

CDPK-mediated signalling pathways: specificity and cross-talk

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Abstract

Plants are constantly exposed to environmental changes and have to integrate a variety of biotic and abiotic stress stimuli. Calcium-dependent protein kinases (CDPKs) are implicated as important sensors of Ca²⁺ flux in plants in response to these stresses. CDPKs are encoded by multigene families, and expression levels of these genes are spatially and temporally controlled throughout development. In addition, a subset of CDPK genes responds to external stimuli. Biochemical evidence supports the idea that CDPKs are involved in signal transduction during stress conditions. Furthermore, loss-of-function and gain-of-function studies revealed that signalling pathways leading to cold, salt, drought or pathogen resistance are mediated by specific CDPK isoforms

Key words: Abiotic and biotic stress, calcium-dependent protein kinases, cross-talk, signalling.

Introduction

Plants are remarkably responsive to a variety of environmental stimuli, including pathogen attack, wounding, cold, and drought stress and fluctuations in incident light. Following the perception of a stress stimulus, various signal transduction pathways are switched on resulting in physiological changes in the plant cell. During the last years, it became increasingly apparent that these signalling pathways are not linear, but are actually part of more complex signalling networks. The challenge of future research will be to understand the individual signalling cascades and their interactions.

Most biotic and abiotic stresses elicit an increase in cytosolic free calcium concentrations (reviewed in Pandey *et al.*, 2000; Sanders *et al.*, 2002; Trewavas and Malhó, 1998). Specific responses to different stimuli could be achieved through variations in the amplitude, duration, location, and frequency of these Ca²⁺-spikes (McAinsh and Hetherington, 1998). As Ca²⁺ is ubiquitous in stress signalling, it may be an important node at which cross-talk between pathways can occur.

Four major families of calcium-binding proteins have been identified in plants: calmodulins, calmodulin-like proteins, calcineurin B-like proteins, and calcium-dependent protein kinases (CDPKs) (Luan *et al.*, 2002; Sanders *et al.*, 2002; Snedden and Fromm, 1998, 2001).

This review will focus on CDPKs, one of the largest subfamilies of plant protein kinases. CDPKs possess a characteristic structure in which an N-terminal serine/threonine protein kinase domain is fused to a carboxy-terminal calmodulin-like domain containing EF-hand calcium-binding sites (Cheng *et al.*, 2002; Harmon *et al.*, 2001). Therefore, CDPKs do not depend on the interaction with exogenous calmodulin but can be activated directly by Ca²⁺ binding. A junction domain between the kinase and calmodulin-like domain functions as a pseudo-substrate autoinhibitor that inhibits phosphorylation in the absence of Ca²⁺ and keeps the CDPK in a state of low activity (Harmon *et al.*, 1994). The N-terminus is highly variable, and some CDPKs contain N-terminal myristoylation or palmitoylation sites that act as membrane anchors (Ellard-Ivey *et al.*, 1999; Martin and Busconi, 2000; Rutschmann *et al.*, 2002). CDPKs comprise a gene family that can be grouped into several subfamilies by phylogenetic criteria. This suggests potential functional diversification such that single isoforms may confer different

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specificities. This update will summarize signalling pathways known to involve CDPKs and will give hints about how specificity and cross-talk within the CDPK-signalling and between signalling pathways could be achieved.

Regulation of CDPK gene expression

The completed *Arabidopsis* genome sequence has revealed 34 genes encoding CDPKs. Sequencing projects in other plants including soybean, tomato, rice, and maize also indicate the presence of multigene families (Harmon *et al.*, 2001), but the reason for such a large number of CDPK genes is not yet known. In this section, the current knowledge of CDPK expression patterns and the stimuli that affect gene expression is discussed (Table 1).

Generally, most CDPK genes are expressed in most of the plant tissues examined. However, some CDPKs display an isoform-specific expression pattern that was not only organ- or tissue-specific but also dependent on growth conditions (reviewed in Hrabak, 2000). For instance, levels for several CDPK mRNAs are down-regulated by light, including *CpCPK1* (*Curcubita pepo*, Ellard-Ivey *et al.*, 1999), *OsCPK2* (*Oryza sativa*, Breviario *et al.*, 1995), *ZmCPK7* and *ZmCPK9* (*Zea mays*, Saijo *et al.*, 1997). These results suggest a possible role for some CDPKs in germination or in response to fluctuations in light intensity.

Changes in both calcium levels and protein phosphorylation, likely to be linked in part by CDPKs, are also required for cold-induced freezing tolerance in plants (Monroy *et al.*, 1993). Exposure to cold temperatures has been correlated with an increase in expression of CDPK genes in various plant species. Interestingly, two CDPKs in alfalfa, *MsCK1* and *MsCK2* were differentially expressed: *MsCK1* showed an induction during cold stress whereas, under these conditions, *MsCK2* gene expression was down-regulated (Monroy and Dhindsa, 1995). In maize, *ZmCPK1* was transcriptionally induced by cold (Berberich and Kusano, 1997) and the rice CDPK gene *OsCPK7* also showed transcriptional activation during high salinity stress (Saijo *et al.*, 2000).

Salt stress was shown to increase CDPK transcripts levels in *Arabidopsis*. Either dehydration or exposure to high concentrations of NaCl induced both *AtCPK10* and *AtCPK11* (Urao *et al.*, 1994). In mung bean (*Vicia faba*), strong induction of *VrCPK1* mRNA was observed in shoots within 2 h after treatment with NaCl (Botella *et al.*, 1996). A similar response to drought or salt stress was observed for *McCDPK1* in common ice plant (*Mesembryanthemum crystallinum*, Patharkar and Cushman, 2000). Interestingly, these CDPK genes as well as the ones described above and below as induced by similar stresses do not necessarily group in the same CDPK subfamilies.

Since phytohormones are implicated in drought and salt-stress signalling, CDPK gene expression was also investigated after treatment with various plant hormones, including gibberellin (GA), auxin (IAA), abscisic acid (ABA), cytokinin or jasmonic acid (JA). Treatment of potato plants with JA resulted in reduced mRNA-levels for *StCPK2* (*Solanum tuberosum*, Ulloa *et al.*, 2002), whereas cytokinin-treatment was reported to induce gene expression of *CsCDPK3* (*Cucumis sativus*, Ullanat and Jayabaskaran, 2002) and *NtCDPK1* (*Nicotiana tabacum*, Yoon *et al.*, 1999). Tobacco *NtCDPK1* was also found to be responsive to GA and ABA (Yoon *et al.*, 1999), whereas mung bean *VrCPK1* was only induced after treatment with IAA (Botella *et al.*, 1996).

Two other important sources for stress in plants are wounding and pathogen attack. Again, CDPKs seem to be involved in both signalling pathways. The first CDPK shown to be induced during wounding or treatment with fungal elicitors was *NtCDPK1* from tobacco. This CDPK gene is also responsive to chitosan and methyl jasmonate, a hormone implicated in disease resistance and also in the wound response (Yoon *et al.*, 1999). *NtCDPK2* and *NtCDPK3*, two other CDPK genes from tobacco, showed mRNA up-regulation after race-specific elicitation as well as osmotic stress (Romeis *et al.*, 2001). Recently, the tomato *LeCDPK1* gene was reported to be transcriptionally induced after wounding or treatment with fungal elicitors (Chico *et al.*, 2002). Another example for the involvement of a CDPK in the defence signalling pathway is *ZmCDPK10*, a maize CDPK which is induced both during a fungal infection and after treatment with fungal elicitors (Murillo *et al.*, 2001).

The constantly growing list of stimuli which regulate CDPK gene expression also includes the induction by mechanical strain for *VrCPK1* (Botella *et al.*, 1996), anoxic stress for *OsCPK2* (Breviario *et al.*, 1995), heat stress for *MsCPK3* (Davletova *et al.*, 2001) and calcium chloride treatment for *NtCDPK1* and *VrCPK1* (Yoon *et al.*, 1999; Botella *et al.*, 1996).

Regulation of CDPK enzyme activity

Whereas the regulation of CDPK gene expression levels during various stress conditions has been described in a variety of plant species, biochemical characterization of the encoded proteins is generally lacking. Often, calcium-dependent protein kinase activities were investigated directly in crude protein extracts, but the corresponding genes were not isolated (Table 1). Various studies described changes in CDPK activities during osmotic stress (Takahashi *et al.*, 1997), cold stress (Martin and Busconi, 2001), elicitation (Allwood *et al.*, 2002), embryogenesis (SwCDPK, Anil *et al.*, 2000) or treatment with sucrose (Iwata *et al.*, 1998) and phytohormones (Abo-El-Saad and Wu, 1995). Biochemical analysis has also

Table 1. Signalling pathways involving CDPKs

| Gene/Protein | Species | Transcriptional activation | Biochemical activation | Literature |
|----------------|--------------------------------------|--|--|---|
| – ^a | French bean | | Fungal elicitor | Allwood <i>et al.</i> , 2002 |
| – ^a | <i>Funaria hygrometrica</i> | Nitrogen, phosphorus and sulphur stress | | Mitra and Johri, 2000 |
| – ^a | Maize | Pollen development | | Estruch <i>et al.</i> , 1994 |
| – ^a | Oat | | Phospholipids | Schaller <i>et al.</i> , 1992 |
| – ^a | Rice | | GA | Abo-El-Saad and Wu, 1995 |
| – ^a | Rice | | Phospholipids | Karibe <i>et al.</i> , 1995 |
| – ^a | Rice | | Cold stress | Martin and Busconi, 2001 |
| – ^a | Tobacco | | Sucrose | Iwata <i>et al.</i> , 1998 |
| AtCPK1 | <i>Arabidopsis</i> | | Phospholipids | Binder <i>et al.</i> , 1994: Harper <i>et al.</i> , 1993 |
| AtCPK10 | <i>Arabidopsis</i> | Drought and salt stress | | Urao <i>et al.</i> , 1994 |
| AtCPK11 | <i>Arabidopsis</i> | Drought and salt stress | | Urao <i>et al.</i> , 1994 |
| CpCPK1 | Zucchini | White light, hypocotyl development ^b | | Ellard-Ivey <i>et al.</i> , 1999 |
| CsCDPK3 | Cucumber | White light, cytokinin (differential induction dependent on plant organ) | | Ullanat and Jayabaskaran, 2002 |
| DcCPK1 | Carrot | | Phospholipids | Farmer and Choi, 1999 |
| LeCDPK1 | Tomato | Fungal elicitor, H ₂ O ₂ , wounding | | Chico <i>et al.</i> , 2002 |
| McCDPK1 | <i>Mesembryanthemum crystallinum</i> | Drought and salt stress | | Patharkar and Cushman, 2000 |
| MsCK1 | Alfalfa | Cold stress | | Monroy and Dhindsa, 1995 |
| MsCK2 | Alfalfa | Cold stress ^b | | Monroy and Dhindsa, 1995 |
| MsCPK3 | Alfalfa | 2,4-D, heat stress | | Davletova <i>et al.</i> , 2001 |
| NtCDPK1 | Tobacco | Ca ²⁺ , GA, ABA, cytokinin, methyl jasmonate, wounding, fungal elicitors, chitosan, salt stress | | Yoon <i>et al.</i> , 1999 |
| NtCDPK2 | Tobacco | Fungal elicitor, osmotic stress | Fungal elicitor, osmotic stress | Romeis <i>et al.</i> , 2001 |
| NtCDPK3 | Tobacco | Fungal elicitor, osmotic stress | | Romeis <i>et al.</i> , 2001 |
| OsCPK1 | Rice | Seed development | | Kawasaki <i>et al.</i> , 1993 |
| OsCPK2 | Rice | White light ^b , anoxic stress ^b , seed development | | Breviaro <i>et al.</i> , 1995; Frattini <i>et al.</i> , 1999 |
| OsCPK7 | Rice | Cold and salt stress | | Saijo <i>et al.</i> , 2000 |
| R-SPSK | Rice | | P _i | Pagnussat <i>et al.</i> , 2002 |
| SPK | Rice | Seed development | | Kawasaki <i>et al.</i> , 1993 |
| StCPK1 | Potato | Tuberization | | Raices <i>et al.</i> , 2001 |
| StCPK2 | Potato | Jasmonic acid ^b | | Ulloa <i>et al.</i> , 2002 |
| SwCPK | Sandalwood | | Embryogenesis, seed development, germination | Anil <i>et al.</i> , 2000 |
| VrCPK1 | Mung bean | Ca ²⁺ , IAA, cycloheximide, mechanical and salt stress | | Botella <i>et al.</i> , 1996 |
| ZmCPK1 | Maize | cold stress | | Berberich and Kusano, 1997 |
| ZmCPK7 | Maize | White light ^b | | Saijo <i>et al.</i> , 1997 |
| ZmCPK9 | Maize | White light ^b | | Saijo <i>et al.</i> , 1997 |
| ZmCPK10 | Maize | Fungal infection and fungal elicitor | | Murillo <i>et al.</i> , 2001 |
| ZmCPKp54 | Maize | | Phospholipids | Szczegieliński <i>et al.</i> , 2000 |

^a CDPK activity investigated in protein crude extract, no gene name available.

^b Suppression of gene transcription.

revealed that specific phospholipids can enhance *in vitro* substrate phosphorylation by CDPKs from oat (Schaller *et al.*, 1992), *Arabidopsis* (AtCPK1, Binder *et al.*, 1994; Harper *et al.*, 1993), carrot (*Daucus carota*, DcCPK1, Farmer and Choi, 1999), and maize (*Zea mays*, ZmCPKp54, Szczegieliński *et al.*, 2000) (Table 1). Some of these phospholipids are known to act as second messengers in plant signal transduction (Munnik *et al.*, 1998) and may elicit their effects, in part, through CDPKs. Interestingly, the phospholipids regulating kinase activity

vary for each of the CDPKs studied, which may provide an added layer of CDPK specificity.

One of the best biologically characterized CDPKs is *NtCDPK2* from tobacco. This enzyme was initially identified in the Cf-9/Avr9 patho-system as a 68/70 kDa calcium-dependent kinase activity that is biochemically activated in response to race-specific elicitation (Romeis *et al.*, 2000). In transient expression assays epitope-tagged *NtCDPK2* showed a stress-induced transition from a resting state to an activated state, which could be

visualized by an electrophoretic mobility shift as had also been described for the 68/70 kDa CDPK. This mobility shift was due to phosphorylation of NtCDPK2. Immuno-complex kinase assays suggested that the shift is correlated with an increased enzymatic activity. Notably, the response of NtCDPK2 to elicitation was more pronounced and sustained compared with an osmotic stress response (Romeis *et al.*, 2001). Thus, the enzyme appears to be involved in both signalling pathways and its specificity is provided by alterations in extent and duration of activation depending on the incoming stimulus.

Specificity of CDPK signalling

Although CDPKs have been implicated to act as key regulators of many signalling pathways, very little is known about which particular CDPK acts as the calcium sensor in each case. Modern techniques such as reverse genetics or ectopic protein expression facilitate the investigation of specific CDPK isoforms in certain signalling pathways (Table 2). In this section it will be described how these techniques were used to elucidate the specificity of certain CDPK signalling pathways.

The mRNA levels for the rice gene *OsCDPK7* increase in response to cold and salt stress, suggesting a function for this CDPK in the corresponding signalling pathways. Remarkably, transgenic rice plants with altered *OsCDPK7* protein levels showed an altered tolerance to cold, drought and salt stress (Saijo *et al.*, 2000). The extent of tolerance of these plants correlated with the level of *OsCDPK7* expression: overexpression increased whereas suppression of *OsCDPK7* expression lowered the stress tolerance.

These results confirmed that *OsCDPK7* has an important role in the tolerance to both cold and salt stress in rice. From previous gene expression data it was assumed that another rice CDPK, *OsCDPK2*, may have a function in seed development or in response to light changes (Breviario *et al.*, 1995; Frattini *et al.*, 1999). Overexpression of the full length *OsCDPK2* in transgenic rice lines confirmed its function in seed development: the seed development in these plants was arrested at a very early stage leading to an overall inhibition of seed formation (Morello *et al.*, 2000). Asano *et al.* (2002) described the involvement of a second rice CDPK, *SPK*, in seed development which is consistent with its specific expression in developing seeds (Kawasaki *et al.*, 1993). As the *SPK* gene expression pattern was very similar to that of enzymes involved in storage starch biosynthesis (of which some are known to be regulated by phosphorylation, Huber *et al.*, 1996), it was suggested that *SPK* may be involved in the regulation of starch biosynthesis. Supporting this assumption, antisense *SPK* rice transformants lacked the ability to accumulate storage products such as starch, resulting in watery seeds with a delayed development (Asano *et al.*, 2002).

The expression of a C-terminally truncated, constitutively active CDPK allele in a maize protoplast system allowed Sheen (1996) to establish a role for the *Arabidopsis AtCPK10* and *AtCPK30* in activating cold, drought and salt stress response pathways. Notably, in these studies *AtCDPK10* and *AtCDPK30* were specifically mediating cold and salt stress signalling, whereas the ectopic expression of other CDPK family members had no effect on the signalling pathway investigated (Sheen,

Table 2. Functional studies for selected CDPK isoforms

| Name | Effect of ectopic reexpression/constitutive activation | Effect of silencing | Literature |
|-------------------|--|---|--|
| AtCPK1 | Increased NADPH oxidase activity ^a | | King <i>et al.</i> , 2001 |
| AtCPK10 | Constitutive activation of ABA-responsive genes ^b | | Sheen, 1996 |
| AtCPK30 | Constitutive activation of ABA-responsive genes ^b | | Sheen, 1996 |
| Maize pollen CDPK | | Disruption of pollen germination ^c | Estruch <i>et al.</i> , 1994 |
| NtCDPK1 | | Defects in cell division and differentiation, constitutive defence response ^d | Lee <i>et al.</i> , 2003 |
| NtCDPK2 | Induced defence responses ^b | Reduced defence responses ^d | Romeis <i>et al.</i> , 2001; unpublished |
| OsCDPK2 | Disruption of seed development ^a | | Morello <i>et al.</i> , 2000 |
| OsCDPK7 | Increased cold/salt/drought tolerance ^a | Decreased cold/salt/drought tolerance ^e | Saijo <i>et al.</i> , 2000 |
| SPK | | Delay in seed development, defect in starch accumulation, reduction of sucrose degradation ^f | Asano <i>et al.</i> , 2002 |

^a Expression of full length protein.

^b Expression of truncated protein.

^c Antisense oligonucleotides.

^d Virus-induced gene silencing.

^e Sense co-suppression.

^f Antisense transgenic lines.

1996). The *AtCPK10* gene expression had previously been shown to be induced by the same stress stimuli (Urao *et al.*, 1994), confirming that this particular *Arabidopsis* CDPK is responsive to changes in the osmotic potential of the environment. In tobacco, both *NtCDPK2* and *NtCDPK3* were transcriptionally up-regulated in response to elicitation and osmotic stress. The hypersensitive response (HR) is a defence response typical in gene-for-gene interactions. Virus-induced gene silencing of the *NtCDPK2/3* gene family resulted in a reduced HR after race-specific elicitation (Romeis *et al.*, 2001). These results suggested that *NtCDPK2* and/or closely related subfamily members were indeed required in a defence-related signalling cascade. Furthermore, by ectopically expressing truncated *NtCDPK2* or *NtCDPK3* variants the specificity of *NtCDPK2* signalling was investigated. *Nicotiana benthamiana* leaves expressing a truncated *NtCDPK2* variant, which only consisted of the variable and kinase domain, responded to a weak abiotic stress stimulus with an HR-like necrosis. In addition, enhanced production of reactive oxygen species (ROS) and an induction of plant defence-related genes were observed. By contrast, an homologous isoform, *NtCDPK3*, was unable to induce such defence responses including the HR-like cell death (AA Ludwig, JDG Jones, T Romeis, unpublished results). This clearly indicates that *NtCDPK2* kinase, but not the closely related *NtCDPK3* protein, is specifically involved in the plant defence response.

Interestingly, the *Arabidopsis* *AtCPK1*, which belongs to the same CDPK subfamily as *NtCDPK2*, has also been implicated in the plant defence response. Overexpression of *AtCPK1* in a heterologous tomato protoplast system resulted in an enhanced NADPH oxidase activity and increased production of ROS (Xing *et al.*, 2001). The release of ROS is one of the earliest responses during a plant defence to pathogen attack and calcium is well known to play an important role in both the production of ROS and the establishment of the hypersensitive response (Blumwald *et al.*, 1998; Piedras *et al.*, 1998). In this scenario, a CDPK functions as a calcium sensor, and the plasma membrane bound NADPH oxidase would be one of its phosphorylation targets (Blumwald *et al.*, 1998; Romeis *et al.*, 2000; Xing *et al.*, 1997).

CDPK-mediated cross-talk between signalling pathways

Cross-talk can be defined as the interaction of two or more different signalling pathways. Various stress stimuli could, for instance, converge at one signalling component, resulting in the same downstream response. Alternatively, different parallel signalling pathways could interact and affect each other's outcome, either in an additive or a negative regulatory way. Usually, when stress signalling pathways are examined, they are

considered in isolation from other stresses to simplify interpretation. Techniques such as silencing or over-expression of certain signalling components may confirm their role in particular pathways, but often, as long as alterations in protein abundance do not result in obvious phenotypic effects, their function in other signalling pathways may still remain unnoticed. As for CDPKs, little is known about if and how they participate in cross-talk between different signalling pathways.

A good example where cross-talk between the signalling pathways seems likely is the response to wound stress (abiotic) and pathogen attack (biotic). Wounding of plant tissue may not only trigger specific responses for tissue healing but, in addition, activate defence responses to prevent further damage caused by pathogen infection. Evidence for the cross-talk between wound- and defence stress responses is accumulating: both trigger the production of reactive oxygen species, activate jasmonate and ethylene phytohormone signalling pathways, and induce the activation of genes coding for basic pathogenesis-related proteins (Kunkel and Brooks, 2002; León *et al.*, 2001; Wasternack and Parthier, 1997). It has been reported that plant-pathogen interactions and wounding may be interlinked at the level of MAPKs (Romeis *et al.*, 1999). Recent data suggest that CDPKs are also multifunctional, being involved in different signalling pathways and potentially acting as switches between these pathways.

NtCDPK2 is activated both by hypo-osmotic stress (infiltration of water) and during the plant defence response (Romeis *et al.*, 2001). Dependent on the incoming stress stimuli, *NtCDPK2* enzyme activation varied in strength and duration (Romeis *et al.*, 2001). It seems that a short and weak *NtCDPK2* activation after an osmotic stress stimulus solely results in the induction of the wound signalling pathway, whereas a much stronger and sustained elicitation may lead to a plant defence response. A functional cross-talk between abiotic and biotic signalling pathways became evident upon overactivation of *NtCDPK2*: *N. benthamiana* leaves expressing a truncated *NtCDPK2* allele showed, upon treatment with a mild abiotic stress stimulus (like wounding with a forceps or infiltration of water), a biotic (pathogen-related) stress response including an HR-like necrosis (AA Ludwig, JDG Jones, T Romeis, unpublished results). It will be of particular interest to learn how this cross-talk correlates with changes in levels of specific phytohormones, in particular JA and ethylene, since both are involved in a plant's wound and pathogen defence response.

Two other CDPKs, *NtCDPK1* from tobacco and *OsCDPK7* from rice, also have been implicated in two different signalling pathways and it is likely that they function as cross-talk mediators between the pathways. *NtCDPK1* is induced after wounding and treatment with phytohormones, high salt or fungal elicitors (Yoon *et al.*, 1999). *N. benthamiana* plants with reduced levels of

NtCDPK1 show severe abnormalities in cell morphology, spontaneous necrotic lesions and increased expression of marker genes for the plant defence response (Lee *et al.*, 2003). Cellular defects caused by abnormal cell division and differentiation might induce programmed cell death in the affected tissue. It can be suggested that plant cell development and defence response are interconnected at the level of *NtCDPK1*. Lee *et al.* (2003) propose that *NtCDPK1* may receive hormone signals to regulate cell division and differentiation. Through interaction with and phosphorylation of the 26S proteasome regulatory subunit *NtRpn3* this CDPK may regulate proteasome activity, thereby adjusting the degradation of regulatory components depending on developmental and environmental stimuli (Lee *et al.*, 2003).

Ectopic expression of *OsCDPK7* conferred both cold and salt/drought tolerance in rice plants (Saijo *et al.*, 2000). Interestingly, *OsCDPK7* overexpression enhanced only the transcription of salt- and drought-responsive, but not cold-responsive target genes. Thus, it was suggested that cold and salt/drought tolerance is promoted through distinct pathways and that both signalling cascades cross-talk at the level of *OsCDPK7*.

So far, there is no experimental proof for an interaction between CDPKs with other signalling pathways. Several studies demonstrate that plant mitogen activated protein kinases (MAPKs) integrate signals arising from diverse stress stimuli (reviewed in Jonak *et al.*, 2002; Zhang and Klessig, 2001). In tobacco, for example, the two MAPKs, salicylic acid-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK), were activated during a plant defence response and also by wounding (Romeis *et al.*, 1999; Seo *et al.*, 1995; Zhang and Klessig, 1998; Zhang and Liu, 2001). It remains to be determined whether important interactions occur between members of the MAPK or CDPK families, and whether cross-talk also occurs between MAPK- and CDPK-dependent pathways.

Conclusions

Signalling pathways have to be regarded as complex networks. Multiple points of convergence and divergence that enable signal integration at different levels, and provide the molecular basis for appropriate downstream responses characterize these signal transduction networks.

CDPK-mediated signalling is envisaged to operate at three levels. Firstly, different stress stimuli can induce specific calcium signatures in certain parts of the cell. Secondly, these variations in calcium concentrations will activate specific CDPK isoforms, which can themselves be differentially expressed within the plant or upon external stimuli. Dependent on the calcium signature, the extent and duration of CDPK enzyme activation will vary, having a direct effect on the phosphorylation status of its

downstream targets. Thirdly, CDPKs most likely participate in cross-talk between signalling pathways.

The major challenge of the future will be to elucidate which CDPK isoform functions in and interacts with which pathway. It was expected that with the completion of the *Arabidopsis* genome project and the availability of knockout libraries, the analysis of CDPK genes implied in certain signalling pathways would be accelerated. However, so far no clear physiological function could be allocated to CDPK isoforms based on the phenotypic analysis of single knockout lines. Due to possible redundancy in CDPK functions (Sheen, 1996), the simultaneous inactivation of highly homologous CDPKs, either by crossing respective single knockout lines or based on an RNAi cosuppression approach, may therefore be necessary. Combined with new technologies like microarrays, researchers will be able to examine the effect of altered CDPK protein levels on the total mRNA expression profile. This will lead to a better understanding of the interaction between signalling pathways in plants.

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References

- Abo-El-Saad M, Wu R. 1995. A rice membrane calcium-dependent protein kinase is induced by gibberellin. *Plant Physiology* **108**, 787–793.
- Allwood EG, Davies DR, Gerrish C, Bolwell GP. 2002. Regulation of CDPKs, including identification of PAL kinase, in biotically stressed cells of French bean. *Plant Molecular Biology* **49**, 533–544.
- Anil VS, Harmon AC, Rao KS. 2000. Spatio-temporal accumulation and activity of calcium-dependent kinases during embryogenesis, seed development, and germination in sandalwood. *Plant Physiology* **122**, 1035–1043.
- Asano T, Kunieda N, Omura Y, *et al.* 2002. Rice SPK, a calmodulin-like domain protein kinase, is required for storage product accumulation during seed development: phosphorylation of sucrose synthase is a possible factor. *The Plant Cell* **14**, 619–628.
- Berberich T, Kusano T. 1997. Cycloheximide induces a subset of low temperature-inducible genes in maize. *Molecular Genetics and Genomics* **254**, 275–283.
- Binder BD, Harper JF, Sussman MR. 1994. Characterization of an *Arabidopsis* calmodulin-like domain protein kinase purified from *Escherichia coli* using an affinity sandwich technique. *Biochemistry* **33**, 2033–2041.
- Blumwald E, Aharon GS, Lam BCH. 1998. Early signal

- transduction pathways in plant–pathogen interactions. *Trends in Plant Science* **3**, 342–346.
- Botella JR, Arteca JM, Somodevilla M, Arteca RN.** 1996. Calcium-dependent protein kinase gene expression in response to physical and chemical stimuli in mungbean (*Vigna radiata*). *Plant Molecular Biology* **30**, 1129–1137.
- Breviario D, Morello L, Gianì S.** 1995. Molecular cloning of two novel rice cDNA sequences encoding putative calcium-dependent protein kinases. *Plant Molecular Biology* **27**, 953–967.
- Cheng SH, Willmann MR, Chen HC, Sheen J.** 2002. Calcium signalling through protein kinases. The *Arabidopsis* calcium-dependent protein kinase gene family. *Plant Physiology* **129**, 469–485.
- Chico JM, Raíces M, Téllez-Iñón MT, Ulloa RM.** 2002. A calcium-dependent protein kinase is systemically induced upon wounding in tomato plants. *Plant Physiology* **128**, 256–270.
- Davletova S, Mesyaros T, Miskolczi P, Oberschall A, Torok K, Magyar Z, Dudits S, Deak M.** 2001. Auxin and heat shock activation of a novel member of the calmodulin-like domain protein kinase gene family in cultured alfalfa cells. *Journal of Experimental Botany* **52**, 215–221.
- Ellard-Ivey M, Hopkins RB, White TJ, Lomax TL.** 1999. Cloning, expression and N-terminal myristoylation of CpCPK1, a calcium-dependent protein kinase from zucchini (*Cucurbita pepo* L.). *Plant Molecular Biology* **39**, 199–208.
- Estruch JJ, Kadwell S, Merlin E, Crossland L.** 1994. Cloning and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase. *Proceedings of the National Academy of Sciences, USA* **91**, 8837–8841.
- Farmer PK, Choi JH.** 1999. Calcium and phospholipid activation of a recombinant calcium-dependent protein kinase (DcCPK1) from carrot (*Daucus carota* L.). *Biochimica et Biophysica Acta* **1434**, 6–17.
- Frattini M, Morello L, Breviario D.** 1999. Rice calcium-dependent protein kinase isoforms OsCDPK2 and OsCDPK11 show different responses to light and different expression patterns during seed development. *Plant Molecular Biology* **41**, 753–764.
- Harmon AC, Gribskov M, Gubrium E, Harper JF.** 2001. The CDPK superfamily of protein kinases. *New Phytologist* **151**, 175–183.
- Harmon AC, Yoo BC, McCaffery C.** 1994. Pseudosubstrate inhibition of CDPK, a protein kinase with a calmodulin-like domain. *Biochemistry* **33**, 7278–7287.
- Harper JF, Binder BM, Sussman MR.** 1993. Calcium and lipid regulation of an *Arabidopsis* protein kinase expressed in *Escherichia coli*. *Biochemistry* **32**, 3282–3290.
- Hrabak E.** 2000. Calcium-dependent protein kinases and their relatives. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **32**, 185–223.
- Huber SC, Huber JL, Liao PC, Gage DA, McMichael RW, Chourey PS, Hannah LC, Koch K.** 1996. Phosphorylation of serine-15 of maize leaf sucrose synthase: occurrence *in vivo* and possible regulatory significance. *Plant Physiology* **112**, 793–802.
- Iwata Y, Kuriyama M, Nakakita M, Kojima H, Ohto M, Nakamura K.** 1998. Characterization of a calcium-dependent protein kinase of tobacco leaves that is associated with the plasma membrane and is inducible by sucrose. *Plant and Cell Physiology* **39**, 1176–1183.
- Jonak C, Ókrész L, Bögre L, Hirt H.** 2002. Complexity, cross talk and integration of plant MAP kinase signalling. *Current Opinion in Plant Biology* **5**, 415–424.
- Karibe H, Komatsu S, Hirano H.** 1995. A calcium- and phospholipid-dependent protein kinase from rice. *Physiologia Plantarum* **95**, 127–133.
- Kawasaki T, Hayashida N, Baba T, Shinozaki K, Shimada H.** 1993. The gene encoding a calcium-dependent protein kinase located near the *sbe1* gene encoding starch branching enzyme I is specifically expressed in developing rice seeds. *Gene* **129**, 183–189.
- Kunkel BN, Brooks DM.** 2002. Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology* **5**, 325–331.
- Lee SS, Cho HS, Yoon GM, Ahn JW, Kim HH, Pai HS.** 2003. Interaction of NtCDPK1 calcium-dependent protein kinase with NtRpn3 regulatory subunit of the 26S proteasome in *Nicotiana tabacum*. *The Plant Journal* **33**, 825–840.
- León J, Rojo E, Sánchez-Serrano JJ.** 2001. Wound signalling in plants. *Journal of Experimental Botany* **52**, 1–9.
- Luan S, Kudla J, Rodriguez-Concepcion M, Yalovsky S, Gruissem W.** 2002. Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. *The Plant Cell* **14**, S389–S400.
- Martin M, Busconi L.** 2000. Membrane localization of a rice calcium-dependent protein kinase (CDPK) is mediated by myristoylation and palmitoylation. *The Plant Journal* **24**, 429–435.
- Martin ML, Busconi L.** 2001. A rice membrane-bound calcium-dependent protein kinase is activated in response to low temperature. *Plant Physiology* **125**, 1442–1449.
- McAinsh MR, Hetherington AM.** 1998. Encoding specificity in Ca²⁺ signaling systems. *Trends in Plant Sciences* **3**, 32–36.
- Mitra D, Johri MM.** 2000. Enhanced expression of a calcium-dependent protein kinase from the moss *Funaria hygrometrica* under nutritional starvation. *Journal of Biosciences* **25**, 331–338.
- Monroy AF, Dhindsa RS.** 1995. Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25 °C. *The Plant Cell* **7**, 321–331.
- Monroy AF, Sarhan F, Dhindsa RS.** 1993. Cold-induced changes in freezing tolerance, protein phosphorylation and gene expression. Evidence for a role of calcium. *Plant Physiology* **102**, 1227–1235.
- Morello L, Frattini M, Gianì S, Christou P, Breviario D.** 2000. Overexpression of the calcium-dependent protein kinase OsCDPK2 in transgenic rice is repressed by light in leaves and disrupts seed development. *Transgenic Research* **9**, 453–462.
- Munnik T, Irvine RF, Musgrave A.** 1998. Phospholipid signalling in plants. *Biochimica et Biophysica Acta* **1389**, 222–272.
- Murillo I, Jaeck E, Cordero MJ, San Segundo B.** 2001. Transcriptional activation of a maize calcium-dependent protein kinase gene in response to fungal elicitors and infection. *Plant Molecular Biology* **45**, 145–158.
- Pagnussat GC, Fiol DF, Salerno GL.** 2002. A CDPK type protein kinase is involved in rice SPS light modulation. *Physiologia Plantarum* **115**, 183–189.
- Pandey S, Tiwari SB, Upadhyaya KC, Sopory SK.** 2000. Calcium signaling: linking environmental signals to cellular functions. *Critical Reviews in Plant Sciences* **19**, 291–318.
- Patharkar OR, Cushman JC.** 2000. A stress-induced calcium-dependent protein kinase from *Mesembryanthemum crystallinum* phosphorylates a two-component pseudo-response regulator. *The Plant Cell* **24**, 679–691.
- Piedras P, Hammond-Kosack KE, Harrison K, Jones JDG.** 1998. Rapid, Cf-9- and Avr9-dependent production of active oxygen species in tobacco suspension cultures. *Molecular Plant–Microbe Interactions* **11**, 1155–1166.
- Raíces M, Chico JM, Téllez-Iñón, MT, Ulloa, RM.** 2001. Molecular characterization of StCDPK1, a calcium-dependent protein kinase from *Solanum tuberosum* that is induced at the onset of tuber development. *Plant Molecular Biology* **46**, 591–601.
- Romeis T, Ludwig AA, Martin R, Jones JDG.** 2001. Calcium-

- dependent protein kinases play an essential role in a plant defence response. *EMBO Journal* **20**, 5556–5567.
- Romeis T, Piedras P, Jones JDG.** 2000. Resistance gene-dependent activation of a calcium-dependent protein kinase (CDPK) in the plant defense response. *The Plant Cell* **12**, 803–815.
- Romeis T, Piedras P, Zhang SQ, Klessig DF, Hirt H, Jones JDG.** 1999. Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. *The Plant Cell* **11**, 273–287.
- Rutschmann F, Stalder U, Piotrowski M, Oecking C, Schaller A.** 2002. LeCPK1, a calcium-dependent protein kinase from tomato. Plasma membrane targeting and biochemical characterization. *Plant Physiology* **129**, 156–168.
- Saijo Y, Hata S, Kyojuka J, Shimamoto K, Izui K.** 2000. Over-expression of a single Ca²⁺-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *The Plant Journal* **23**, 319–327.
- Saijo Y, Hata S, Sheen J, Izui K.** 1997. cDNA cloning and prokaryotic expression of maize calcium-dependent protein kinases. *Biochimica et Biophysica Acta* **1350**, 109–114.
- Sanders D, Pelloux J, Brownlee C, Harper JF.** 2002. Calcium at the crossroads of signalling. *The Plant Cell* **14**, S401–S417.
- Schaller GE, Harmon AC, Sussman MR.** 1992. Characterization of a calcium- and lipid-dependent protein kinase associated with the plasma membrane of oat. *Biochemistry* **31**, 1721–1727.
- Seo S, Okamoto M, Seto H, Ishizuka K, Sano H, Ohashi Y.** 1995. Tobacco MAP kinase: a possible mediator in wound signal transduction pathways. *Science* **270**, 1988–1992.
- Sheen J.** 1996. Ca²⁺-dependent protein kinases and stress signal transduction in plants. *Science* **274**, 1900–1902.
- Snedden WA, Fromm H.** 1998. Calmodulin, calmodulin-related proteins and plant responses to the environment. *Trends in Plant Science* **3**, 299–304.
- Snedden WA, Fromm H.** 2001. Calmodulin as a versatile calcium signal transducer in plants. *New Phytologist* **151**, 35–66.
- Szczegieliński J, Liwosz A, Jurkowski I, Loog M, Dobrowolska G, Ek P, Harmon AC, Muszyńska G.** 2000. Calcium-dependent protein kinase from maize seedlings activated by phospholipids. *European Journal of Biochemistry* **267**, 3818–3827.
- Takahashi K, Isobe M, Muto S.** 1997. An increase in cytosolic calcium ion concentration precedes hypo-osmotic shock-induced activation of protein kinases in tobacco suspension culture cells. *FEBS Letters* **401**, 202–206.
- Trewavas AJ, Malhó R.** 1998. Ca²⁺ signalling in plant cells: the big network! *Current Opinion in Plant Biology* **1**, 428–433.
- Ullanat R, Jayabaskaran C.** 2002. Distinct light-, cytokinin- and tissue-specific regulation of calcium-dependent protein kinase gene expression in cucumber (*Cucumis sativus*). *Plant Science* **162**, 153–163.
- Ulloa RM, Raíces M, MacIntosh GC, Maldonado S, Téllez-Iñón, MT.** 2002. Jasmonic acid affects plant morphology and calcium-dependent protein kinase expression and activity in *Solanum tuberosum*. *Physiologia Plantarum* **115**, 417–427.
- Urao T, Katagiri T, Mizoguchi T, Yamaguchi-Shinozaki K, Hayashida N, Shinozaki K.** 1994. Two genes that encode Ca²⁺-dependent protein-kinases are induced by drought and high-salt stresses in *Arabidopsis thaliana*. *Molecular Genetics and Genomics* **244**, 331–340.
- Wasternack C, Parthier B.** 1997. Jasmonate-signalled plant gene expression. *Trends in Plant Science* **2**, 302–307.
- Xing T, Higgins VJ, Blumwald E.** 1997. Race-specific elicitors of *Cladosporium fulvum* promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. *The Plant Cell* **9**, 249–259.
- Xing T, Wang XJ, Malik K, Miki BL.** 2001. Ectopic expression of an *Arabidopsis* calmodulin-like domain protein kinase-enhanced NADPH oxidase activity and oxidative burst in tomato protoplasts. *Molecular Plant-Microbe Interactions* **14**, 1261–1264.
- Yoon GM, Cho HS, Ha HJ, Liu JR, Lee HSP.** 1999. Characterization of NtCDPK1, a calcium-dependent protein kinase gene in *Nicotiana tabacum*, and the activity of its encoded protein. *Plant Molecular Biology* **39**, 991–1001.
- Zhang S, Klessig DF.** 1998. The tobacco wounding-activated mitogen-activated protein kinase is encoded by SIPK. *Proceedings of the National Academy of Sciences, USA* **95**, 7225–7230.
- Zhang S, Klessig DF.** 2001. MAPK cascades in plant defence signaling. *Trends in Plant Science* **6**, 520–527.
- Zhang S, Liu Y.** 2001. Activation of salicylic acid-induced protein kinase, a mitogen-activated protein kinase, induces multiple defence responses in tobacco. *The Plant Cell* **13**, 1877–1889.