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Plant Protein Kinases in Signal Transduction: A Synoptic Review

Nazir Ahmad Malla and Mudasir Bashir
Department of Botany, Government Degree College Bijbehara-India
Department of Botany, Government Degree College Bandipora- Kashmir-India

(Corresponding author: Nazir Ahmad Malla)
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ABSTRACT: A protein kinase is a enzyme that modifies other proteins by adding phosphate groups to them. This results in a functional change of the target protein by changing enzyme activity, cellular location, or association with other proteins. Cells can interact to environmental fluctuations by transduction of extracellular signals, to produce intracellular responses. Membrane-impermeable signal molecules are recognized by receptors, which are localized on the plasma membrane of the cell. Binding of a ligand can result in the stimulation of an intrinsic enzymatic activity of its receptor or the modulation of a transducing protein. This review discusses the various protein kinases and their role in plants.

INTRODUCTION

Eukaryotes are in possession of great cell complexity. This high level of complexity is well accomplished and maintained by a cascade of much complicated mechanisms termed “Cell Signaling” in common.

Proteins form the basic building blocks of an organism. More interestingly it’s one of the most important signaling and signal receiving molecule. There occurs a continuous activation and deactivation of proteins during cell signaling. The presence of a phosphate group alters the shape of the active site of the enzyme (protein) rendering it active and thus increasing its catalytic activity. Enzymes that transfer phosphate group to other proteins are called as Protein Kinases and they are known to regulate diverse

Kinases are enzymes that transfer a phosphoryl group from a nucleoside triphosphate. Kinases catalyze phosphoryl group transfer reactions by an in line mechanism. Kinases transfer a phosphoryl group from ATP to another substrate or from an energy rich intermediate to ADP.

Protein Kinases were first brought into the picture by Fisher and Krebs. “The Converting Enzyme” as they had termed it was found to transfer a phosphate group from ATP to one of the amino acid that make up the glycogen phosphorylase molecule. They had elucidated the mechanism of activity of protein kinase, a phosphorylating kinase which in turn phosphorylates phosphorylase and also dephosphorylates it. In the year 1992 they were awarded the Nobel Prize for Physiology and medicine for the discovery of reversible protein phosphorylation as a regulatory mechanism of the cellular metabolism. The term Protein Kinase was coined later. A combined activity of protein kinase and protein phosphatases regulates the response to a stimulus. The energy thus required is derived from the free energy of ATP hydrolysis.

Higher plants possess genes encoding for putative receptor kinases (Receptor-like kinases, RLK). A completely sequenced *Arabidopsis* genome contains over 600 genes encoding RLKs (Shiu and Bleecker, 2001).

Thus use receptor kinase signaling commonly to respond to vast arrays of stimuli to modulate gene expressions. Although only a handful of RLKs have been shown to have defined biological functions, their roles in development, self-incompatibility response, and defense against pathogens. Receptor protein tyrosine kinase is the specific receptors for protein kinases mediated cell signaling.

Plant Receptor like Kinases are classified into sub-families based on the structural feature of the extra cellular domain, which is thought to act as a ligand-binding site. S-domain class (S-RLKs) posses an extra cellular S-domain homologous to the self-incompatibility-locus glycoprotein (SLG) of *Brassica oleracea* (Nasrallah *et al.,* 1988). The S-domain consists of 12 conserved cysteine residues. The S-domain possess the PTDT-box, which has a conserved WQSFBXPTDTFL sequence (X= non conserved amino acids; F= aliphatic amino acid). In *Brassica*the S-RLK gene is physically linked to the S-locus (Nasrallah*et. al.,* 1988). It has been seen that the S-RLK primarily functions as a receptor for the pollen-derived ligand, SCR (S-locus cysteine rich protein) during the self-incompatibility recognition process between pollen and stigma. The SLG protein is required for a full manifestation of the self-incompatibility response (Takasaki*et. al.,* 2000). Isolation of several S-RLK genes from self-compatible plant species and their expression in vegetative tissues indicate that S-RLKs play a developmental role in addition to self-incompatibility. One of the S-RLKs of *Brassica* is implicated in plant defense response (Pastugalia *et. al.,* 1997).

LRR-RLKs comprise the largest class of plant RLKs. LRRs (leucine-rich repeats) are tandem repeats of 24 amino acids with conserved leucines. LRRs have been found in a variety of proteins with diverse functions, from yeast, flies, humans, and plants, and are implicated in protein-protein interactions. Several LRR-RLKs have been shown to play critical roles in development. Those include ERECTA which regulates organ shape, CLAVATA1 which controls cell differentiation at the shoot meristem, HAESA, which regulates floral abscission process, and BR11, which is involved in brassinosteroid perception (Torii *et. al.,* 1996; Clark *et. al.,* 1997; Li and Chory 1997; Jinn *et. al.,* 2000). On the other hand, the rice gene *Xa21* confers race-specific resistance to *Xanthomonasoryzae* (Song *et. al.,* 1995). Therefore, LRR-RLKS also play a role in disease resistance. Interestingly, the tomato *Cf disease* resistance gene products, which confer a race-specific resistance to *Cladospriumfulvum,* contain extra cellular LRR domains but lack the cytoplasmic protein kinase domain (Jones and Jones, 1997).

The maize (*Zea mays*) *CRINKLY4 (CR4)* gene product possesses TNFR (tumor-necrosis factor receptor)-like repeats, that has a conserved arrangement of six cysteine, and seven repeats of ~39 amino acids that display a weak similarity to the RCC GTPase (McCarthy and Chory, 2000). *CR4* is required for a normal cell differentiation of the epidermis. The *Arabidopsis* genome contains several genes related to *CR4* (McCarthy and Chory, 2000).

The *ArabidopsisLecRK1* gene product possesses an extra cellular domain homologous to carbohydrate-binding proteins of the legume family (Harve*et. al.,* 1996). It is involved in a perception of oligosaccharide-mediated signal transduction. The *Arabidopsis* genome contains >30 genes belonging to Lectin-RLKs several genes coding for Lectin-RLKs (McCarthy and Chory, 2000).

Calmodulin is the paradigm for EF-hand proteins that are involved in signal transduction (Kawasaki *et. al*., 1998). The binding of Calcium to Calmodulin induces conformational changes that expose hydrophobic binding sites that interact with target protein thus altering the activity of those proteins. A particular important class Calmodulin target proteins is Protein kinases, which regulate the activities of structural proteins and enzymes which, in turn, regulate cellular process such as ion transport, metabolism and gene expression. In addition to several types of protein kinases that are regulated by Calmodulin (CaMK family). Protein kinases Cs (PKCs) contain Calcium binding C2 domain instead of EF-hand. *Arabidopsis* plants have neither homologs of PKC nor any protein kinases that contain a C2 domain in the same polypeptide chain as the Protein Kinase domain, but they do have kinases that are regulated *via* EF-hand protein or domains. EF-hand proteins in plants include Calmodulin, Calcineurin B-like proteins (called CBLs or SCaBP), calcium-dependent protein kinases (CDPKs), all of which have been recently reviewed (Zielinski, 1998; Harmon *et.al.*, 2001; Snedden and Fromm, 2001; Hrabak *et. al*., 2003). The protein kinases activity of CDPK, which belongs to the CDPK/SnRK family of protein kinases, is stimulated by the binding of calcium to its intrinsic Calmodulin-like domain.

Unlike CDPK, Calmodulin and calcineurin B-like proteins have no enzymatic activity of their own, but they have recently been shown to interact with protein kinases that are also members of the CDPK/SnRK family. These findings suggest that plant have a large variety of calcium regulated protein kinases, which have the potential for decoding and transducing the wide variety of calcium signals that have been observed in plants.
Protein kinases have been classified into five classes, each of which is further divided into groups and families. The CDPK/SnPK protein kinases are in class 4 group2, and they include four families, Family1, CDPKs and CRKs (CDPK-related kinases) , Family 2 PPCKs (Phosphoenol pyruvate carboxylase kinase), Family 3, CCaMKs (Calcium and Calmodulin regulated kinases) and CaMK (Calmodulin binding protein kinases) and Family 4,SnRKs(SNF1-related Protein Kinases), Type 1,2 and 3 , kinases within each of the families have catalytic domains that are more similar to each other than to those of other families .Calcium acts as an universal secondary messenger that responds to abiotic stresses. The fluctuation in cytosolic Calcium levels can be sensed by Calcium-dependent protein kinases (CDPKs), which relay the signals by modifying the phosphorylation status of the substrate protein. The majority of the 84 *Arabidopsis* protein kinases that are in the family (Hrabak *et. al*., 2003) are potentially regulated by Calcium. Calcium – dependent protein kinases (CDPKs) sense the calcium concentration changes in plant cells and play important roles in signaling pathways for disease resistance and various stress responses as indicated by emerging evidences.
The symbiotic plant-bacteria interactions between rhizobia and legume root nodules involve the exchange of signals that triggers specific cellular and developmental programs in both. Symbiosis is initiated by the infection of legume host by rhizobia, resulting in formation of root nodules which are newly formed plant organs that host the bacteria. The nodules provide a microenvironment for the bacteria, in which symbiotic interactions occurs involving transfer of carbon and energy sources from the plant to the bacteria; the rhizobia are nitrogen-fixing bacteria, converting atmospheric nitrogen into ammonium which serves as a nitrogen source for the plant. Recently it has been reported that an amino-acid cycle of mutual exchanges exists, in which the plant provides amino-acids to the bacteria, which in return cycles amino-acids back to the plant in the form of Asparagine precursor, and thus actually act like plant organelles.
It is generally realized that metabolites exuded by the plant root activate production of specific compounds in rhizobia, termed Node factors (Nf), which act as signals for the induction of a developmental program in the plant root, which includes root hair deformation and the alternate production of nodules. Both the bacterial and root cells undergo a comprehensive change in their transcriptional program and cellular activity when symbiosis is engaged. These factors are receptor protein kinases points to a conserved signal transduction mechanism that operates by responding to specific signals, both activatory and inhibitory, that ultimately affect gene expression.

DISCOVERY OF NEW PROTEIN CONTROLLING HORMONE TRANSPORT IN PLANT

Hormones control development and metabolic processes not only in humans and animals, but also in plants. The substance auxin plays the most important role here. These phyto hormones promote root growth and ensure that flowers and leaves grow in the right places on plants.
The fast that auxin is carried from cell to cell by transport proteins is a long established fact. However, researcher at the Techische Universitat Munchen (TUM) has now discovered an additional protein that also plays a part in distributing the hormones. If this “controller” is not present, transport proteins do not seem to function correctly. The phytohormone auxin is responsible for ensuring that a plant follows a perfect end in most cases, predictable blueprint through its entire life-cycle. Auxin is produced by plants and localized in what are known as founder cells, the cells that grow into roots, leaves or flowers. Proteins first transport the auxin from a plant cell to the gap between cells; from here, it is taken to the neighboring cells by specific import proteins. It is discovered that a further protein is involved in auxin transport. This protein caught the biologist’s attention during investigation of protein kinases on a thale cress model plant (*Arabidopsis thaliana*).
Protein kinases act as a trigger switch, regulating the activity of other proteins. They do this by modifying them through the addition of a phosphate group. In this case, they noticed that the protein kinases which were being examined were distributed in the plant cell in a pattern that was strikingly similar to that of the protein for auxin.

At the same time it was shown that the plants which did not possess these protein kinases were able to grow roots in unusual places, whereas normal thale cress plants were not able to lead the conclusion that the new protein kinase is capable of modifying the export protein for auxin and thus controls the hormone transport path. The work has therefore discovered a new dimension to the molecular transport of auxin.

Auxin action was found to be dependent on dynamic gradients of this hormone generated by PIN protein-vacillated polar auxin transport (PAT). These PIN protein posses' auxin efflux activity and direct PAT through their asymmetric sub-cellular localization. The polar PIN localization is dynamically maintained and regulated through cyclic trafficking of pin loaded vesicles along the actin-cytoskeleton between endosomal compartment and the plasma membrane.

The protein kinase PINOID (PID) as a regulator of the apico-basal polar localization of PIN proteins, as above threshold levels of this signaling enzyme direct PIN traffic to the upper (apical) side of plant cells. To identify the components that contribute to the PID-binding protein (PBPs) using the two-hybrid system in yeast. Two Calcium-binding proteins were identified which interact with the PID protein kinase probably to regulate the activity of these protein in response to changes in Calcium level. This provides the first molecular evidence for the involvement of Calcium in auxin-regulated plant development. A third PID-binding protein, PBP2, is a BTB-POZ domain protein that possibly link PID activity with the brefeldin. A sensitive and cytoskeleton dependent recycling of PIN-vesicle between endosomal compartment and plasma membrane. The functional analysis of the PBPs and the search for phosphorylation targets for PID are current approaches to further elucidate the role of the PID-protein kinase in auxin regulated plant development.

A new protein kinase activity, which was named the mitogen-activated protein kinase (originally microtubule-associated protein kinase), abbreviated MAP Kinase (this kinase is also sometimes called the extra cellular receptor-activated protein kinase or ERK). MAP Kinase was activated at a pretty late step in the signaling pathway (so-called upstream events) and some step occurred later in the pathway as a result of the activity of MAP Kinase (naturally called downstream events). MAP Kinase is normally inactivated, but when the cell is stimulated by a mitogen, MAP Kinase gets activated by being phosphorylated, on both a threonine and tyrosine residue, by an enzyme called MAPK Kinase by some people and MEK by other. (MEK is another acronym for MAP and ERK Kinase). MEK, a MAP Kinase, is upstream of MAP Kinase MEK I is also inactive mostly unless it is phosphorylated by another serine-threonine kinase, called Raf, which is farther upstream of MAPK. The active MEK can in turn activate MAP Kinase which activates a whole slot of other proteins by phosphorylation.

The first evidence that NO modulates the activation of a memberof the plant SNF1-related protein kinase 2 (SnRK2) subfamilyhas been reported recently (Lamotte *et. al.*, 2006). Plant SnRKs are classified into three subfamilies: SnRK1, SnRK2, and SnRK3,the SnRK2 and SnRK3 subfamilies being specific to plants (Harmon, 2003).Members of the SnRK2 subfamily function in abiotic stress signaling and include the tobacco 42 kDa protein kinase NtOSAK (*Nicotiana tabacum* Osmotic Stress-Activated Protein Kinase (Mikolajczyk *et. al.*, 2000). NtOSAK is activated very rapidly in response to osmotic stressthrough phosphorylation of two serine residues (154 and 158) located within the enzyme activation loop (Burza *et. al.*, 2006).

Proteinkinasesof the SnRK2 subfamily are activated by osmolytes and some of them by ABA as well, highlighting a role for these enzymes ina general response to osmotic stress (Boudsocq *et. al.,* 2004; Kobayashi *et. al.,* 2004). The SnRK2 kinases present in guardcells, AAPK (ABA-Activated Protein Kinase) from *Vicia faba* and its *Arabidopsis* orthologue SnRK2.6/OST1/SRK2E play an important role in ABA signaling in response to drought and regulate stomataclosure under low humidity stress (Li *et. al.*, 2000; Mustilli *et. al.*, 2002; Yoshida *et. al.*, 2002). It has been shown that the other *Arabidopsis*ABA-dependent SnRK2 kinase, SRK2C/SnRK2.8, improves plant droughttolerance, probably by promoting the up-regulation of stress-responsivegenes expression, including *DREB1A/CBF3* encoding a transcriptionfactor that broadly regulates stress-responsive genes (Umezawa *et. al.*, 2004).

The field of nitric oxide in plant biology was born almost many years ago, when it was first revealed that this free radicalgas is involved in defense responses (Delledonne *et. al.*, 1998;Durner*et. al.*, 1998). Since that time, NO has been shown tofunction as a ubiquitous molecule with diverse physiologicalroles. Although much has been learnt about NO, several issuesconcerning its action remain outstanding.

It is necessary to define the physiological relevance of these modulations and to understand how interplays between NO andCa^{2+} guide the cell toward a specific response. Such tasks willrequire functional analysis of the molecular mechanisms thatrelay NO-dependent Ca^{2+} signals. Finally, it should be keptin mind that pharmacological evidence for cADPR involvement in mediating NO-induced Ca^{2+} mobilization has been obtained, but the direct measurement of cellular cADPR levels is urgently required. Although in its infancy, research into the signaling functions of NO in plants is advancing rapidly and there shouldsoon be a much better understanding of this most unusual signalingagent.

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