

Cyclin dependent protein kinases and stress responses in plants

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Plants have to adjust, grow and establish themselves in various changing environmental conditions. Additionally, the sessile life-style of plants requires the development of response mechanisms for their adaptation in such environmental cues. Under biotic and abiotic stress, plant growth is negatively affected mainly as a result of cell cycle inhibition. The perception of stress involves the activation of signaling cascades that result in a prolonged S-phase and delayed entry into mitosis. Although the molecular interactions that link the cell cycle machinery to perception of stress are not fully understood, recent studies indicated the involvement of Cyclin Dependent Kinases (CDKs) in the plant response machinery. CDKs are core cell cycle regulators but their activity has been implicated in additional diverse cellular processes. Here we review the impact of different types of abiotic stress on plant cell cycle progression and CDK activity, and discuss the contribution of CDK function in the signaling control of stress tolerance.

Introduction

As a consequence of their lifestyle, plants are immobile for most of their lifetime and, lacking the mobility of the majority of animals, they have to cope with wide changes in their local environment—they cannot move to a more favourable one. Their successful adaptation to this sessile life-style can be attributed to their ability to adapt and respond to different types of biotic and abiotic stress. Here, the term stress is used to refer to factors that could negatively affect performance and/or physiology. These factors are generally classified into two broad groups, biotic and abiotic. Biotic stress refers to the damaging impact of living organisms, such as bacteria, fungi, viruses, animals and weeds while abiotic stress includes diverse environmental conditions like high temperatures, drought, salinity and UV light (Fig. 1). Exposure to stress can lead to a variety of responses, the extreme manifestation of which is the synthesis of reactive oxygen species (ROS) that can lead to cell death.¹

Stress responses have been extensively studied due to their economic implications, as they can adversely influence the physiology, growth and crop productivity of plants.² There is now added urgency in understanding stress responses as climate change is

likely to lead to environmental deterioration and has raised serious concerns about future food production. As well as breeding more stress tolerant varieties of crops, it is important to understand the genetic and molecular basis of the stress response.³ To this end, stress inducible genes have identified and the complex signalling cascades that mediate stress tolerance are being dissected.^{4–7} One of the key responses to moderate (i.e., non-lethal) stress is the inhibition of cell proliferation and cell growth. While this response can lead to reduced yield in crops, it has a more general adaptive significance in that the resultant plant is more likely to survive. In this article, we briefly review the molecular response to abiotic stress but focus on the possible roles of Cyclin Dependent Kinases (CDKs), a group of protein kinases that include key cell cycle regulators, with particular emphasis on agronomic importance. Animal CDKs are well known for their role in the cell's response to genotoxic and other cell cycle specific stresses that cause different types of DNA damage.⁸ In yeast, the Pho85 CDK protein regulates transcriptional response to a variety of stress conditions^{9,10} and specifically modulates the nuclear localization of Rim101, a key transcription factor in response to alkaline stress.¹¹ There is an increasing body of evidence that plant CDKs also play similar roles in the plant stress response mechanism.

Plant Response Mechanisms to Abiotic Stress: The Role of Protein Kinases

Appropriate perception and rapid response to stressful conditions is thought to contribute to tolerance. The regulatory mechanisms that confer stress tolerance involve the induction of stress-responsive genes. The first group comprises proteins that operate directly in stress tolerance and include late-embryogenesis-abundant (LEA) proteins.¹² LEA proteins are also produced during the normal maturation of seeds. They appear to protect proteins and membranes and assist in the renaturation of damaged proteins and, during seed maturation, they may act as a hydration buffer.¹³ The second group of induced genes includes regulatory molecules, such as protein kinases, involved in signal transduction cascades.¹²

Ca²⁺-dependent protein kinases (CDPKs) and sucrose non-fermentation 1 (SNF1)-related kinases (SnRKs) are activated by ABA-dependent and ABA-independent signaling pathways and regulate stress responsive gene expression, including ABA-responsive transcription factors and LEA genes.^{14–22} Recently,

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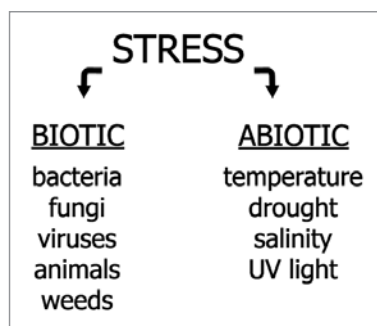


Figure 1. Types of biotic and abiotic stress that affect plant development.

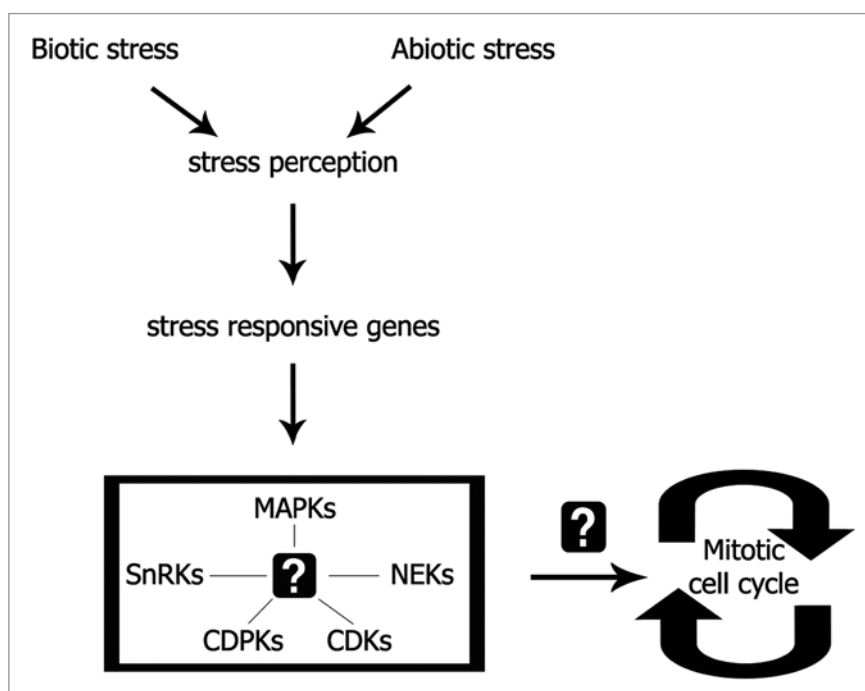


Figure 2. Participation of protein kinases in the plant stress-defense machinery. Crosstalk regulatory pathways among the different kinase modules and integration of their function into cell cycle progression remain to be elucidated.

members of the wheat SnRK2 family were implicated in the regulation of enhanced abiotic stress tolerance and growth under normal and stress conditions. Additionally, overexpression of those genes favored root development, benefited water and nutrient uptake and did not retard the growth of transgenic plants, indicating their strong potential in transgenic breeding assays for the improvement of abiotic stress tolerance in crops.^{23,24}

The induction of mitogen-activated protein kinase (MAPK) cascades transduce stress stimuli into cellular responses. MAPK cascades are widespread in eukaryotes and the typical module generally consists of a MAPK kinase kinase (MAPKKK), a

MAPK kinase (MAPKK) and a MAPK. For example, a MAPK module (consisting of MEKK1 (MAPKKK), MEK1 (MAPKK) and MPK4/MPK6 (MAPK)) transduces abiotic stress signals to confer tolerance in *Arabidopsis* by altering the expression of 152 genes that have diverse functions in signal transduction, cellular defense and stress metabolism.²⁵⁻²⁷ Furthermore, overexpression of the *Arabidopsis* MPK3 and rice MAPK5 resulted in enhanced biotic and abiotic stress resistance.^{28,29} Although these cascades are often portrayed as a linear hierarchy, the multiplicity of molecular components at each level within the cascade offers the possibility of crosstalk between pathways and may allow integration and optimization of the organism's response to diverse stimuli. Indeed, the Ca^{2+} -dependent protein kinase CPK3 was recently proposed to mediate stress response at the post translational level through the regulation of membrane-localized target proteins, following the immediate MAPK-dependent transcriptional acclimation to salt stress.³⁰

More recently, a member of the NIMA (never in mitosis A)-related kinases (NEKs) was implicated to osmotic stress response and regulation of plant growth. Animal and fungi NEKs were reported to regulate cell cycle progression.^{31,32} On the other hand, the function of their plant counterparts was restricted to developmental processes like morphogenesis of epidermal cells, flowering, fruit and shoot development and vascular tissue formation.³³⁻³⁷ Nevertheless, the *Arabidopsis* AtNEK6 was shown to associate with the novel Abscisic Acid (ABA)-signaling component ARIA (Arm repeat protein interacting with ABF), a protein that interacts with ABF-transcription factors and regulates ABA-dependent stress responsive gene expression.³⁸⁻⁴⁰ The authors suggested that AtNEK6 participates in ABA and salt stress response during germination, indicating that protein kinases with diverse functions are involved in the plant stress response mechanism (Fig. 2).

Several protein kinases involved in stress tolerance are stimulated by ABA, a hormone with a well-documented role in stress response.⁴¹ ABA biosynthesis is triggered by stress and in turn activates a variety of stress-inducible genes corresponding to different types of stress. Additionally, ABA may link abiotic stress response with plant development since members of the *Arabidopsis* SnRK2 family are strongly activated by ABA and regulate growth and reproduction.^{42,43}

Introduction to the CDK Protein Kinase Family

CDKs are a large family of serine/threonine protein kinases with an important role in ensuring that cells progress in an orderly fashion over the different stages of cell division.^{44,45} Almost all

CDKs require binding with a cyclin for their activity. Cyclins, so-called due to their cyclical appearance and disappearance during the cell cycle,⁴⁶ function as positive regulatory subunits while the active site of the kinase complex is on the CDK component. CDKs were genetically defined in yeasts as cell division cycle (*cdc*) mutants, *cdc2* for *S. pombe* and *CDC28* for *S. cerevisiae*.⁴⁷⁻⁵⁰ Although the prototypical CDK1/A has a primary function as a central cell cycle regulator, the other groups have been implicated in additional diverse cellular processes like regulation of transcription, translation and mRNA processing.^{51,52}

Higher eukaryotes possess a number of *cdc2/CDC28*-related genes,⁵³ now known as CDKs, with the different classes designated by numbers (animals) or letters and numbers (plants). Thus, in Arabidopsis, seven different classes of CDKs named CDKA through CDKF have been described based on their cyclin binding motif. In addition, there is a class of CDK-like proteins designated as CKLs.⁵⁴ The CDKB family is unique to plants and contains 2 sub-classes, B1 and B2. Members of the B1 group are expressed from the onset of S phase until mitosis while those belonging to the B2 sub-class are expressed during G₂ to M transition. Additionally, the members of this CDK family seem to control the balance between mitotically dividing and endoreduplicating cells.⁵⁵⁻⁵⁹ The C-class contains two genes, *CDKCI* and *CDKC2*, that are expressed constantly during the cell cycle.⁵⁴ The plant CDKC-CycT complex was reported to phosphorylate the C-terminal domain (CTD) of RNA Polymerase II (RNAP II), suggesting a role similar to the human CDK9-CycT complex of the P-TEFb transcription factor.^{60,61} Recently, CDKC function has been implicated in the regulation of mRNA processing in the nucleus.⁶² CDKD genes belong to the family of CDK-activating kinases (CAKs). One role is to regulate activation of CDKs through phosphorylation of a conserved residue within the T-loop (Thr161 in CDKA1). There are four potential CAK orthologs in Arabidopsis. The three members of the D-class are closely related to the human CDK-activating kinase CDK7 and the fourth potential CDKD gene has been proposed to define a distinct class, designated as CDKF1.^{63,64} The G-class of Arabidopsis CDKs is closely related to the human galactosyltransferase-associated protein kinase p58/GTA, an isoform of human CDK11 with a characteristic PITSLRE motif,^{54,65} suggesting a role in regulation of transcription and mRNA processing.⁵² Finally, the CDK-like proteins (CKLs) encompass 15 members (CKL1-15) that are more closely related to each other than to any of the other *cdc2*-related classes and their function is still unknown.⁵⁴ Different members of the CDK have, therefore, diverse roles in cell cycle progression and in regulating gene expression. As regulators of these essential processes, they are good candidates as targets of stress responsive signalling cascades. In the next section we discuss the evidence for this.

Abiotic Stress Negatively Affects CDK Function

Stress negatively affects plant growth—plants grown under stressful conditions are almost always smaller than they would be under favorable conditions. Often the reduction in plant size can be attributed to a reduction in cell number, although in many

cases, cell growth is also affected. Post proliferative cell enlargement is modulated in response to stress by the plant growth hormone gibberellin.⁶⁶ Reduction in cell number has been attributed to inhibition of CDK activity, resulting in cell cycle arrest at the G₁/S and G₂/M checkpoints, prolonged S-phase progression or delayed entry into mitosis.⁶⁷ In parsley cell suspensions, the expression of mitotic CDK and cyclin genes was suppressed following treatment with both UV irradiation and fungal elicitors.⁶⁸ Although the perception mechanisms differ, the end result is very similar.

In the following section, we discuss three types of abiotic stress with agronomic importance to the farmers; heat stress, drought stress and salt stress and the effect of such stresses on CDK activity.

Heat stress. Transient exposure to elevated temperatures can disturb reproductive development in many crop species leading to reduced yields. Prolonged exposure can lead to irreversible damage, mainly due to increased tissue temperature and excessive evaporation demands.⁶⁹ At the molecular level, heat stress was reported to affect the organization of microtubules, cell division and eventually results to the production of reactive oxygen species.⁶⁹

Under heat stress, proliferating cells generally accumulate at G₁/S and G₂/M, as it was originally shown in studies with the model protozoa species of Tetrahymena.⁷⁰ It was also suggested that heat shock could be applied for the synchronization of cell populations.^{71,72} However, heat stress leads to increased permeability of mitochondrial membranes, resulting in ATP depletion and disruption of phosphorylation-dependent signaling cascades.⁷³ Thus, heat-stressed animal cells arrest at G₁/S transition due to inhibition of CDK-mediated phosphorylation and inactivation of the retinoblastoma protein (Rb), which is required for S-phase progression.⁷⁴ Additionally, heat stress was reported to enhance inhibitory phosphorylation of CDKs by inhibitors and reduce the expression of cyclins, suggesting an alternative regulatory mechanism of CDK function.⁷⁵⁻⁷⁸

In plants, the molecular mechanisms that regulate CDK function in response to heat stress are beginning to emerge. Mild heat stress (30°C) induced a transient cell cycle arrest at G₁/S or G₂/M in BY2 cell suspensions, depending on the stage at which the stress was applied.⁷⁹ The expression of CycA and CycB and, as a consequence, the activity of their corresponding CDKs was transiently inhibited. On the other hand, application of very high temperature (55°C) activated a signaling pathway that resulted in programmed cell death.^{80,81} The EL2 protein from rice (*Oryza*; E2L) may couple cell cycle progression to cold stress response pathway. *Oryza*; E2L belongs to a plant specific family of CDK inhibitors that inhibit the activity of CDKA1 during G₁/S transition, both directly, and indirectly through binding to CycD.⁸² This gene was transcriptionally induced by low temperatures but remained constant under heat stress. Similarly, cold stress was reported to induce the rice transcription factor OsMYB3R-2 that resulted to higher transcript levels of several G₂/M phase specific genes, including OsCycB1;1. Additionally, plants overexpressing OsCycB1;1 displayed enhanced resistance to cold stress, suggesting that this gene is involved in the regulation of cell cycle

progression under chilling conditions.⁸³ Such findings indicate that response to different temperature-related stresses might involve different molecular components.

Drought stress. Increasing temperatures reduces plant water content by increasing the rate of respiration, and can have a negative impact on plant development and productivity. Under water deficit, plants experience drought stress that stimulates a series of physiological and biochemical responses including stomatal closure, inhibition of cell growth, repression of photosynthesis and activation of respiration.¹² At the cellular level, plants deploy a mechanism of water stress response and adaptation that involves the expression and/or repression of genes with various functions including different types of transcription factors, protein kinases, phosphatases, metabolic enzymes and cis-acting elements.⁸⁴⁻⁸⁶

Drought-stressed pea leaves displayed reduced rates of cell division and cell expansion, depending on the developmental stage of the leaves.⁸⁷ Drought-stressed maize roots displayed longer (larger) meristematic cells suggesting that entry into mitosis is delayed, while the rate and zone of cell division were reduced at the root meristem.⁸⁸ The decrease of mitotic activity following imposition of drought stress has been reported in root meristems.^{89,90} The link with reduced CDK activity was provided by studies on wheat leaves where a reduction in mesophyll cell division was correlated with inhibition of CDKA1 activity. CDKA was phosphorylated on Tyr-15, a post-translational modification known to reduce kinase activity. Additionally, cyclin expression was also reduced and cells accumulated in G₁ or G₂.⁹¹ Similarly, mild water deficit reduced both the cell division rate and CDKA1 activity almost by half in maize leaves. However, the amount of CDKA1 protein remained unaffected implying again that post-translational regulation of CDKA1 was responsible.⁹² On the other hand, water stress affected maize CDKA1 activity at the transcriptional and post transcriptional level and resulted to inhibition of endosperm development and, to a less extent, of the endoreduplication cycle.⁹³ It was suggested that early endosperm development, during which mitosis predominates, is more sensitive to stress compared to later stages where cells are committed to repeated rounds of S-phase, in the absence of mitosis that results to DNA replication.

Salt stress. Salt contamination of the soil is another major agricultural problem that takes much land out of cultivation and marginalizes even more. This is a particular problem in hot areas with insufficient rain or poor drainage and currently affects almost 20% of the cultivated and nearly half of the irrigated land worldwide.⁹⁴ Excess soluble salts disturb the natural process of water uptake by plants and elevated salt concentrations in the soil can draw out water from plant cells resulting to wilting or even premature death. Some plants known as halophytes can flourish in salt concentrations higher than 400 mM.^{95,96}

In general, high salinity causes ion toxicity, nutritional disorders, water deficit and oxidative stress to plants.^{94,97} Additionally, it can adversely affects all aspects of plant development and limits productivity of crop species, disturbing cell cycle progression and differentiation.⁹⁸⁻¹⁰¹ More specifically, salt stress decreased the transcript levels of CDKA/CDKB and *CycA/CycB* resulting in transient downregulation of mitotic activity in the shoot and root

apex of Arabidopsis plants and cell cycle arrest. As a result, fewer cells of smaller size were produced resulting to a smaller meristem and limited growth.^{102,103} However, CDKA and CDKB proteins levels were minimally affected, suggesting post-translational level regulation. The period of reduced proliferative activity was considered adaptive, since the promoter activities and transcripts of the cell cycle genes eventually returned to their original levels.

Participation of Non Cell Cycle CDKs in the Plant Stress Response

CDKs with non-cell cycle functions are also involved in the stress response. *Oryza*; *CDKCI* was proposed to be involved in the plant salt stress response mechanism through an ABA-signaling pathway. The transcript level of the rice gene *Oryza*; *CDKCI* was increased by ABA treatment, unlike the levels of mitotic CDKB2;1 transcript that were considerably decreased.¹⁰⁴ Furthermore, elevated NaCl also triggered increased expression of *Oryza*; *CDKCI* in cell suspensions, while the activity of *Oryza*; *CDKB2;1* was transiently reduced but recovered. The authors concluded that *Oryza*; *CDKCI* expression might be a primary response to salt stress and could participate in the transcriptional regulation of salt-induced genes for the induction of defense and/or tolerance in a saline environment.¹⁰⁴ Such a regulatory role is certainly possible as the Arabidopsis CDKC gene has been implicated in the regulation of mRNA processing. CDKC co-localizes with splicing factor SRp34 and their spatial organization was dependent on CDKC kinase activity.⁶² Reversible phosphorylation is important for the functional distribution and dynamics of plant SR-splicing factors^{105,106} so it is likely that CDKC is an important regulator. Abiotic stress modulates the splicing profiles of SR-splicing factors resulting in the production of different isoforms with (possibly) distinct functions. Stress may affect their phosphorylation status and result to altered ratios of their splicing variants and other pre-mRNAs that participate in the acquisition of stress tolerance.^{107,108} Indeed, non-functional forms of stress-inducible genes were accumulated in plant cells under non-stress conditions, while alternatively spliced functional forms of the same genes were found under abiotic stress.^{109,110} All the above suggest that CDKC contributes to plant stress tolerance by regulating the phosphorylation of SR-splicing factors, which in turn triggers the alternative splicing of their pre-mRNAs and of stress-related genes, resulting to the induction of stress response.

Conclusion

Plants have developed highly sophisticated stress tolerance mechanisms to better adapt to a variable environment, from which they cannot escape. Such mechanisms involve complex signaling cascades that facilitate the perception of stress signals and the activation of hundreds of stress-related genes and transcription factors.¹¹¹⁻¹¹³ A common cellular response upon stress application is transient inhibition of cell cycle progression or even cell cycle exit. The signaling pathways that integrate abiotic stress with cell division control remains to be fully elucidated, but evidence suggests that post-transcriptional modulation of CDK

kinase activity is important for transient inhibition. Other kinases including CDKs with functions other than cell cycle control also are crucial in the stress response. Such findings suggest novel intrinsic functions for CDKs in the regulation of plant response upon biotic and abiotic stress.

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