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Review

Structure and functions of plant calcium-dependent protein kinases

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Calcium ions as second messengers play an essential role in many important cellular processes. In plants, transient changes in calcium content in the cytosol (calcium signatures) have been observed during growth, development and under stress conditions. Such diverse functions require many different calcium sensors. One of the largest and most differentiated group of calcium sensors are protein kinases, among them calcium-dependent protein kinases (CDPKs) which were identified only in plants and protists. CDPKs have a regulatory domain which is able to bind calcium ions. For regulation of CDPKs activities not only calcium ions but also specific phospholipids and autophosphorylation are responsible. CDPKs have many different substrates, which reflects the diversity of their functions. Potential protein substrates of CDPK are involved in carbon and nitrogen metabolism, phospholipid synthesis, defense responses, ion and water transport, cytoskeleton organization, transcription and hormone responses. Presently, participation of CDPKs in stress signal transduction pathways (e.g., cold, drought, high salinity, wounding) is intensively studied in many laboratories. An intriguing, but still not fully clarified problem is the cross-talk *via* CDPKs among different signaling pathways that enables signal integration at different levels and ensure appropriate downstream responses.

Keywords: calcium, plant protein kinases, signal transduction, stress responses, cross-talk

ROLE OF CALCIUM IONS AS SENSORS IN PLANT SIGNAL TRANSDUCTION

In plants, calcium ions are the second messenger coupling physiological responses to external and developmental signals (Reddy & Reddy, 2004). Changes in cytosolic free calcium ion concentration are apparent during transduction of abiotic stimuli including light, low and high temperature, touch, hyperosmotic and oxidative stresses and also in the case of biotic stimuli including fungal elicitors and nodulation (Nod) factors (Rudd & Franklin-Tong, 2001; Sanders *et al.*, 2002). It has been suggested that information encrypted in the stimulus can induce an increase of free calcium ions in the cytosol and helps to define the response. The stimulus-specific increases in free cytosolic Ca^{2+} are called calcium signatures (Evans *et al.*, 2001). Any given Ca^{2+}-mediated cellular process begins with the generation of such signal-specific calcium signature in the cytoplasm (or nucleus) by a synchronized activity of channels, pumps and transporters. Changes in cy-

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Abbreviations: ABA, abscisic acid; ACC, 1-amino-cyclopropane-1-carboxylic acid; CBL, calcineurin B-like proteins; CaM, calmodulin; CaMKs, Ca^{2+}/calmodulin dependent kinases; CaM-LD, calmodulin like-domain; CCaMKs, Ca^{2+} or Ca^{2+}/calmodulin regulated kinases; CDPK/SnRK, calcium-dependent protein kinase/sucrose non fermenting related kinases family; CRKs, CDPK related kinases; ER, endoplasmic reticulum; EST, expressed sequence tag; GA, gibberelic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; LysoPC, L-α-lysophosphatidylcholine; MAPKs, mitogen-activated protein kinases; MeJA, methyl jasmonate; PA, phosphatidic acid; (1,2-diacyl-*sn*-glycero-3-phosphate); PC, phosphatidylcholine; (1,2-diacyl-*sn*-glycero-3-phosphocholine); PE, L-α-phosphatidylethanolamine; PEPRKs, PPCK-related kinases; PI, phosphatidylinositol; (1,2-diacyl-*sn*-glycero-3-phospho-(1-D-*myo*-inositol)); PPCKs, phosphoenolopyruvate carboxylase kinases; PRs, pathogenesis-related genes; PS, phosphatidylserine; (1,2-diacyl-*sn*-glycero-3-phospho-L-serine); SnRKs 1, 2, 3, SNF1-related kinases, type 1, 2 and 3; WR, wound-related genes.

tosolic free calcium ion level are sensed by a specific set of proteins named calcium sensors. The first class of sensors without any responder domains (e.g., calmodulin and calcineurin B-like proteins) bind Ca^{2+} and undergo conformational changes that in turn regulate the activity/function of a variety of target proteins or regulate gene expression. Such sensors are called ''sensor relays''. Sensors of the second group are called ''responders'' as they have effector domains (e.g., protein kinase or phospholipase domain) through which they relay the message to their downstream targets (Reddy & Reddy, 2004).

The Ca^{2+} binding EF-hand motif is the predominant calcium sensor. This motif is highly conserved. The EF-hand motif is a 29 aa helix-loop-helix structure which resembles that of a hand having the index finger and thumb (the two helices) extended at roughly right angles from the rest of the fingers, which are curled towards the palm (the loop) (Harmon, 2003). The loop in each EF-hand motif consists of 12 residues with a pattern of conserved residues that coordinate the calcium ion. The EF-hand modules bind Ca^{2+} ions in the loops and undergo large conformational changes that result in the exposure of hydrophobic pockets, which in turn facilitates interactions of the protein with a variety of protein partners. The presence of pairs of EF-hand motifs in proteins increases their stability and affinity for calcium (Reddy & Reddy, 2004). A study of 300 EF-hand proteins from all kingdoms of eukaryotes has shown that most of them bind Ca^{2+} with K_ds in the range of 0.1 to 1 μM (Kawasaki *et al.*, 1998). The EF-hand proteins found in plants include calmodulin (CaM), calcineurine B-like proteins (called CBL/SCaBPs) and protein kinases (Hrabak *et al.*, 2003).

PLANT PROTEIN KINASES AS CALCIUM SENSORS

Plant protein kinases are a large and differentiated group of calcium sensors. The CDPK/SnRK family of protein kinases was defined by a sequence similarity analysis of 1264 plant protein kinase sequences (Hrabak *et al.*, 2003).

All members of the CDPK/SnRK superfamily have kinase domains of similar length and sequence, and all have a similar general organization, with the kinase domains at or near the N-terminus, junction domains followed by the regulatory domains (Fig. 1). The major distinguishing features of the nine subfamilies are the primary structures of the terminal domains and the mode of kinase regulation (Harmon, 2003).

Five CDPK/SnRK subfamilies (CDPK, CCaMK, CaMK, CRK and SnRK3) are known to be regulated (or potentially regulated) directly or indirectly by calcium ions. Two subfamilies (CDPK and CCaMK)

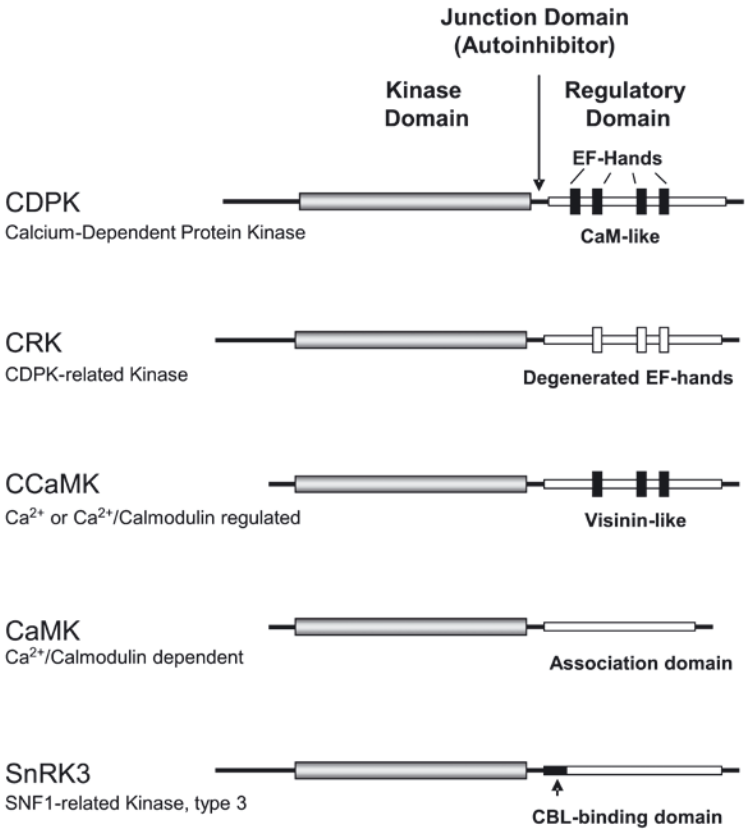

Figure 1. Diagram of kinase structures for members of the CDPK-SnRK superfamily.
Black boxes within the regulatory domain — functional EF-hands; white boxes within the regulatory domain — non-functional EF-hands; (adapted from Harmon, 2003; Harper *et al.*, 2004)

contain EF-hands at their C-terminal domains, three subfamilies (CCaMK, CaMK and CRK) bind calmodulin, and one subfamily (SnRK3) binds calcineurin B-like proteins (CBL).

CDPKs subfamily — CDPKs (calcium-dependent protein kinases) are monomeric proteins, with a molecular mass of 40 to 90 kDa. CDPKs consist of five domains, an amino-terminal variable domain, kinase domain, autoinhibitory domain, regulatory domain (CaM-LD — calmodulin-like domain) and a C-terminal domain of variable length (Harmon, 2003). CDPKs are unique owing to the presence of CaM-LDs that are able to couple the calcium sensor directly to its responder (kinase). This group of kinases was first discovered in plants. While structurally analogous kinases have been found in alveolates, the major line of protists, CDPKs have not been found in members of other phylogenetic branches, including fungi, insects, and mammals (Sanders *et al.*, 2002).

CRKs subfamily — CRKs (CDPK-related kinases) have the same general structure as the CDPKs, but they have an apparently degenerated calmodulin-like domain. Of the eight *Arabidopsis* CRKs, three (3, 5 and 8) are predicted to have a pair of EF-hands but it is still not known if they are functional (Harmon, 2003).

CCaMKs subfamily — CCaMKs (calcium or calcium/calmodulin regulated kinases) have the same general structure as CDPKs, but they have calcium-binding domains with three EF-hands and they resemble the calcium-binding brain protein visinin rather than calmodulin. These enzymes are expressed in tobacco and lilly, but genes encoding CCaMK have not been found in *Arabidopsis* (Harmon *et al.*, 2001). CCaMKs bind not only calcium but also calmodulin. The calmodulin-binding site is located at the C-terminal side of the autoinhibitory domain (Ramachandiran *et al.*, 1997).

CaMKs subfamily — CaMKs (calmodulin-dependent protein kinases) are prevalent in animals; the only example of a putative plant CaMK is from apple (Watillon *et al.*, 1993). Like in the case of CCaMKs, *Arabidopsis* does not have a gene for CaMK. These kinases are about 100 aa shorter than CCaMK and do not have any EF-hands (Harmon *et al.*, 2001). Recombinant apple CaMK was shown to bind calmodulin, but the effect of calcium/calmodulin on the enzyme activity was not determined (Watillon *et al.*, 1993).

SnRK3s subfamily — The SnRK3s (SNF1-related kinases, type 3), also called CIPKs (calcineurin B-like interacting protein kinase) or PKS (protein kinases related to SOS2), consist of a catalytic, autoinhibitory and C-terminal domains. *Arabidopsis* contains 25 SnRK3s, and many have been shown to interact with one of ten members of a family of EF-hands proteins called CBLs or SCaBP (calcineurin B-like/SOS3-like calcium binding proteins). The carboxy-terminal domain of the SnRK3s has no similarity to other members of the CDPK/SnRK family.

The CDPK/SnRK superfamily includes also four subfamilies (PPCK, PEPRK, SnRK1 and SnRK2) which are either known, or are predicted, not to be regulated by calcium ions (Harmon, 2003).

CDPKS

Structure and isoforms

Among the five domains of CDPKs, the N- and C-terminal ones are variable. The N-terminal domains of CDPKs vary in length from 40 to 180 amino acids, and there is no high homology in the sequence between family members (Harmon, 2003). While none of the CDPKs appear to be integral membrane proteins, a significant percentage of CDPKs have potential myristoylation sites at the beginning of their highly variable N-terminal domains. Myristoylation and palmitoylation at the N-terminus have been implicated in CDPK-membrane associations. Additionally, the PEST sequence has been localized at the N-terminus of some CDPKs (the PEST sequence is a proline, glutamine, serine and treonine-rich region which was found in proteins undergoing rapid proteolytic degradation). The conserved kinase domain is typical for serine/threonine kinases, and the activation loop (between subdomains VII and VIII) contains acidic residues which make it unnecessary for the loop to be phosphorylated in order to activate the kinases. Adjacent to the catalytical domain is an autoinhibitory domain that contains a pseudosubstrate sequence that can interact with the active site and inhibit the activity. The last well conserved domain is the regulatory domain (calmodulin-like domain) which contains four EF-hands and is able to bind four moles of calcium per mole of enzyme. A relatively short C-domain follows the regulatory domain.

CDPKs are encoded by multigene families, for example in *Arabidopsis thaliana* there are 34 genes for CDPKs. A genome-wide analysis of *Arabidopsis* CDPKs provides an over-view of the diversity of this large multigene family and shows that gene duplication and subsequent divergence generated CDPKs with distinct functions. Information available from genomic sequencing as well as from expressed sequence tag (EST) projects, indicates the presence of multigene families of CDPKs in other plants, including soybean *(Glycine max*), tomato (*Lycopersicon esculentum*), rice (*Oryza sativa*), and maize (*Zea mays*) (Cheng *et al.*, 2002). The relatively well known three CDPK isoforms (α, β, γ) from soybean differ from

each other in their RNA expression patterns and biochemical and kinetic properties, including calcium binding properties. This supports the hypothesis that CDPKs, in spite of being members of a single family, play distinct roles in mediating various responses to Ca^{2+} signals (Lee *et al.*, 1998).

Substrate specificity

A growing list of potential CDPKs substrates supports the view that conventional CDPKs are involved in the regulation of diverse cellular functions. In early studies two phosphorylation motifs named Simple 1 and 2 were revealed (Roberts & Harmon, 1992; Neumann *et al.*, 1996). Further analysis of peptide substrates has led to the recognition of four distinct motifs which define substrate specificity determinants outside the classic motifs (Table 1).

Regulation of CDPK activity

Regulation by calcium ions

The first and most important regulator of CDPKs activity are calcium ions. Due to the variation in the number and amino-acid sequence of the EF-hand motifs, CDPKs may differ in their affinity for Ca^{2+}. For example, soybean isoforms α and γ display Ca^{2+} activation thresholds that differ by more than ten-fold (isoform α shows a very low threshold around 60 nM calcium). Thus, a low-magnitude Ca^{2+} spike may selectively activate CDPKα, and a much higher magnitude spike would activate both isoforms (Lee *et al.*, 1998).

Presently two models of activation of CDPK by calcium exist, as illustrated in Fig. 2. In the first, CaM-LD (calmodulin like-domain) which is composed of two globular EF structural domains (N-lobe, C-lobe), each containing a pair of Ca^{2+} binding sites, is joined by a flexible tether to the kinase domain (Christodoulou *et al.*, 2004). Upon stimulation by calcium ions, CaM-LD binds to the junction domain and displaces the autoinhibitor (model I). In the second model (model II), which is supported by current evidences, CaM-LD is pre-bound to the junction at basal levels of calcium ions (Harper *et al.*, 2004). It has been suggested that under *in vivo* basal calcium ion concentrations, the C-terminal lobe is loaded with Ca^{2+} and is pre-bound to the junction domain. This implies that activation occurs through Ca^{2+} binding to the N-terminal lobe of CaM-LD. Consistent with this prediction, the N-terminal lobe displays a K_d for calcium ions around 1 μM, as expected for a kinase regulated by typical cytosolic Ca^{2+} signals. The conformation of the N-terminal lobe presumably changes in response to Ca^{2+} binding and somehow induces a conformational change in the autoinhibitory/kinase interaction.

Regulation by reversible phosphorylation

Apart from calcium ions, reversible phosphorylation and/or autophosphorylation may also regulate CDPK activity (Cheng *et al.*, 2002). Phospho-regulation has been observed in the case of NtCDPK2. Phosphorylation of NtCDPK2 by an upstream kinase results in a 10- to 200-fold increase in maximum Ca^{2+}-stimulated activity (Romeis *et al.*, 2000; 2001). This phospho-activation occurs in response to certain pathogens, possibly through MAPK (mitogen-activated protein kinase) cascades. However, the upstream kinase and the site of phospho-regulation have not been determined (Harper *et al.*, 2004).

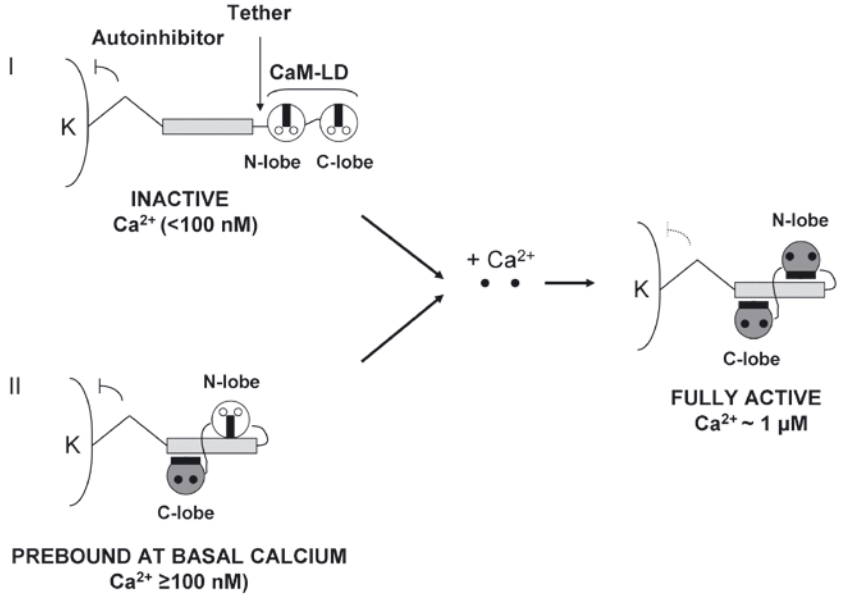

Figure 2. Activation models for CDPKs.
K, kinase domain; CaM-LD, calmodulin-like domain; white dots within N- and C-lobes, empty Ca^{2+} binding sites; black dots within N- and C-lobes, Ca^{2+} binding sites with bound Ca^{2+} (modified from Harper *et al.*, 2004; Harper & Harmon, 2005).

Autophosphorylation has been observed in both native and recombinant CDPKs. Although phosphorylation sites in many CDPKs have been defined, still little is known about the effects of autophosphorylation on CDPK function *in vitro*, and almost nothing is known about its physiological or mechanistic role *in vivo* (Hegeman *et al.*, 2006). For example, *in vitro* autophosphorylation is prerequisite for activation of a ground nut (*Arachis hypogea*) AhCDPK (Chaudhuri *et al.*, 1999), but contrary to that it can also cause inhibition of the WbCDPK activity, which was observed in the winged bean (*Psophocarpus tetragonologus*). In addition to that, a soluble phospho-Ser phosphatase from winged bean shoots activates *in vitro* (by dephosphorylation) autophosphorylated WbCDPK (Ganguly & Singh, 1999). It is thought that this action reverses the inhibitory effect of autophosphorylation.

In almost all studied cases autophosphorylation is Ca^{2+}-dependent. An interesting exception is WbCDPK, the autokinase activity of which is apparently calcium-independent (Saha & Singh, 1995). Also interesting is that a second isoform of CDPK from that organism, designated WbPK (winged bean protein kinase), is the only reported example of a CDPK that does not exhibit an autokinase activity (Ganguly & Singh, 1998).

Regulation by phospholipids

Biochemical analysis has revealed that in the presence of calcium ions also certain phospholipids can enhance *in vitro* protein phosphorylation by CDPKs from oat (*Avena sativa*) (Schaller *et al.*, 1992), *Arabidopsis* (AtCPK1) (Harper *et al.*, 1993), carrot (*Daucus carota*, DcCPK1) (Farmer & Choi, 1999) and maize (*Zea mays*, ZmCPK11) (Szczegielniak *et al.*, 2000; 2005). The stimulation of CDPK activity by membrane lipids suggests that membrane association may be a factor that contributes to CDPK activity. Furthermore, as can be seen in Table 2 the phospholipids regulating the kinase activities are different for different CDPKs. Because of the differential specificity of the phospholipids stimulating each CDPK, the effects will likely have physiological relevance. There is evidence that some of these phospholipids do act as second messengers in plant signal transduction, and, therefore, might elicit their effects through CDPKs (Cheng *et al.*, 2002).

Regulation by 14-3-3 proteins

Data accumulating during the recent decade has indicated that phosphorylation alone is not always sufficient to complete the transduction of a regulatory signal. In many cases, the phosphorylated target protein must associate with a specialized adapter protein. The most known adapter protein is 14-3-3, which is able to complete the regulatory action of phosphorylation. The 14-3-3 protein was first identified as a brain protein, now it is known that it is ubiquitous in all eukaryotes (Moorhead *et al.*, 1999).

Table 1. Motifs phosphorylated by CDPKs.

Subscripts denote the position of residues relative to the phosphorylation site, which is bold residues (S or S/T). Basic — R or K; X — any residue; Z — any residue except R; ϕ — hydrophobic residue (Harper & Harmon, 2005, modified).

Motif	Sequence	Reference
Simple 1	$Basic_{-3}$-X_{-2}-X_{-1}-**S/T**$_0$	Roberts & Harmon, 1992
Simple 2	**S**$_0$-X_{+1}-$Basic_{+2}$	Neumann *et al.*, 1996
Motif 1		
minimal	ϕ_{-5}-X_{-4}-$Basic_{-3}$-X_{-2}-X_{-1}-**S**$_0$-X_{+1}-X_{+2}-X_{+3} –ϕ_{+4}	Neumann *et al.*, 1996
optimal	$Basic_{-6}$ ϕ_{-5}-X_{-4}-$Basic_{-3}$-X_{-2}-X_{-1}-**S**$_0$-X_{+1}-X_{+2}-X_{+3}-ϕ_{+4}-$Basic_{+5}$	Huang & Huber 2001
Motif 2	$Basic_{-9}$-$Basic_{-8}$-X_{-7}-$Basic_{-6}$- ϕ_{-5}-X_{-4}-X_{-3}-X_{-2}-X_{-1}- **S/T**$_0$-X_{+1}-$Basic_{+2}$	Huang *et al.*, 2001; Huang & Huber, 2001
Motif 3	ϕ_{-1}- **S/T**$_0$-ϕ_{+1}-X_{+2}-$Basic_{+3}$-$Basic_{+4}$	Sebastià *et al.*, 2004
Motif 4	A/L_{-5}-X_{-4}-R_{-3}-X_{-2}-X_{-1}-**S**$_0$-X_{+1}-R_{+2}-Z_{+3}-R_{+4}	Loog *et al.*, 2000

Table 2. Efect of different phospholipids on activity of CDPK.

AtCDPK1, *Arabidopsis thaliana* (Harper *et al.*, 1993); DcCPK1, *Daucus carota* (Farmer & Choi, 1999); ZmCPK11, *Zea mays* (Szczegielniak *et al.*, 2005); CDPK, *Avena sativa* (Schaller *et al.*, 1992).

CDPK	PA	PS	PI	PC	LysoPC	PE	Crude extract
AtCPK1	0	0	++	0	++	0	++
DcCPK1	++	+	+	n.d.	0	++	n.d.
ZmCPK11	++	++	+	0	0	n.d.	+
Oat CDPK	n.d.	+	+	+	+	n.d.	++

n.d., not determined; 0, no effect; "+", stimulation of activity; "++", strong stimulation of activity; Crude extract, mixture of lipids isolated from particular plant homogenate; PA, PS, PI, PC, LysoPC, PE, commercial phospholipids.

In plants there are many isoforms of 14-3-3 proteins, for example 12 isoforms in *Arabidopsis* (Sehnke *et al.*, 2002). Three different 14-3-3 isoforms have been demonstrated to specifically bind and activate AtCPK1 *in vitro* in the presence of calcium ions. There was no stimulatory effect of 14-3-3 in the absence of calcium ions. Thus, 14-3-3 does not merely mimic Ca^{2+} but rather provides an additional stimulation once the enzyme is activated (Camoni *et al.*, 1998). Calcium ions may be needed in part to induce autophosphorylation of CDPK, because 14-3-3 proteins typically regulate the activities of many enzymes by binding to specific phosphorylated residues. Although the specific sites of AtCPK1 autophosphorylation and its interaction with 14-3-3 are unknown, AtCPK1 contains one site within the N-terminus similar to the most common 14-3-3 consensus binding site, R-S/T-X-S-X-P, where the underlined Ser is phosphorylated. Such putative 14-3-3 binding sites are also present in two other *Arabidopsis* CDPKs, AtCPK24 and AtCPK28 (Cheng *et al.*, 2002).

Subcellular localization and organ expression of CDPKs

In plants, CDPKs are present as both soluble and membrane-anchored isoforms. CDPKs exhibit multiple locations, including the cytosol, nucleus, plasma membrane, endoplasmatic reticulum, peroxisomes, mitochondrial outer membrane and oil bodies (Harper *et al.*, 2004). In *A. thaliana*, 9 of 34 isoforms have been localized either in the nucleus, cytoplasm, or associated with the plasma membrane (Lu & Hrabak, 2002; Dammann *et al.*, 2003). In *Nicotiana tabacum*, NtRpn3, a regulatory subunit of 26S proteasome, was identified as protein interacting with NtCDPK1. Both proteins are co-localized in the nucleus, nucleus periphery, and around the plasma membrane (Lee *et al.*, 2003). The 26S proteasome represents the first example of a specific protein complex containing CDPK (Harper *et al.*, 2004).

In addition to the diverse subcellular distribution of CDPKs, there is evidence that some of them can change locations in response to stress. Such a situation was observed in the ice plant (*Mesembryanthemum crystallinum*), where expressed in leaves isoform of McCPK1 tagged with green fluorescent protein (GFP) showed a pronounced shift in localization from the plasma membrane to the nucleus in response to a salt or dehydratation stresses (Patharkar & Cushman, 2000; 2006).

Transcripts of CDPKs have been found in every studied plant and plant organ. In many cases CDPKs are strongly expressed in proliferating tissues. In *N. tabacum NtCDPK1* is mainly expressed in the rapidly proliferating tissues including shoot and root meristems and developing flower buds. Another tobacco CDPK (*NtCPK4*) is expressed additionally in rapidly growing tissues such as root tip, lateral root primodia, vasculature in leaf and anther, suggesting that it might be related to cell differentiation and particular metabolic function (Lee *et al.*, 2003; Zhang *et al.*, 2005). The highest level of expression of cytosolic *OsCDPK13* from rice was also detected in young tissues (Abbasi *et al.*, 2004). The highest transcript level of maize CDPK, *ZmCPK11*, was observed in seedlings, also rapidly growing tissues (Szczegielniak *et al.*, 2005). Transcripts of *CDPKs* have been detected in seeds and pollen. Rice CDPK (*SPK*) is expressed uniquely in the endosperm of immature seed (Asano *et al.*, 2002). The expression of one of maize *CDPK* genes is pollen-specific and is temporally restricted to the late stages of pollen development (Estruch *et al.*, 1994).

Plant organs grown in the light or in darkness exhibit different expression and activity of some CDPKs. The presence of varying levels of CpCPK1 activity among different organs of light- and dark-grown roots, hypocotyls, hooks, and cotyledons of zucchini (*Cucurbita pepo* L.) was demonstrated. The level of *CpCPK1* mRNA isolated from zucchini seedlings was higher in dark-grown than in their light-grown counterparts. In dark-grown tissue, expression was the highest in the hypocotyls followed by hooks and roots, whereas little or no expression was detected in dark-grown cotyledons. In light-grown tissue, expression was greatly reduced (Ellard-Ivey *et al.*, 1999). Significant differences between etiolated and light-grown tissues was observed also in apple (*Malus domestica* Borkh. cv. Braeburn) and maize. A detailed study in both light-grown and etiolated maize plants revealed that expanding tissues, such as rapidly growing tips, leaves, and coleoptiles had particularly high levels of $p67^{CDPK}$ activity. Mature etiolated leaves had less activity, but it was still higher than in mature light-grown leaves. An inactivating effect of light was confirmed where $p67^{CDPK}$ activity dropped in etiolated secondary leaves after 8 h of their exposure to light (Barker *et al.*, 1998). Exposure to light was found to differentially regulate the transcript level of *CsCDPK3* isolated from various organs of cucumber (*Cucumis sativus*). The transcript level decreased in light-grown hypocotyls and roots, whereas in cotyledons light had an up-regulatory effect (Ullanat & Javabaskaran, 2002). This means that light-regulated transcription of *CDPKs* is organ-specific. In maize, the transcript levels of *ZmCDPK7* and *ZmCDPK9* were higher in etiolated than in light-grown leaves (Saijo *et al.*, 1997). In rice plants overexpressing *OsCDPK2*, in etiolated leaves, stems and flowers the higher level of the transcript was detected (Morello *et al.*, 2000). Also in wheat seedlings (Sharma *et al.*, 1997), and in seedlings of the

short-day plant *Pharbitis nil* (Jaworski *et al.*, 2003), activity of CDPKs was higher in etiolated tissues.

Putative functions of CDPKs

The CDPK superfamily members, as expected for a family of multifunctional kinases with many substrates, have been implicated in many biological phenomena (Table 3) (Harper & Harmon, 2005).

In vitro phosphorylation reactions make it possible to identify potential CDPK protein substrates involved in carbon and nitrogen metabolism, phospholipid synthesis, defense responses, ion and water transport, cytoskeleton organization, transcription, fertilization and proteasome regulation (Reddy & Reddy, 2004). For example, the phosphorylation by CDPK of enzymes that are involved in carbon and nitrogen metabolism might coordinate the supply of carbon skeletons and ammonia needed for synthesis of carbon stores or amino acids (Huber & Hardin, 2004). Consistent with this, the accumulation of starch was disrupted in rice seeds by antisense knockdown of an endosperm-specific CDPK (Asano *et al.*, 2002). Phosphorylation by CDPKs of such enzymes as phenylalanine ammonia lyase and 3-hydroxy-3-methylglutaryl-CoA reductase might stimulate the production of defense compounds in response to pathogen attack, or the regulation of membrane-associated ion and water transporters, which might alter signal transduction, transport of nutrients and turgor (Harmon *et al.*, 2000). Another example is phosphorylation of ACC synthase, which catalyses the rate-determining step in the biosynthesis of ethylene, a gaseous hormone that is produced in response to developmental signals and various stresses (Sebastià *et al.*, 2004).

Role in rearrangements of cytoskeleton organization

Dynamic cytoskeleton reorganization is essential for a variety of cell functions, mainly proliferation and differentiation. The actin cytoskeleton in eukaryotic cells forms a dynamic network that can reorganize in response to intracellular and extracellular signals. These reorganizations are coordinated by stimulus-responsive actin-modulating proteins. To this group belong profilins and actin-depolym-

Table 3. Known potential CDPKs substrates.

Substrates were identified through *in vitro* phosphorylation and are grouped according to their biological functions (Cheng *et al.*, 2002; Reddy & Reddy, 2004; modified).

Putative substrates	CDPK source	References
Carbon metabolism		
Sucrose phosphate synthase	Spinach	McMichael *et al.*, 1995a, b
Sucrose synthase	Soybean	Nakai *et al.*, 199; Zhang & Chollet, 1997
Phosphoenolpyruvate carboxylase	Maize and soybean	Ogawa *et al.*, 1998
Nitrogen metabolism		
Nitrate reductase	Spinach	Douglas *et al.*, 1998; McMichael *et al.*, 1995a
Phospholipid metabolism		
PI 4-kinase activator	Carrot	Yang & Boss, 1994
Ethylene synthesis		
ACC synthase	Maize	Sebastià *et al.*, 2004
Defense response		
Components in Cf9/Avr9-induced HR	Tobacco	Romeis *et al.*, 2001
Phenylalanine ammonia lyase	French bean	Cheng *et al.*, 2001
Ion and water transport		
Aquaporins	Spinach	Huang *et al.*, 2001
Plasma membrane H^+-ATPase	Oat and maize	Camoni *et al.*, 1998; Harmon *et al.*, 1996
Potassium channel KAT1	Broad bean	Li *et al.*, 1998; Berkowitz *et al.*, 2000
Ca^{2+}-ATPase (ACA2)	Arabidopsis	Hwang *et al.*, 2000
Cytoskeleton organization		
Actin-depolymerizing factor	French bean	Allwood *et al.*, 2001
Transcription		
Pseudoresponse regulator	Ice plant	Patharkar & Cushman, 2000
Proteasome regulation		
26S proteasome regulatory factor	Tobacco	Lee *et al.*, 2003
Fertilization regulation		
Self-incompability RNases	Tobacco	Kuntz *et al.*, 1996
RNase activity		
Ser acetyltransferase	Soybean	Harmon, 2003

erizing factor (ADF). It was known earlier that the reversibly phosphorylated vertebrate ADF does not bind G- and F-actin and is consequently inactive in *in vitro* polymerization/depolymerization assays. Studies of Smertenko and co-workers (1998) on actin depolymerization in maize revealed that the maize actin-depolymerizing factor (ZmADF3) can be phosphorylated on Ser-6 by a protein kinase in a Ca^{2+}-dependent manner. An analogue of phosphorylated ZmADF3, in which Ser-6 was replaced by a negatively charged residue (aspartate) did not bind G- or F-actin and had a negligible effect on the rate of disassembly of actin. This means that reversible phosphorylation of Ser-6 controls the functioning activity of actin. Results of another group working on regulation of actin tension in soybean cells confirmed that phosphorylation may be necessary for maintenance of F-actin interactions with either myosin filaments or actin-binding proteins (Grabski *et al.*, 1998).

Role in growth, development and differentiation

A wide variety of growth and developmental processes in plants are regulated by Ca^{2+} fluxes (Hepler *et al.*, 2001). It has been shown that abiotic stresses such as oxidative and hypoosmotic stress increase the cytoplasmic Ca^{2+} level in synchronized BY-2 cells, which in turn causes a delay of cell cycle progression (Sano *et al.*, 2006). The environmental stimuli and signaling events that trigger and regulate plant embryogenesis are largely unknown, however, studies carried out on a carrot system have shown that Ca^{2+} enhances embryogenetic frequency and its deprivation arrests somatic embryo formation. In addition, the accumulation of a 55-kDa soluble calcium dependent protein kinase (swCDPK) has been observed only in somatic/zygotic embryos, endosperm and seedlings of sandalwood (*Santalum album*). The developmentally regulated, tissue-specific expression of this CDPK isoform suggests its involvement in early developmental processes such as embryogenesis, seed development and germination (Anil & Rao, 2000; 2001).

The exclusive expression of a CDPK named SPK in the endosperm of immature rice seeds suggested its involvement in biosynthetic pathways of storage products (Asano *et al.*, 2002). This hypothesis was confirmed by a study of antisense SPK transformants, which lacked the ability to accumulate storage products such as starch and produced watery seed with a large amount of sucrose instead, as a result of an inhibition of sucrose utilization. This finding suggests that SPK is a sucrose synthase kinase that may be important for supplying substrates for the biosynthesis of storage products.

Germination and growth of the pollen tube require intense cytoplasmic streaming and cytoskeleton reorganization. Because these processes are thought to be Ca^{2+}-dependent the pollen-specific maize CDPK protein may serve a very specific function during germination and growth of the pollen tube by acting as a stimulus-response protein (Estruch *et al.*, 1994). This hypothesis was confirmed by a study of cells of pollen tubes of *Agapanthus umbellatus*. It was found that the pollen tubes exhibited a higher protein kinase activity in the apical region, whereas nongrowing cells showed uniform distribution of the kinase activity (Moutinho *et al.*, 1998).

Tuberization is a complex process that results in differentiation of specialized shoot (the stolon) into a storage organ (the tuber). A wide variety of environmental and hormonal stimuli are known to be involved in the induction of tuberization (MacIntosh *et al.*, 1996). Also, intracellular Ca^{2+} is necessary for tuber development and calmodulin antagonists can inhibit this process. Participation of CDPK isoforms in tuber development was studied during potato (*Solanum tuberosum* L.) tuber formation. Two CDPK activities (StCDPK1 and StCDPK3) with different substrate specificity and cellular distribution were found associated with early (thin) or induced (swelling) stolons. Differential expression of *StCDPK3* (only transcribed in early elongating stolons) and *StCDPK1* (expressed upon swelling of the stolon tip) confirmed the existence of specific CDPK isoforms with restricted patterns of expression and activity during the events leading to tuber formation. This data suggest that sequential activation of specific CDPKs with distinct biochemical properties and subcellular localization could be essential for the co-ordination of multiple Ca^{2+} signals triggered upon tuberization (Raices *et al.*, 2003).

Role in regulation of transcription

In contrast to yeast and animals, little is known about the regulation of plant transcription factors by reversible phosphorylation. It has been demonstrated that in wheat, a bZIP (basic/leucine-zipper motif) transcription factor (named HBP-1a) can be phosphorylated at three Ser residues: 261, 265 and 269 in a Ca^{2+}-dependent manner by a kinase present in the nuclear extract (Meshi *et al.*, 1998). DNA-binding analysis of mutants in which Ser was substituted by Glu indicated that the phosphorylation of residues 265 and 269 prevents HBP-1a from binding DNA. This suggests that the phosphorylation regulates the function of HBP-1a at the level of DNA binding at least (Meshi, 1998).

The regulation of *trans*-acting factors by reversible phosphorylation could be a key post-translational modification responsible for stability, migration from the cytosol to the nucleus, oligomeriza-

tion of the factors themselves, and for interactions with proteins of the transcriptional machinery. Ca^{2+}-dependent phosphorylation is responsible for modulation of the DNA-binding activity of GT-1, a *trans*-acting factor (Maréchal *et al.*, 1999). GT-1 is a light-modulated DNA-binding protein interacting with BoxII *in vitro* in *rbcS-3A* promoter in pea plants. Analysis of recombinant GT-1 mutants has demonstrated that phosphorylation of Thr-133 is accountable for a 10–20-fold stimulation of DNA-binding activity. Treatment with calf intestine alkaline phosphatase of extracts prepared from light-grown plants (but not from etiolated ones) reduced the GT-1 DNA-binding activity, suggesting that GT-1 may act as a molecular switch modulated by Ca^{2+}-dependent phosphorylation and dephosphorylation in response to light signals.

It has been shown that cold shock causes an increase in cytosolic free calcium ion concentration in *A. thaliana* (Knight *et al.*, 1996; Knight & Knight, 2000). Inhibition of this increase causes a partial inhibition of *kin1* (cold-induced gene) expression, which in connection with *kin2* expression is probably important for cold acclimation of plants. W7, an antagonist of CDPKs and calmodulin, not only decreased the cold-induced expression of *kin* genes but also prevented the cold acclimation of *Arabidopsis* plants. These results suggest that CDPKs may couple the Ca^{2+} response to downstream events, leading to metabolic adjustment to the cold and to the development of freezing tolerance (Tähtiharju *et al.*, 1997). The involvement of calcium ions in the regulation of plastid-encoded photosynthesis-related genes (*psbA, psaA,* and *rbcL*) in rice leaves was studied by Grover and co-workers. The observed significant decrease in the transcript level of *psbA*, *psaA* and *rbcL* in response to calcium ion channel blockers (nifedipine and verapamil) and protein phosphatase inhibitors (okadaic acid and sodium fluoride) may suggest that phosphorylation/dephosphorylation plays a role during biogenesis of leaves chloroplasts (Grover *et al.*, 1998).

Role in hormone responses

CDPKs are known to modulate Ca^{2+}-dependent plant responses caused by phytohormones, mostly gibberellins, cytokinins and auxins. One of the first documented examples of gibberellin influence on the activity of CDPK was observed in gibberellin-treated rice seeds. The activity of partly purified rice CDPK was 10-fold higher in GA-treated seeds than in untreated ones (Abo-El-Saad & Wu, 1995). Additionally, GA treatment caused an increase of the transcript level of another CDPK from rice seedlings, *OsCDPK13* (Yang *et al.*, 2003). In tobacco, accumulation of *NtCPK4* transcript was also observed after GA treatment, reaching a maximum after 60 min and returning to the initial level during the next 2 h (Zhang *et al.*, 2005). An influence of GA was observed in aleurone cells of barley (*Hordeum vulgare*) expressing an inactivated (D140N) version of HvCDPK1. Secretion, cell vacuolation, and vacuolar acidification were inhibited, while GA-induced gene expression was not affected by the inactive version of HvCDPK1. Therefore, HvCDPK1 may play a role in regulating post-transcriptional events in protein secretion (McCubbin *et al.*, 2004). A different regulation by plant hormones was observed in rice roots, where the activity of a 45 kDa CDPK decreased previously by cold was recovered by exogenous abscisic acid (ABA). This may suggest that the 45 kDa protein kinase activity regulated by ABA is involved in the cold-stress response in rice (Komatsu *et al.*, 2001). Studies of ACPK1 from the mesocarp of grape barriers (*Vitis vinifera x V. labrusca*) revealed that ABA stimulates both the activity of the kinase and mRNA level, but not at the same time. The rapid stimulation of the ACPK1 activity after 15 min of ABA treatment without an increase of the ACPK1 protein level suggests involvement of the enzyme in mediating some rapid ABA-responsive events (ABA could probably act on post-translational modification of ACPK1). A much slower *de novo* ABA-stimulated synthesis of ACPK1 implicates its role in regulating a development-related ABA-signaling pathway (Yu *et al.*, 2006). An influence of GA and ABA treatment was observed also in tobacco, where accumulation of *NtCDPK1* mRNA occurred 12 h after treatment with the phytohormones (Yoon *et al.*, 1999).

Auxins also influence the expression and activity of CDPKs. For example, in tobacco leaves treated with indole-3-acetic acid (IAA) a slight induction of *NtCDPK1* transcript was observed (Yoon *et al.*, 1999), while after the treatment of mung bean plants (*Vigna radiata*) a significant increase in the transcript level occurred, reaching a maximum 6 h after the treatment (Botella *et al.*, 1996). Another example of CDPK transcript accumulation after auxin (2,4D) treatment was observed in microcallus suspension of alfalfa (*Medicago sativa*) during embryogenic induction. The maximal level of *MsCPK3* transcript was observed after 2 h (Davletova *et al.*, 2001). Activity of 50 kDa CDPK was detected in extracts from cucumber hypocotyls after first day of IAA treatment and it was extended to the third day of continuous treatment. It has been suggested that, together with NO, which also increases the activity of this CDPK, IAA plays a role in a Ca^{2+}-mediated signaling pathway that regulates CDPK activity leading to adventitious root formation (Lanteri *et al.*, 2006). Cytokinins affect the transcript level of *CsCDPK3* from cucumber, and this effect differs between organs. Treatment with benzyladenine (a synthetic cytokinin) up-regulated the transcript level of this

kinase in cotyledons in opposition to a down-regulatory effect in roots and a lack of a significant effect in hypocotyls (Ullanat & Jayabaskaran, 2002). An interesting observation was made during studies on rice BRI1, a receptor of a group of brassinosteroids (BRs), and its influence on MAPK and CDPK activity. After exogenous application of brassinolide both MAPK and CDPK were activated. In control as well as in *OsBRI1* antisense-expressing rice plants only an increase of CDPK (in contrast to MAPK) activity was observed. These results suggest that brassinosteroids cause activation of CDPK *via* a receptor different from BRI1 identified for MAPK activation (Sharma *et al.*, 2001).

It is also known that Ca^{2+}-dependent protein phosphorylation is involved in plant response triggered by the gaseous plant hormone ethylene which regulates a variety of physiological processes including fruit ripening, leaf abscission and senescence as well as response to environmental stresses such as wounding and pathogen invasion (Kwak & Li, 1997; Ludwig *et al.*, 2005).

Role in plant stress signal transduction and defense responses

In response to a particular stress, e.g. cold, drought, high salinity or wounding, plants exhibit recognizable cytoplasmic calcium ion elevations decoded by CDPKs (Knight & Knight, 2001). Elevation in transcription level as well as an increase of the activity of CDPKs has been observed as a response to different stresses. In roots and shoots of 10-day-old rice seedlings, after cold or salt treatment the level of *OsCDPK7* transcript was increased. Overexpression of *OsCDPK7* made transgenic rice lines more tolerant to cold, drought and salt stress, therefore OsCDPK7 seems to play a role in the tolerance to different types of stresses in rice (Saijo *et al.*, 2000). In tobacco leaves the *NtCDPK1* gene is transcriptionally regulated not only by phytohormones (ABA, GA and cytokinin or methyl jasmonate) and Ca^{2+}, but also by abiotic and biotic stimuli such as NaCl, wounding, fungal elicitors and chitosan. The induction occurs quite rapidly; the level of *NtCDPK1* mRNA increases after 1–2 h treatment with fungal elicitors, chitosan or NaCl. However, the duration of the elevation of the mRNA level appeared to vary depending on the treatment (Yoon *et al.*, 1999). Transcript of another tobacco CDPK, *NtCPK4*, was induced not only by a phytohormone (GA) but also by NaCl treatment. The maximal transcript level was observed 30 min after NaCl treatment and returned to basal level in 2 h (Zhang *et al.*, 2005). Northern blot analysis of total RNA isolated from dehydrated *Arabidopsis* plants indicated that mRNAs corresponding to the *AtCDPK1* and *AtCDPK2* genes are rapidly induced by drought and high-salt stress but not by low temperature or heat stress. These findings suggest that a change in the osmotic potential of the environment can serve as a trigger for the induction of *AtCDPK1* and *AtCDPK2* (Urao *et al.*, 1994). An increase in the activity of a 51 kDa cytoplasmic CDPK was observed in rice plants treated with cold, salt or drought stresses (Li & Komatsu, 2000). The activity of another CDPK from rice, a membrane-bound 56-kDa CDPK, was increased by cold treatment (Martin & Busconi, 2001). Cold treatment also caused an accumulation of *OsCDPK13* transcript in 2-week-old rice plants (Abbasi *et al.*, 2004). Not only cold, but also heat shock can affect CDPKs transcript level. Thirty minutes after heat shock (37°C) an induction of expression of *MsCPK3* from alfalfa was observed. This is a unique example of such induction, not observed in other plant species studied (Davletova *et al.*, 2001).

NtCDPK1 from tobacco is a CDPK which is induced transiently during wounding (Ludwig *et al.*, 2004). The *NtCDPK1* transcript appeared within 2 h, went down at 11 h and disappeared at 28 h after wounding (Yoon *et al.*, 1999). An increase in the transcript level after mechanical strain treatment (manually bent downward) was also observed in leaves of 10-day-old mung bean plants, where induction of *VrCDPK1* expression was observed 30 min after treatment reaching a maximum after 60 min and then remaining constant up to 3 h (Botella *et al.*, 1996). In tomato plants subjected to mechanical wounding, a transient increase in the *LeCDPK1* mRNA steady-state level was detected not only locally at the site of the injury, but also systemically in distant non-wounded leaves. Analysis of *LeCDPK1* mRNA in whole plants subjected to wounding showed that the kinase expression in injured leaves reaches a maximum at 4 h whereas in neighboring leaflets or distal leaves its mRNA appeared later (after 8–12 h or 18 h, respectively). The increase observed in *LeCDPK1* mRNA upon wounding correlates with an increase in the amount and activity of a soluble CDPK detected in extracts of tomato leaves, suggesting that the kinase is a part of mechanisms involved in plant responses to abiotic stresses (Chico *et al.*, 2002). Similar responses were also observed in maize, where wounding induced accumulation of the *ZmCPK11* transcript in both wounded and neighboring leaves (after 3 h and 6 h, respectively). These results indicate that monocot, alike dicot plants are able to respond to wounding in a systemic way (Szczegielniak *et al.*, 2005).

Wounding commonly occurs as a result of abiotic stress factors such as wind, rain, and hail or is caused by phytopathogens and herbivores. Wounding presents a constant threat to plant survival because it not only physically destroys plant tissues, but also provides a pathway for pathogen

invasion (Cheong *et al.*, 2002). Because plants do not have specialized cells devoted to wound healing such as those found in mammals, some plant cells have evolved towards the capacity of making each cell competent for the activation of defense responses. These wound-activated responses are directed to healing of the damaged tissues and to the activation of mechanisms that prevent further damage (León *et al.*, 2001). Such mechanisms largely depend on the transcriptional activation of wound-responsive (WR) genes. Several components involved in the transduction of wound signals have been identified, mostly in tomato. Oligosaccharides, the peptide systemin, the phytohormones: JA, ABA and ethylene together with electrical signals have been shown to play a role in the signaling network leading to the activation of proteinase inhibitors and other WR genes (Rojo *et al.*, 1999). Elevation of intracellular levels of free calcium ions and changes in the pattern of phosphorylation catalysed by CDPKs are also parts of the wound-activated responses in plants.

Elicitor-induced calcium ion influx and changes of CDPKs activity and their transcript levels have been reported for many pathogen-plant interactions as one of the earliest responses required for further downstream signaling. Transcript accumulation of *NtCDPK1* from tobacco was reported upon nonrace-specific elicitation with chitin fragments (Yoon *et al.*, 1999). In tobacco cells that expressed the resistance gene from tomato (*Cf-9*) as a transgene, *NtCDPK2* was activated upon elicitation with the corresponding fungal-derived avirulence gene product Avr9 (Romeis *et al.*, 2000). Interestingly, the enzyme activation was accompanied by a phosphorylation-dependent transition of NtCDPK2 from a non-elicited into an elicited enzyme form, which could be visualized as a shift in electrophoretic mobility in immunoblot and in-gel kinase assays (Romeis *et al.*, 2001). Another example of a CDPK involved in pathogen defense response is ZmCPK10 from maize. This kinase is transcriptionally activated in response to both fungal infection (*Fusarium moniliforme*) and treatment with its elicitors. Activation of *ZmCPK10* gene is extremely rapid, its transcript could be detected 5 min after elicitation and reached maximum levels 30 min after treatment. The activation of this kinase gene is accompanied by an increase in the level of PR transcripts. The PRs are the pathogenesis-related proteins whose expression is induced in tissues in response to fungal infection and treatment with fungal elicitors. Interestingly, the notable cell-type-specific pattern of *ZmCPK10* gene expression fully correlates with the pattern of PR gene expression in both fungus-infected and elicitor-treated maize tissues (Murillo *et al.*, 2001).

Participation of CDPKs in cross-talk between signal transduction pathways

Cross-talk can be defined as an interaction of two or more signaling pathways. The interaction of these signaling pathways *via* common components can cause the same response to different stimuli or alternatively different signaling pathways could interact and affect negatively or positively each other's outcome. Although the participation of CDPKs in different signaling pathways is relatively well

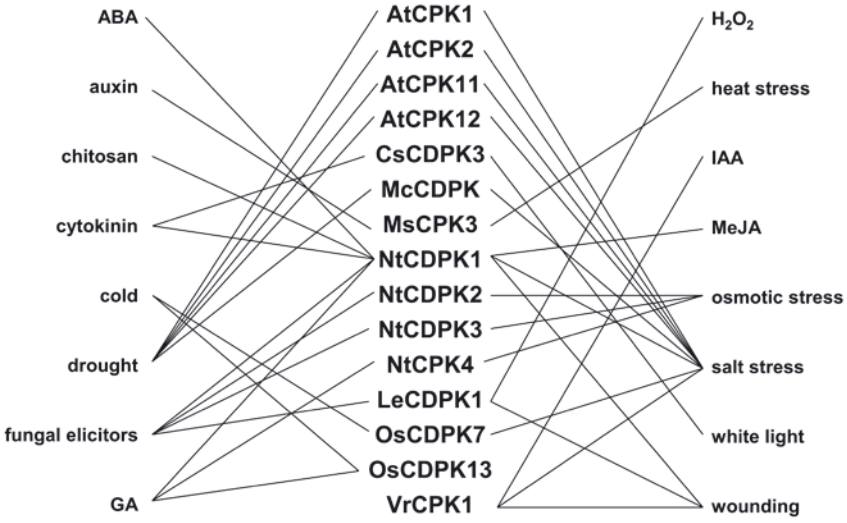

Figure 3. Cross-talk of signaling pathways involving CDPKs.
AtCPK1, AtCPK2, AtCPK10, AtCPK11 are from *Arabidopsis* (Urao *et al.*, 1994); CpCPK1 is from zucchini (Ellard-Ivey *et al.*, 1999); CsCDPK3 is from cucumber (Ullanat & Javabaskaran, 2002); LeCDPK1 is from tomato (Chico *et al.*, 2002); McCDPK1 is from crystalline ice plant (Patharkar & Cushman, 2000); MsCPK3 is from alfalfa (Davletova *et al.*, 2001); NtCDPK1, NtCDPK2, NtCDPK3, NtCPK4 are from tobacco (Yoon *et al.*, 1999; Romeis *et al.*, 2001; Zhang *et al.*, 2005); OsCDPK7, OsCDPK13 are from rice (Saijo *et al.*, 2000; Yang *et al.*, 2003; Abbasi *et al.*, 2004); VrCPK1 is from mung bean (Botella *et al.*, 1996); (modified from Ludwig *et al.*, 2004).

documented (Fig. 3) still little is known about their role in the cross-talk between these pathways. Furthermore, considering the presence of many different CDPK isoforms induced by specific calcium signatures differently localized in the cell, triggered by different stimuli, this cross-talk is very complex and hard to elucidate. NtCDPK2 illustrates this complexity. NtCDPK2 is considered to be the integrating part of two different pathways activated by both hypo-osmotic stress and by pathogen attack (*Cladosporium fulvum*) (Romeis *et al*., 2001). Depending on the stress stimuli, NtCDPK2 activation varies in strength and duration. It seems that a short and weak activation of the enzyme after osmotic stress solely results in the induction of the wound signaling pathway, whereas a much stronger and sustained elicitation may lead to a plant defense response. Other CDPKs are also thought to function as cross-talk mediators between different pathways. For example, NtCDPK1 is induced not only by wounding but also by phytohormone treatment, high salt or fungal elicitors (Lee *et al*., 2003). A reduced level of *NtCDPK1* in *N. benthamiana* plants results in severe abnormalities in cell morphology, spontaneous necrotic lesions and increased expression of marker genes for the plant defense response (*PR1a, SAR8.2a* and *HIN1*). It has been proposed that NtCDPK1 receives hormone signals to regulate cell division and differentiation. Overexpression of *OsCDPK7* conferred both cold and salt/drought tolerance to rice plants (Saijo *et al*., 2000). Interestingly, *OsCDPK7* overexpression enhanced only the transcription of the salt- and drought-responsive, but not the cold-responsive, target genes. Thus, it was suggested that cold and salt/drought tolerance are promoted through distinct pathways which can cross-talk *via* OsCDPK7.

In conclusion, the CDPKs are points of convergence that enable signal integration at different levels and provide appropriate downstream responses (Ludwig *et al*., 2004).

The major challenge for the future will be to elucidate the functions of CDPKs in particular signaling pathways and also in the cross-talk in the complex signaling network. To achieve this goal CDPK studies should combine traditional biochemical methods with molecular biology technologies. Studies concerning the effects of different stress stimuli, the character of calcium signatures activating the enzymes and potential targets of CDPKs should help to understand plant signal transduction.

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