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 Ca\(^{2+}\) 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 Malho, 1998). Specific responses to different stimuli could be achieved through variations in the amplitude, duration, location, and frequency of these Ca\(^{2+}\)-spikes (McAinsh and Hetherington, 1998). As Ca\(^{2+}\) 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 Ca\(^{2+}\) binding. A junction domain between the kinase and calmodulin-like domain functions as a pseudo-substrate autoinhibitor that inhibits phosphorylation in the absence of Ca\(^{2+}\) 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
specifications. 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). NtCPK2 and NtCPK3, 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 LeCPK1 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 (SwCPDK, Anil et al., 2000) or treatment with sucrose (Iwata et al., 1998) and phytohormones (Abo-El-Saad and Wu, 1995). Biochemical analysis has also
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, Szczegielniak 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

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**Table 1. Signalling pathways involving CDPKs**

<table>
<thead>
<tr>
<th>Gene/Protein</th>
<th>Species</th>
<th>Transcriptional activation</th>
<th>Biochemical activation</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>-a</em></td>
<td>French bean</td>
<td>Nitrogen, phosphorus and sulphur stress</td>
<td>Fungal elicitor</td>
<td>Allwood et al., 2002</td>
</tr>
<tr>
<td><em>-a</em></td>
<td>Funaria hygrometrica</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
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<td><em>-a</em></td>
<td>Maize</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>-a</em></td>
<td>Oat</td>
<td>Pollen development</td>
<td>Phospholipids</td>
<td>Schaller et al., 1992</td>
</tr>
<tr>
<td><em>-a</em></td>
<td>Rice</td>
<td>Pollen development</td>
<td>GA</td>
<td>Abo-El-Saad and Wu, 1995</td>
</tr>
<tr>
<td><em>-a</em></td>
<td>Rice</td>
<td>Pollen development</td>
<td>Phospholipids</td>
<td>Karibe et al., 1995</td>
</tr>
<tr>
<td><em>-a</em></td>
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<td>Pollen development</td>
<td>Cold stress</td>
<td>Martin and Busconi, 2001</td>
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<td><em>-a</em></td>
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<td>Pollen development</td>
<td>Sucrose</td>
<td>Iwata et al., 1998</td>
</tr>
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<td>AtCPK1</td>
<td>Arabidopsis</td>
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<td>Phospholipids</td>
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<td>AtCPK10</td>
<td>Arabidopsis</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AtCPK11</td>
<td>Arabidopsis</td>
<td>Pollen development</td>
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<td>-</td>
</tr>
<tr>
<td>CpCPK1</td>
<td>Zucchini</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CsCDPK3</td>
<td>Cucumber</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DcCPK1</td>
<td>Carrot</td>
<td>Pollen development</td>
<td>Phospholipids</td>
<td>Farmer and Choi, 1999</td>
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<td>LeCDPK1</td>
<td>Tomato</td>
<td>Pollen development</td>
<td>-</td>
<td>Chico et al., 2002</td>
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<td>McCDPK1</td>
<td>Mesembryanthemum crystallinum</td>
<td>Pollen development</td>
<td>-</td>
<td>Patharkar and Cushman, 2000</td>
</tr>
<tr>
<td>McCK1</td>
<td>Alfalfa</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>McCK2</td>
<td>Alfalfa</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>McCPK3</td>
<td>Alfalfa</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
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<td>NtCDPK1</td>
<td>Tobacco</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NtCDPK2</td>
<td>Tobacco</td>
<td>Pollen development</td>
<td>Fungal elicitor, osmotic stress</td>
<td>Romeis et al., 2001</td>
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<td>NtCDPK3</td>
<td>Tobacco</td>
<td>Pollen development</td>
<td>Fungal elicitor, osmotic stress</td>
<td>Romeis et al., 2001</td>
</tr>
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<td>OsCPK1</td>
<td>Rice</td>
<td>Pollen development</td>
<td>-</td>
<td>Kawasaki et al., 1993</td>
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<tr>
<td>OsCPK2</td>
<td>Rice</td>
<td>Pollen development</td>
<td>-</td>
<td>Breviario et al., 1995; Frattini et al., 1999</td>
</tr>
<tr>
<td>OsCPK7</td>
<td>Rice</td>
<td>Pollen development</td>
<td>-</td>
<td>Saijo et al., 2000</td>
</tr>
<tr>
<td>R-SPSK</td>
<td>Rice</td>
<td>Pollen development</td>
<td>-</td>
<td>Kawasakai et al., 1993</td>
</tr>
<tr>
<td>SPK</td>
<td>Rice</td>
<td>Pollen development</td>
<td>-</td>
<td>Raices et al., 2001</td>
</tr>
<tr>
<td>ScCPK1</td>
<td>Potato</td>
<td>Pollen development</td>
<td>-</td>
<td>Ulloa et al., 2002</td>
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<tr>
<td>ScCPK2</td>
<td>Potato</td>
<td>Pollen development</td>
<td>Embryogenesis, seed development, germination</td>
<td>Anil et al., 2000</td>
</tr>
<tr>
<td>SwCPK</td>
<td>Sandalwood</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VrCPK1</td>
<td>Mung bean</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
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<tr>
<td>ZmCPK1</td>
<td>Maize</td>
<td>Pollen development</td>
<td>-</td>
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<tr>
<td>ZmCPK7</td>
<td>Maize</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
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<tr>
<td>ZmCPK9</td>
<td>Maize</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
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<tr>
<td>ZmCPK10</td>
<td>Maize</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
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<tr>
<td>ZmCPKp54</td>
<td>Maize</td>
<td>Pollen development</td>
<td>-</td>
<td>-</td>
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</table>

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

*b* Supression of gene transcription.
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 the 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

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

<sup>a</sup> Expression of full length protein.
<sup>b</sup> Expression of truncated protein.
<sup>c</sup> Antisense oligonucleotides.
<sup>d</sup> Virus-induced gene silencing.
<sup>e</sup> Sense co-suppression.
<sup>f</sup> Antisense transgenic lines.
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
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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