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Ca^{2+}/Calmodulin-Dependent NAD Kinase and Glutamate Decarboxylase: Their Roles in Plant Responses to The Environment

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Plant cells encounter a variety of environmental stress conditions which result in fluxes in cytosolic calcium. Calcium plays a key role as a second messenger in plants and is involved in physiological responses to many environmental stimuli. The biochemical targets of calcium signals are a set of structurally related calcium-modulated proteins. Calmodulin is a calcium-modulated protein and interacts with many enzymes and proteins. Plants have been shown to contain Ca^{2+}/calmodulin-dependent NAD kinase and glutamate decarboxylase. Recent evidence indicates that Ca^{2+}/calmodulin-dependent plant NAD kinase and glutamate decarboxylase are involved in responses to a variety of environmental stimuli by producing active oxygen species and gamma-aminobutyric acid, respectively. In addition, the transgenic tobacco plants expressing calmodulin or calmodulin-dependent enzyme raise the possibility of generation of crop plants more resistant to the environmental stresses.

Calcium and Calmodulin

Calcium plays a key role as a signal molecule in the regulation of many cellular activities in eukaryotes (Hepler and Wayne, 1985: Rasmussen, 1989, 1990; Berridge and Irvine, 1989; Berridge, 1990). In animals, calcium is believed to be involved in the regulation of muscle contraction and relaxation, mitotic events, egg fertilization, neurotransmitter release, and many others (Rasmussen, 1990; Berridge, 1990). In plants, calcium has been implicated in the regulation of a variety of growth and developmental processes such as cell motillty, chromosome motion, cell proliferation, and germination (Hepler and Wayne, 1985; Hepler, 1990; Roberts and Harmon, 1992). Calcium is a second messenger in plant signal transduction and is involved in the physiological responses to a variety of environmental stimuli (Snedden et al., 1995). The targets of calcium signals are calcium-modulated proteins. All of these calcium-modulated proteins have a common helix-loop-helix motif known as the EF-hand. These structures allow high affinity calcium binding and undergo a change in activity that is related to a calcium-induced change in conformation (Strynadka and James, 1989; Roberts and Harmon, 1992).

Since the first detection of calmodulin from bovine brain as an activator of phosphodiesterase (Kakiuchi et al., 1970: Cheung, 1970), calmodulin has been found widely distributed in eukaryotes ranging from fungi to humans. A number of calmodulin target proteins have been identified (Klee and Vanaman, 1982; Manalan and Klee, 1984; Roberts et al., 1986; Cohen and Klee, 1988; Roberts and Harmon, 1992). Therefore, it is believed that calmodulin, which itself has no intrinsic enzymatic activity, mediates its cellular regulation through interaction with target enzymes or other nonenzymic proteins. Thus far, over 25 calmodulin-dependent enzymes and calmodulin-binding proteins in animals have been identified and characterized (Cohen and Klee, 1988; Rhoads and Friedberg, 1997). Several calmodulin-dependent enzymes and calmodulin-binding proteins have been detected in plant systems. Among the groups of calmodulin-dependent enzymes detected in plants are NAD kinase, glutamate decarboxylase, Ca^{2+}-ATPase and a nuclear nucleoside triphosphatase (Roberts and Harmon, 1992; Snedden and Fromm, 1998). Among other groups of calmodulin-binding proteins identified in plants are 30,000 to 33,000 M_r protein from pea and spinach (Roberts et al., 1983), and 13,000 M_r protein from germinating radish seed embryos (Cocucci and Negrini, 1988). Additionally, Brawley and Roberts (1989) identified 45,000 M_r and 72,000 M_r calmodulin-binding proteins that are found predominantly in sperm and zygotes, respectively, of the marine brown algae *Fucus vesiculosus*. Interestingly, the 72,000 M_r pro-

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tein was not detectable in the unfertilized egg and started to appear 1 hr postfertilization and maintained during early embryogenesis. Thus, there is precedent for developmental regulation of calmodulin target proteins. However, precise roles for calmodulin and the calmodulin-binding proteins in the systems are unknown. To understand the mechanism by which calmodulin and calmodulin target proteins exert their roles in plant systems, it is necessary to identify the activities of these calmodulin receptor proteins.

Comparison of the amino acid sequences between vertebrate and plant calmodulins shows over 90% sequence identity (Roberts and Harmon, 1992). An interesting finding is that one organism can have more than one type of calmodulin (Roberts and Harmon, 1992; Snedden and Fromm, 1998). For example, calmodulin genes encoding calmodulin isoforms and calmodulin-like proteins have been detected and sequenced from *Arabidopsis* and barley (Braam and Davis, 1990; Zielinski et al., 1990; Ling et al., 1991; Perera et al., 1991). The deduced amino acid sequences of the two cDNAs of two calmodulin isoforms from *Arabidopsis* leaf showed 97% sequence homology (Ling et al., 1991). These two calmodulin isoforms differ by four amino acid substitutions which do not occur within the four calcium-binding domains. Genomic Southern blot assays revealed that they are derived from two different genes. The reason for multiple isotypes is unknown, but the expression of the genes may be differently controlled to meet cell requirements. Braam and Davis (1990) detected and sequenced three touch-induced (TCH) cDNAs that encode calcium modulated proteins with homology to calmodulin in *Arabidopsis*. TCH 1 cDNA encodes *Arabidopsis* calmodulin 2 (Ling et al., 1991). TCH 2 and TCH 3 also encode proteins with 44% and 70% amino acid sequence identities to calmodulin, respectively. The finding showing the existence of calmodulin isoforms and calmodulin-like proteins within the same organism may suggest that they have different roles in the regulation of cellular processes. In this regard, it is of interest to note that soybean calmodulin isoform, SCaM-4 which differs at 32 amino acid residues from soybean calmodulin SCaM-1, was unable to activate pea NAD kinase. However, the SCaM-4 was able, like SCaM-1, to activate mammalian 3',5'-cyclic nucleotide phosphodiesterase (Lee et al., 1997).

In plants, it has been shown that the levels of calmodulin also vary from tissue to tissue depending on the developmental stages (Muto and Miyachi, 1984; Allan and Trewavas, 1985; Zielinski, 1987). In general, higher amounts of calmodulin have been found from young meristematic tissues compared with mature tissues. Analyses of the germinated embryos (Cocucci and Negrini, 1988; Hernandez-Nistal, 1989; Oh et al., 1992) show that the levels of calmodulin can vary depending on the state of plant development. The germinated embryos have significantly increased calmodulin levels compared with undifferentiated cells or non-germinated embryos. The developmentally different distribution between tissues may reflect a close relationship between calmodulin level and plant cell growth and development. In this regard it is important to note that even minor changes in calmodulin levels can drastically influence cell growth (Rasmussen and Means, 1987, 1989).

Ca^{2+}/calmodulin-dependent NAD Kinase

Since the first demonstration of plant NAD kinase activation by Ca^{2+}/calmodulin (Muto and Miyachi, 1977; Anderson and Cormier, 1978), Ca^{2+}/calmodulin-dependent NAD kinases have been found in many plant tissues (Roberts and Harmon, 1992; Oh and Yun, 1999), in sea urchin eggs (Epel et al., 1981), and in human neutrophils (Williams and Jones, 1985). Among these enzymes, pea NAD kinase is well characterized. Roberts et al. (1990) obtained a purified enzyme from pea seedlings with apparent molecular weight ranging from 50,000 to 55,000 based on SDS-PAGE analysis and molecular exclusion chromatography. The purified enzyme is completely dependent upon the presence of added calcium and calmodulin. The enzyme is particularly sensitive to the state of posttranslational calmodulin methylation (see below for discussion).

NAD kinase catalyzes the phosphorylation of NAD to NADP using ATP as a co-substrate (McGuinness and Butler, 1985). NAD and NADP are utilized in different metabolic pathways (Lowenstein, 1961; Roberts et al., 1990). NAD(H) is mainly used in catabolic reactions for energy production. NADP(H) is mainly used in biosynthetic reactions and in certain catabolic pathways such as the pentose phosphate pathway. Availability of NADP is the rate limiting step for certain pathways including the pentose phosphate pathway (Yamamoto, 1963). Therefore, calcium fluxes and the Ca^{2+}/calmodulin-dependent NAD kinase could be important in triggering a rapid shift of metabolism in response to external signals by modulating NAD/NADP ratios.

Epel (1964, 1981) observed that there are increases of free Ca^{2+} and a burst of NADPH after fertilization of sea urchin eggs. Ca^{2+}/calmodulin-dependent NAD kinase was proposed to be responsible for this increase (Epel et al., 1981). The kinase was proposed to be necessary to provide cellular NADP(H) for reductive biosynthetic processes or for protection of the egg from oxidative stress (Epel, 1964; Epel et al., 1981; Shapiro, 1991). Williams and Jones (1985) found another Ca^{2+}/calmodulin-dependent NAD kinase from human neutrophils. NADPH is utilized by NADPH oxidase in the production of superoxide for bactericidal activity in the neutrophils (Baggiolini and Wymann,

1990). Therefore, the elevation of NADP by calmodulin-dependent NAD kinase could be necessary to provide a rapid increase in substrate NADPH for reductive processes in these cells.

It is becoming apparent that similarities exist between the oxidative burst reactions in animal and plant defense responses. NADPH oxidase activities have been also detected in plants and are elevated in response to stress and elicitor treatment (Mehdy, 1994; Auh and Murphy, 1995). In addition, antibodies against the neutrophil oxidase subunits cross-reacted with similar proteins in plant extracts (Dwyer et al., 1996). In plants, oxidative burst reaction that generates active oxygen species (AOS) such as superoxide and H_2O_2 is proposed to be involved in several responses to pathogen and environmental stresses (Mehdy, 1994). Among the responses are cell wall lignification, cross-linking of cell wall proteins and the expression of defence response genes (Harding et al., 1997). Plant NAD kinase is widely distributed in plants (Roberts and Harmon, 1992). Recently, its potential role during the oxidative burst response in plant has been evaluated. We found that transgenic tobacco plants and cells expressing a mutant calmodulin that is incapable of being methylated at a specific amino acid residue show an enhanced ability to produce H_2O_2 in response to various stimuli such as harpin, incompatible bacteria and mechanical stresses (Harding et al., 1997). This response was paralleled by an enhanced basal level of NADPH as well as a more rapid and higher accumulation of NADPH when challenged with a stimulus. These data show that calmodulin is a target of calcium fluxes in response to elicitor or environmental stress. Also the data provide the first evidence that plant NAD kinase may be a downstream target which potentiates AOS production by altering NAD(H)/NADP(H) homeostasis (Harding et al., 1997; see below for further discussion).

Significance of Calmodulin Methylation to NAD Kinase Regulation

ε-N-trimethyllysine is a posttranslational modification that is found at position 115 of many calmodulins (Roberts et al., 1986). This site is methylated by a calmodulin: lysine N-methyltransferase (Morino et al., 1987; Oh and Roberts, 1990). Evidence from plants and plant cells shows that the methylation state of calmodulin varies depending on the growth and developmental state of the cells (Oh and Roberts, 1990; Oh et al., 1992). Thus, unmethylated calmodulin can exist in an undegraded state *in vivo*. With respect to the regulatory activities of calmodulin, unmethylated calmodulins are not significantly different from methylated calmodulins in their ability to bind calcium and activate a number of enzymes (Robert et al., 1986). However, one calmodulin-dependent enzyme, plant NAD kinase, is sensitive to calmodulin methylation (Roberts et al., 1990; Oh and Yun, 1999). For example, NAD kinase purified from pea has a specific activity of 18 μmole NADP/min/mg protein with saturating unmethylated calmodulin in the presence of calcium and saturating levels of NAD and ATP (Roberts et al., 1990). However, the specific activity of the enzyme measured with methylated calmodulin was 2 to 4 μmole NADP/min/mg protein under the same conditions. These results raise the possibility that selective calmodulin activator activities could be attenuated by posttranslational methylation of lysine-115. For example, the methylation of calmodulin could result in an overall reduction in NAD kinase activation whereas other regulatory targets may not be similarly affected. To investigate the functional significance of lysine methylation in the control of nicotinamide coenzyme metabolism and to elucidate specific molecular targets of calcium and calmodulin in plant defence responses, we have generated transgenic tobacco plants expressing a dominant-acting calmodulin mutant (VU-3 calmodulin, lys → arg 115). VU-3 calmodulin differs from endogenous plant calmodulin in that it can not be methylated. As a result it activate NAD kinase to an activity that is at least 3-fold higher than trimethylated calmodulin (Roberts et al., 1992). Analyses of leaf tissues of transgenic VU-3 plants showed 4-fold higher levels of NADPH and 2-fold higher levels of H_2O_2 than normal control plants (Harding et al., 1997). In addition, cells expressing VU-3 calmodulin showed a stronger active oxygen burst that occurred more rapidly than in normal control cells challenged with various stimuli. For example, the purified elicitor protein harpin from *Pseudomonas syringae* pathovar 61 (He et al., 1993) showed a faster rate and a quicker onset of AOS release in VU-3 cells than normal control cells (Harding et al., 1997). VU-3 cells also showed a more enhanced response upon actual infection by *Pseudomonas syringae* pathovar 61 (Harding et al., 1997). Seeds from the VU-3 transgenic tobacco plants are much less susceptible to fungal contamination than seeds from normal tobacco plants (Oh et al., unpublished data). These observations support a model of AOS production in relation to pathogen infection (Fig. 1).

Ca^{2+}/calmodulin-dependent Glutamate Decarboxylase

Glutamate decarboxylase (GAD) catalyzes the conversion of glutamate to γ-aminobutyric acid (GABA). The presence of GAD activity and GABA in plants has been known for at least half a century (Styranarayan and Nair, 1990). The role of GABA in plants is still obscure, whereas its involvement as an inhibitory neurotransmitter in animals is well understood (Bown and Shelp, 1989; Styranarayan and Nair, 1990).

There is a considerable literature demonstrating that

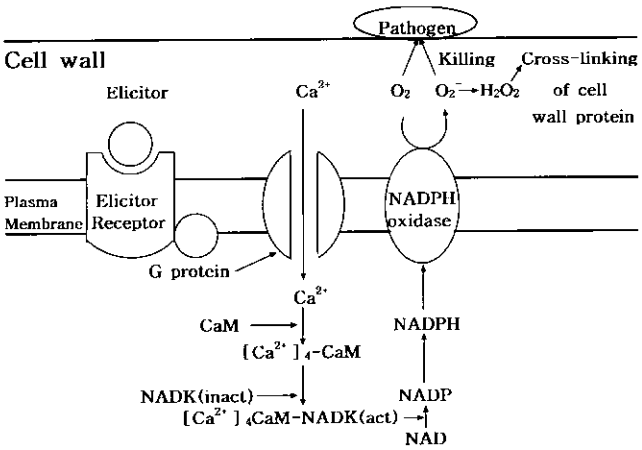

Fig. 1. Model of AOS production in relation to pathogen infection. Plant cells release AOS following elicitor treatment. Membrane associated oxidases such as NADPH oxidase have been proposed to be responsible for the production of AOS in response to the stimulus. Calcium fluxes can aid in the production of reductant for this reaction through the stimulation of Ca^{2+}/calmodulin-dependent NAD kinase. This model is modified from the model (Mehdy et al., 1994) based on our recent findings (Harding et al., 1997). CaM, calmodulin.

GABA is accumulated rapidly and largely in a variety of plant tissues under several environmental stress conditions such as mechanical stimulation, damage, cold shock, heat shock, hypoxia, cytosolic acidification, darkness, water stress, phytohormones, and drought stress (Bown and shelp, 1997; Serraj et al., 1998). It has been suggested that GABA is part of an adaptive response to cytosolic acidosis (Crawford et al., 1994). However reduction of cytosolic pH does not seem to be a prerequisite for stimulation of GABA synthesis (Crawford et al., 1994). This suggests that other factors except cytosolic pH are involved in the activation of GAD in plant cells. Interestingly, many of the same stresses that induce GABA production in plants also cause increases in cytosolic Ca^{2+} levels (Knight et al., 1991). Transient elevations in cytosolic Ca^{2+} levels are transmitted through Ca^{2+}-modulated proteins such as calmodulin. For the first time, Ca^{2+}-stimulated GAD activity was observed by screening a cDNA expression library from petunia with ^{35}S-labeled calmodulin (Baum et al., 1993). A cDNA coding for a Ca^{2+}-dependent calmodulin-binding protein was isolated and the recombinant protein showed GAD activity (Baum et al., 1993).

Although several forms of GAD from a variety of sources have been described (Erlander and Tobin, 1991; Bao et al., 1995), only plant GAD showed Ca^{2+}/calmodulin-dependent activation (Ling et al., 1994; Snedden et al., 1995; Oh and Yun, 1999). The activity assays of partially purified GAD from faba bean roots showed a 50% stimulation by the addition of 100 μM Ca^{2+}, a 100% stimulation by the addition of 100 μM Ca^{2+} plus 100 nM calmodulin, and no appreciable stimulation by calmodulin in the absence of added Ca^{2+} (Ling et al., 1994). GAD partially purified from various soybean tissues was stimulated 2- to 8-fold in the presence of Ca^{2+}/calmodulin at pH 7.0 (Snedden et al., 1995). GAD partially purified from tobacco plants showed 3.8-fold activation by Ca^{2+}/calmodulin (Oh and Yun, 1999). The differences in the level of Ca^{2+}/calmodulin activation of GAD from different tissues and sources may reflect different degrees of contamination by calmodulin or proteolysis of GAD or multiple forms of GAD (Snedden et al., 1995; Baum et al., 1996; Zik et al., 1998).

In order to test these, we expressed a cloned tobacco GAD gene in *E. coli*, which lacks calmodulin (Yun and Oh, 1998). The gene-encoded 56-kD protein interacted strongly with a monoclonal antibody against the petunia GAD and showed almost complete Ca^{2+}/calmodulin dependency for activity (Yun and Oh, 1998; Oh and Yun, 1999). When the GAD activity of the tobacco gene-encoded protein was assayed at pH 7.0 without the addition of calcium and calmodulin, the activity was less than 1.0% of that measured with the addition of 2.5 mM Ca^{2+} and 200 nM calmodulin. In addition, the activity was not stimulated by the addition of 2.5 mM Ca^{2+} or 200 nM calmodulin alone. Petunia recombinant GAD was also inactive in the absence of Ca^{2+} and calmodulin. But it was stimulated to high levels of activity by the addition of exogenous calmodulin in the presence of calcium at pH 7.0-7.5 (Snedden et al., 1996). In addition, a monoclonal antibody directed against the carboxyl-terminal region, which contains the calmodulin-binding domain of GAD, was able to fully activate in a dose-dependent manner in the absence of calcium and calmodulin. However, an antibody recognizing an epitope outside of this region was unable to activate GAD (Snedden et al., 1996). These data indicate that plant GAD is a Ca^{2+}/calmodulin-dependent enzyme and Ca^{2+}/calmodulin or antibody binding to the domain of GAD can remove an autoinhibitory function of the region and activates GAD.

Roles of GAD and GABA in Plants

GAD activity and GABA have been detected in all plant tissues analyzed (Styranarayan and Nair, 1990). Plant GAD and GABA levels are developmentally regulated (Kajimura et al., 1991; Chen et al., 1994). For example, developmental changes in the abundance of GAD mRNA and the 58-kD GAD were observed in petunia flowers and leaves and during seed germination (Chen et al., 1994). Transgenic tobacco plant expressing a mutant petunia GAD lacking the calmodulin-binding domain exhibits extremely high GABA levels, low Glu levels, and severe morphological abmormalities such as short stems (Baum et al., 1996). The GAD activity

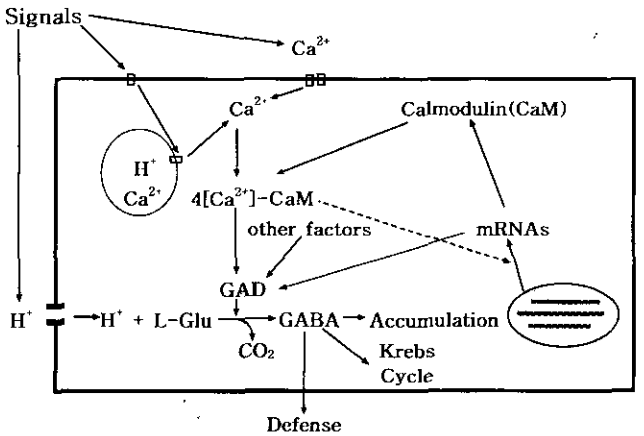

Fig. 2. Model of GABA production in plants. Plant cells produce GABA following environmental stimuli. Calcium fluxes can aid in the production of GABA through the stimulation of Ca^{2+}/calmodulin-dependent GAD. This model is modified from the model of Snedden et al. (1995).

in extracts of the plants was found to be Ca^{2+}-independent (Baum et al., 1996). However, GAD plants which expressed the full-length petunia GAD showed higher GABA levels and lower Glu levels than those of wild-type plants and Ca^{2+}-dependent activation of GAD activity, but indistinguishable morphology from wild-type (Baum et al., 1996). These data demonstrate that regulation of GAD activity by Ca^{2+} and calmodulin is necessary for normal plant growth and development.

GAD activity and GABA levels are known to be rapidly enhanced by a variety of environmental stresses such as anaerobiosis, mechanical shock, cold shock, darkness, heat shock, water stress and drought stress (Bown and Shelp, 1997; Serraj et al., 1998) (Fig. 2). As a widely distributed and highly accumulated nonprotein plant amino acid, GABA has been postulated to have roles in nitrogen metabolism and storage, and in the plant's defence against phytophagous insects. High concentrations of GABA are found in nitrogen-fixing nodules of a number of legumes (Bown and Shelp, 1997). GABA concentration in the nodules of soybean was significantly higher than that in either roots or leaves (Serraj et al., 1998). Housley et al. (1979) reported that higher concentrations of GABA were found in nodulated plants than in non-nodulated ones. Also higher levels of calmodulin were found in nodule extracts than in root extracts (Oh, 1992; Ling et al., 1994). It was also shown that GABA in nutrient solutions can function as a sole nitrogen source for the growth of isolated roots of *Pinus serotina* (Barnes and Naylor, 1959).

In animals, GABA functions as a major inhibitory neurotransmitter by modulating the conductance of ion channels (Zhang and Jackson, 1993). Initially, Wallace et al. (1984) proposed that GABA accumulation may be a defence mechanism against phytophagous insects. Stimulation of the mechanical damage resulting from phytophagous activity increased soybean leaf GABA 10- to 25-fold within 1 to 4 min to values 2.15 μmol GABA g^{-1} fresh weight. Introducing GABA into a synthetic diet to this level reduced the growth rates, developmental rates, and survival rates of cultured phytophagous larvae of the oblique-banded leaf roller (Ramputh and Bown, 1996). In addition, more than 90% of oblique-banded leaf-roller larvae were found on light-green expanding leaves of apple trees, which produce lower GABA levels than dark-green mature leaves when mechanically damaged. In insect larvae, the GABA-gated Cl^- channels in the neuromuscular junctions of body wall muscles are exposed to solutes in the hemolymph. If the neuromuscular junctions of body wall muscles are exposed to GABA, the GABA-gated Cl^- channels in the junctions are not protected by a blood-brain barrier (Ramputh and Bown, 1996; Bown and Shelp, 1997). Many commercially employed insecticides are agonists or antagonists of the GABA-gated Cl^- current, and are thought to disrupt normal neuromuscular activity (Ramputh and Bown, 1996; Bown and Shelp, 1997). Therefore, GABA ingestion that raises levels in the hemolymph may have a similar effect.

Conclusions and Future Perspectives

NAD kinase and glutamate decarboxylase are well characterized enzymes among the Ca^{2+}/calmodulin-dependent activities detected in plants. In plants, NAD kinase and glutamate decarboxylase appear to be regulated following environmental stimulation leading to the synthesis of active oxygen species and gamma-aminobutyric acid, respectively. Evidence shows that many environmental stresses cause fluxes in cytosolic Ca^{2+} and the increased Ca^{2+} stimulates Ca^{2+}/calmodulin-dependent NAD kinase and glutamate decarboxylase. Transgenic tobacco cells overexpressing a mutant calmodulin that is incapable of being methylated at lysine 115 showed a stronger active oxygen burst that occurred more rapidly than in normal control cells challenged with various stimuli. In addition, the transgenic tobacco cells showed an enhanced response upon actual infection by *Pseudomonas syringae* pathovar 61. Evidence indicates that glutamate decarboxylase and GABA are involved in nitrogen metabolism and storage, and in the plant's defence against phytophagous insects. There is also evidence that regulation of glutamate decarboxylase activity by Ca^{2+} and calmodulin is necessary for normal plant growth and development. Integrated studies in model systems, such as genetically engineered plants, may provide further insight into the mechanism of defence of plants during pathogen infection, phytophagous attack and other environmental stresses.

References

Allan, E. F. and Trewavas, A. J. 1985. Quantitative changes in calmodulin and NAD kinase during early cell development in the root apex of *Pisum Sativum* L. *Planta* 165:493-501.

Anderson, J. M. and Cormier, M. J. 1978. Calcium-dependent regulator of NAD kinase in higher plants. *Biochem. Biophys. Res. Commun.* 84:595-602.

Auh, C.-K. and Murphy, T. M. 1995. Plasma membrane redox enzyme is involved in the synthesis of O_2^- and H_2O_2 by *Phytophthora* elicitor stimulated rose cells. *Plant Physiol.* 107: 1241-1247.

Baggiolini, M. and Wymann, M. P. 1990. Turning on the respiratory burst. *Trends Biochem. Sci.* 15:69-72.

Bao, J., Cheung, W. Y. and Wu, J.-Y. 1995. Brain L-glutamate decarboxylase. *J. Biol. Chem.* 270:6464-6467.

Barnes, R. L. and Naylor, A. W. 1959. Effect of various nitrogen sources on growth of isolated roots of *Pinus serotina*. *Physiol. Plant* 12:82-89.

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

Baum, G., Lev-Yadun, S., Fridmann, Y., Arazi, T., Katsnelson, H., Zik, M. and Fromm, H. 1996. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. *EMBO J.* 15:2988-2996.

Berridge, M. J. and Irvine, R. F. 1989. Inositol phosphate and cell signalling. *Nature* 341:197-204.

Berridge, M. J. 1990. Calcium oscillations. *J. Biol. Chem.* 265: 9583-9586.

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. *Developmental Biol.* 131:313-320.

Bown, A. and Shelp, B. 1989. The metabolism and physiological roles of 4-aminobutyric acid. *Biochem.(Life Sci. Adv.)* 8:21-25.

Bown, A. and Shelp, B. 1997. The metabolism and functions of γ-aminobutyric acid. *Plant Physiol.* 115:1-5.

Chen, Y., Baum, G. and Fromm, H. 1994. The 58-kilodalton calmodulin-binding glutamate decarboxylase is a ubiquitous protein in petunia organs and its expression is developmentally regulated. *Plant Physiol.* 106:1381-1387.

Cheung, W. Y. 1970. Cyclic 3',5'-nucleotide phosphodiesterase: Demonstration of an activator. *Biochem. Biophys. Res. Commun.* 38:533-538.

Cocucci, M. and Negrini, N. 1988. Changes in the levels of calmodulin and of a calmodulin inhibitor in the early phases of radish (*Raphanus sativus* L.) seed germination: Effect of ABA and fusicoccin. *Plant Physiol.* 88:910-914.

Cohen, P. and Klee, C. B eds. 1988. Calmodulin: Molecular Aspects of Cellular Regulation, Vol. 5, Elsevier Biomedical Press, Amsterdam.

Crawford, L. A., Bown, A. W., Breitkreuz, K. E. and Guinel, F. C. 1994. The synthesis of γ-aminobutyric acid in response to treatments reducing cytosolic pH. *Plant Physiol.* 104:865-871.

Dwyer, S. C., Legendre, L., Low, P. S. and Leto, T. L. 1996. Plant and human neutrophil oxidative burst complexes contain immunologically related proteins. *Biochim. Biophys. Acta* 1289:231-237.

Epel, D. 1964. A primary metabolic change of fertilization: Interconversion of pyridine nucleotides. *Biochem. Biophys. Res. Commun.* 17:62-68.

Epel, D., Patton, C., Wallace, R. W. and Cheung, W. Y. 1981. Calmodulin activates NAD kinase of sea urchin eggs: An early event of fertilization. *Cell* 23:543-549.

Erlander, M. J. and Tobin, A. J. 1991. The structural and functional heterogeneity of glutamic acid decarboxylase: a review. *Neurochem. Res.* 16:215-226.

Harding, S. A., Oh, S.-H. and Roberts, D. M. 1997. Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. *EMBO J.* 16:1137-1144.

He, S. Y., Huang, H.-C. and Collmer, A. 1993. *Pseudomonas syringae* pv. syringae Harpin pss: a protein that is secreated via the *Hrp* pathway and elicits the hypersensitive response in plants. *Cell* 73:1255-1266.

Hepler, P. K. and Wayne, R. O. 1985. Calcium and plant development. *Ann. Rev. Plant Physiol.* 36:397-439.

Hepler, P. K. 1990. Dose calcium regulate events through amplitude modulation? *Curr. Top. Plant Biochem. Physiol.* 9:1-9.

Hernandez-Nistal, J., Rodriguez, D., Nicols, G. and Aldasoro, J. J. 1989. Abscisic acid and temperature modify the levels of calmodulin in embryonic axes of *Cicer arietinum*. *Physiologia Plantarum* 75:255-260.

Housley, T. L., Schrader, L. E., Miller, M. and Setter, T. L. 1979. Partitioning of ^{14}C-photosynthate, and long distance translocation of amino acids in preflowering and flowering, nodulated and nonnodulated soybeans. *Plant Physiol.* 64:94-98.

Kajimura, K., Iwamoto, Y., Yoshida, S., Yamasaki, K., Tanaka, R., Suzuki, S., Nakazawa, H. and Yoneda, K. 1991. Studies on cultures of *Astragalus mongholicus*(I): amino acids composition in seeds, young plantlets and cell cultures. *Shoyakugaku Zasshi* 45:293-298.

Kakiuchi, S., Yamazaki, R. and Nakajima, H. 1970. Properties of heat-stable phosphodiesterase activating factor isolated from brain extract. *Proc. Natl. Acad. Sci.* USA 46:587-592.

Klee, C. B. and Vanaman, T. C. 1982. Calmodulin. *Adv. Prot. Chem.* 35:213-321.

Knight, M. R., Campbell, A. K., Smith, S. M. and Trewavas, A. J. 1991. Transgenic plant aequorin reports the effect of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature* 352: 524-526.

Lee, S. H., Seo, H. Y., Kim, J. C., Lee, M. S., Heo, W. D., Chung, W. S., Lee, K. J., Kim, M. C., Cheong, Y. H., Choi, J. Y. and Cho, M. J. 1997. Differential activation of NAD kinase by plant calmodulin isoforms: The critical role of domain I. *J. Biol. Chem.* 272:9252-9259.

Ling, V., Perera, I. and Zielinski, R. E. 1991. Primary structure of

Arabidopsis calmodulin isoforms deduced from the sequences of cDNA clones. *Plant Physiol.* 96:1196-1202.

Ling, V., Snedden, W. A., Shelp, B. J. and Assmann, S. M. 1994. Analysis of a soluble calmodulin binding protein from fava bean roots: Identification of glutamate decarboxylase as a calmodulin-activated enzyme. *Plant Cell* 6:1135-1143.

Lowenstein, J. M. 1961. Reduction and oxidation in mammalian biosynthesis. *J. Theoret. Biol.* 1:98-103.

Manalan, A. S. and Klee, C. B. 1984. Calmodulin. *Adv. Cyclic Nucleotide Res.* 18:227-273.

McGuinness, E. T. and Butler, J. R. 1985. NAD kinase - A review. *Int. J. Biochem.* 17:1-11.

Mehdy, M. 1994. Active oxygen species in plant defence against pathogens. *Plant Physiol.* 105:467-472.

Morino, H., Kawamoto, T., Miyake, M. and Kakimoto, Y. 1987. Purification and properties of calmodulin-lysine N-methyltransferase from rat brain cytosol. *J. Neurochem.* 48:1201-1208.

Muto, S. and Miyachi, S. 1977. Properties of protein activator of NAD kinase from plants. *Plant Physiol.* 59:55-60.

Muto, S. and Miyachi, S. 1984. Production of an antibody against spinach calmodulin and its application to radioimmunoassay for plant calmodulin. *Z. Pflanzenphysiol. Bd.* 114:421-431.

Oh, S.-H., and Roberts, D. M. 1990. Analysis of the state of post-translational calmodulin methylation in developing pea plants. *Plant Physiol.* 93:880-887.

Oh, S.-H., Steiner, H.-Y., Dougall, 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.

Oh, S.-H. 1992. *In vitro* and *in vivo* studies on posttranslational calmodulin methylation in plants. *Ph.D. Dissertation*, Univ. of Tennessee, Knoxville.

Oh, S.-H. and Yun, S. J. 1999. Effects of various calmodulins on the activation of glutamate decarboxylase and nicotinamide adenine dinucleotide kinase isolated from tobacco plants. *Agric. Chem. Biotechnol.* 42:19-24.

Perera, I., Szymanski, D., Gawienowski, M. and Zielinski, R. 1991. Calmodulin is encoded by multiple genes in *Arabidopsis*. *Plant Physiol. Suppl.* 96:128.

Ramputh, A. and Bown, A. W. 1996. Rapid γ-aminobutyric acid synthesis and inhibition of the growth and development of oblique-banded leaf-roller larvae. *Plant Physiol.* 111:1349-1353.

Rasmussen, C. D. and Means, A. R. 1987. Calmodulin is involved in regulation of cell proliferation. *EMBO J.* 6:3961-3968.

Rasmussen, C. D. and Means, A. R. 1989. Calmodulin is required for cell-cycle progression during G1 and mitosis. *EMBO J.* 8: 73-82.

Rasmussen, C. D., Means, R. L., Lu, K. P., May, G. S. and Means, A. R. 1990. Characterization and expression of the unique calmodulin gene of *Aspergillus nidulans*. *J. Biol. Chem.* 265: 13767-13775.

Rhoads, A. R. and Friedberg, F. 1997. Sequence motifs for calmodulin recognition. *FASEB J.* 11:331-340.

Roberts, D. M., Zielinski, R. E., Schleicher, M. and Watterson, D. M. 1983. Analysis of suborganellar fractions from spinach and pea chloroplasts for calmodulin-binding proteins. *J. Cell. Biol.* 97:1644-1647.

Roberts, D. M., Lukas, T. J. and Watterson, D. M. 1986. Structure, function, and mechanism of action of calmodulin. *CRC Crit. Rev. Plant Sci.* 4:311-339.

Roberts, D. M., Oh, S.-H., Besl, L., Weaver, C. D. and Stacey, G. 1990. Attenuation of calmodulin-dependent NAD kinase activation by posttranslational methylation. *Curr. Top. Plant Biochem. Physiol.* 9:67-84.

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

Serraj, R., Shelp, B. J. and Sinclair, T. R. 1998. Accumulation of γ-aminobutyric acid in nodulated soybean in response to drought stress. *Physiologia Plantarum* 102:79-86.

Shapiro, B. M. 1991. The control of oxidant stress at fertilization. *Science* 252:533-536.

Snedden, W. A., Arazi, T., Fromm, H. and Shelp, B. J. 1995. Calcium/calmodulin activation of soybean glutamate decarboxylase. *Plant Physiol.* 108:543-549.

Snedden, W. A., Koutsia, N., Baum, G. and Fromm, H. 1996. Activation of a recombinant petunia glutamate decarboxylase by calcium/calmodulin or by a monoclonal antibody which recognizes the calmodulin binding domain. *J. Biol. Chem.* 271:4148-4153.

Snedden, W. A. and Fromm, H. 1998. Calmodulin, calmodulin-related proteins and plant responses to the environment. *Trends in Plant Science* 3:299-304.

Stayanarayan, V. and Nair, P. M. 1990. Metabolism, enzymology and possible roles of 4-aminobutyrate in higher plants. *Phytochemistry* 29:367-375.

Strynadka, N. C. and James, M. N. G. 1989. Crystal structures of the helix-loop-helix calcium-binding proteins. *Annu. Rev. Biochem.* 58:951-998.

Wallace, W., Secor, J. and Schrader, L. E. 1984. Rapid accumulation of γ-aminobutyric acid and alanine in soybean leaves in response to abrupt transfer to low temperature, darkness, or mechanical manipulation. *Plant Physiol.* 75:170-175.

Williams, M. J. and Jones, H. P. 1985. Calmodulin-dependent NAD kinase of human neutrophils. *Arch. Biochem. Biophys.* 237:80-87.

Yamamoto, Y. 1963. Pyridine nucleotide content in higher plants: Effect of age of tissue. *Plant Physiol.* 38:45-54.

Yun, S. J. and Oh, S.-H. 1998. Cloning and characterization of a tobacco cDNA encoding calcium/calmodulin-dependent glutamate decarboxylase. *Mol. Cells* 8:125-129.

Zhang, S. J. and Jackson, M. B. 1993. GABA-activated chloride channels in secretory nerve endings. *Science* 259:531-534.

Zik, M., Arazi, T., Snedden, W. A. and Fromm, H. 1998. Two isoforms of glutamate decarboxylase in *Arabidopsis* are regulated by calcium/calmodulin and differ in organ distribution. *Plant Mol. Biol.* 37:967-976.

Zielinski, R. E. 1987. Calmodulin mRNA in barley (*Hordeum Vulgare* L.): Apparent regulation by cell proliferation and light. *Plant Physiol.* 84:937-943.

Zielinski, R. E., Ling, V. and Perera, I. 1990. Structure and expression of genes encoding calcium-modulated proteins in higher plants. *Curr. Top. Plant Biochem. Physiol.* 9:141-152.