Calcium Homeostasis in Plants: Role of Calcium Binding Proteins in Abiotic Stress Tolerance

Giridhar Pandey1, M K Reddy1, Sudhir K Sopory1 and Sneh Lata Singla-Pareek1*

1Plant Molecular Biology Laboratory, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067, India
2Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

A majority of environmental signals of varied types as well as varied intensities are perceived at the membrane level in a cell. In case of multicellular organisms like plants or animals, this ‘perception’ not only needs to be transferred to the actual centre of controlling and responding unit i.e. the nucleus but sometimes from one cell to another cell which may just be lying close enough or even at an appreciable distance e.g. ‘root-shoot communications’. In contrast to processes of cell-to-cell communication in a plant system, which is just beginning to be elucidated, the mechanism of ‘transfer’ of this information from outer surface to the core controlling units has been an active area of research since past few decades. Abundant reports do exit in literature, which support a kind of ‘cascading mechanism’ for this purpose. It is now a well-established fact that a divalent cation i.e. Ca2+ plays an extremely important role in this process. The fluctuations in the level of Ca2+ at a given time in a given cell organelle is the crucial factor determining the activation stage of some special proteins, which have been proposed to have a high affinity for Ca2+. Such proteins are known as Ca2+-binding proteins (CaBPs). Under environmental abuses, the level and activation of CaBPs play an important role in bringing about the ‘ignition’ of ‘protective’ as well as ‘defensive’ mechanisms, which ultimately are reflected in the form of the physiological adaptations in the plant as a whole system. The present review is an attempt to highlight the importance of CaBPs in plants under abiotic stress conditions.

Keywords: abiotic stresses, adaptations, Ca2+, CaBPs, signal transduction, stress tolerance, transgenic plants

Introduction

Calcium plays a key role in plant growth and development. Changes in cellular Ca2+ are perceived by specific Ca2+ binding proteins (CaBPs), which by modulating their targets regulate an astonishing variety of cellular processes. The Ca2+ cation is now firmly established as an intracellular second messenger that couples a wide range of extra cellular stimuli to characteristic responses in plants. Ever since, a stimulus induced increase in the concentration of cytosolic Ca2+ (represented as [Ca2+]cyt) in higher plants has been reported, there has been a massive increase in the number of signalling systems known to use [Ca2+]cyt as an intracellular second messenger (Trewavas & Malho, 1998; Sander et al, 1999; Trewavas, 1999; Pandey et al, 2000; Reddy, 2001; Rudd & Franklin-Tong, 2001). The implication is that calcium flow constitutes a large network which actually connects the environment, cytoplasm, vesicles, organelles, nucleus and in higher species—the organs.

The changes in the [Ca2+]cyt in response to any specific signal vary in the frequency, amplitude and spatial domains—leading to the concept of ‘calcium signatures’. This term is used to describe the specific pattern or ‘finger print’ of Ca2+ release and propagation. ‘Calcium signatures’ have been proposed to allow signal specificity by precise control of alterations in spatial, temporal and concentration of [Ca2+]cyt. They encode specific information, which is relayed to the downstream elements (or effectors) for translation into an appropriate cellular response.

The levels of calcium are delicately balanced by the presence of ‘calcium stores’ like vacuoles, which release calcium whenever required. Several specific pumps/channel (through which Ca2+ can move in and out), and a plethora of CaBPs (also called as Ca2+ sensors), which are involved in either performing some complex functions or involved in buffering of calcium (i.e. calcium homeostasis) have been identified. The role of calcium as an important signal molecule and the various components involved in maintaining calcium homeostasis has been reviewed by several workers (Sander et al, 1999; Trewavas, 1999; Knight, 2000; Pandey et al, 2000; Reddy, 2001; Rudd & Franklin-Tong, 2001).

*Author for correspondence:
Tel: +91-11-6181242; Fax: +91-11-6162316
E-mail: snehpareek@hotmail.com
At a given time, even under favourable conditions, the physiological reactions taking place in a cell (be it uni- or multi-cellular) are very complex, which in turn indicate the involvement of physiological cation in a host of cellular proceedings. Nonetheless, the cellular physiology becomes even more complex under the induced conditions.

Environmental stresses are known to affect crop yield in a major way. Such stresses commonly encountered by plants lead to a rapid transient elevation in concentration of $[\text{Ca}^{2+}]_{\text{cy}}$ (Knight et al., 1991; Bush, 1995), which then transduces the signal further by acting as a second messenger. The alteration in cellular calcium signals lead ultimately to the increased expression of stress responsive genes that encode proteins of protective function and hence confer stress tolerance (Knight et al., 1996, 1997). In this context, understanding of Ca$^{2+}$ homeostasis under unfavourable environmental conditions presents a major challenge. A number of studies have been attempted to address to the following questions:

(a) How Ca$^{2+}$ concentrations are affected in a microenvironment under stress conditions?

(b) How this change in Ca$^{2+}$ concentration, in turn, brings about changes in concentration of downstream molecules i.e. CaBPs?

(c) How perturbations in the level of CaBPs help the system to counteract the damaging effects of environmental stresses?

(d) Ultimately, how can this response be manipulated to address the problem of increasing the crop yield under stress conditions?

The authors have attempted to review the work done to elucidate the nature and function of various CaBPs with special reference to their role in abiotic stress tolerance.

**Calcium Signalling in Plants: An Overview**

In this part, the authors have briefly prefaced on the aspect as to how the Ca$^{2+}$ homeostasis is brought about under favourable environmental conditions. The later part of this section describes how this machinery is affected under stress conditions.

**Mechanism of Ca$^{2+}$ Regulation**

An ensemble of Ca$^{2+}$ transporter proteins maintains cellular Ca$^{2+}$ homeostasis. Functionally, these transporters fall into two classes—those that mediate efflux from the cytoplasm (the Ca$^{2+}$/H$^+$ antiporters) and —those that mediate influx into the cytoplasm (the Ca$^{2+}$ channels) as follows:

(a) **Efflux Transporters**

Basicallly, two types of efflux transporters operate in a cell: the Ca$^{2+}$ ATPase and the Ca$^{2+}$/H$^+$ antiporter. A brief discussion on each has been provided below.

(i) **Ca$^{2+}$ ATPases.** Plant cells contain a diverse group of primary ion-pumps, the Ca$^{2+}$ ATPases, which belong to the super family of P-type ATPases that directly use ATP to drive ion translocation. Two distinct Ca$^{2+}$ pump families have been proposed based on protein sequence identities (Axelsen & Palmgren, 1998). Members of the type II A and II B families, include the ER-type and plasma membrane-type Ca$^{2+}$ pumps, respectively. Previously, ER and plasma membrane-type pumps were distinguished by three criteria: (a) localization to either the ER or the plasma membrane; (b) differential sensitivity to inhibitors (e.g. ER-type is inhibited by cyclopiazonic acid and thapsigargin); and (c) direct activation of plasma membrane type pumps by CaM (Evans & Williams, 1998).

Several genes encoding type II A (ER type) pumps have been cloned from plants, including LCA1 (Ly-copersicum Ca$^{2+}$ ATPase) from tomato (Wimmers et al., 1992), OCA1 from rice (Chen et al., 1997) and ECA1/ACA3 from Arabidopsis (ER-type Ca$^{2+}$ ATPase/Arabidopsis Ca$^{2+}$ ATPase isoform 3; Liang et al., 1997). There is evidence that ECA1p/ACA3p is located in the ER. A unique sub-family of CaM-dependent Ca$^{2+}$ ATPases has been recently identified. One of the isoforms of this family, ACA2p from Arabidopsis, has been also found to reside in the ER. This was confirmed by tagging the gene with GFP and transforming the Arabidopsis plants. Confocal and computational analysis confirmed that it was located in the ER which makes it distinct from all other CaM-regulated pumps identified in plants and animals (Hong et al., 1999).

Further, three plant genes encoding type II B (plasma membrane-type) pumps have been reported: ACA1 and ACA2 from Arabidopsis (Huang et al., 1993a; Harper et al., 1998) and BCA1 from B. oleracea (Malmstrom et al., 1997). In general, the plant proteins are distinguished from animal plasma membrane-type pumps by (1) localization at membranes other than the plasma membrane, and (2) a unique structural arrangement with putative auto-inhibitory domains at the N-terminus instead of the C-terminus.
Based on membrane fractionation and immunodetection with an anti-ACA1 polyclonal antibody (Huang et al., 1993a), it has been shown that ACA1p is localized in the plastid inner envelope membrane. BCA1p appears to be present in the vacuolar membrane, since it showed correspondence with a peptide sequence obtained from a purified vacuolar-ATPase (Malmstrom et al., 1997). ACA2p was shown to fractionate with the ER, as indicated by immunodetection of ACA2p in membrane fractions and this was confirmed by cytological visualization of an ACA2p tagged with a C-terminal green fluorescent protein (Harper et al., 1998; Hong et al., 1999).

(ii) \(\text{Ca}^{2+}/\text{H}^+\) antiporters. They are different from \(\text{Ca}^{2+}\)-ATPases as they do not require ATP and are not sensitive to vanadate. The first plant \(\text{Ca}^{2+}/\text{H}^+\) antiporter to be cloned and functionally expressed was CAX1p (Calcium exchanger 1; Hirschi et al., 1996). The gene was identified by its ability to restore growth on a high \(\text{Ca}^{2+}\) medium to a yeast mutant defective in vacuolar \(\text{Ca}^{2+}\) transport. Antiporters are shown to exist mainly on the tonoplast (vacuolar membrane) and are involved in the maintenance of vacuolar \(\text{Ca}^{2+}\) stores (Blackfords et al., 1990). There are some evidences for the presence of \(\text{Ca}^{2+}/\text{H}^+\) antiporters on other membrane, such as the plasma membrane (Kasai & Muto, 1990).

(b) Influx Transporters

The steep electrochemical gradient for \(\text{Ca}^{2+}\) influx into the cytosol is known to be tightly regulated. Although \(\text{Ca}^{2+}\) influx can occur through a pump or an antiporter operating in reverse, transporters appear to operate far away from thermodynamic equilibrium and are likely to function only in the export of \(\text{Ca}^{2+}\). A wheat cDNA clone, LCT1 (low-affinity cation transporter 1), complements yeast mutants defective in \(\text{Ca}^{2+}\) influx (Schachtman et al., 1997; Clemens et al., 1998). Although the sequence of LCT1 provides no clues to its likely relationship to previously identified ion-channels, an exciting possibility is that LCT1 provides a physiologically significant pathway for \(\text{Ca}^{2+}\) uptake in plants. Based on the state of the channel, influx transporters are classified into five classes of \(\text{Ca}^{2+}\) channels in the animal system (Tsien & Tsien, 1990). Of these, voltage-operated, second messenger-operated, and mechanically operated, have been identified in plants and are as follows:

(i) Voltage gated channels. At least two major classes of \(\text{Ca}^{2+}\) channels reside on the plasma membrane (White, 1998): (1) Maxi-cation channels are relatively nonselective with respect to cation and possess a high single channel conductance (White, 1993, 1994); and (2) Voltage-dependent cation channel 2 is relatively more selective for cations and exhibits as a blocker single-channel conductance (White, 1994; Pineros & Tester, 1995, 1997; Sander et al., 1999). Both the channels have been characterized mostly thoroughly in cereal crops. They exist in a variety of other cell types and tissues like carrot, parsley suspension cultures (Thuleau et al., 1994) and Arabidopsis mesophyll and root cells (Ping et al., 1992; Thion et al., 1998). Different pharmacological compounds like verapamil, bepridil, nifedipine, etc. either block or promote specifically the activities of these channels in various calcium mediated responses.

(ii) Ligand gated channels. These channels are predominantly localized on the vacuolar endomembranes. At least four different \(\text{Ca}^{2+}\) permeable channel types have been localized in the vacuolar membrane (Allen & Sanders, 1997). Two of these channels are ligand gated; one by inositol 1,4,5-trisphosphate (IP3) (Schumaker & Sze, 1987; Alexandre et al., 1990) and the other by cADP-ribose (cADPR) (Allen et al., 1995). The pharmacological properties of plant cADPR gated channels resemble those of ryanodine receptors (Muir & Sanders, 1996). Microinjection of IP3 and cADPR into guard cells revealed that both compounds have the capacity to elevate cytosolic calcium, thereby demonstrating that IP3 and cADPR channels are functional in plants (Gilroy et al., 1991; Leckie et al., 1998). A property of IP3 receptors and ryanodine receptors in animal cells is their capacity to get activated by \([\text{Ca}^{2+}]_{\text{cyt}}\). This response is thought to underlie \(\text{Ca}^{2+}\) induced \(\text{Ca}^{2+}\) release (CICR), which can be fundamental to the amplification of \(\text{Ca}^{2+}\) signals (Taylor & Traynor, 1995). Neither IP3 nor cADPR gated currents across the vacuolar membrane of plants are activated by \([\text{Ca}^{2+}]_{\text{cyt}}\) (Allen & Sanders, 1994; Leckie et al., 1998), despite the presence of waves, oscillation and spikes of \([\text{Ca}^{2+}]_{\text{cyt}}\), which are suggestive of CICR.

(iii) Stretch activated channels. These are \(\text{Ca}^{2+}\) permeable channels activated by tension and found on the plasma membrane of plants (Cosgrove & Hedrich, 1991; Ding & Pickard, 1993; Pickard & Ding, 1993). They have been proposed to be involved in turgor regulation (Cosgrove & Hedrich, 1991), thigmotropic responses (Braam, 1992; Braam & Davis, 1990) and responses to temperature (Ding & Pickard, 1993) and hormones (Pickard & Ding, 1993; Bush, 1995).
**Ca**²⁺ Homeostasis under Stress Conditions

The authors have discussed in detail the various types of cellular machineries and their functioning, which control the entry and exit of Ca**²⁺** in the cytoplasm. Basically, for signal transduction, small bursts of Ca**²⁺** are flushed into the cytoplasm from two large Ca**²⁺** stores; one is extracellular (cell wall, which is a huge reserve of Ca**²⁺**) and other is intracellular (a minor ER component and a major vacuolar store). However, under stress conditions, a major challenge for the cell will be to maintain these machineries equally functional. The survival of a cell will ultimately depend on to what an extent or a degree, a cell is able to maintain these processes functionally close to the unstressed conditions.

The changes in the cytosolic calcium concentration have been reported in various stress conditions. Involvement of calcium has also been shown to modulate the expression of virulence genes of the pathogen (Flego et al., 1997) and in cell-to-cell communication (Trucker & Boss, 1996). Cold shock response has been studied in case of maize suspension culture cells (Campbell et al., 1996) and *Arabidopsis* seedlings (Knight et al., 1996) where a biphasic i.e. fast and slow prolonged increase in calcium concentration was observed. In cold sensitive tobacco and cold resistant *Arabidopsis* seedlings, the plant seems to have a cold calcium memory and depicted a plant specific calcium signature in response to cold acclimation. Heat shock induced changes in Ca**²⁺** level have also been recorded and correlated with thermotolerance in tobacco seedlings (Gong et al., 1998).

Response of plants to hypoxia, studied in *Arabidopsis* seedlings, was a biphasic pattern of calcium change (Sedbrook et al., 1996). Similarly, a change in cytoplasmic calcium level was seen during water stress in ageotropic pea mutants (Takano et al., 1997) and senescence in detached parsley leaves (Huang et al., 1997). Hypoosmotic shock to tobacco cells induces a biphasic cytosolic response of calcium change (Cessna et al., 1998).

In *Arabidopsis* seedlings, response to drought and stress was mediated via changes in calcium level. A transient increase in calcium could be blocked with calcium chelator - EGTA or channel blocker - lanthanum. Elicitor induced signalling was also mediated via calcium ions (Gelli & Blumwald, 1997) into tomato protoplasts. Also, oligonucleotide elicitor mediated signalling is mediated via calcium ion (Ebel, 1998). Even ozone mediated signalling involved a transient increase in cytosolic calcium level (Clayton et al., 1999).

Several mechanical stimuli like wind, touch and gravity are known to mediate a change in cytoplasmic calcium concentration (Bjorkman & Cleland, 1991; Halley et al., 1995). A regres test to show the involvement of cytosolic Ca**²⁺** in gravitropic responses has been performed in *Arabidopsis* roots (Legue et al., 1997; Sinclait et al., 1996).

Not only increase in calcium, but also its relationship to gene expression has been shown in a number of instances. Salt or mannitol induces expression of three genes, p5cs (which encodes Δ¹-pyrroline-5-carboxylate synthetase—the first enzyme of the proline biosynthesis pathway), rab18 and iti 78 (both of these encode protein of unknown function). Mannitol stimulated expression could be inhibited by pretreatment with lanthanum, but in case of salt stimulated expression, lanthanum could inhibit expression of p5cs only. Calcium chelator – EGTA and calcium channel blocker - gadolinium and verapamil blocked mannitol induction of p5cs thus further confirming the role of calcium in this response. Also, the involvement of calcium via IP₃ mediated release from vacuoles has been shown in this case (Knight et al., 1997).

A transgenic approach has utilized the sea urchin photoprotein, aequorin, as a calcium indicator in plant tissues (Knight et al., 1991). This protein consists of an apoprotein and a luminophore and is, therefore, a proteinaceous luminiscent reporter of Ca**²⁺**. Aequorin has been introduced into tobacco using the cDNA and constitutively expressed under the CaMV 35S promoter. Using these plants, it has been possible to determine changes in [Ca**²⁺**], (intracellular free calcium) in response to a number of external stimuli. Using various Ca**²⁺** channel blockers, the signals, such as cold shock, fungal elicitor, touch and wind, leads to a transient increase in [Ca**²⁺**]; but the source of Ca**²⁺**, either extracellular or intracellular, is dependent on stimulus (Knight et al., 1992).

Cold calcium signalling in *Arabidopsis* has been involved in two cellular pools and a change in calcium signature after acclimation to give rise to "cold memory" (Knight et al., 1996, 1997). Intracellular Ca**²⁺** increase following cold shock requires extracellular calcium and may be derived from a Ca**²⁺** influx mediated by plasma membrane Ca**²⁺** channels (Polinsensky & Braam, 1996). The cold up-regulation of at least a subset of TCH genes, which encode CaM related proteins, requires an intracellular Ca**²⁺** increase.
In apoaequorin transformed tobacco cells, influx of Ca\(^{2+}\) was required to communicate oxidative burst signal but not maintain the defense response, which suggest that Ca\(^{2+}\) pulses serve frequently, but not invariably, to transduce an oxidative burst signal (Chandra et al, 1997).

Ca\(^{2+}\) mediated signal transduction in stomata guard cells has also been addressed. In Nicotiana plumbaginifolia, aequorin expression was specifically targeted to the guard cells. Changes in the Ca\(^{2+}\) levels in response to ABA, mechanical and low temperature promoted rapid stomatal closure were monitored. Elevations of guard cell [Ca\(^{2+}\)]\(_{cyt}\) play a key role in the transduction of all the stimuli (Wood et al, 2000). However, there was a striking difference in the magnitude and kinetic of all the responses. Studies using Ca\(^{2+}\) channel blockers and calcium chelator further suggested that mechanical and ABA signals primarily mobilize Ca\(^{2+}\) from the intracellular store(s) whereas the influx of extracellular Ca\(^{2+}\) is a key component in the transduction of low temperature signals.

Although the roots respond to cold, drought and salt stress with increase in cytoplasmic free calcium, the role(s) of various functionally diverse cell types that comprise the root is not known. Transgenic Arabidopsis with enhancer trapped GAL4 expression in specific cell types was used to target the aequorin, fused to a modified yellow fluorescent protein (YFP) – to enable in vivo measurement of changes in cytosolic free Ca\(^{2+}\) concentrations in specific cell types during acute cold, osmotic and salt stresses. In response to acute cold stress, all cell types displayed rapid [Ca\(^{2+}\)]\(_{cyt}\) peaks while the endodermis and pericycle displayed prolonged oscillations in [Ca\(^{2+}\)]\(_{cyt}\) in response to osmotic and salt stress (Kiegle et al, 2000).

Thus, different abiotic stresses can elicit very distinct Ca\(^{2+}\) signatures that are affected by the cell type, kinetics, amplitude and duration of the increased Ca\(^{2+}\) and the source of the mobilizable Ca\(^{2+}\) store. These studies highlight the importance of Ca\(^{2+}\) signalling in a wide range of plant responses to perturbations in the environmental conditions. Still much remains to be learned in signal perception mechanisms, particularly the nature of receptors for these types of signals, it is clear that they can be sensed at the cell surface or intracellularly. In both cases, the information can somehow be transduced into a Ca\(^{2+}\) signature that is in some way used to direct the specific responses of the plant to the stimulus.

### Calcium Binding Proteins (CaBPs) in Plants

The conversion of the Ca\(^{2+}\) signal into gene expression or a physiological response is dependent on the nature of the Ca\(^{2+}\) signal and on downstream response components. This involves various CaBPs and kinases. Thus, a Ca\(^{2+}\) dependent ‘conformation switch’ performs these functions: activation of enzymatic or other biochemical activities of the proteins and association or dissociation with their target molecules.

The diverse functions mediated by Ca\(^{2+}\) are carried out by a large number of CaBPs, which serve as Ca\(^{2+}\) sensors (Ohta et al, 1995; Reddy, 2001). CaBPs sense an increase in the [Ca\(^{2+}\)]\(_{cyt}\) levels which decode Ca\(^{2+}\) signal. Once the Ca\(^{2+}\) sensors decode the elevated [Ca\(^{2+}\)]\(_{cyt}\), the Ca\(^{2+}\) levels are restored back to its resting state by either efflux of Ca\(^{2+}\) to the cell exterior and/or sequestration into cellular organelles such as vacuoles, ER and mitochondria. Thus, CaBPs play a key role in decoding Ca\(^{2+}\) signatures and transducing signals by activating specific targets and pathways.

Several CaBPs have been identified and characterised in both the animal (Celio, 1996) and the plant (Pouvaiah & Reddy, 1993; Zielinski, 1998; Reddy, 2001) systems. In plants, Ca\(^{2+}\) sensors can be grouped into the following five major classes as follows: (a) CaM; (b) CaM-like and other EF-hand containing CaBPs; (c) Ca\(^{2+}\)-regulated protein kinases; (d) Ca\(^{2+}\)-modulated protein phosphatases; and, (e) CaBPs without EF-hand motifs. The members of first three classes of Ca\(^{2+}\) sensors contain a common structural motif(s), ‘EF hand’, which is a helix-loop-helix structure that binds a single Ca\(^{2+}\) ion with high affinity (Roberts & Harmon, 1992). These motifs typically occur in closely linked pairs, interacting through antiparallel β-sheets. This arrangement is actually the basis for cooperativity in Ca\(^{2+}\) binding. Different CaBPs differ in the number of EF hand motifs and their affinity to bind Ca\(^{2+}\); some of the CaBPs have Ca\(^{2+}\)-binding affinities in the nanomolar to micromolar range. Binding of Ca\(^{2+}\) to the Ca\(^{2+}\) sensor results in a conformational change in the sensor that alters its interaction with the other proteins, which modulate their function/activity or modulates its own activity.

#### (a) Calmodulin (CaM)

CaM is a highly conserved, most well characterized, ubiquitously expressed CaBP in eukaryotes (Snedden & Fromm, 1998; 2001). It is a small molecular weight (16.7 kDa), acidic, heat-stable protein of 148 amino acids with four EF-hand motifs that...
bind to four Ca$^{2+}$ ions. The binding of Ca$^{2+}$ to CaM results in conformational change in such a way that the hydrophobic pockets of CaM are exposed in each globular end which can then interact with target proteins (O’ Neil & DeGrado, 1990; Rhoads & Friedberg, 1997).

CaM has been shown to be a multi-functional protein. The comparison of various cDNA sequences and alignment of amino acid sequences from various plant and animal CaMs shows a very high degree of homology, indicating existence of a parallel Ca$^{2+}$/CaM signalling pathway in plants and animals. The involvement of CaM has been shown during processes of cell cycle (Vantard et al., 1985), cell growth and embryogenesis (Oh et al., 1992), germination of seed embryo (Cocucci & Negrini, 1988), differentiation of treachery elements (Kobayashi & Fukuda, 1994), cell proliferation (Perera & Zielinski, 1992) and phytochrome mediated signalling pathways (Lam et al., 1989; Neuhaus et al., 1993). Besides, responsiveness of CaM gene(s) to various stimuli and their spatially and temporally regulated expression confirms their importance in Ca$^{2+}$ signalling pathways (Galaud et al., 1993).

Plants have a large number of CaM isoforms whereas no isoform could be detected in animals despite the presence of a multigene family. Presence of multigene family of CaM in plants i.e. two each in rice, Petunia and Vigna; three in maize; five in soybean; six in Arabidopsis, eight in potato and existence of a number of isoforms that contain a few conservative changes i.e. four in Arabidopsis; three in wheat and four in soybean (Ling et al., 1991; Liu et al., 1991; Gwieniowski et al., 1993; Lee et al., 1995; Takezawa et al., 1995; Yuang et al., 1996), possibly contributes to the diversity and specificity of Ca$^{2+}$/CaM mediated signalling. These small changes in the CaM isoforms may contribute to differential interaction of each isoform with the target protein. CaM genes are expressed differentially in response to different stimuli (Snedden & Fromm, 1998; Zielinski, 1998). Such differential regulation is possibly one of the mechanisms, for the cells to fine-tune Ca$^{2+}$ signalling.

In plants, the expression pattern of CaM isoforms is spatially regulated. In Arabidopsis, ACAM 1 was most abundant transcript in leaves and developing siliques (Ling et al., 1991) while ACAM 3 was expressed preferentially in aerial tissues except floral buds (Perera & Zielinski, 1992; Antosiewicz et al., 1995). The levels of ACAM 4, 5 and 6 were considerably lower than the other isoforms. In roots, only ACAM 1 could be detected (Perera & Zielinski, 1992; Gwieniowski et al., 1993). In Brassica also, the level of CaM transcript was higher in leaves and shoot apical meristem than in root tips or root elongation zone (Chye et al., 1995). Maize CaM isoforms, ZMCAM1 and ZMCAM2, were differentially expressed in different tissues (Breton et al., 1995). In potato, PCM1 isoform was highly expressed in stolon tip, moderately in roots and very low in leaves, while PCM6 showed a steady state expression level in all the tissues except roots (Takezawa et al., 1995).

The expression of CaM mRNA was also found to be different in different tissues of soybean (Lee et al., 1995). In Arabidopsis, in a study of transcriptional activation of all the six CaM genes in response to touch show that two of the isoforms are not regulated by touch whereas the other four show differences in their response (Verma & Upadhyaya, 1998). This shows that the level of expression of CaM varies in different parts of a plant, and the isoforms also behave differently thus providing the much needed diversity and specificity to Ca$^{2+}$ signal.

Various other external stimuli as light (Jena et al., 1989; Braam & Davis, 1990; Botella & Arteca, 1994), auxin (Jena et al., 1989; Botella & Arteca, 1994; Okamoto et al., 1995) and ethylene (Braam & Davis, 1990), also bring about differential expression of CaM and related gene(s). Similarly, in barley aleurone protoplasts, where Ca$^{2+}$ mediates GA and ABA effects, a modulation of CaM gene expression and protein level could be detected (Gilroy, 1996). Specific soybean CaM isoforms, SCaM-4 and SCaM-5, are activated by infection or pathogen derived elicitors and participate in Ca$^{2+}$ mediated induction of plant disease resistance response, whereas other SCaM genes encoding highly conserved CaM isoforms showed no effect indicating strikingly the differential regulation of CaMs in plants (Heo et al., 1999).

A strategy for elucidating specific molecular targets of Ca$^{2+}$ and CaM in plant defense responses has been developed. A dominant-acting CaM mutant, VU-3, was used to investigate the oxidative burst and nicotinamide coenzyme fluxes after various stimuli (cellulase, harpin, incompatible bacteria, osmotic and mechanical) that elicit plant defense responses in transgenic tobacco cell cultures. VU-3 CaM differs from endogenous plant CaM in that it cannot be methylated post-translationally, and as a result, it hyperactivates CaM-dependent NAD kinase. Cells ex-
pressing VU-3 CaM exhibited a stronger active oxygen burst that occurred more rapidly than in normal control cells challenged with the same stimuli. Increase in NADPH levels were also greater in VU-3 cells and coincided both in timing and magnitude with development of the active oxygen species (AOS) burst. These data show that CaM is target of calcium fluxes in response to elicitor or environmental stress, and provided 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).

**CaM binding proteins (CBPs).** CaM is multifunctional because of its ability to interact with and regulate the activity of various target proteins, which is mediated by CBPs in plants. The CaM-binding motifs from different CBPs form characteristic basic amphipathic α-helices with several positive residues on one side and hydrophobic residues on the other side. However, the amino acid sequences of the CBP in different CaM target proteins are not conserved per se (Rhoads & Friedberg, 1997).

CaM regulates the activation of large number of diverse proteins implicated in a wide variety of cellular processes such as kinases, nitric oxide synthase, myosin light chain kinase, phosphorylase kinase, calcineurin phosphatase, plasma membrane Ca\(^{2+}\) ATPases and many other enzymes (Billingsley et al., 1990). Role of these proteins has been extensively reviewed (Zeilinski, 1998; Reddy, 2001). Interestingly, many CBPs have no homologs in animals.

CaM stimulates small nuclear NTPases. As nuclear NTPases also get stimulated during phytochrome signalling, it was postulated that CaM may be playing a role along with Ca\(^{2+}\) in phytochrome mediated signalling through nuclear NTPases (Hsieh et al., 1996).

Another important protein with which CaM binds to and stimulates its activity is glutamate decarboxylase (GAD). This enzyme has homologues in animals but lack CaM binding motif, suggesting that they are regulated by CaM only in plants. GAD has been purified from different plants (Ling et al., 1994) and exists as many isoforms. It catalyses decarboxylation of glutamate, thereby producing CO\(_2\) and gamma-aminobutyrate, a very important component of many metabolic pathways. Transgenic Petunia plants, harbouring a mutant GAD lacking the CaM binding site, showed several abnormalities confirming CaM regulation of GAD activity (Baum et al., 1996). As GAD activity gets stimulated by hypoxia and some other stress signals, it indicates that besides its role in controlling various metabolic processes, it might also be involved in CaM regulated stress signalling (Baum et al., 1993, 1996; Gallego et al., 1995; Snedden et al., 1996).

NAD-kinase, which catalyzes the conversion of NAD to NADP, was shown to be regulated by CaM in plants, whereas in animal systems no such regulation was observed. It is vital to living organisms especially when energy is in demand under stress conditions. It plays a role in oxidative burst and in the formation of active oxygen species, which are involved in plant defense against pathogens (Harding et al., 1997). NAD kinases are also regulated by CaM isoforms (Lee et al., 1995). In certain cases, light and some other stimuli have been shown to stimulate the activity of NAD kinases. Using transgenic plants which over express CaM and its mutants and by giving different stress or elicitor signals such as cellulase, heparin and osmotic or mechanical stress to such transformed plants, it was found that CaM is the target of Ca\(^{2+}\) fluxes, and NAD kinase could be the downstream target for this Ca\(^{2+}\)/CaM mediated signalling pathways (Harding et al., 1997; Lee et al., 1997).

The other important CBPs are the endoplasmic reticulum and tonoplast located Ca\(^{2+}\)ATPases and slow vacuolar ion channels which have been involved in a number of calcium signalling processes (Askerlund, 1997; Harper et al., 1998). Several CBPs that show similarities to ion channels have been isolated from barley (Schuurink et al., 1998), tobacco (Arazi et al., 1999, 2000) and Arabidopsis (Kohler et al., 1999; Kohler & Neuhaus, 2000). Such proteins reside in the plasma membrane and show sequence similarity to cyclic nucleotide gated channels from animals and inward rectifying K\(^{+}\) channels from plants. Constitutive overexpression of one of these proteins, NtCBP4, in sense and antisense orientation in tobacco, exhibited a normal phenotype under normal growth conditions. However, NtCBP4 sense transgenic plants showed improved tolerance to Ni\(^{2+}\) and hypersensitivity to Pb\(^{2+}\) (Arazi et al., 1999, 2000) suggesting a role for this CaM-binding channel in metal tolerance.

By using \(^{15}\)S-labeled CaM, it was found that it can bind to various microtubular motors in specific ways. A novel CaM-binding microtubule motor protein was isolated from Arabidopsis (Reddy et al., 1996a; 1996b) and tobacco (Wang et al., 1996a). This kinesin-like CaM-binding protein (KCBP) is distinct from...
all other KLPs in having a CaM binding domain adjacent to its motor domain and appears to be ubiquitous in plants. KCBP interacts with tubulin subunits (Song et al., 1997). The binding of KCBP with tubulin is regulated by Ca\(^{2+}\)/CaM i.e. in presence of Ca\(^{2+}\)/CaM, the motor domain with the CaM binding domain does not bind to tubulin and this modulation is abolished in the presence of antibodies specific to CaM binding domain of KCBP. This CaM-dependent modulation of KCBP interaction with tubulin suggests regulation of KCBP function by calcium (Narasimhulu et al., 1997) as well as in cell division. During cell division, these proteins have been found to be associated with preprophase band, mitotic spindle and phragmoplast. Association with microtubular motor arrays in dividing cells suggests that this negative end directed motor protein is likely to be involved in the formation of microtubular arrays and/or functions associated with these structures (Bowser & Reddy, 1997). The myosin heavy chain binding cDNA also contains a putative CaM binding site (Kinkema & Schiefelbein, 1994). This shows that CaM is involved in intracellular transport processes in cells.

Heat shock proteins also contain CaM binding domains (Li et al., 1994; Lu et al., 1995). One of such CBPs, NtCBP48, contains a centrally located putative transmembrane domain and a nuclear localization sequence motif (Lu et al., 1995). Besides, few transcription factors of basic helix-loop-helix family (Corneliussen et al., 1994), basic amphipathic \(\alpha\)-helix (Dash et al., 1997) also contain CaM binding domains. In Arabidopsis, ACAM-3 promoter binds to the leucine zipper family of transcription factor TGA3, while CaM itself acts as an enhancer of TGA3 binding with its own cis-elements (Szymanski et al., 1996). Different CaM isoforms differentially enhance binding of TGA3 promoter to ACAM 3 and also to cauliflower nuclear proteins. All this suggests that Ca\(^{2+}\) mediated signalling coupled to gene expression could be mediated via CaM and CaM binding transcription factors and could lead to specificity of the response.

AtCNGC1 and AtCNGC2 have CaM and a putative cyclic nucleotide-binding domain (Köhler et al., 1999), and interact with yeast CaM. In plants, Ca\(^{2+}\)/CaM is involved in stabilizing cortical microtubules at low Ca\(^{2+}\) concentration and destabilizing the same at higher Ca\(^{2+}\) concentration (Cyr 1991, Fisher et al., 1996). Two CBPs that show different sensitivities to Ca\(^{2+}\) are implicated in evoking these two opposing effects of CaM on cortical microtubules. Elongation factor-1\(\alpha\) (EF-1\(\alpha\)), a CaM-binding microtubule associated protein, stabilizes microtubules. Ca\(^{2+}\)/CaM has been shown to inhibit EF-1\(\alpha\)-promoted microtubule stabilization (Moore et al., 1998). An auxin-induced gene product binds CaM, suggesting the involvement of CaM in auxin action (Yang & Poovaiah, 2000). Two maize proteins, CBP-1 and CBP-5, and a multidrug resistant protein also bind CaM in a Ca\(^{2+}\)-dependent manner (Reddy et al., 1993; Wang et al., 1996b). However, their function is not known. A pollen specific CBP from maize, MPCBP (Safadi et al., 2000), is a novel CBP and has no homolog in non-plant systems. Binding of superoxide dismutase to CaM-Sepharose column is indicative of its regulation by CaM (Gong & Li, 1995).

(b) CaM like Ca\(^{2+}\) Regulated Proteins

In addition to CaM, plants contain numerous CaM-like proteins whose function in Ca\(^{2+}\) signalling pathway(s) is not fully characterized as compared to that of CaM. CaM-like proteins differ from CaM in containing more than 148 amino acids and one to six EF-hand motifs with limited homology to CaM (Snedden & Fromm, 1998). Hence, it is likely that such proteins are functionally distinct from CaM and are involved in controlling different Ca\(^{2+}\) mediated cellular functions.

Calcineurin in calcineurin B-like (CBL) protein, a new family of CaBPs, is a Ca\(^{2+}\)/CaM dependent protein phosphatase involved in Ca\(^{2+}\) signalling in animals and yeast. However, the biochemical identity of calcineurin remains elusive. In Arabidopsis, AtCBL (Kudla et al., 1999), which shares maximum homology to the regulatory subunit of mammalian calcineurin B, shows significant similarity with another CaBP, the neuronal calcium sensor in animals. AtCBL contains typical EF-hands motifs. Interaction of AtCBL1 and calcineurin B complemented the salt sensitive phenotype in a yeast calcineurin B mutant. Cloning of cDNA revealed a family of at least six genes in Arabidopsis encoding highly similar but functionally distinct CaBPs (AtCBL proteins).

(c) Ca\(^{2+}\) Modulated Protein Kinases in Plants

The role of protein kinases and phosphatases in plant signal transduction has been extensively reviewed (Stone & Walker, 1995; Sopory & Munshi, 1998). Kinases are the main trigger proteins, which transduce signal by phosphorylating other proteins/kinase(s). A large number of protein kinases
exist in plants. Some of these kinases are similar to the kinases present in animal systems while others are specific to plants. Calcium regulates following three different families of protein kinases in plants: (i) calcium dependent protein kinases (CDPKs) which require only Ca\(^{2+}\) for their activity; (ii) Ca\(^{2+}\)/CaM dependent protein kinases (CCaMKs) which along with Ca\(^{2+}\) also require CaM for their activity; and, (iii) Ca\(^{2+}\)/lipid dependent protein kinases (PKCs) which require lipids along with Ca\(^{2+}\) for activity.

(i) **CDPKs.** These are widely distributed most abundant and well-characterized kinases and their existence is ubiquitous in plants (Sopory & Munshi, 1998; Harmon et al., 2000). CDPKs are plant specific protein kinases as no CDPK homologue has been reported from the animal systems as yet. These kinases require micromolar concentration Ca\(^{2+}\) for their activity and have no requirement of CaM or lipids.

The CDPKs have a unique structure as the N-terminal protein kinase domain is fused with C-terminal auto-regulatory domain and a CaM like domain(CaMLD), which has four Ca\(^{2+}\) binding EF-hand or helix-loop-helix motif (Balty & Venis, 1988; Harper et al., 1991; Suen & Choi, 1991). The region that joins the kinase domain to the CaM-like region (junction region) corresponds to the autoinhibitory/CaM-binding region of CaM K II (Ca\(^{2+}\)/CaM-dependent protein kinase II) and prevents kinase activity in the absence of Ca\(^{2+}\) (Harper et al., 1991). Binding of Ca\(^{2+}\) to CDPK affects conformation of the kinase and relieves the inhibition caused by the autoinhibitory region (Harmon et al., 2000).

The N-terminal domain of CDPKs is variable and provides specificity to different CDPK isoforms. Ca\(^{2+}\) directly binds to the CDPK and stimulates the kinase activity. These enzymes show autophosphorylation and many fold stimulation with Ca\(^{2+}\). The CDPK from groundnut shows that autophosphorylation is a prerequisite for the activation of GnCDPK (Dasgupta, 1994; Chaudhari et al., 1999). They are both soluble as well as membrane bound and have been reported from organelles also. Several CDPKs have putative myristoylation sites indicating that myristoylation of CDPKs may regulate the association of CDPKs with membranes (Harmon et al., 2000). Although CaM does not stimulate these kinases, different CaM inhibitors affect the activity of these CDPKs, possibly due to the existence of CaMLD. The autoregulatory domain keeps the activity of the enzyme at basal level.

Under *in vitro* conditions, the kinases get activated by binding to Ca\(^{2+}\) and use various proteins as substrates like histone, casein, phosvitin, BSA and few synthetic peptides but *in vivo* substrates are not known in many cases. Genes for various CDPKs have been cloned and some of them belong to multigene family (Biermann et al., 1990; Ali et al., 1994; Breviaro et al., 1995; Thummel et al., 1995; Hrabak et al., 1996; Redhead & Palme, 1996). There are over 40 CDPKs in the *Arabidopsis* genome. Furthermore, CDPKs differ in their affinity for Ca\(^{2+}\). In *Arabidopsis*, the AtCDPK1 differs from AtCDPK2 in its Ca\(^{2+}\) stimulated activity although both of them possess four EF hand motifs.

Besides Ca\(^{2+}\), lipids are involved in the regulation of CDPK activity (Harper et al., 1993; Binder et al., 1994). A carrot CaM-like domain protein kinase, DcCPK1, resembles animal PKC in its activation by Ca\(^{2+}\) and certain phospholipids suggesting that lipids regulate the activity of some CDPKs and perform specific biological functions in plants (Farmer & Choi, 1999). CDPKs have been reported to play a role in diverse cellular processes ranging from ion transport to gene expression. Ion channels, enzymes involved in metabolism, cytoskeletal proteins and DNA binding proteins have been identified as CDPK substrates (Harmon et al., 2000).

Further, there are certain CDPK-related kinases (CRKs) that are similar to CDPKs except that the CaM–like region is poorly conserved with degenerate EF-hands. Atleast seven CRKs have been found in *Arabidopsis*, however, regulation and function of these kinases are not yet known (Harmon et al., 2000).

(ii) **CCaMKs.** It is a group of calcium-dependent kinases, which in addition to calcium also require CaM for their activity. Thus CaM, besides acting directly, could also exert its effect by binding to protein kinases and modulating their activities. In animal systems, CaM activates Ca\(^{2+}\)/CaM dependent kinase I, II and III, which regulate a wide variety of physiological processes involving Ca\(^{2+}\) mediated signalling (Colbran et al., 1989).

Since, importance of Ca\(^{2+}\)/CaM kinases in animal systems is very well established, attempts were made to look for a parallel signalling pathway in plant systems as well. Some indirect studies (Salimath & Marme, 1983; Blowers & Trewavas, 1987) predicted existence of kinases family in plants which was eventually confirmed by cloning of various cDNA homologues from different plant systems e.g. carrot
(Suen & Choi, 1991), apple (Watillon et al., 1992, 1993, 1995), lily (Patil et al., 1995; Takezawa et al., 1996) and maize (Lu et al., 1996). All homologues show a considerable similarity with their animal system counterparts at the cDNA level. Sequence analysis revealed the presence of an N-terminal catalytic domain, a centrally located CaM-binding domain and a C-terminal visinin-like domain containing only three EF hands. Biochemically, Ca\(^{2+}\)/CaM stimulates CCaMK activity. In the absence of CaM, Ca\(^{2+}\) promotes autophosphorylation of CCaMK. The phosphorylated form of CCaMK possesses more kinase activity than the non-phosphorylated form (Takezawa et al., 1996).

The kinase cDNA homologue from apple encodes a single polypeptide (mol wt, 46.5 kDa) with serine/threonine catalytic domain and an adjacent Ca\(^{2+}/\)CaM binding regulatory domain. It shows considerable homology to corresponding regions of mammalian multi-functional Ca\(^{2+}/\)CaM protein kinase II (Watillon et al., 1995). Ca\(^{2+}/\)CaM dependent kinase gene from lily anthers is a chimeric gene containing a neural visinin like Ca\(^{2+}\) binding domain fused with a CaM binding domain. The amino-terminal region of the encoded protein contains all the eleven conserved sub-domains characteristics of serine/threonine protein kinase. The CaM binding region has high homology (79%) to the subunit of mammalian Ca\(^{2+}/\)CaM dependent protein kinase (Patil et al., 1995).

Biochemical properties of lily Ca\(^{2+}/\)CaM dependent kinase, studied by over-expressing its cDNA in E. coli, show that it is a non-conventional, novel Ca\(^{2+}/\)CaM kinase, which shows a dual regulation with Ca\(^{2+}\) and CaM. Autophosphorylation of the protein is only Ca\(^{2+}\) dependent while both Ca\(^{2+}\) and CaM regulate substrate phosphorylation, though neither of them modulates the kinase activity individually (Takezawa et al., 1996). Using the yeast two-hybrid system, to obtain genes coding for the proteins interacting with this kinase, a cDNA clone, which shows very high similarity to EF-1α, has been obtained. The kinase phosphorylates EF-1α in a calcium/CaM dependent manner suggesting its direct role in the regulation of gene expression (Wang & Poovaiah, 1999). Similar CCaMK genes (TCCaMK-1 and TCCaMK-2) cloned from tobacco show differential regulation by CaM isoforms (Liu et al., 1998).

MCKI, a Ca\(^{2+}/\)CaM kinase homologue from maize roots (Lu et al., 1996; Lu & Feldman, 1997), contains all the eleven conserved subdomains, characteristic of protein kinase catalytic domain and all the conserved amino acid residues. It shares sequence homology with yeast CMK1 (42%), rat CaMK II (37%) and apple CBI (34%). It is expressed in root caps, which is the site of perception of both light and gravity signals. Since MCKI is expressed both in light and dark grown tissue, it appears not to be directly regulated by light but has been shown to be involved in gravitropic responses. However, biochemical properties of this kinase have not been studied till now. A kinase (mol wt, 72 kDa), characterized biochemically from Zea mays, belongs to the serine/threonine family of protein kinases and shows a dual regulation by calcium and CaM. The substrate phosphorylation is calcium dependent and addition of exogenous CaM stimulates it further, whereas autophosphorylation is only calcium dependent (Pandey & Sopory, 1998).

Thus, homologues of both conventional and novel Ca\(^{2+}/\)CaM dependent protein kinases exist in plants. As these kinases are involved in a wide range of signalling processes in animals also, hence their important role is envisaged in Ca\(^{2+}\) signalling pathways in plants.

(iii) PKCs. These kinases belong to the serine/threonine family of protein kinases, and in addition to Ca\(^{2+}\) require phospholipids for their activity. In mammalian system, they are basically involved in various regulatory processes (Nishizuka, 1992), playing a pivotal role in signal transduction involving receptor-mediated hydrolysis of PIP\(_2\), which produces IP\(_3\) and DAG. DAG is an activator of PKC type kinases, while IP\(_3\) releases calcium from intracellular stores. Such kinases are present in both membrane and soluble fraction, and show some selectivity towards the lipids, which are required for their stimulation.

In plants, some of these types of purified kinases have similar activities as their animal system counterparts (Baron-Marting & Scherer, 1989; Komatsu & Hirano, 1993; Honda et al., 1994; Nanmori et al., 1994; Karibe et al., 1995; Chandok & Sopory, 1998). Although these kinases have been reported from various plant systems, their exact role in calcium mediated signalling is not known and further work is needed in this direction. A cPKC activity in maize has been characterized in great detail and its role in light mediated nitrate reductase (NR) gene induction has been studied (Chandok & Sopory, 1998).

(d) Ca\(^{2+}\) Modulated Protein Phosphatases in Plants

A Ca\(^{2+}\) modulated protein phosphatase has been
discovered through the analysis of a calcium signalling pathway mutant. A mutation at the ABI1 (abscisic acid insensitive) locus in Arabidopsis thaliana caused a reduction in the sensitivity to the plant hormone, abscisic acid. The sequence of ABI1 predicts a protein composed of an N-terminal domain that contains motif for an EF-hand Ca\(^{2+}\) binding site and a C-terminal domain with similarities to protein serine/threonine phosphatase 2C. The C-terminal of ABI1 can partially complement temperature sensitive growth defect of a Saccharomyces cerevisiae protein phosphatase 2C mutant and this also shows phosphatase activity in vitro (Leung et al., 1994; Meyer et al., 1994; Bertauche et al., 1996). These suggest that the ABI1 protein is a Ca\(^{2+}\)-modulated phosphatase and functions to integrate ABA and Ca\(^{2+}\)-signals with phosphorylation dependent response pathway.

Protein phosphatase 2C (PP2C) is a class of ubiquitous and evolutionarily conserved serine/threonine PP involved in stress responses in yeasts, mammals, and plants (Shenolikar, 1994; Hunter, 1995). Mutational analysis of two Arabidopsis thaliana PP2Cs, encoded by ABI1 and AtPP2C, involved in the plant stress hormone abscisic acid (ABA) signalling in maize mesophyll protoplasts. Consistent with the crystal structure of the human PP2C, the mutation of two conserved motifs in ABI1 (predicted to be involved in metal binding and catalysis) abolished PP2C activity. Surprisingly, although the DGH177-179KLN mutant lost the ability to be a negative regulator in ABA signalling, the MED141-143IGH mutant still inhibited ABA-inducible transcription, perhaps through a dominant interfering effect. Moreover, two G to D mutations near the DGH motif eliminated PP2C activity but displayed opposite effects on ABA signalling. The G174D mutant had no effect but the G180D mutant showed strong inhibitory effect on ABA-inducible transcription. Based on the results that a constitutive PP2C blocks but constitutive Ca\(^{2+}\) dependent protein kinases (CDPKs) activate ABA responses, the MED141-143IGH and G180D dominant mutants are unlikely to impede the wild type PP2C and cause hyper phosphorylation of substrates. In contrast, these dominant mutants could trap cellular targets and prevent phosphorylation by PKs required for ABA signalling. The equivalent mutations in AtPP2C showed similar effects on ABA responses. This study suggests a mechanism for the action of dominant PP2C mutants that could serve as valuable tools to understand protein-protein interactions mediating ABA signal transduction in higher plants (Sheen, 1998). Stress response in plants involves changes in the transcription of specific genes. The constitutively active mutants of two related Ca\(^{2+}\)-dependent protein kinases, CDPK1 and CDPK1a, activate a stress-inducible promoter, by passing stress signals. Six other plant protein kinases, including two distinct CDPKs, fail to mimic this stress signalling (Sheen, 1996). Furthermore, the activation is abolished by a CDPK1 mutation in the kinase domain and diminished by a constitutively active protein phosphatase 2C that is capable of blocking response to the stress hormone, abscisic acid.

(e) CaBPs without EF-Hand Motifs

There are several proteins that bind Ca\(^{2+}\) but do not contain EF-hand motifs, like calreticulin, centrin, annexin, etc.

(i) Calreticulin (CRT). It is a Ca\(^{2+}\) sequestering protein in the ER and functions as a chaperone (Michalak et al., 1998; Baluska et al., 1999). It is a major calcium storage protein (mol wt, 48 - 55 kDa) located mainly in the endoplasmic reticulum and also in the nucleus and/or cytoplasm of some cells. The cDNA for several CRT have been cloned from maize, barley, tobacco and Ricinus communis (Chen et al., 1994; Kwiatkowski et al., 1995; Dresselhaus et al., 1996; Coughlan et al., 1997; Borisjuk et al., 1998). Several other homologues have been detected in other plants e.g. CRT-like protein in tobacco cells (Denecke et al., 1995; Drolillard et al., 1997), spinach (Navazio et al., 1996) and pea (Hassan et al., 1995). The functional role of CRT in post-translational processing and translocation processes has been suggested apart from its postulated function in cellular Ca\(^{2+}\) homeostasis (Borisjuk et al., 1998).

The Ca\(^{2+}\) status of the endoplasmic reticulum can be altered by the overexpression of a CaBP (CRT) in transgenic plants. A 2.5-fold increase in CRT led to a 2-fold increase in ATP-dependent Ca\(^{2+}\) accumulation in the ER-enriched fraction of the transgenic plant indicating that altering the production of CRT affects the ER Ca\(^{2+}\) pool (Persson et al., 2001). Such plants were able to retain chlorophyll when transferred from Ca\(^{2+}\)-depleted medium to Ca\(^{2+}\)-containing medium thereby enhancing the survival of plants grown in low Ca\(^{2+}\) medium.

(ii) Arabidopsis GF14 protein. It shows more than 60% identity with mammalian brain 14-3-3 proteins at
the amino acid sequence level and is apparently associated with G-box DNA binding protein complex (Lu et al., 1994). Arabidopsis GF14α binds calcium and its C-terminal domain contains a potential EF-hand motif. GF14α is phosphorylated by Arabidopsis protein kinase activity at a serine residue(s) in vitro. Therefore, GF14α protein has biochemical properties consistent with potential signalling roles in plants.

(iii) Centrin. It is an acidic CaBP of 20 kDa and was first identified in the green alga, Tetraselmis striata, as a component of striated flagellar roots. Basal body complexes isolated from Chlamydomonas reinhardtii contain protein whose cDNA shows high homology to centrin, which is strongly related to CDC31 gene product of S. cerevisiae (Schiebel & Bornens, 1995). Centrin has been reported from higher plants, Atriplex nummularia (Zhu et al., 1992) and animals, mouse (Ogawa & Shimizu, 1993) and humans (Lee & Huang, 1993). In some organisms,centrins are also known as caltractins. Although centrin from different species seems to have different functions, it could well be that they act by a common underlying molecular mechanism. Centrins probably drastically change their conformation upon binding to Ca\(^{2+}\) and neither performs contraction (in Chlamydomonas) or transduction of signal for duplication of the SPB (in S. cerevisiae) (Schiebel & Bornens, 1995).

(f) Other CaBPs

CaBPs have also been identified as caldesmon-like protein from pollen tubes of Ornithogalum virens (Krauze et al., 1998) and from vacuoles of celery (Apetum graveolens L.) (Randall, 1992). The genes (cDNA) for other CaBPs from several plants have also been cloned. Some of these cDNA are novel e.g. NaCl induced CaBP from Arabidopsis, AtCP1 (Jang et al., 1998), a CaBP from Brussica, FCP, which is predominantly expressed in pistil and anthers (Furuyama & Dzelzkalns, 1999), ABA and osmotic stress induced CaBP from germinating rice seedlings (Frandsen et al., 1996), a 22 kDa CaBP (CaBP-22) from Arabidopsis, which is related to CaM (Ling & Zielinski, 1993).

In Atriplex nummularia, multiple transcripts of CaBP showed differential regulation by environmental stimuli and development (Zhu et al., 1996). In Arabidopsis, a homologue of neutrophil NADPH oxidase gp91phox subunit encoding a plasma membrane CaBP containing EF-hand motif (respiratory burst oxidase homologue A—rbohA) (Keller et al., 1998) and in slime mold, Dictyostelium discoideum, a novel CaBP, Calfumirin-1 (CAF-1), which shows specific expression during transition of cells from growth to differentiation, have been reported.

In bean, Hra 32 (hypersensitive reaction associated), a CaBP (mol wt, 17 kDa) that specifically accumulates during HR (hypersensitive response), has been identified. Hra 32 has four putative EF-hand calcium-binding domains (Jakobek et al., 1999). Therefore, several CaBPs have been identified from plants but their in vivo function has not been shown.

(i) Annexin. Higher plants contain annexins (Boustead et al., 1989), which have been purified and characterized from a variety of plant sources. Analyses of the deduced proteins encoded by annexin cDNA indicate that the majority of these annexins possess the characteristic four repeats of 70 to 75 amino acids and motif proposed to be involved in Ca\(^{2+}\) binding. Like animal annexins, plant annexins bind Ca\(^{2+}\) and phospholipids and are abundant proteins, but the number of distinct plant annexin genes may be considerably fewer than that found in animals. Various members of the annexin family in plants may play roles in secretion and/or fruit ripening, as they show interaction with the enzyme callose (1,3-beta-glucan) synthase, possess intrinsic nucleotide phosphodiesterase activity, bind to F-actin and/or have peroxidase activity (Clark & Roux, 1995).

(ii) Calnexin. Of the few calnexin genes identified in plants, the first calnexin(CNX1P), reported in Arabidopsis, encodes for a protein of 65.5 kDa, and is 48% identical to dog calnexin (Huang et al, 1993b). Calnexin is 64 kDa protein, which co-purified with oat vacuolar ATPase B subunit and interacts with both subunit A and B of vacuolar ATPase (Li et al., 1998). A pea calnexin, PsCNX, cloned and characterized recently (Ehtesham et al., 1999), binds to ATP and contains ATPase activity. In addition, casein kinase II phosphorylates it in vitro. The function of calnexin in plants is not yet known.

(iii) Phospholipases (PLA, PLC, PLD). Phospholipid catabolism is essential for cell function and encompasses a variety of processes including metabolic channeling of unusual fatty acids, membrane reorganization and degradation, and the production of secondary messenger. Phospholipases are grouped into classes depending upon their general site of cleavage. Multiple isoforms of two phospholipases have been identified. Their different activities were observed during different physiological consequences
in the context of the plant development and in response to environmental stress (Chapman, 1998). Of the several phospholipases reported from plants, almost all of them have C2-domain and require Ca\(^{2+}\) for their activities (Hirayama et al., 1995, 1997; Shi et al., 1995; Singer et al., 1997; Wang, 1997; Munnik et al., 1998).

(iv) Copines. Paramaecum tetraurelia copine (mol wt, 55 kDa) is calcium dependent phospholipid binding protein, which contains C2 domain. In plants, copine gene is reported in Arabidopsis thaliana. In ciliates, the role of copine is suggested in the membrane trafficking. Current sequence databases indicate the presence of multiple copine homologues in green plants, Arabidopsis thaliana, nematodes, and humans (Creutz et al., 1998).

**Role of CaBPs in Stress Tolerance**

Various stimuli, which act by modulation of Ca\(^{2+}\) concentration, affect the expression of different CaM related gene(s). The presence of their multiple isoforms in plants add further complexity to the Ca\(^{2+}\) mediated network and points to their differential sensitivity to elevated cytosolic Ca\(^{2+}\) levels in response to different stress stimuli. For example, in case of mechanical signals such as rain, wind and touch, differential expression of various CaM related genes was observed and the pattern of expression of such genes was reported to be spatially and temporally regulated (Braam & Davis, 1990; Ito et al., 1995; Polisensky & Braam, 1996). Other CaBPs have also been shown to be responsive to various environmental stimuli. The possible involvement of CaBPs in stress response is summarized below.

- Studies with salt overly sensitive (SOS) mutants of Arabidopsis indicate a key role for Ca\(^{2+}\) in salt stress signalling (Wu et al., 1996, Liu & Zhu 1997, Liu et al., 2000). SOS mutants are hypersensitive to Na\(^+\) and Li\(^+\) and are unable to grow on low K\(^+\) culture medium. The abnormal growth patterns in the presence of NaCl could be ameliorated by the addition of increased levels of Ca\(^{2+}\) in the same medium. The deduced amino acid sequence of SOS3 gene product shows its close affinity to CaBPs, specifically calcineurin B like Ca\(^{2+}\) sensor and neuronal calcium sensors (NCS) of animals, which can stimulate protein phosphatases or inhibit protein kinases (Liu & Zhu, 1998). Also, SOS3 gene is predicted to contain three potential Ca\(^{2+}\) binding sites. These versatile proteins participate in ion transport phenomena in other organisms, which concluded that SOS3 mediates the interaction of potassium, sodium and calcium i.e. control the K\(^+/Na^+\) transport system via a Ca\(^{2+}\)-regulated pathway (Bressan et al., 1998; Epstein, 1998; Liu & Zhu, 1998). The SOS3 has been found to physically interact with and to activate a protein kinase SOS2 (Shi et al., 1999; Halfter et al., 2000). Without SOS3, SOS2 has virtually no activity as tested on several peptide substrates, while it is able to phosphorylate peptides in the presence of SOS3. The binding of SOS3 and SOS2 appears to be independent of free calcium, however, the SOS2 phosphorylation of peptide substrates requires calcium. Mutations in the Arabidopsis salt genes, SOS3 and SOS2, cause Na\(^+\) and K\(^+\) imbalance and render plants more sensitive towards growth inhibition by high Na\(^+\) and low K\(^+\) environments. Also in these mutants, the operation of high affinity potassium transporter is suppressed (Liu & Zhu, 1998). Both abnormal functions are mitigated or abolished by high external calcium concentration, suggesting that in mutants there is impairment in the signalling pathway essential for the normal function of calcium in mitigating salt stress. The SOS3 gene is predicted to encode a CaBP with an N-myristoylation signature sequence. Both N-myristoylation and calcium binding are crucial for SOS3 function in plant salt tolerance (Ishitani et al., 1999). The SOS2 gene has been isolated and identified as a serine/threonine type protein kinase (Liu et al., 2000), which forms another potential candidate for engineering abiotic stress tolerance. The functional domains in protein kinase SOS2, that interacts with SOS3 and is required for plant salt tolerance, have also been identified (Guo et al., 2001). SOS3 interaction with and activation of SOS2 kinase is very consistent with genetic evidence that the two genes are both the positive regulators of salt tolerance and function in the same pathway (Halfter et al., 2000). These two proteins together define a previously unknown regulatory pathway, the SOS pathway, for plant Na\(^+\) tolerance. It has been suggested that high Na\(^+\) stress is sensed either externally or internally and somehow leads to an increase in cytosolic free Ca\(^{2+}\) concentration. SOS3 binds to Ca\(^{2+}\) and activates the protein kinase SOS2. Activated SOS3-SOS2 kinase complex is necessary for increased...
expression of SOS1—a putative Na\(^+\)/H\(^+\) antiporter at plasma membrane (Shi et al., 2000), and perhaps the other transporter genes under salt stress. The SOS3/SOS2 pathway may also regulate the activities of SOS1 and other transporters at the post-translational level. This gene expression and transporter activity regulation brings about homeostasis of ions such as Na\(^+\) and K\(^+\) and consequently plant tolerance to Na\(^+\) stress (Zhu, 2000).

- In *Arabidopsis* (apart from 6 CaMs), there are other CaM-like genes including the TCH genes that are induced in response to various mechanical, chemical and environmental stimuli (Braam et al., 1997). Further, a protein, TCH3, has been found to be 324 amino acids long and contains six EF hand motifs (Sistrunk et al., 1994).

- A cDNA sequence encoding a 27 kDa CaM-like protein, EFA27, has been isolated from ABA-treated rice seedlings (Frandsen et al., 1996). It contains a single EF hand motif and the expression of EFA 27 is induced in response to salt and dehydration stress, and to ABA signal. In *Arabidopsis*, there are several EFA27 gene homologues (Frandsen et al., 1996), suggesting the existence of similar proteins in phylogenetically distant species.

- Another CaBP, AtCP1, from *Arabidopsis* contains three EF hand motifs and binds Ca\(^{2+}\) (Jang et al., 1998). The AtCP1 gene transcripts are also highly inducible by NaCl treatment but not by ABA treatment indicating the specificity of this unique CaBP in responding to stress factors.

- The transcripts of *Arabidopsis* CDPK genes, AtCDPK1 and AtCDPK2, are highly inducible by drought and salinity but not by low temperature or heat stress, suggesting the specificity of CDPK’s induction in response to different stress factors (Urao et al., 1994). A CDPK from *Vigna radiata* is highly inducible by wounding, CaCl\(_2\), IAA and NaCl treatments (Botella et al., 1996). AtCDPK1 and AtCDPK1a are involved in regulating the expression of stress-inducible genes (Sheen, 1996). The Ca\(^{2+}\) regulated phosphorylation has been found to be necessary for stress-induced gene expression as phosphatases have been found to counteract these responses. An immunohomologue of this Ca\(^{2+}\)/CaM dependent kinase has been identified in *Pisum sativum*, which is involved in light and stress mediated signalling (Pandey & Sopory, unpublished).

- AICBL1 mRNA expressed preferentially in stems and roots and its level increases in response to specific stress signals such as drought, cold, and wounding, whereas AICBL2 and AICBL3 are constitutively expressed (Kudla et al., 1999).

- Calcineurin B-like (CBL) -interacting protein kinases (CIPK 1 to 4) belong to the serine/threonine class of kinases and show high homology to protein kinases, SNF1 and AMPK from yeast and mammalian systems, respectively. SOS2 (CBL4) and other CBEs activate CIPK/SIPK in a Ca\(^{2+}\)-dependent manner, suggesting that CBL/SIPK complex is likely to regulate Na\(^+\) and K\(^+\) homeostasis through phosphorylation. Chips/Spikes represent a novel family of CaBP kinases in plants. The interaction of CBL proteins with protein kinases is very surprising since the CBL proteins are known to activate a protein phosphatase in animals. In addition to CaBP kinase, there is some evidence for the role of a Ca\(^{2+}\)-regulated phosphatase in salt tolerance.

- Ectopic expression of constitutively active yeast calcineurin has been shown to confer salt tolerance in tobacco (Bressan et al., 1998; Pardo et al., 1998).

- The authors have found the presence of homologue(s) of a *Entamoeba histolytica* CaBP (EhCaBP) and EhCaBP stimulated kinase(s) in plants, which may be involved in alternate calcium signalling pathway regulating development and adaptation under unfavorable environmental conditions (Pandey et al., 2002).

- PhospholipaseD (PLD) and its product phosphatidic acid have a role in stress signalling. PLD has a putative catalytic domain and a C2 domain that is involved in Ca\(^{2+}\)/phospholipid binding. It has been found that the antisense expression of *Arabidopsis thaliana* PLDa in transgenic *Arabidopsis* plants leads to retardation of senescence, which is promoted by ethylene and ABA (Fan et al., 1997). The retardation of the senescence was demonstrated by delayed leaf yellowing, lower ion leakage, greater photosynthetic activity, and higher content of chlorophyll and phospholipids in the PLDa antisense plants than in those of the wild type. Another PLD, AtPLD8, cloned from the cDNA library prepared from dehydrated *Arabidopsis thaliana*, has been found to accumulate in response to dehydration and high salt stress (Katagiri et al., 2001).
Manipulation of CaBPs for Developing Abiotic Stress Tolerance

Studies have focussed towards the over and under expression of the genes encoding CaBPs employing the contemporary tools and techniques of genetic engineering. Transgenic plants for CaBPs like calcineurin, Ca\(^{2+}/\text{H}^+\) antiporter, CDPK, glyoxalase I, and Eh-CaBP have been raised with improved stress tolerance as follows:

(a) Calcineurin (CaN)

The altered expression of a calcium stress-signalling component, Ca\(^{2+}/\text{CaM}\)-dependent protein phosphatase calcineurin (PP2B), has successfully worked for raising salt tolerant plants in Arabidopsis. CaN has been suggested to be an integral component of a salt stress signal transduction pathway which has been found to be regulating the influx and efflux of Na\(^{+}\) that ultimately affects salt tolerance.

Pardo et al (1998), have co-expressed a truncated form of catalytic subunit and the regulatory subunit of yeast CaN in transgenic tobacco plants to reconstitute a constitutively activated phosphatase in vivo. Several different transgenic lines expressing CaN, exhibited substantial tolerance to NaCl. It has also been suggested that the CaN functions mainly in roots to regulate the movement of ions from the apoplasm to the symplast, which controls the ion content of the xylem sap, which in turn, is transported to the shoots by the strength of the transpirational sink. Root growth was found to be less perturbed than shoot growth by NaCl in plants expressing CaN. Also, NaCl stress survival of control shoots was enhanced substantially when grafted onto roots of plants expressing CaN, further implicating a significant function of the phosphatase in the preservation of root integrity during salt shock.

(b) Glyoxalase I (Gly I)

Besides phosphatases, there are enzymes that are directly regulated by calcium and CaBP. One such enzyme that authors have identified is glyoxalase I which is involved in methylglyoxal detoxification and maintaining glutathione homeostasis. The authors have earlier purified Gly I (Deswal et al, 1993) and shown that it is a calcium-binding enzyme and its activity is modulated by calcium/CaM (Deswal & Sopory, 1999).

To determine the functional significance of Gly I in plants, authors cloned its gene (Veena et al, 1999) and raised transgenic plants via Agrobacterium mediated transformation. The transgenic plants over-expressing Gly I showed tolerance to salt and heavy metals (zinc). The wild type plants showed an early bleaching as compared to the Gly I overexpressors. The tolerance level of transgenic plants to zinc was dependent on the expression level of Gly I protein. The biochemical basis of this tolerance mechanism is not clear. However, during stress, the levels of methyl glyoxal that is a toxic compound increases and that leads to decreased cell division (Szent-Gyorgi & Egyud, 1966; Scaife, 1969) and causes cell death (Kalapos et al, 1991). A system producing more glyoxalase could, therefore, convert methyl glyoxal into non-toxic form, thus preventing the system from its cytotoxic effects during stress condition. Besides detoxification of methylglyoxal, the glyoxalase system could also play a role in providing tolerance under stress by recycling glutathione that would be trapped spontaneously by methyl glyoxal to form hemi thio-acetal (Creighton et al, 1988; Thornalley, 1990), thereby maintaining the glutathione homeostasis.

(c) Ca\(^{2+}/\text{H}^+\) Antiporter (CAX 1 or Calcium Exchanger 1)

The changes in the cytosolic Ca\(^{2+}\), which are tightly regulated in plants, have been implicated in converting numerous signals into adapted responses. Ca\(^{2+}\) efflux from the cytosol modulates Ca\(^{2+}\) concentrations in the cytosol, loads Ca\(^{2+}\) into intracellular compartments and supplies Ca\(^{2+}\) to organelles for supporting biochemical functions. Vacuolar ion transporter, CAX 1, is thought to be a key mediator of these processes.

CAX 1 transcript was found to be highly induced in response to exogenous Ca\(^{2+}\). Transgenic tobacco plants expressing CAX 1 displayed symptoms of Ca\(^{2+}\) deficiencies, including hypersensitivity to ion imbalances, such as increased magnesium and potassium ions and to cold shock, but increasing the Ca\(^{2+}\) in the media abrogated these sensitivities (Hirschi, 1999). Tobacco plants expressing CAX 1 were found to show an increased Ca\(^{2+}\) accumulation and altered activity of the CAX 1. In a similar study, yeast vacuolar Ca\(^{2+}/\text{H}^+\) antiporter (VCX 1) was expressed in Arabidopsis plants, which displayed increased sensitivity to sodium and other ions, which could be suppressed by addition of calcium to the media (Hirschi et al, 2001). These results emphasize that regulated expression of Ca\(^{2+}/\text{H}^+\) antiporter activity is critical for normal growth.
and adaptation to certain stresses and could be a valuable tool to experimentally dissect the role of \( \text{Ca}^{2+} \) transport around the plant vacuole.

Further, expression of another calcium exchanger, CAX 2 from *Arabidopsis* in tobacco, has been found to regulate metal transport from the cytosol to the vacuole. Tobacco plants expressing CAX 2 accumulated more \( \text{Ca}^{2+} \), \( \text{Cd}^{2+} \) and \( \text{Mn}^{2+} \) and were more tolerant to elevated \( \text{Mn}^{2+} \) levels. Expression of CAX 2 in tobacco increased \( \text{Cd}^{2+} \) and \( \text{Mn}^{2+} \) transport in isolated root tonoplast vesicles (Hirschi *et al.*, 2000). These results suggest that CAX 2 has a broad substrate range and its modulation may be an important component of future strategies to improve plant ion tolerance and of plant's potential use in phytoremediation.

**d) CDPK**

As discussed earlier, the cytoplasmic \( \text{Ca}^{2+} \) levels in plant cells increase rapidly in response to multiple stress stimuli, including cold, salt and drought. Following this \( \text{Ca}^{2+} \) influx, relay of signals is likely to be mediated by combinations of protein phosphorylation/dephosphorylation cascades. It is presumed that members of the CDPK family in plants perform the majority of \( \text{Ca}^{2+} \) stimulated protein phosphorylation predominantly. Despite the potential importance of CDPKs, the physiological function of a specific pathway has not been elucidated so far.

The selected members of CDPK family are involved in activation of stress/ABA-responsive promoter (Sheen, 1996). Transgenic rice plants have been generated with altered levels of this protein. The extent of tolerance to cold and salt/drought stresses of such plants correlated well with the level of OsCDPK7 expression (Saijo *et al.*, 2000).

Overexpression of this protein in the transgenic rice enhanced induction of some-stress responsive genes in response to salinity/drought, but not to cold. Thus, the downstream pathways leading to cold and salt/drought tolerance are different from each other. It has also been suggested that at least two distinct pathways commonly use a single CDPK, maintaining the signalling specificity through unknown post-translational regulation mechanisms. Thus, simple manipulation of CDPK activity has great potential with regard to stress tolerance.

**e) EhCaBP Kinase**

Apart from CaM, there are several other CaBPs, CBPs and kinases in plants that are performing various functions. Authors have identified a different class of protein kinase activity in plants e.g., a protein kinase activity from *Brassica juncea* that is stimulated by a structural, but not functional homologue of CaM purified from *E. histolytica* [EhCaBP — a CaBP with 4 calcium binding sites (EF-hand motif)] like CaM.

The \( \text{Ca}^{2+}/\text{EhCaBP} \) stimulated protein kinase, BjCCaBPK, gets stimulated over 6-fold by EhCaBP (10.5 nM) but not by CaM when used at equimolar concentration. Moreover, this kinase also did not bind CaM-Sepharose. There was no inhibition of the kinase activity in presence of W-7 (a CaM antagonist), KN-62 (a specific calcium/CaM kinase inhibitor) and anti-CaM antibodies. Even staurosporine (a protein kinase C inhibitor) had no effect on the BjCCaBPK activity. Furthermore, a CaM-kine specific substrate, Syn tide-2, proved to be a poor substrate for the BjCCaBPK compared with histone III-S. The phosphorylation of histone III-S involved serine residues. Based on the differences in the properties of various previously reported kinases, authors have suggested that BjCCaBPK may be a novel protein kinase which does not fall in the CDPKs, CaM kinases, and PKC categories and has an affinity towards a CaBP like EhCaBP (Deswal *et al.*, 2000).

To elaborate the role of EhCaBP homologue(s) in plants, transgenic approach has been followed. The EhCaBP expressing plants were found to grow under high salt concentration (200 mM NaCl), suggesting that the expression of EhCaBP leads to tolerance under higher salinity level (Pandey *et al.*, 2002). Whether the similar CaBPs are involved in stress tolerance mechanism in tolerant varieties needs to be looked into in future studies.

**Conclusions**

In living organisms, the cellular events following stimulus perception and culminating in physiological response are referred to, broadly, as signal transduction. As sessile organisms, plants cope with abiotic (cold, drought, etc.) and biotic (pathogen attack) stress through cellular adaptations that are coordinated by an array of signal transduction pathways. Plants, like other eukaryotes, use spatially and temporally complex patterns of calcium ion fluxes to communicate, intracellularly, information on the nature of a stimulus. How cells interpret the information encoded in calcium signals remains unclear but is known to involve CaBPs such as CaM. CaM, in response to calcium, binds to and regulates the activities
of a wide assortment of proteins (enzymes, ion channels and pumps, cytoskeletal components, etc.), thereby orchestrating signalling pathways. Plants are unique among eukaryotes in possessing numerous CaM isoforms and evidence is emerging that CaMs play important roles during stress-responsive signalling. The current focus of research involves identifying and characterizing the downstream protein targets of CaMs and delineating the signalling pathways under their control. Though a plethora of other CaBPs have been identified and characterized, transgenic studies for only selective CaBPs have been attempted till date. Certaining the use of specific mutants to ascertain the role of other CaBPs in enhancing stress tolerance can also be of great advantage. Such work will provide a basic knowledge of how plant cells cope with stress and will lay the foundation for engineering specific pathways to improve stress tolerance in crop plants.

References


Baltz H N & Venis M A, 1988. Calcium dependent protein kinase from apple fruit membranes is CaM independent but has CaM like properties. Planta, 176, 91-97.


Baum G et al., 1996. CaM binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. EMBO J, 15, 2988-2996.


Hunter T, 1995. Protein kinases and phosphatases, the yin and yang of protein phosphorylation. Cell, 80, 225-236.


Ling V & Zielinski R E, 1993. Isolation of an Arabidopsis cDNA sequence encoding a 22 kDa calcium-binding protein (CaBP-22) related to CaM. Plant Mol Biol, 22, 201-214.


Ueno T et al, 1994. Two genes that encode Ca(2+)-dependent protein kinases are induced by drought and high-salt stresses in Arabidopsis thaliana. Mol Gen Genet, 244, 331-340.


