

Update on Signal Transduction

Plant Protein Kinase Families and Signal Transduction¹

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Enzymes of the eukaryotic protein kinase superfamily catalyze the reversible transfer of the γ -phosphate from ATP to amino acid side chains of proteins. Protein kinase function can be counteracted by the action of phosphoprotein phosphatases. Phosphorylation status of a protein can have profound effects on its activity and interaction with other proteins. An estimated 1 to 3% of functional eukaryotic genes encode protein kinases, suggesting that they are involved in many aspects of cellular regulation and metabolism. In plants, protein phosphorylation has been implicated in responses to many signals, including light, pathogen invasion, hormones, temperature stress, and nutrient deprivation. Activities of several plant metabolic and regulatory enzymes are also controlled by reversible phosphorylation. As might be expected from this diversity of function, there is a large array of different protein kinases. Purification of protein kinases and their subsequent cloning, facilitated by the PCR and advances in homology-based cloning techniques, as well as functional analyses, including complementation of conditional yeast mutants and positional cloning of mutant plant genes, has already led to identification of more than 70 plant protein kinase genes. However, the precise functional roles of specific protein kinases and phosphatases during plant growth and development have been elucidated for only a few.

This update will focus on the eukaryotic protein kinases that have been classified by sequence similarity into related families (Hanks and Hunter, 1995). The predominant features of the five major plant protein kinase families will be discussed, and the similarities and differences between the plant protein kinases and the other eukaryotic protein kinases will be highlighted. This analysis has led to some interesting observations regarding signal transduction in higher plants.

THE EUKARYOTIC PROTEIN KINASE SUPERFAMILY

Enzymes belonging to the eukaryotic protein kinase superfamily are related by homologous protein kinase catalytic domains. Typically, eukaryotic protein kinases have been subdivided into those that phosphorylate Ser and/or

Thr and those that phosphorylate Tyr. Recently, protein kinases related to the prokaryotic His kinase family, which function in two-component sensory regulatory systems, have been identified in eukaryotes. These include the *Arabidopsis thaliana* *ETR1* gene, which is involved in ethylene sensing (Chang et al., 1993), the *Saccharomyces cerevisiae* *SLN1* gene (Hughes, 1994), which is involved in sensing nutrient information, and the mammalian mitochondrial branched chain α -keto acid dehydrogenase and pyruvate dehydrogenase kinases (Popov et al., 1994). Since these putative His kinases belong to a family of genes distinct from the eukaryotic protein kinase superfamily, they will not be discussed here (for a review, see Swanson et al., 1994).

The catalytic domains of eukaryotic protein kinases are 250 to 300 amino acids in length with alternating regions of high and low conservation. There are 12 conserved regions (Fig. 1A), referred to as subdomains, some of which contain invariant or nearly invariant residues (Hanks and Quinn, 1991). Crystal structure determinations of protein kinases demonstrate the importance of these conserved residues in catalysis and conservation of overall three-dimensional structure (for a review, see Wei et al., 1994). Using a phylogenetic analysis based on alignment of protein kinase catalytic domains, Hanks and Hunter (1995) have classified the superfamily into five main groups: (a) the "AGC" group, consisting of the cyclic nucleotide-dependent family (PKA and PKG), the PKC family, and the ribosomal S6 kinase family; (b) the "CaMK" group, consisting of calcium-/calmodulin-dependent kinases and the SNF1/AMP-activated protein kinase; (c) the "CMGC" group, containing the CDK, the MAPK, GSK-3, and CKII families; (d) the "conventional PTK" group, and (e) the "other" group. This classification scheme is useful because it groups protein kinases into families with similar sequences, as well as into functionally related groups.

Abbreviations: CaMK, calcium-/calmodulin-dependent protein kinase; CDK, cyclin-dependent kinase; CDPK, calmodulin-like domain protein kinase; CKII, casein kinase II; ERK, extracellular-regulated protein kinase; GSK, glycogen synthase kinase; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; MBP, myelin basic protein; PKA, PKC, PKG, protein kinase A, C, and G, respectively; PTK, protein Tyr kinase; RLK, receptor-like kinase; RTK, receptor Tyr kinase; SNF, Sac nonfermenting; SRK, S-receptor kinase; *Tsl*, *TOUSLED*.

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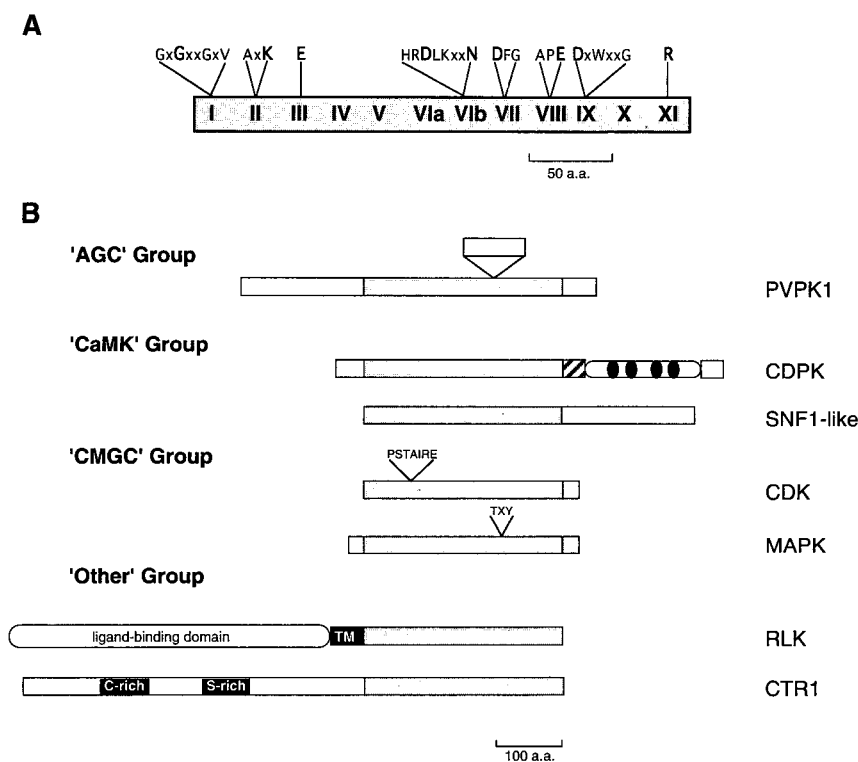


Figure 1. A, Typical 300-amino acid protein kinase catalytic domain. The 12 conserved subdomains are indicated by Roman numerals. Consensus sequences found in some subdomains are shown with invariant residues indicated in bold (Hanks and Quinn, 1991; Wei, et al., 1994). B, A schematic diagram of some major families of plant protein kinases showing their relevant features. The kinase catalytic domains are indicated by a shaded region. The AGC group (PKA, PKG, and PKC) is not well represented in plants. However, a large family of plant kinases, represented by *PVPK-1*, has an approximately 80-amino acid insert within the kinase catalytic domain and are categorized in this group. The CaMK group includes the CDPKs and SNF1-like protein kinases. CDPKs are characterized by an N-terminal kinase catalytic domain, a central autoinhibitory junction domain (diagonal stripes), and a C-terminal calmodulin-like domain with four calcium-binding helix-loop-helix or EF-hand domains (filled ovals). SNF1-like kinases have an N-terminal protein kinase domain and a less-conserved C-terminal region that may be involved in regulation of activity or interaction with other proteins. The CMGC group includes the CDK family characterized by a conserved PSTAIRE motif in subdomain III of the kinase catalytic domain and the MAPK family with the dual phosphorylation motif Thr-X-Tyr upstream of subdomain VIII. Protein kinases that do not fall into any of these families include the RLKs and the Raf homolog CTR1. RLKs have a large, extracellular N-terminal domain proposed to be involved in ligand binding, a central transmembrane region (filled box; TM), and a C-terminal protein kinase catalytic domain. CTR1 has an N-terminal extension proposed to function in regulation of protein kinase activity and interaction with other signaling molecules. Many other plant kinases not represented by these major families are described in the text. a.a., Amino acids.

CLASSIFICATION OF PLANT PROTEIN KINASES

The plant protein kinases will be discussed on the basis of the categories defined by Hanks and Hunter (1995). Because of the large number of cloned plant protein kinases and space limitations, all published reports will not be included, but references to recent reviews will be provided wherever possible. Representative examples of the major families of plant protein kinases and their relevant features will be the focus of this review.

AGC Group

The AGC group is represented by the cyclic nucleotide-dependent kinases (PKA and PKG) and the calcium-phospholipid-dependent kinases (PKC). A common theme in this group is regulation by second messengers (i.e. cAMP,

cGMP, diacylglycerol, and Ca^{2+}). The denominating members of this group are not well represented in plants. There is little evidence for prototypical cyclic nucleotide-dependent kinases in higher plants. Reports of cAMP in plants have been disputed (Spiteri et al., 1989), although cGMP may function in phytochrome responses (Bowler et al., 1994). Classical PKC has not been cloned from plants; however, there are numerous reports of calcium-dependent protein kinases and several have been cloned (see "CDPKs" below).

Despite a lack of evidence for any of the major AGC kinases, several plant protein kinases have been identified that are classified as members of the AGC group. The first plant protein kinase cloned, *PVPK-1* (Lawton et al., 1989), belongs to this group. *PVPK1* is representative of a large group of plant protein kinases with similarity to PKA. A

distinguishing feature of the PVPK1 family is a 70- to 90-amino acid insert between two of the conserved catalytic subdomains (Fig. 1B). Genes encoding PVPK1-related protein kinases, currently unique to higher plants, have turned up in several screens for protein kinases from different plants, but the function of these protein kinases remains a mystery.

Not all members of the AGC group are directly regulated by second messengers. The ribosomal S6 protein kinases are classified into the AGC group. In plants, the Arabidopsis genes *atpk1* and *atpk2* (Zhang et al., 1994), which are putative ribosomal protein kinases, also belong to the AGC group.

CaMK Group

The CaMK group of protein kinases includes the calcium-/calmodulin-dependent and SNF1/AMP-activated protein kinase families. Like the AGC group kinases, regulation by second messengers is common for this family. A calmodulin-binding protein kinase with strong homology to CaMKII has been cloned from apple (Watillon et al., 1993), but calcium-dependent, calmodulin-independent CDPKs seem to be the predominant calcium-dependent protein kinases in plants. The other major family in the CaMK group, the *SNF1* homologs, have been identified in plants.

CDPKs

Transient increases in intracellular calcium have been observed in plant cells under many conditions (Gilroy and Trewavas, 1994). These observations have stimulated substantial interest in identification of calcium-dependent protein kinase activities in plant extracts. In most cases calcium-dependent protein kinases from plants do not require calmodulin, phospholipids, or diacylglycerol and thus differ from both the CaMK and PKC families prevalent in mammals (Roberts and Harmon, 1992). The first calcium-dependent protein kinase, CDPK- α , was cloned from soybean and encodes a 508-amino acid polypeptide. The N-terminal half has homology with protein kinase catalytic domains of the CaMK family, and the C-terminal half shows homology to calmodulin with four helix-loop-helix, or EF-hand, Ca^{2+} -binding sites (Harper et al., 1991). A 31-amino acid junction region (Fig. 1B) between the kinase and calmodulin-like domains is proposed to function as an autoinhibitory domain (Harmon et al., 1994; Harper et al., 1994).

CDPKs have been identified in many plant species and are encoded by multigene families. Soluble, membrane-associated, and cytoskeleton-associated forms have been reported. Putative endogenous substrates of CDPKs include the plasma membrane H^+ -ATPase and the soybean nodule transport and channel protein homolog, nodulin 26 (Roberts and Harmon, 1992). Even though the CDPKs are the predominant Ca^{2+} -dependent protein kinases found in plant cells, an understanding of their physiological role(s) remains elusive.

SNF1-Like Kinases

S. cerevisiae SNF1 is a Ser-Thr protein kinase required for carbon catabolite derepression. *SNF1* homologs have been identified in several plant species (Fig. 1B) and, in some cases, have been shown to be functional homologs of yeast *SNF1*. Rye *RKIN1* and tobacco *NPK5* complement *snf1* mutants, which are unable to utilize Suc and other sugars as a carbon source (Alderson et al., 1991; Muranaka et al., 1994). This observation has led to the suggestion that plant SNF1-like protein kinases have a role in carbon metabolism.

Some plant *SNF1*-like kinase genes have been shown to be transcriptionally regulated by environmental stimuli. *PKABA1* transcript levels increase in response to low levels of ABA and water stress in wheat (Anderberg and Walker-Simmons, 1992). The mRNA levels of a wheat *SNF1* homolog, *wpk4*, increase upon exposure to light and cytokinin, as well as nutrient deprivation (Sano and Youssefian, 1994).

SNF1 homologs are also found in mammalian systems. These protein kinases are activated by elevated AMP levels imposed under stress conditions and have a role in control of lipid metabolism by phosphorylation and subsequent inactivation of acetyl-CoA carboxylase and hydroxymethylglutaryl-CoA reductase (Carling et al., 1994). Taken together, current evidence suggests that the SNF1 family of protein kinases may function in cellular responses to environmental conditions via the regulation of key metabolic pathways (Hardie, 1994).

CMGC Group

The CMGC group includes the CDK, MAPK, GSK-3, and CKII families. All four of these families have been identified in plants. In contrast to the AGC and CaMK group kinases, which are regulated by second messengers, the CMGC group kinases act further downstream in phosphorylation cascades. Regulatory phosphorylation sites are commonly found in the region between subdomains VII and VIII, a region known as the "activation loop" (Wei et al., 1994) (Fig. 1A).

CDKs

Progression through the cell cycle in eukaryotes is controlled by protein kinase complexes consisting of a regulatory subunit, cyclin, and a catalytic subunit, CDK. Cyclin homologs are present in plants, and CDKs, which have a conserved PSTAIRE amino acid motif, have been isolated from several different plant species (Fig. 1B). Functional complementation has demonstrated that some plant CDK homologs can rescue cell-cycle defects in yeast mutants, whereas others cannot (for a review, see Doerner, 1994). In addition to their interaction with cyclins, CDKs are themselves regulated by protein phosphorylation. Phosphorylation of the plant CDKs has not been fully investigated, but the regulatory phosphorylation sites found in animal or yeast CDKs are conserved in plant CDK homologs, suggesting that they are subject to similar regulation. Phosphorylation of a Thr residue is universally required for kinase activation, whereas phosphorylation of a Tyr resi-

due serves an inhibitory function in humans and *Schizosaccharomyces pombe* but not *S. cerevisiae*. Whether plant CDKs undergo a similar mode of regulation is currently unclear.

MAPKs

MAPKs, also known as ERKs, are Ser-Thr protein kinases activated by dual phosphorylation on Thr and Tyr residues. The enzyme responsible for this dual phosphorylation, MAPKK, represents an unusual class of eukaryotic protein kinases that will phosphorylate on Ser, Thr, and Tyr residues. Activating phosphorylations occur in the conserved Thr-X-Tyr motif in the activation loop of MAPKs (Fig. 1B). MAPKs have been implicated in the regulation of gene expression and cell division.

Many MAPK homologs have been cloned from plants. The alfalfa MAPK, MsERK1, exhibits kinase activity toward MBP, the conventional MAPK substrate (Duerr et al., 1993). MAPK homologs from Arabidopsis, ATMPK1 and ATMPK2, also have MBP kinase activity. Further evidence that these proteins are typical MAPKs is provided by enhanced MBP kinase activity upon addition of purified *Xenopus* MAPKK. Moreover, auxin treatment leads to accumulation of putative MAPKK activity, which stimulates both phosphorylation of the recombinant MAPK and its MBP kinase activity (Mizoguchi et al., 1994). These data suggest that plant MAPKs, as in other organisms, may be involved in cell proliferation.

GSK-3 Homologs

GSK-3 family members have been identified in mammals, *Drosophila*, yeast, and plants. Mammalian GSK-3, implicated in hormonal responses, is functionally homologous to a *Drosophila* gene, *shaggy/zeste-white 3*, which is required for specifying cell fate and polarity during embryo development. The plant members of this family are encoded by small multigene families. Three alfalfa homologs, MsKs, are differentially expressed during development (Pay et al., 1993). At least five genes, ASKs, encode GSK-3 homologs in Arabidopsis. Two recombinant ASKs autophosphorylate on Ser, Thr, and Tyr. This Tyr phosphorylation may be significant, because phosphorylation of a specific Tyr residue is required for maximal enzyme activity of mammalian GSK-3. A Tyr residue is found at an equivalent position in the plant homologs. Recombinant ASKs phosphorylate MBP and phosphatase inhibitor-2, consistent with the substrate specificity of other GSK-3 family members (Bianchi et al., 1994). The identification of multiple GSK-3 homologs with different expression patterns suggests an important role for these protein kinases in plant development.

CKII

Regulation of cell division, DNA replication, and gene expression have been linked to the multifunctional enzyme CKII. A critical role for CKII is evidenced in yeast by the lethality of disruptions of both copies of the catalytic protein kinase subunits (Padmanabha et al., 1990). CKII from

yeast and mammals exists as a tetrameric complex consisting of two α catalytic subunits and two regulatory β subunits, $\alpha_2\beta_2$. In plants, however, both monomeric and oligomeric forms of CKII have been purified. Counterparts of both the catalytic protein kinase α and regulatory β subunits have been cloned from plants (Dobrowolska et al., 1991; Mizoguchi et al., 1993; Collinge and Walker, 1994). Studies using the recombinant Arabidopsis subunits have demonstrated that a tetrameric $\alpha_2\beta_2$ complex can be formed and that the regulatory β subunit stimulates catalytic activity. Both native and recombinant CKII phosphorylate and promote the DNA-binding activity of G-box-binding factor 1, a transcription factor that binds to the plant G-box promoter element found in several inducible plant promoters (Klimczak et al., 1995). Thus, CKII may serve a critical function in transcriptional regulation in plants.

Conventional PTK Group

PTKs are an important family of regulatory enzymes in higher eukaryotes. This family of protein kinases is specific for Tyr and should be considered distinct from the dual specificity kinases that have been identified in plants. No plant protein kinases have been identified that fall into the PTK group. The sequences that distinguish between the Ser-Thr-specific and Tyr-specific protein kinases are found in subdomains VI and VIII (Fig. 1A). In subdomain VI, the consensus D-L-K-P-E-N indicates Ser-Thr specificity, whereas D-L-R/A-A-A/R-N suggests Tyr specificity. In subdomain VIII, P-I/V-K/R-W-T/M-A-P-E is found in Tyr kinases, whereas the less-conserved G-T/S-X-X-Y/F-X-A-P-E is associated with Ser-Thr kinases (Hanks et al., 1988).

The lack of conventional PTKs does not imply that Tyr phosphorylation is not important in plants. CDK, MAPK, and GSK-3 homologs, which are regulated by Tyr phosphorylation in other eukaryotes, may also be regulated by Tyr phosphorylation in plants. Although the protein kinases responsible for this Tyr phosphorylation have not been identified, conservation of the regulatory sites in the plant protein kinases suggest that Tyr phosphorylation may play an important physiological role in higher plants.

Other Group

Most protein kinases, including many of the cloned plant protein kinases, do not fall into any of the four families described above and are therefore classified in the Other group. The Other group from plants contains unique protein kinases as well as protein kinases widespread in eukaryotes.

RLKs

The RLKs make up a continually expanding family of plant kinases that are predicted to have structural features (Fig. 1B) similar to the RTKs. RTKs are transmembrane proteins that recognize an extracellular signal, in the form of a polypeptide ligand. Ligand-binding leads to autophosphorylation on the cytoplasmic kinase domain, a require-

ment for propagation of the signal. Genes from plants have been cloned that encode proteins with the same overall features of the RTKs with the notable exception that the catalytic domains are Ser-Thr specific rather than Tyr specific (Fig. 1B).

Many members of the RLK family have been identified in plants. The RLK protein kinase catalytic domains share a high degree of homology, but the extracellular domains are very divergent. Therefore, RLKs have been classified into several groups according to their extracellular domains (for a review, see Walker, 1994). The abundance of RLKs cloned from plants and their diverse expression patterns imply a common signaling mechanism in response to many different types of signals (Walker, 1994). The functions of most of the RLKs are unknown, but one subgroup of the RLK family, the *Brassica* SRKs, play a role in self-incompatibility. Alterations in SRKs found in self-compatible lines of *Brassica* support the proposition that SRK activity is required for self-incompatibility (Walker, 1994). However, neither the pollen component, presumably the ligand, nor the mechanism of signaling downstream are currently known.

Catalytic domains closely related to the RLKs are found in the predicted soluble protein kinases Pto and Fen. These Ser-Thr kinases were identified by map-based cloning in tomato mutants resistant to the bacterial pathogen *Pseudomonas syringae* pv *tomato* (Pto) and sensitive to the organophosphorous insecticide fenthion (Fen) (Martin et al., 1993, 1994). Pto and Fen have no apparent membrane-spanning domains but have potential N-terminal myristoylation sites and may therefore be membrane associated. Because they are predicted to act early in their respective signal transduction pathways, they may be components of a membrane receptor complex.

Another protein kinase from tobacco, NPK15, has a catalytic domain closely related to the RLKs. Although NPK15 has no apparent extracellular domain, it does possess an N-terminal hydrophobic region that could function in membrane anchoring (Ito et al., 1994).

Proposed subcellular localization and functions of these related kinases, the transmembrane RLKs, the "soluble" Pto and Fen, and NPK15, suggests that these enzymes could mediate the early steps of signal recognition and transduction. In fact, the mammalian protein kinases closely related to the plant RLK group, the Raf kinases, require membrane localization for activation of a "cytoplasmic kinase cascade" (see below).

CTR1

CTR1 is a plant protein kinase belonging to the Raf family. Raf kinases have been implicated in signaling from RTKs, seven-transmembrane-domain receptors, and cytokine receptors. CTR1 was identified as a gene in which a mutation leads to constitutive activation of the ethylene triple response. CTR1 is proposed to be a negative regulator of ethylene signal transduction because of the recessive nature of the mutation and analysis of several mutant alleles that suggest that the protein kinase catalytic activity is required for this negative regulation (Kieber et al., 1993). Although the catalytic domain of CTR1 shows considerable

sequence identity with the Raf family, the N-terminal region, which is believed to serve a negative regulatory function, has significantly weaker homology. A Ser-rich region implicated in regulation of Raf kinases and a Cys-rich region implicated in interaction with other proteins are present in CTR1, but the spacing of the Cys's is not consistent with formation of a zinc finger structure (Fig. 1B).

Tsl

Evidence for protein phosphorylation in plant morphogenesis comes from analysis of an Arabidopsis developmental mutant. *Tsl* is required for proper initiation and development of organ primordia. Mutations of the *Tsl* locus result in abnormalities in floral organs and leaf morphology. *Tsl* encodes a novel Ser-Thr protein kinase with little similarity to other kinases (Roe et al., 1993). The novelty of this plant protein kinase may reflect the uniqueness of plant morphological development.

PLANT PROTEIN KINASES AND SIGNAL TRANSDUCTION

Organisms are subjected to a wealth of external stimuli and endogenous developmental signals that must be recognized and translated into cellular responses. This is especially true for plants, which, because of their sessile nature, must adapt to changing environmental conditions. Comparison of protein kinases found in plants and other eukaryotes reveals some interesting parallels and incongruities between plant and animal signal transduction mechanisms. Many plant protein kinases are found ubiquitously in other eukaryotes (e.g. SNF1, CKII, MAPK, and CDK). Other kinases are conspicuously absent from plants, such as the cyclic nucleotide-dependent protein kinases and conventional PTKs, in spite of numerous attempts to isolate them by homology-based methods. Conversely, higher plants have unique protein kinases distinct from those found in most eukaryotes (e.g. RLKs, CDPKs, Tsl, and the PVPK1 family).

An intriguing pattern emerges by comparing the plant protein kinases with the entire eukaryotic superfamily. In plants, the protein kinases implicated in the earlier events of signal transduction are mediated by unique protein kinases, but these signals converge into pathways utilizing more highly conserved protein kinases that are universal in eukaryotes. The differences observed in the initial steps of signal transduction pathways may reflect the divergence of developmental and environmental signals to which plants must respond. Protein kinases responsible for signal recognition appear to differ (RLKs versus RTKs), as do the second-messenger-regulated protein kinases (CDPKs versus PKC or CaMK). For example, a common mechanism for transmitting signals across the plasma membrane in eukaryotes, with the notable exception of plants and yeast, involves the activation of RTKs. Ligand binding results in autophosphorylation on specific Tyr residues (van der Geer et al., 1994). Although RTKs are of fundamental importance in cell-surface signaling in many eukaryotes, the

equivalent function in plants may be fulfilled by RLKs, which differ from RTKs in catalytic specificity.

Second-messenger-regulated protein kinases prevalent in other eukaryotes, PKA, PKG, and PKC, have not been identified in plants. However, second messengers are important in signal transduction in plant cells. Cytosolic Ca^{2+} levels are altered in response to a variety of stimuli such as phytohormone treatment, temperature stress, pathogen invasion, and light (for a review, see Gilroy and Trewavas, 1994). These alterations in Ca^{2+} are often accompanied by changes in protein phosphorylation. However, calcium-dependent phosphorylation in plants is catalyzed primarily by CDPKs rather than by PKC or CaMK. By utilizing CDPKs, which have a calcium-binding, calmodulin-like domain on the same polypeptide as a protein kinase catalytic domain, plants may have surpassed the need for kinases homologous to CaMK or PKC.

Although plants mediate early signaling events through unconventional molecules, they share a common element in eukaryotic signal transduction. Components of the highly conserved cytoplasmic kinase cascade have been identified in *S. cerevisiae*, *S. pombe*, *Drosophila*, mammals, and higher plants. These pathways are involved in multiple physiological processes, including response to mating pheromone in both *S. cerevisiae* and *S. pombe*, control of cell division (*S. cerevisiae*), osmosensing (*S. cerevisiae*), development (*Drosophila*), and response to growth factors or stress (mammals) (Davis, 1993). Typically, three constituents are involved: (a) MAPKs, which are Ser-Thr protein kinases activated by dual phosphorylation on Thr and Tyr by (b) the bifunctional MAPKK, which in turn is activated by (c) a MAPKKK. The final element of this pathway, MAPK, has a variety of endogenous substrates in other systems, including transcription factors. Many MAPK homologs have been cloned from plants (see "CMGC Group"). Moreover, other members of the cytoplasmic kinase cascade have been identified in plants, including a MAPKK and a MAPKKK homolog from tobacco. Southern blot analysis of genomic DNA from several plant species reveals multiple homologs of MAPKKs and MAPKKKs (Shibata et al., 1995). Additional support for a cytoplasmic kinase cascade in plants comes from the identification of a Raf homolog, CTR1, involved in ethylene signaling. Raf is considered an MAPKKK, since it phosphorylates and activates MAPKK (Daum et al., 1994), suggesting that ethylene signaling may utilize the cytoplasmic kinase cascade.

The universal existence of homologous protein kinases may reflect fundamental functions required of all eukaryotic cells. However, there are plant protein kinases, such as the PVPK1 family and the novel Tsl, that have no known counterparts in other eukaryotes. The presence of these kinases may reflect unique features of plant growth and development.

PERSPECTIVES

Plant signal transduction is, and will continue to be, a very exciting field. Further identification and characterization of plant protein kinases and their interactions will lead to insights into the mechanisms controlling plant growth

and development. Genetic approaches, which were instrumental in identifying *CTR1*, *Pto*, *Fen*, and *Tsl*, provide clues to function and are likely to yield a plethora of new kinases and other signaling molecules. Biochemical approaches will be useful in the identification of the specific kinases involved in regulation of the activities of plant enzymes (Huber et al., 1994). Molecular approaches will not only continue to yield new protein kinases, they can also be used to identify signaling molecules and targets (Stone et al., 1994). Untangling the inherent complexities of signal transduction in plants will ultimately require a combination of genetic, biochemical, and molecular approaches to place protein phosphorylation in a physiological context.

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