. E. Pilet, Ed.), Springer-Berlin, Heidelberg, pp. 115-128.
- Pilet, P. -E., (1983). Control of root growth by endogenous IAA and ABA. In *Proceedings of British Plant Growth Regulator Group* (M. B. Jackson, A. D. Steed, Eds.), London, pp. 15-24.
- Polya, G. M., and Davies, J. R. (1982). Resolution of calcium-calmodulin-activated protein kinase from wheat-germ. *FEBS Lett.* 150: 167-171.
- Poovaiah, B. W., and Reddy, A. S. N. (1987). Calcium messenger system in plants. *CRC Crit. Rev. Plant Sci.* 6: 47-103.
- Poovaiah, B. W., and Reddy, A. S. N. (1990). Calcium and signal transduction: Involvement of calcium-dependent protein phosphorylation and turnover of inositol phospholipids. In *Second Messengers and Phosphoinositides in Plants* (J. Morre, Ed.), Alan R. Liss, New York, pp. 335-349.
- Poovaiah, B. W., and Reddy, A. S. N. (1993). Calcium and signal transduction in plants. *Crit. Rev. Plant Sci.* 12: 185-211.
- Poovaiah, B. W., McFadden, J. J., and Reddy, A. S. N. (1987a). The role of calcium ions in gravity signal perception and transduction. *Physiol. Plant.* 71: 401-407.
- Poovaiah, B. W., Reddy, A. S. N., and McFadden, J. J. (1987b). Calcium messenger system: Role of protein phosphorylation and inositol phospholipids. *Physiol. Plant.* 69: 569-573.
- Raghothama, K. G., Reddy, A. S. N., Friedmann, M., and Poovaiah, B. W. (1987). Calcium-regulated in vivo protein phosphorylation in *Zea mays* L. root tips. *Plant Physiol.* 83: 1008-1013.
- Ranjeva, R., and Boudet, A. M. (1987). Phosphorylation of proteins in plants: Regulatory effects and potential involvement in stimulus/response coupling. *Ann. Rev. Plant Physiol.* 38: 73-93.
- Ranjeva, R., Refeno, G., Boudet, A. M. and Marme, D. (1980). Activation of plant quinate: NAD⁺ 3-oxidoreductase by calcium and calmodulin. *Proc. Natl. Acad. Sci. USA* 80: 5222-5226.
- Rasmussen, H., and Barrett, P. Q. (1984). Calcium messenger system: an integrated view. *Physiol. Rev.* 64: 938-984.
- Reddy, A. S. N. (1994). Calcium as a messenger in stress signal transduction. In *Handbook of Plant and Crop Physiology* (M. Pessarakli, Ed.), Marcel Dekker, New York, pp. 719-732.
- Reddy, A. S. N., and Poovaiah, B. W. (1987). Inositol 1,4,5-trisphosphate induced calcium release from corn coleoptile membranes. *J. Biochem.* 101: 569-573.
- Reddy, A. S. N., Chengappa, S., and Poovaiah, B. W. (1987a). Auxin-regulated changes in protein phosphorylation in pea epicotyls. *Biochem. Biophys. Res. Commun.* 144: 944-950.
- Reddy, A. S. N., McFadden, J. J., Friedmann, M., and Poovaiah, B. W. (1987b). Signal transduction in plants: Evidence for the involvement of calcium and turnover of inositol phospholipids. *Biochem. Res. Commun.* 149: 334-339.
- Reddy, A. S. N., Takezawa, D., Fromm, H., and Poovaiah, B. W. (1993). Isolation and characterization of two cDNAs that encode for calmodulin-binding proteins from corn root tips. *Plant Sci.* 94: 109-117.
- Reddy, A. S. N., Safadi, F., Beyette, J. and Mykles, D. L. (1994). Calcium-dependent proteinase activity in root cultures of *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 199: 1089-1095.
- Roberts, D. M., Lukas, T. J., and Watterson, D. M. (1986). Structure, function, and mechanism of action of calmodulin. *CRC Crit. Rev. Plant Sci.* 86: 311-339.
- Roux, S. J., and Serlin, B. S. (1987). Cellular mechanisms controlling light-stimulated gravitropism: role of calcium. *CRC Crit. Rev. Plant Sci.* 5: 205-236.
- Roux, S. J., and Slocum, R. D. (1982). Role of calcium in mediating cellular functions important for growth and development in higher plants. In *Calcium and Cell Function, Vol. III.* Academic Press, New York, pp. 409-453.
- Sack, F. D., Suyamoto, M. M., and Leopold, A. C. (1985). Amyloplast stimulation kinetics in gravistimulated maize roots. *Planta* 165: 295-300.
- Sakai-Wada, A. and Shimako, Y. (1993). Ultrastructural studies on the Ca²⁺ localization in the dividing cells of the maize root tip. *Cell Struct. Func.* 18: 389-397.
- Salimath, B. P., and Marme, D. (1983). Protein phosphorylation and its regulation by calcium and calmodulin in membrane fractions from zucchini hypocotyls. *Planta* 158: 560-568.
- Sandelius, A. S., and Sommarin, M. (1986). Phosphorylation of phosphatidylinositols in isolated plant membranes. *FEBS Lett.* 201: 282-286.
- Schafer, A., Bygrave, F., Matzenauer, S., and Marme, D. (1985). Identification of a calcium- and phospholipid-dependent protein kinase in plant tissue. *FEBS Lett.* 187: 25-28.

Schumaker, K.
vesicles of
Scott, T. K. (197
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Ed.), *Acade*
Sikela, J. M., an
of a cDNA
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- Schumaker, K. S., and Sze, H. (1987). Inositol 1,4,5-trisphosphate releases Calcium from vacuolar membrane vesicles of oat roots. *J. Biol. Chem.* 262: 3944-3946.
- Scott, T. K. (1972). Auxins and roots. *Ann. Rev. Plant Physiol.* 23: 235-258.
- Sievers, A., and Hensel, W. (1982). The nature of graviperception. In *Plant Growth Substances* (P. F. Wareing, Ed.), Academic Press, London, pp. 479-506.
- Sikela, J. M., and Hahn, W. E. (1987). Screening an expression library with a ligand probe: Isolation and sequence of a cDNA corresponding to a brain calmodulin-binding protein. *Proc. Natl. Acad. Sci. USA* 84: 3038.
- Slocum, R. D., and Roux, S. J. (1983). Cellular and subcellular localization of calcium in gravistimulated coleoptiles and its possible significance in the establishment of tropic curvature. *Planta* 157: 481-492.
- Stinemetz, C. L., and Evans, M. L. (1986). The effect of calmodulin antagonists on the asymmetric redistribution of Ca²⁺ and IAA following gravistimulation. In *Proceedings of the American Society of Gravitational and Space Biology, 2nd Annual Symposium of the NASA Space Biology Program, Charlottesville, V. A., p. 9*
- Stinemetz, C. L., and Kuzmanoff, K. M., Evans, M. L., and Jarrett, H. W. (1987). Correlation between calmodulin activity and gravitropic sensitivity in primary roots of maize. *Plant Physiol.* 84: 1337-1342.
- Stirling, D. A., Welch, K. A., and Stark, M. J. R. (1994). Interaction with calmodulin is required for the function of Spc110p, an essential component of the yeast spindle pole body. *EMBO J.* 13: 4329-4342.
- Szuts, E. Z., Wood, S. F., Reid, M. S., and Fein, A. (1986). Light stimulates the rapid formation of inositol trisphosphate in squid retinas. *Biochem. J.* 240: 929-932.
- Takezawa, D., Liu, Z. H., An, G., and Poovaiah, B. W. (1995). Calmodulin gene family in potato: developmental and touch-induced expression of the mRNA encoding a novel isoform. *Plant Mol. Biol.* 27: 693-703.
- Torrey, J. G. (1976). Root hormones and plant growth. *Ann. Rev. Plant Physiol.* 27: 435-59.
- Veluthambi, K., and Poovaiah, B. W. (1984a). Calcium-promoted protein phosphorylation in plants. *Science* 223: 167-169.
- Veluthambi, K., and Poovaiah, B. W. (1984b). Calcium- and calmodulin-regulated phosphorylation of soluble and membrane proteins from corn coleoptiles. *Plant Physiol.* 76: 359-365.
- Veluthambi, K., and Poovaiah, B. W. (1986). In vitro and in vivo protein phosphorylation in *Avena sativa* L. coleoptiles: Effects of Ca²⁺, calmodulin antagonists and auxin. *Plant Physiol.* 81: 836-841.
- Widada, J. S., Asselin, J., Colote, S., et al. (1989). Cloning and deletion mutagenesis using direct protein interaction on an expression vector. *J. Mol. Biol.* 205: 455-458.
- Wilkins, M. B. (1979). Growth-control mechanisms in gravitropism. In *encyclopedia of Plant Physiology. New Series, Vol. 7* (W. Haupt and M. E. Feinleib, Eds.), Springer-Verlag, Berlin, pp. 601-626.
- Williamson, R. E. (1981). Free Ca²⁺ concentration in the cytoplasm: A regulator of plant cell function. *What's New Plant Physiol.* 12: 45-48.
- Williamson, R. E., and Ashley, C. C. (1982). Free Ca²⁺ and cytoplasmic streaming in the alga *Chara*. *Nature* 296: 647-651.
- Zbell, B., and Walter, C. (1987). About the search for the molecular action of high-affinity auxin-binding sites on membrane-localized rapid phosphoinositide metabolism in plant cells. In *Plant Hormone Receptors* (D. Klambt, Ed.), Springer-Verlag, Berlin, pp. 141-153.
- Zocchi, G., Rogers, S. A., and Hanson, J. B. (1983). Inhibition of proton pumping in corn roots is associated with increased phosphorylation of membrane proteins. *Plant Sci. Lett.* 31: 215-221.