J. Plant Biol. 40 (3) : 190~197 (1997)

This paper was presented at the 11th Symposium on Plant Biotechnology entitled "Plant Genes and Genetic Resources" organized by Gynheung An, held July 4-5, 1997, by the Botanical Society of Korea

Calcium-Mediated Signaling in Plants: Calmodulin and Ca^{2+}/Calmodulin-Dependent Protein Kinase

Poovaiah, B.W.[1,*], W. Wang[1], D. Takezawa[2], P.V. Sathyanarayanan, and G. An[3]

[1]*Laboratory of Plant Molecular Biology and Physiology, Department of Horticulture, Washington State University, Pullman, Washington 99164-6414, USA*

[2]*Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan*

[3]*Department of Life Sciences, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, Koera*

Plants contain multiple genes that code for calmodulin (Zielinski *et al.*, 1990; Perera and Zielinski, 1992; Reddy *et al.*, 1991; Poovaiah *et al.*, 1992; Botella and Arteca, 1994; Takezawa *et al.*, 1995). The role of these multiple genes in Ca^{2+}/calmodulin-mediated signal transduction is not clearly understood. The amino acid comparison of different calmodulin genes from potato, *Arabidopsis*, barley and chick are shown in Fig. 1. Studies have shown that several calmodulin and calmodulin-related genes are responsive to signals. Various physical and chemical signals have been shown to induce mRNAs corresponding to calmodulin and camodulin-related genes. Using a potato calmodulin cDNA as a probe, we have (Jena *et al.*, 1989) investigated the effect of auxin and light on calmodulin gene expression. For example, exposure of dark grown Merit corn root tips to light increased the calmodulin mRNA level (Jena *et al.*, 1989). Takezawa *et al.* (1995) tested the effect of touch stimulation. Lee *et al.* (1995) have isolated a calmodulin isoform from soybean that activates calmodulin-dependent enzymes in a differential manner. In *Arabidopsis*, Braam and Davis (1990) have shown that 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 was identified as a calmodulin and *TCH* 2 and *TCH* 3 were identified as calmodulin-related genes.

Fig. 1. Amino acid sequence comparisons of different calmodulin genes (PCM1, 5, 6 and 8) from potato with *Arabidopsis* CaM-2 (Ling *et al.*, 1991), barley CaM-1 (Ling and Zielinski, 1989), and chick calmodulin (Putkey *et al.*, 1983). The sequences in the fourth Ca^{2+}-binding region toward the C-terminus are shown. Asterisks indicate the position of amino acids involved in Ca^{2+}-binding (from Poovaiah *et al.*, J. Plant Physiol., 149: 553-558, 1996).

MANIPULATION OF CALMODULIN LEVELS IN TRANSGENIC PLANTS

The role of calmodulin in plant growth and development can be studied by overexpressing or blocking the expression of calmodulin. Transient overproduction of calmodulin levels in transformed mouse cells accelerated cell proliferation. Furthermore, decreased calmodulin levels by antisense RNA resulted in arrest of the cell cycle (Rasmussen and Means, 1989). To study the consequences of altered levels of calmodulin on plant growth and development, we produced independent transgenic potato plants carrying potato calmodulin cDNA (PCM-1) in sense or antisense orientation driven by the CaMV 35S and patatin promoters. These transgenic plants exhibited

*Corresponding author: Fax +1-509-335-8690
© 1997 by Botanical Society of Korea, Seoul

striking differences in growth and development including tuberization and their responsiveness to environmental signals (Poovaiah *et al.*, 1996). To study the regulation of PCM-1, transgenic potato plants carrying the PCM-1 promoter fused to the β-glucuronidase (GUS) reporter gene were produced. GUS expression was found to be developmentally regulated and touch responsive, indicating a correlation between the expression of PCM1 and GUS mRNAs (Takezawa *et al.*, 1985). Roberts *et al.* (1992) addressed the functional significance of calmodulin methylation by generating transgenic plants expressing normal (VU-1) and the methylation mutant (VU-3) calmodulins. VU-1 and VU-3 are identical except that VU-3 cannot be methylated. Introduction of these foreign calmodulins resulted in a two-fold increase in the calmodulin level, and the amount of foreign calmodulin was found to be equivalent to that of endogenous calmodulin. Transgenic plants experssing calmodulin the methylation mutant (VU-3) showed decreased stem internode, reduced seed and pollen viability, and reduced seed production. However, the transformants expressing VU-1 were found to be indistinguishable from control plants. The phenotypic differences in the transgenic plants containing VU-3 were attributed to mutant calmodulin.

Ca^{2+}/CALMODULIN-BINDING PROTEINS

Calmodulin, upon binding to Ca^{2+}, interacts with a number of enzymes and other proteins called calmodulin-binding proteins that play a key role in plant growth and development (Poovaiah and Reddy, 1987, 1993; Roberts and Harmon, 1992). A number of calmodulin-binding proteins have been isolated, characterized, and identified in animals (Bachs and Carafoli, 1987; Bachs *et al.*, 1990; Colbran and Soderling, 1990; Klee, 1991; Soderling, 1994; Perrino *et al.*, 1995). This information has increased our understanding of how Ca^{2+} and calmodulin regulate the various biochemical and molecular processes that eventually lead to a physiological response. The calmodulin-binding proteins that have been identified in plants include NAD kinase, Ca^{2+} ATPase, nuclear NTPases, and protein kinases (Poovaiah Reddy, 1993). Reddy *et al.* (1993) isolated and characterized two calmodulin-binding proteins from corn root tips, one of which is signal responsive. Lu and Harrington (1994) have also cloned cDNAs that encode calmodulin-binding proteins in tobacco, the expression of which is reduced by heat shock treatment. Calmodulin-binding proteins with sequence similarity to the *E. coli* enzyme, glutamate de-

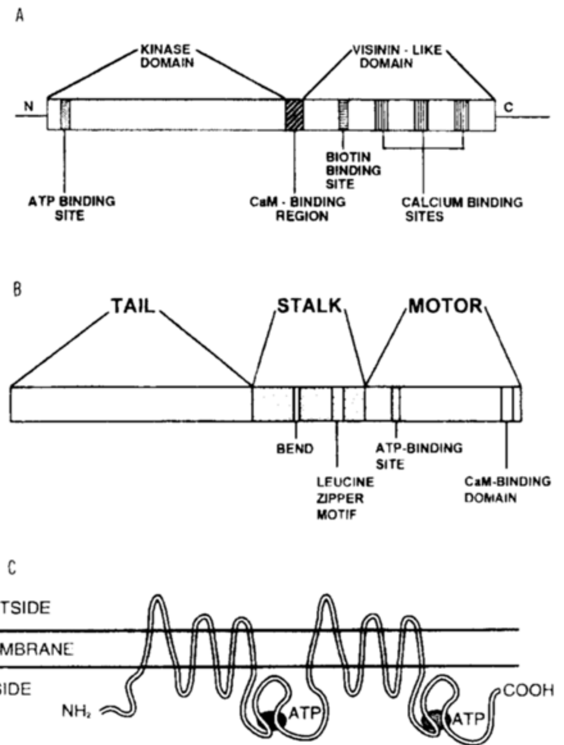

Fig. 2. Structural features of different calmodulin-binding proteins: calcium/calmodulin-dependent protein kinase (CCaMK) (A); calmodulin-binding kinesin-like protein (TCK1) (B); homolog of mammalian multidrug resistant P-glycoprotein (PMDR1) (C).

```
CCaMK     311  LIEPEVVSRLRSFNARRKLRAAAIASVLSS  340
               :   | |: |: |||||||::| ::::|::
CaMKIIα   281  MHRQETVDCLKKFNARRKLKGAILTTMLAT  310
```

Fig. 3. Comparison of amino acid sequences surrounding the CaM-binding sites of CCaMK and the α-subunit of CaMKII.

carboxylase (GAD) which converts glutamate to γ-aminobutyric acid (GABA) have been reported (Baum *et al.*, 1993; Ling *et al.*, 1994). GAD levels are known to change in response to environmental stresses. Calmodulin-dependent protein kinases with homology to mammalian CaM KII have been reported in plants (Poovaiah *et al.*, 1992; Watillon *et al.*, 1993). Recently, a chimeric Ca^{2+}/calmodulin-dependent protein kinase with a visinin-like Ca^{2+}-binding domain was colned and characterized in this laboratory (Patil *et al.*, 1995; Takezawa *et al.*, 1996a). The structural features of CCaMK are shown in Fig. 2A. A comparison of CCaMK and the α-subunit of CaMKII, is shown in Fig. 3. See the section on biochemical properties of CCaMK for addition details. Two other genes (*TCK1* and *PMDR1*) that encode for calmodu-

lin-binding proteins that are not kinases have also been cloned in our laboratory (Wang *et al.*, 1996a, b). The structural features of these three calmodulin-binding proteins are shown in Fig. 2.

TCK1: The *TCK1* cDNA encodes a protein with 1265 amino acid residues. Its structural features are very similar to those of known kinesin heavy chains and kinesin-like proteins from plants and animals, with one distinct exception. Unlike other known kinesin-like genes from plants and animals, TCK1 contains a novel calmodulin-binding domain which distinguishes it from all other known kinesin proteins (Wang *et al.*, 1996a). *E. coli*-expressed TCK1 binds calmodulin in a Ca^{2+}-dependent manner. In addition to the presence of a calmodulin-binding domain in the motor domain at the carboxyl-terminal, it also has a leucine zipper motif in the stalk region (Fig. 2B). The amino acid sequence at the carboxyl-terminal of TCK1 has striking homology with the mechanochemical motor domain of kinesins. The motor domain has ATPase activity that is stimulated by microtubules. Southern blot analysis revealed that *TCK1* is coded by a single gene. Expression studies indicated that *TCK1* is expressed in all of the tissues tested. Its expression was highest in the stigma and anther, especially during the early stages of anther development. Our results suggest that Ca^{2+}/calmodulin may an important role in the function of this microtubule-associated motor protein and may be involved in the regulation of microtubule-based intracellular transport.

PMDR1: A homology of the multidrug resistance (MDR) gene was obtained while screening a potato stolon tip cDNA expression library with ^{35}S-labeled calmodulin. The mammalian MDR gene codes for a membrane-bound P-glycoprotein (170-180 kDa) which imparts multidrug resistance to cancerous cells (Gottesman & Pastan, 1993). The potato cDNA (PMDR1) codes for a polypeptide of 1,313 amino acid residues (approximately 144 kDa) and its structural features are very similar to the MDR P-glycoprotein (Fig. 2C). The N-terminal half of the PMDR1 encoded protein shares striking homology with its C-terminal half, and each half contains a conserved ATP-binding site and six putative transmembrane domains (Wang *et al.*, 1996b). Southern blot analysis indicated that potato has one or two MDR-like genes. PMDR1 mRNA is constitutively expressed in all organs studies with higher expression in the stem and stolon tip.

Ca^{2+} AND Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN PHOSPHORYLATION

Protein phosphorylation is one of the major mechanisms by which eukaryotic cells transduce extracellular signals to intracellular responses (Cohen, 1992). Ca^{2+} and Ca^{2+}/CaM-dependent protein kinases are involved in amplifying and diversifying the action of Ca^{2+}-mediated signal (Veluthambi and Poovaiah, 1984; Edelman *et al.*, 1987; Colbran and Soderling, 1990; Schulman, 1993). In animals, Ca^{2+}/CaM-dependent protein kinases are known to play a pivotal role in cellular regulation (Nairn *et al.*, 1985; Colbran and soderling, 1990; Hanson and Schulman, 1992). Several types of CaM-dependent protein kinases (CaM kinases, phosphorylase kinase, and myosin light chain kinase) have been well characterized in mammalian systems (Fujisawa, 1990; Colbran and soderling, 1990; Klee, 1991; Mochizuki *et al.*, 1993). Although very little is known about Ca^{2+}/CaM-dependent protein kinases in plants (Poovaiah *et al.*, 1992; Watillon *et al.*, 1992, 1993), Ca^{2+}-dependent, but CaM-independent protein protein kinases (CDPKs) have been well documented (Harmon *et al.*, 1987; Harper, *et al.*, 1991; Roberts and Harmon, 1992; Roberts, 1993; Stone and Walker, 1995). Extracellular signals, either directly or through second messengers, regulate the activity of protein kinases which in turn regulate the acivity of their substrates by phosphorylation. Studies in animal systems indicate that CaM-dependent protein kinases are central to Ca^{2+}-mediated signal transduction pathways (Colbran and Soderling, 1990). Amplifcation and diversity in the action of some signals is achieved by phosphorylation and dephosphorylation of proteins (Cohen, 1985). Many key regulatory proteins undergo phosphorylation, resulting in conformational changes in these proteins eventually leading to altered biological properties.

The structure of CCaMK and its regulation independently by Ca^{2+} and Ca^{2+}/CaM makes it distinct from other kinases. The catalytic and CaM-binding domains of CCaMK have high homology to corresponding domains of CaMKII, a well characterized Ca^{2+}/CaM-dependent protein kinase in animals. CaM-dependent protein kinases are known to be maintained in an inactive state by the interaction of the catalytic region with an autoinhibitory domain located on the same polypetide (Colbran, 1993). Binding of Ca^{2+}/CaM relases the catalytic site of these kinases from the autoinhibitory domain. Removal of the autoinhibitory domain of rat brain CaMKII by deletion or truncation converts the enzyme to Ca^{2+}/CaM-independent form (Hagiwara *et al.*, 1991). This was further confirmed by creating a series of substitutions of CaMKII and measuring their Ca^{2+}/CaM-independent

activity (Cruzalegui *et al.*, 1992). CaMKII is known to autophosphorylate at Thr-286 upon binding Ca^{2+}/CaM, which further disrupts the interaction of the autoinhibitory region with the catalytic site. Afer CaM disassociates from an autophosphorylated CaMKII, the subunits modified by autophosphorylation remain partially active. Site-directed mutagenesis showed that a mutant CaMKII which has Ala-286 does not exhibit Ca^{2+}-independent activity after dissociation of CaM. Furthermore, replacement of Thr-286 with negatively charged amino acids mimics the effect of autophosphorylation (Fong *et al.*, 1989).

BIOCHEMICAL CHARACTERIZATION OF CCaMK

The biochemical characterization of this novel kinase (CCaMK) revealed that it is modulated by Ca^{2+} and Ca^{2+}/calmodulin (Takezawa *et al.*, 1996). CCaMK contains all eleven major conserved subdomains of the catalytic domain of serine/threonine kinases. Sequence comparisons revealed that CCaMK has high similarity to mammalian Ca^{2+}/CaM-dependent protein kinases, especially in the kinase and CaM-binding domains (amino acid residues 1-338). The CaM-binding region of CCaMK (FNARRKLRyAAAIASVL, residues 323-338) is similar to the CaM-binding domain (FNARRKLKGAILTTML, residues 293-309) of α-subunit of mammalian CaMKII. The sequence downstream of the CaM-binding region of CCaMK (amino acid residues 339-520) does not have significant similarity to known Ca^{2+}/CaM-dependent protein kinases. Further analysis of this region revealed the presence of three Ca^{2+}-binding EF-hand motifs that had high homology (52-54% similarity; 32-35% identity) to frequenin, neurocalcin, hippocalcin, and visinin-like neural Ca^{2+}-binding proteins. These proteins are members of a family of Ca^{2+}sensitive regulators, each containing three Ca^{2+}-binding EF-hand motifs. The structural features of CCaMK indicate that it is a chimeric Ca^{2+}-and Ca^{2+}/CaM-dependent protein kinase with two distinct regulatory domains; a CaM-binding domain and a visinin-like Ca^{2+}-binding domain. Fig. 4A and 4B show Ca^{2+}/calmodulin-dependent and independent activity of wildtype and mutant CCaMKs. We have observed a calmodulin isoform-specific effect on autophosphorylation and substrate phosphorylation (Liu *et al.*, unpublished data).

The mechanisms of CCaMK activation by calcium and calcium/calmodulin were investigated using various deletion mutants. The use of deletion mutants of CCaMK lacking either one, two, or all three calcium-

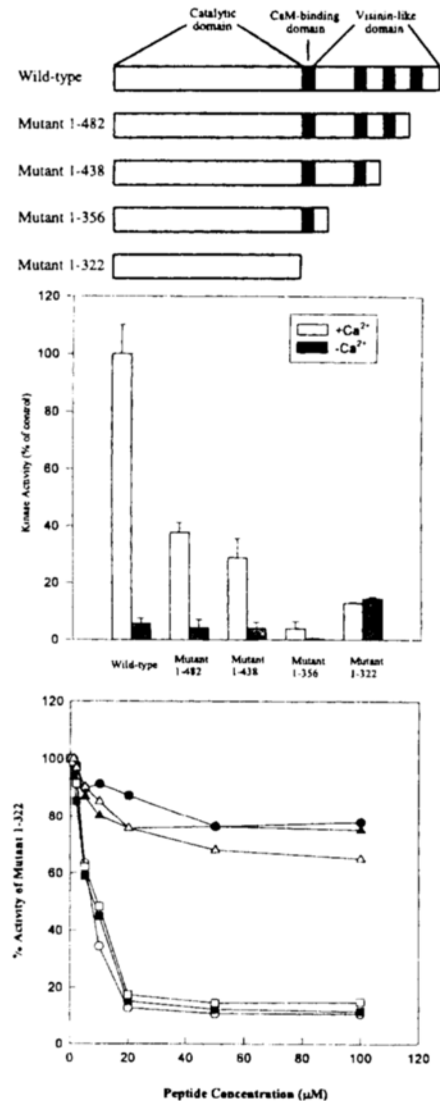

Fig. 4. A. Schematic diagrams of wildtype CCaMK and the deletion mutants. B. calcium/calmodulin-dependent and independent activity of wildtype and mutant CCaMKs. The kinase activity of CCaMK and its mutants were assayed in the presence of 0.5 mM $CaCl_2$ plus 1 μM calmodulin (open bars), or 2.5 mM EGTA (solid bars). The mean values and standard deviation were calculated from three independent experiments (from J. Biochem., Ramachandiran *et al.*, 121: 984-990, 1997). C. Effect of various synthetic autoinhibitory peptides on the activity of the constitutively active CCaMK mutant (1-322). Mutant CCaMK both the visinin-like domain and the calmodulin-binding domain (200 ng) was assayed in the presence of 0.5 mM calcium, 100 μM GS peptide and indicated concentrations of synthetic peptides corresponding to amino acids 328 to 340 (●) 322-340 (○), 317-340 (■) or 311-340 (□), 322-333 (▲) or 317-333 (△) for 2 min at 30°C under standard assay condition. The activity is represented as a percentage of control activity without the inhibitory peptides (from J. Biochem., Ramachandiran *et al.*, 121: 984-990, 1997).

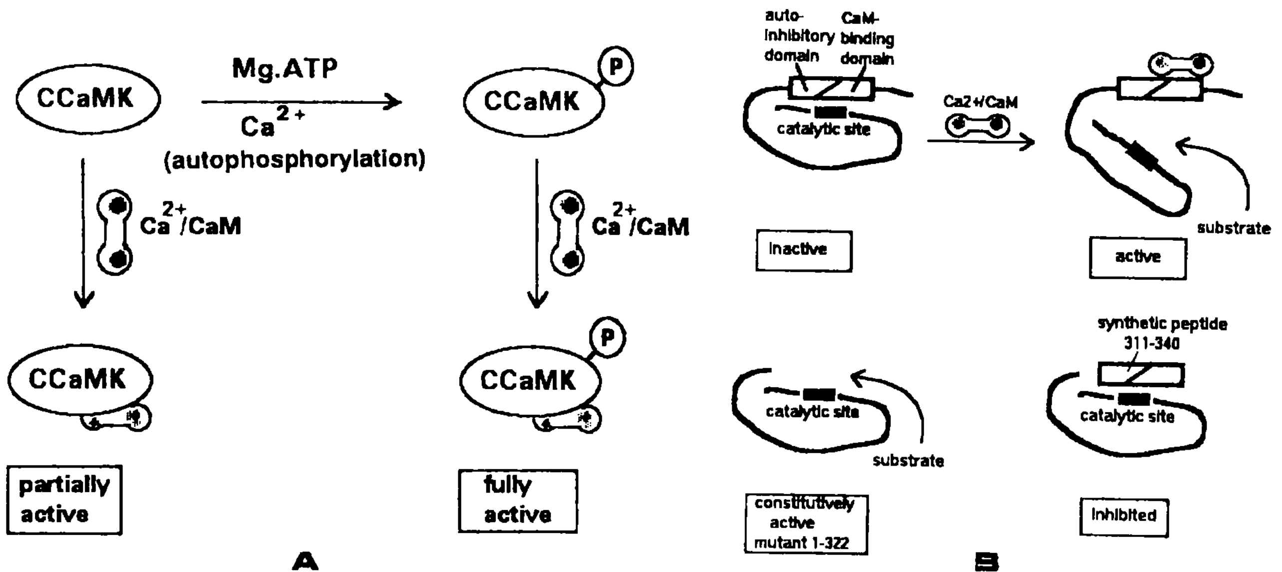

Fig. 5. Proposed model showing regulation of CCaMK by Ca^{2+} and Ca^{2+}/calmodulin (A), and autoinhibitory domain (B).

Fig. 6. A. Homology model of the kinase domain and calmodulin binding domain of CCaMK. CCaMK has a bilobate structure and the C-terminal CaM binding regulatory element is displayed in green. The five antiparallel beta sheets constitute the catalytic site and includes the ATP-binding region. The model was drawn using MOLSCRIPT (Kraulis, 1991). B. Model of CaMK I from X-ray crystallography (Goldberg *et al.*, 1996). C. Homology model of the visinin-like domain of CCaMK based on the NMR strucure of different calcium-binding proteins. The three EF hands are shown in orange.

binding EF-hands indicated that all three calcium-binding sites in the visinin-like domain were crucial for the full calcium/calmodulin-dependent kinase activity. As each calcium-binding EF hand was deleted, there was a gradual reduction in calcium/calmodulin-dependent kinase activity from 100% to 4%. Another mutant (amino acids 1-322) which lacks both the visinin-like domain containing three EF hands and the calmodulin-binding domain was constitutively acitive, indicating the presence of an autoinhibitory domain around the calmodulin-binding domain. By using various synthetic peptides and the constitutively active mutant, we have shown that CCaMK contains an autoinhibitory domain within the residues 322-340 which overlaps its calmodulin-binding domain (Fig. 4C). Kinetic studies with both ATP and the GS peptide substrate suggest that the autoinhibitory domain of CCaMK interacts only with the peptide substrate binding motif of the catalytic domain, but not with the ATP-binding motif (Ramachandiran *et al.*, 1997). Pro-

posed models for the regulation of CCaMK by Ca^{2+} and Ca^{2+}/calmodulin, and the autoinhibitory domain are shown in Fig. 5.

HOMOLOGY MODELING OF THE KINASE, CALMODULIN-BINDING, AND THE VISININ-LIKE DOMAINS OF CCaMK

The unique structural features and the structure/function relationships of CCaMK were studied by homology modeling. The homology model of the kinase and calmodulin-binding domain was built based on its homology with CaMK I. At the amino acid level CCaMK has 31% sequence identity and 68% similarity with CaMK I. Overall, the kinase domain and the calmodulin-binding domain of CCaMK are structurally similar to CaMK I (Fig. 6A-C). The initial results indicated that there was high conservation of these two kinases and the structure surrounding the catalytic core could be relatively plastic. The presence of the visinin-like domain with different structural features suggests a role as a calcium sensor element (antenna) in mediating kinase activity (Sathyanarayanan *et al.*, unpublished results).

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation Grant MCB 96-30337, and the National Aeronautics and Space Administration Grant NAG5-4841.

LITERATURE CITED

Bachs, O. and E. Carafoli. 1987. Calmodulin and calmodulin-binding proteins in liver cell nuclei. *J. Biol. Chem.* **262**: 10786-10790.

Bachs, O., L. Lanini, J. Serratosa and M.J. Coll. 1990. Calmodulin-binding proteins in the nuclei of quiescent and proliferatively activated rat liver cells. *J. Biol. Chem.* **265**: 18595-18600.

Baum, G., Y. Chen, T. Arazi, H. Takatsuji and H. Fromm. 1993. A plant glutamate decarboxylase containing a calmodulin binding domain. cloning, sequence and functional analysis. *J. Biol. Chem.* **268**: 19610-19617.

Bottella, J.R. and R.N. Arteca. 1994. Differential expressio of two calmodulin genes in response to physical and chemical stimuli. *Plant Mol. Biol.* **24**: 757-766.

Braam, J. and R.W. Davis. 1990. Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-reated genes in *Arabidopsis*. *Cell* **60**: 357-364.

Cohen, P. 1985. The role of protein phosphorylation in the hormonal control of enzyme activity. *Eur. J. biochem.* **151**: 439-448.

Colbran, R.J. and T.R. Soderling. 1990. Calcium/calmodulin-dependent protein kinase II. *Curr. Top. Cell Regul.* **31**: 181-221.

Colbran, R.J. 1993. Inactivation of of Ca^{2+}/camodulin-dependent protein kinase II by basal autophosphorylation. *J. Biol. Chem.* **268**: 7163-7170.

Cruzalegui, F.H., M.S. Kapiloff, J.-P. Morfin, B.E. Kemp, M.G. Rosenfeld and A.R. Means. 1992. Regulation of intrasteric inhibition of the multifunctional calcium/calmodulin-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **89**: 12127-12131.

Edelman, A.M., D.K. Bluementhal, E.G. Krebs. 1987. Protein serine/threonine kinases. *Ann. Rev. Biochem.* **56**: 567-613.

Fong, Y.-L., W.L. Taylor, A.R. Means and T.R. Soderling. 1989. Studies of the regulatory mechanism of Ca^{2+}/calmodulin-dependent protein kinase II. *J. Biol. Chem.* **264**: 16759-16763.

Fujisawa, H. 1990. Calmodulin-dependent protein kinase II. 1990. *BioEssays.* **12**: 27-29.

Goldberg, J., A. Nairn and J. Kuriyan. 1996. Structural basis for the autoinhibition of calcium/calmodulin-dependent protein kinase I. *Cell* **84**: 875-887.

Gottesman, M.M., and I. Pastan. 1993. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* **62**: 385-427.

Hanson, P.I. and H. Schulman. 1992. Neuronal Ca^{2+}/calmodulin-dependent protein kinases. *Annu. Rev. Biochem.* **61**: 559-601.

Harmon, A.C., C. Putnam-Evans, and M.J. Cormier. 1987. A calcium-dependent but calmodulin-independent protein kinase from soyben. *Plant Physiol.* **83**: 830-837.

Harper, J.F. M.R. Sussman, G.E. Schaller, C. Putnam-Evans, H. Charbonneau and A.C. Harmon. 1991. A calcium-dependent protein kinase with a regulatory domain similar to camodulin. *Science* **252**: 951-954.

Jena, P.K., A.S.N. Reddy and B.W. Poovaiah. 1989. Molecular cloning and sequencing of a cDNA for plant calmodulin: Signal-induced changes in the expression of calmodulin. *Proc. Natl. Acad. Sci. USA* **86**: 3644-3648.

Klee, C.B. 1991. Concerted regulation of protein phosphorylation and dephosphorylation by calmodulin. *Neurochem. Res.* **16**: 1059-1065.

Kraulis, P.J. 1991. MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. *J. Applied Crystallography.* **24**: 946-950.

Lee, S.H., J.C. Kim, M.S. Lee, W.D. Heo, H.Y. Seo, H. W. Yoon, J.C. Hong, S.Y. Lee, J.D. Bahk, I. Hwang and M.J. Cho. 1995. Identification of a novel divergent calmodulin isoform from soybean which has differential ability to activate calmodulin-dependent enzymes. *J. Biol. Chem.* **270**: 21806-21812.

Ling, V., I. Perera and R.E. Zielinski. 1991. Primary structures of *Arabidopsis* calmodulin isoforms deduced from the sequences of cDNA clones. *Plant Physiol.*

96: 1196-1202.

Ling, V., W.A. Sendden, B.J. Shelp and S.M. Assmann. 1994. Analysis of a soluble calmodulin binding protein from fava bean roots: Identification of glutamate decarboxylase as a calmodulin-activated enzyme. *Plant Cell* **6**: 1135-1142.

Ling, V. and R.E. Zielinski. 1989. Cloning of cDNA sequences encoding the calcium-binding protein, calmodulin, from barley (*Hordeum vulgare* L.). *Plant Physiol.* **90**: 714-719.

Lu, Y. and H.M. Harrington. 1994. Isolation of tobacco cDNA clones encoding calmodulin-binding proteins and characterization of a known calmodulin-binding domain. *Plant Physiol. Biochem.* **32**: 413-422.

Mochizuki, H., T. Ito and H. Hidaka. 1993. Purification and characterization of Ca^{2+}/calmodulin-dependent protein kinase V from rat cerebrum. *J. Biol. Chem.* **268**: 9143-9147.

Nairn, A.C., H.C. Hemmings, Jr. and P. Greengard. 1985. Protein kinases in the brain. *Annu. Rev. Biochem.* **54**: 931-976.

Patil, S., D. Takezawa and B.W. Poovaiah. 1985. Chimeric plant calcium/calmodulin-dependent protein kinase gene with a neural visinin-like calcium-binding domain. *Proc. Natl. Acad. Sci. USA* **92**: 4797-4801.

Perera, I.Y. and R.E. Zielinski. 1992. Structure and expression of the *Arabidopsis* CaM-3 calmodulin gene. *Plant Mol. Biol.* **19**: 649-664.

Perrino, B.A., L.Y. Ng and T.R. Soderling. 1995. Calcium regulation of calcineurin phosphatase activity by its β subunit and calmodulin: Role of the autoinhibitory domain. *J. Biol. Chem.* **270**: 340-346.

Poovaiah, B.W. and A.S.N. Reddy. 1987. Calcium messenger system in plants. *CRC Cri. Rev. Plant Sci.* **6**: 47-103.

Poovaiah, B.W. and A.S.N. Reddy. 1993. Calcium and Signal Transduction in plants. *Crit. Rev. Plant Sci.* **12(3)**: 185-211.

Poovaiah, B.W., A.S.N. Reddy, G. An, Y.J. Choi and Z. Q. Wang. 1992. Calmodulin gene expression and Ca^{2+}/calmodulin-dependent protein kinase II in plants. *In* Progress in Plant Growth Regulation. Karssen, C.M., VanLoon, L.C., and Vreugdenhil, D., Eds.. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 691-702.

Poovaiah, B.W., D. Takezawa, G. An, and T.-J. Han. 1996. Regulated expression of a calmodulin isoform alters growth and development in potato. *J. Plant Physiol.* **149**: 553-558.

Poovaiah, B.W., D. Takezawa, S. Ramachandiran, Z.H. Liu, s. Patil and V. Paranjape. 1996. An anter-specific Ca^{2+}/calmodulin-dependent protein kinase with a novel visinin-like Ca^{2+}-binding domain. *In* Current Topics in Plant biochemistry, Physiology, and Molecular Biology, Phosphorylation-Dephosphorylation of Plant Proteins, University of Missouri, Columbia. p. 16.

Putkey, J.A., K.F. Ts'us, T. Tanaka, L. Lagace, J.P. Stein, E.C. Lai and A.R. Means. 1983. Chicken calmodulin genes: a species comparison of cDNA sequences and isolation of a genomic clone. *J. Biol. Chem.* **258**: 11864-11870.

Ramachandiran, S., D. Takezawa, W. Wang and B.W. Poovaiah. 1997. Functional domains of plant chimeric calcium/calmodulin-dependent protein kinase: Regulation by autoinhibitory and visinin-like domains. *J. Biochem.* **121**: 984-990.

Rasmussen, C.D. and A.R. Means. 1989. Calmodulin is required for cell-cycle progression during G_1 and mitosis. *EMBO J* **8**: 73-82.

Reddy, A.S.N., D. Takezawa, H. Fromm and B.W. Poovaiah. 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., Z.Q. Wang, Y.J. Choi, G. An, A.J. Czernik and B.W. Poovaiah. 1991. Calmodulin gene expression and calcium/calmodulin dependent protein kinase II in plants. *In* Proc. Cold Spring Harbor Symp. Plant Signal transduction, Cold Spring Harbor Laboratory, Cold Spring harbor, NY.

Roberts, D.M. and A.C. Harmon. 1992. Calcium-modulated proteins: Targets of intracellular calcium signals in higher plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43**: 375-414.

Roberts, D.M. 1993. Protein kinases with calmodulin-lkie domains: novel targets of calcium signals in plants. *Curr. Opin. Cell Biol.* **5**: 242-246.

Schulman, H. 1993. The multifunctional Ca^{2+}/calmodulin-dependent protein kinases. *Curr. Opin. Cell Biol.* **5**: 247-253.

Soderling, T.R. 1994. Calcium-dependent protein kinases in learning and memory. *Advances in second messengers and phosphoprotein research.* **30**: 175-189.

Stone, J.M. and J.C. Walker. 1995. Plant protein kinase families and signal transduction. *Plant Physiol.* **108**: 451-457.

Takezawa, D., Z.H. Liu, G. An and B.W. Poovaiah. 1995. Calmodulin gene family in pototo: developmental and touch-induced expression of the mRNA encoding a novel isoform. *Plant Mol. Biol.* **27**: 693-703.

Takezawa, D., S. Ramachandiran, V. Paranjape and B. W. Poovaiah. 1996. Dual regulation of a chimeric plant serine/threonine kinase by calcium and calcium/calmodulin. *J. Biol. Chem.* **271**: 8126-8132.

Veluthambi, K. and B.W. Poovaiah. 1984. Calcium-promoted protein phosphorylation in plants. *Science* **223**: 167-169.

Wang, W. D. Takezawa, S.B. Narasimhulu, A.S.N. Reddy and B.W. Poovaiah, B.W. 1996a. A novel kinesin-like protein with a calmodulin-binding domain. *Plant Mol. Biol.* **31**: 87-100.

Wang, W., D. Takezawa and B.W. Poovaiah. 1996b. A potato cDNA encoding a homologue of mammalian multidrug resistant P-glycoprotein. *Plant Mol. Biol.* **31**: 683-687.

Watillon, B., R. Kettmann, Ph. Boxus, and A. Burny. 1992. Cloning and characterization of an apple (*Malus domestica* L. Borkh) cDNA encoding a calmodulin-binding protein domain similar to the calmodulin-bind-

ing region of type II mammalian Ca^{2+}/calmodulin-dependent protein kinase. *Plant Sci.* **81**: 227-235.

Watillon, B., R. Kettmann, Ph. Boxus and A. Burny. 1993. A calcium/calmoduling-bindign serine/threonine protein kinase homologous to the mammalian type II calcium/calmodulin-dependent protein kinase is expressed in plant cells. *Plant Physiol.* **101**: 1381-1384.

Zielinski, R.E., V. Ling and I. Perera. 1990. Structure and expression of genes encoding calcium-modulated proteins in higher plants. *In* Current Topics in Plant biochemistry and Physiology, Vol. 9, Plant Protein Phosphorylation, Protein Kinases, Calcium and Calmodulin, Randall, D.D. and Belvin, D.G., Eds., University of Missouri, Columbia, p. 141.