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Topical Review

Unusual Membrane-Associated Protein Kinases in Higher Plants

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Abstract. Plant genomes encode a variety of protein kinases, and while some are functional homologues of animal and fungal kinases, others have a novel structure. This review focuses on three groups of unusual membrane-associated plant protein kinases: receptor-like protein kinases (RLKs), calcium-dependent protein kinases (CDPKs), and histidine protein kinases.

Animal RLKs have a putative extracellular domain, a single transmembrane domain, and a protein kinase domain. In plants, all of the RLKs identified thus far have serine/threonine signature sequences, rather than the tyrosine-specific signature sequences common to animals. Recent genetic experiments reveal that some of these plant kinases function in development and pathogen resistance.

The CDPKs of plants and protozoans are composed of a single polypeptide with a protein kinase domain fused to a C-terminal calmodulin-like domain containing four calcium-binding EF hands. No functional plant homologues of protein kinase C or Ca^{2+}/calmodulin-dependent protein kinase have been identified, and no animal or fungal CDPK homologues have been identified.

Recently, histidine kinases have been shown to participate in signaling pathways in plants and fungi. ETR1, an *Arabidopsis* histidine kinase homologue with three transmembrane domains, functions as a receptor for the plant hormone ethylene. G-protein-coupled receptors, which often serve as hormone receptors in animal systems, have not yet been identified in plants.

Key words: CDPK — Histidine kinase — *Arabidopsis* — RLK — Phosphorylation — Calcium

Introduction

Living creatures of all types, from archaebacteria to zebras, have evolved mechanisms to sense and respond to changes in the environment. Peptide hormones, G-protein-coupled receptors, G-proteins, intracellular calcium, lipids, cyclic nucleotides, protein kinases, and transcription factors are just some of the diverse machinery that may be utilized in these signaling pathways. We understand how some of these molecules transduce signals, but the specific role of many of these molecules is not yet known.

With the advent of whole genome sequencing we are just beginning to be able to compare the plethora of pathways and proteins used by different organisms to transduce signals. These signaling pathways can be quite similar, or differ dramatically. For example, all organisms appear to use protein kinases and transcription factors of some sort, although the structures of the specific molecules involved may differ. Histidine kinases have been shown to play a role in prokaryotic, plant, and fungal signal transduction. On the other hand, prokaryotes lack many signaling molecules found in eukaryotes, such as receptor protein kinases and heterotrimeric G-proteins. Higher plants appear to have some signaling molecules that are similar to those in animals and fungi, but also have some distinctive molecules not found in these organisms.

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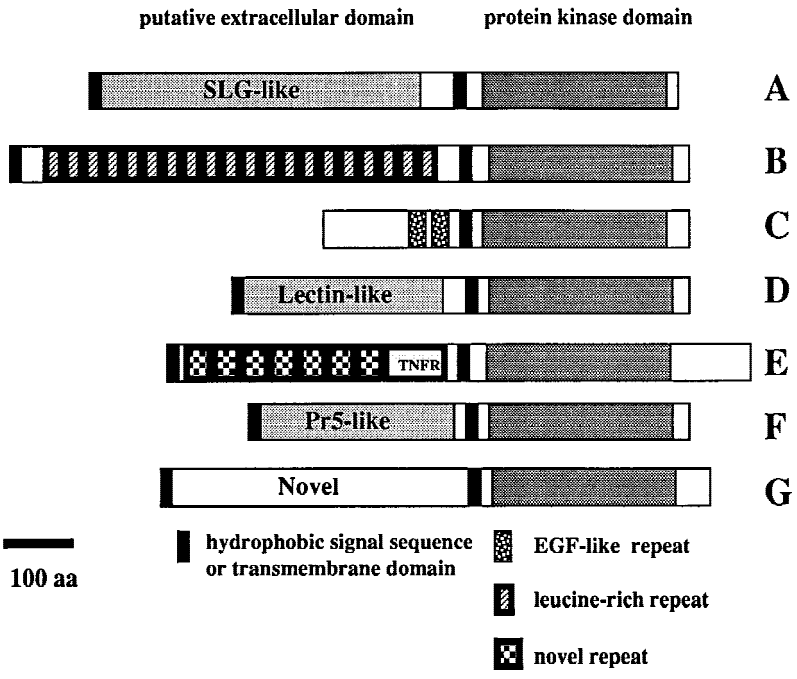

Fig. 1. Modular structure of plant receptor protein kinases. Schematic diagrams of the following plant receptor-like protein kinases are shown: (*A*) SRK, a *Brassica S*-locus receptor kinase with homology to the S-locus glycoprotein (SLG) [63]; (*B*) ERECTA, an *Arabidopsis* receptor protein kinase with 20 leucine-rich repeats [107]; (*C*) WAK1, an *Arabidopsis* wall-associated receptor kinase with two EGF-like repeats [50]; (*D*) lecRK1, an *Arabidopsis* receptor kinase with homology to putative carbohydrate-binding lectins [38]; (*E*) CRINKLY4, a maize receptor kinase with a region of homology to the tumor necrosis factor receptor (TNFR) and seven novel repeats [4]; (*F*) PR5K, an *Arabidopsis* receptor protein kinase with homology to pathogenesis-related (PR5) proteins [112]; and (*G*) CrRLK1, a novel receptor protein kinase from *Catharanthus roseus* (Madagascar periwinkle) [91]. The wheat *Lr10* locus receptor kinase is not shown. All of the deduced proteins contain a single transmembrane domain and a protein kinase domain, and all have a hydrophobic signal sequence except for WAK1.

Protein kinases are one of the major classes of signal transducers. 113 conventional protein kinase genes (about 2% of all yeast genes) have been identified in the *Saccharomyces cerevisiae* genome [43], and there may be more than 1,000 protein kinases encoded by a single mammalian genome [42]. Protein kinases catalyze the transfer of the γ-phosphate of ATP to the amino acid side chain of a protein. The side chain specificity of protein kinases provides a convenient way to classify protein kinases. ''Conventional'' members of the protein kinase superfamily are structurally related, and can be placed into two major categories: serine/threonine specific, and tyrosine specific [43, 33]. A few of these proteins, called dual-specificity kinases, are capable of phosphorylating serine, threonine, and tyrosine [104, 43]. Histidine kinases are ''nonconventional'' protein kinases which autophosphorylate on an active site histidine residue. These kinases do not have protein sequence homology to conventional eukaryotic protein kinases [43].

Although much work has been done on protein kinases in yeast and animal systems, relatively little is known about the biological functions of protein kinases in plants. In particular, a number of protein kinases of novel structure have been identified in plants [97]. This review will focus on three groups of membrane-associated plant protein kinases: receptor-like protein kinases (RLKs), calcium-dependent protein kinases (CDPKs) and histidine kinase homologues.

Receptor-like Protein Kinases

Structure

Receptor-like protein kinases are composed of a putative extracellular domain, a transmembrane domain, and a protein kinase domain (Fig. 1). In animals, many receptor protein kinases contain a tyrosine-specific kinase domain [29, 59], although there are some examples, such as Transforming Growth Factor-β receptor [55], and activin [20] in which the kinase domain has serine/threonine specificity. All plant RLK cDNAs published to date encode proteins with serine/threonine protein kinase signature sequences rather than tyrosine-specific signature sequences. These receptor kinases fall into several subclasses [10, 109] based on the structure of their putative extracellular domains (Fig. 1): the S-locus receptor-like protein kinases have distinctive extracellular domains homologous to the S-locus glycoprotein [62], some receptor-like protein kinases such as Xa21 or TMK1 have extracellular domains with varying numbers of *L*eucine-*R*ich *R*epeats (LRRs) [107], while the *W*all-*A*ssociated receptor protein *K*inase, WAK1, has two epidermal growth factor-like repeats in its extracellular domain [50].

Several new plant RLKs with different types of extracellular domains have been identified recently are are also illustrated in Fig. 1. The CRINKLY4 receptor protein kinase has an extracellular domain with homology to tumor necrosis factor receptor as well as seven novel repeated motifs [4]. CrRLK1 is a receptor-like protein kinase from the Madagascar periwinkle plant. The putative extracellular domain of this receptor kinase homologue appears to be novel [91], but the catalytic domain of this kinase is similar to that of *Pto* which is involved in tomato disease resistance. The Pto protein kinase lacks a transmembrane domain, but has a putative myristoylation motif [60]. A receptor kinase tightly linked to the wheat *Lr10* disease resistance locus has a deduced extracellular domain with some regions of similarity to

the S-locus receptor kinases, but lacks a set of conserved cysteine residues in the S-domain, suggesting that this kinase may be a member of a new group of receptor kinases [26]. PR5K, a receptor protein kinase from the model plant *Arabidopsis thaliana* has a putative extracellular domain homologous to PR5 proteins which are involved in pathogenesis [112]. Perhaps the most interesting of these newly reported receptor kinases are the lectin receptor kinases, or LRKs which have an extracellular domain homologous to carbohydrate binding lectins [38, 100]. It is likely that there will be even more classes of plant receptor protein kinases identified, especially as the *Arabidopsis* genome is sequenced.

Function

Much remains to be learned about receptor protein kinase signal transduction in plants. Although quite a few plant receptor kinases have been cloned, no receptor kinase ligands have been isolated. In animal systems, soluble, membrane-associated, and extracellular matrix proteins have all been identified as receptor protein kinase ligands [29]. The identification of plant receptor protein kinases with lectin-like extracellular domains suggests carbohydrates as candidate ligands for these receptor kinases.

Some work has been done with respect to the subcellular residence of plant receptor protein kinases. TMK1, which contains leucine-rich repeats, has been shown to be glycosylated and associated with membranes [84]. PRK1, which also has leucine-rich repeats, was shown by Western blotting to be associated with microsomal membranes [61]. WAK1, the receptor kinase with EGF-like repeats, appears to be tightly associated with plant cell walls and may provide a transduction link between the cytosol and the extracellular wall matrix [37].

Classical genetics has provided clues as to the function of some plant receptor kinases. Lesions in some of these kinases have been shown to be disruptive to normal plant development. The *Brassica* S-locus receptor kinase (SRK) is involved in signaling between pollen cells and stigma cells [62, 63, 76]. There are at least five *Arabidopsis* RLKs of the S-locus type which have expression patterns distinct from the *Brassica* S-locus RLKs, suggesting that these proteins may be involved in processes other than self-incompatibility [10]. The maize *crinkly4* mutation alters cell differentiation, resulting in morphological phenotypes such as rough, crinkly leaves and aleurone defects. A *crinkly4* allele tagged with a transposable element revealed this gene to be a novel receptor-like protein kinase containing an extracellular cysteine-enriched domain with homology to the tumor necrosis factor receptor [4]. The *erecta* mutation in *Arabidopsis* causes several morphological changes in organ shape, including short petioles, blunt fruits, and a compact inflorescence. This mutation is caused by a lesion in a gene encoding a leucine-rich repeat receptor protein kinase [107]. Resistance to the rice pathogen *Xanthomonas oryzae* is conferred by the *Xa21* gene, a receptor-like kinase with a serine/threonine type protein kinase domain and leucine-rich repeats [93]. The *clavata1* mutant of *Arabidopsis* possesses larger than normal floral and shoot meristems, apparently due to the accumulation of undifferentiated cells [17]. This phenotype, like that of *erecta* and *Xa21*, is caused by a lesion in a receptor protein kinase homologue with a putative extracellular domain containing leucine-rich repeats.

Plant receptor protein kinases do not have the signature sequences associated with animal tyrosine-specific protein kinases, but rather possess serine/threonine signature sequences. Making biochemical conclusions based on amino acid sequence analysis is fraught with peril, however. Several plant receptor protein kinases have been expressed in heterologous systems, and phosphoamino acid analysis shows autophosphorylation on serine and/or threonine, but not on tyrosine [91, 106, 14]. However a petunia receptor protein kinase has been shown to autophosphorylate on serine and tyrosine [61], suggesting that this kinase may have dual specificity. As yet, no plant receptor protein kinases have been shown to have tyrosine specificity. This is in striking contrast to the animal receptor protein kinases, the vast majority of which have tyrosine specificity [29].

Little is known about the downstream signal transduction pathways activated by plant receptor protein kinases. TGF-β receptor kinase, a serine/threonine receptor kinase, has been well characterized in animal systems [55]. In this case, there are two types of TGF-β receptor kinases, Type I and Type II. Type II binds the TGF-β ligand, and then this complex can bind the Type I receptor, forming a heterodimer in which TGFR II can cross-phosphorylate TGFR I. The subsequent signaling steps have not been clearly defined, although both heterotrimeric G-proteins and the Ras/Raf/MAPK cascade have been implicated [51]. An analogous system has not been found in plants.

Some interesting work by Stone et al. shows that RLK5, an *Arabidopsis* leucine-rich repeat containing serine/threonine receptor kinase of unknown function, interacts with a protein phosphatase in vitro [96]. This interaction is dependent upon autophosphorylation of the receptor kinase. Phosphorylation-dependent interaction is extremely important in receptor tyrosine kinase interaction with proteins containing SH2 (Src homology 2) domains in animals [83, 94]. The discovery of an analogous plant signaling mechanism, in which a specific binding domain interacts with phosphoserine or phosphothreonine residues, provides our first glimpse at the downstream components of the receptor-like protein kinase signal transduction pathway in higher plants.

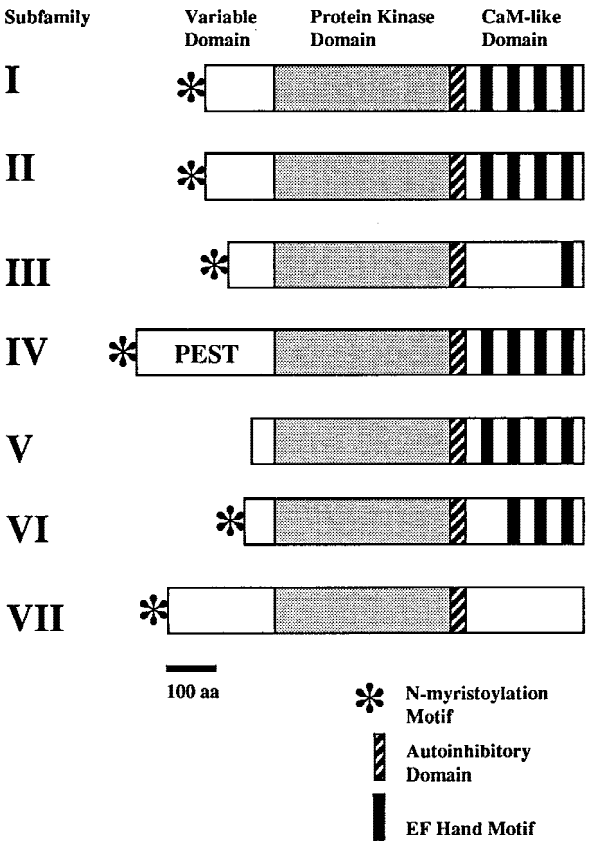

Fig. 2. Modular structures of the seven plant CDPK subfamilies. CDPKs have protein kinase catalytic domains with serine/threonine signature sequences. The variable domain differs in size and structure in the different CDPK subfamilies [40]. Many CDPKs have N-terminal myristoylation motifs, which may allow for post-translational addition of a fatty acid moiety to the N-termini of these isoforms. Members of one CDPK subfamily have PEST motifs which are associated with rapid protein turnover [79]. The autoinhibitory domain separates the kinase and calmodulin-like domains [35]. The calmodulin-like domain often has four calcium-binding EF hands, although the members of some CDPK subfamilies have fewer consensus EF hands.

Protein Kinases with Calmodulin-like Domains

Structure

Plants possess a novel calcium-dependent, calmodulin-independent protein kinase referred to as a *C*alcium-*D*ependent *P*rotein *K*inase or *C*almodulin-like *D*omain *P*rotein *K*inase (CDPK). For a review, *see* Roberts and Harmon [80]. These calcium-stimulated kinases consists of a single polypeptide (Fig. 2) with a protein kinase domain and a C-terminal calmodulin-like domain containing four calcium-binding EF hand motifs [36]. CDPKs have been identified in several protozoans including *Paramecium* and *Plasmodium*, however, these proteins are not encoded by the *Saccharomyces* genome [43], and have not been identified in animals. Currently there are fourteen *Arabidopsis* CDPK sequences deposited in Genbank and an analysis of *Arabidopsis* expressed sequence tags indicates the presence of six additional genes bringing the total number of *Arabidopsis* CDPK genes to a minimum of twenty, in a plant with one of the smallest genomes. Maize has nine identified CDPK isoforms, and rice has three thus far.

While plants have many CDPK genes, clear plant homologues of classical protein kinase C, an important calcium-stimulated kinase in animal and fungal systems, have not been identified. The vertebrate protein kinase C (PKC) family contains a large number of isoforms, and these isoforms can be grouped into functionally similar subfamilies [65]. For example, the conventional calcium-stimulated protein kinase C isozymes (α, $\beta 1$, $\beta 2$, γ) have a calcium binding site in the C2 domain, while the novel and atypical groups of protein kinase C isozymes (δ, ε, η, θ, ζ, λ) have alterations in this site, and are not directly regulated by calcium [64].

It is unclear whether or not plants have functional homologues of the Ca^{2+}/calmodulin-stimulated protein kinases of animals and fungi. These kinases are not stimulated by calcium directly, but are activated by a Ca^{2+}/calmodulin complex. There is an apple gene with some similarity to animal Ca^{2+}/calmodulin-stimulated protein kinases, but this protein has not been shown to possess Ca^{2+}/calmodulin-stimulated protein kinase activity [113, 114, 115]. A *Lilium* protein has been reported to possess Ca^{2+}/calmodulin-stimulated protein kinase activity, however this protein possesses a visinin-like domain containing three consensus EF hands, making it structurally distinct from animal Ca^{2+}/calmodulin-dependent protein kinases [70, 103].

Phylogenetic analysis of CDPK sequences (Fig. 3) is revealing. One striking aspect of this phylogram is that some *Arabidopsis* CDPK isoforms appear to be much more similar to CDPK isoforms in other plant species, including monocots, than they are to other *Arabidopsis* isoforms. These CDPKs form subfamilies which share common motifs. A schematic diagram showing the motifs for each subfamily is shown in Fig. 2.

We hypothesize that these subfamilies have different biochemical and/or functional properties. For example, the half-life of a protein may be significantly different depending on whether or not the protein has a PEST domain, which are regions enriched in the amino acids proline, glutamate, serine and threonine [79]. Both soluble and membrane-associated CDPK activity has been observed [86]. The presence or absence of a myristoyl moiety could affect membrane association of these kinases and might explain this observation. Six out of the seven CDPK subfamilies have conserved myristoylation motifs, while one subfamily does not. Myristoylation can affect protein-protein interactions, protein-membrane interactions [32], and can confer calcium-modulated association with membranes in some EF hand containing proteins such as hippocalcin [49] and neurocalcin [25]. Myristoylation has also been shown to in-

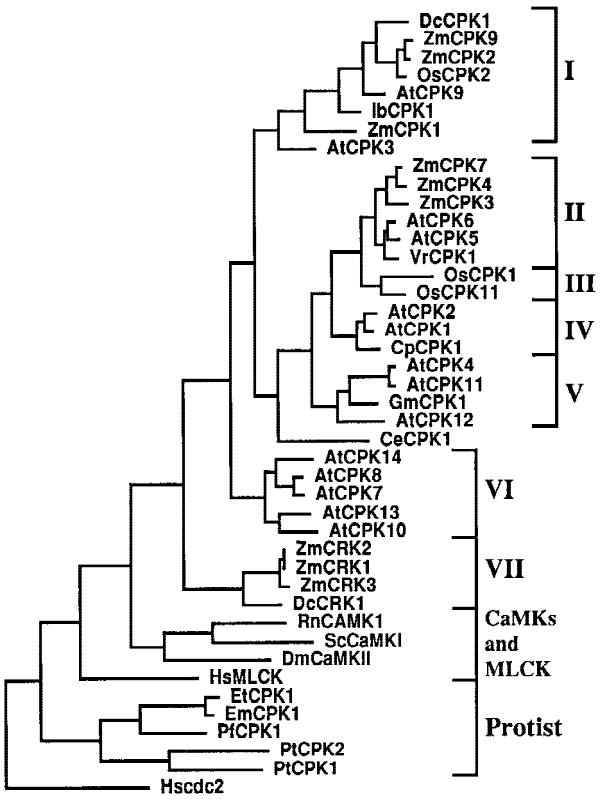

Fig. 3. CDPK Phylogram. This is a minimum length phylogram showing the relationships between the amino acid sequences of all known CDPKs and certain other protein kinases of interest. Only the protein kinase domains (Subdomain I-XI) of each of the genes was used [33]. Alignments were performed using CLUSTALW [105] and the tree was then generated using PAUP 3.1.1 software [101]. 100 random trees were used to initiate the four step minimal tree search strategy [66]. The human cdc2 kinase was used as an outgroup for the purpose of rooting the tree [69]. The x-axis is proportional to the number of amino acid changes between proteins. In the phylogram, the first letter of the genus and species from which the gene is derived is used as a prefix, followed by either CPK (the genetic designation for CDPK) or CRK which stands for CDPK-related kinase [28]. The plant species are maize (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), mungbean (*Vigna radiata*), carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*), zucchini (*Curcurbita pepo*), a unicellular algae (*Chlamydomonas eugamatos*), and *Arabidopsis thaliana.* Protozoan CDPK sequences are from *Eimeria maxima, Eimeria tenella, Parmecium tetraurelia,* and *Plasmodium falciparum.* Sequences encoding calcium/calmodulin dependent protein kinases from *Rattus norvegicus, Drosophila melanogaster,* and *Saccharomyces cerevisiae,* as well as *Homo sapiens* myosin light chain kinase and cdc2 were included for comparison. Sequences of the following genes can be found in the indicated reference: AtCPK1 [35]; AtCPK2, AtCPK4, AtCPK5, AtCPK6, AtCPK7, and AtCPK9 [40]; AtCPK10 and AtCPK11 [108]; AtCPK3, AtCPK8, and AtCPK12 [39]; OsCPK1 [46]; OsCPK2 and OsCPK11 [11]; GmCPK1 [36]; VrCPK1 [9]; ZmCPK1 [24]; DcCPK1 [98]; ZmCPK2 and ZmCPK3 [102]; ZmCRK1 and ZmCRK3 [28]; CeCPK1 [92]; EmCPK1 and EtCPK1 [22]; ZmCRK2 [57]; DcCRK1 [56]; PtCPK1 and 2 [48]; PfCPK1 [118]; ZmCPK4 [5]; Hscdc2 [54]; ScCAMKI [71]; DcCAMKII [16]; RnCAMKI [15]; ZmCPK7 and ZmCPK9 [81]. AtCPK13 (U54615), AtCPK14 (U90439), CpCPK1 (U90262) and IbCPK1 (D87707) are deposited in Genbank with the indicated accession numbers.

duce cooperative calcium binding in the EF hand-containing protein recoverin [2].

The consensus EF hands in the CDPK subfamilies vary in number from zero to four, which could result in different binding affinities for calcium or altered cooperativity of calcium binding. Mutations in conserved calmodulin residues have such effects [110, 95]. These CDPKs may be activated by different thresholds of calcium or have different specific activities at a given calcium concentration. Comparison of calcium sensitivities of AtCPK1 and AtCPK4, both of which have four consensus EF hands, show AtCPK1 to be five times more sensitive to calcium than AtCPK4 [82]. All heterologously expressed CDPKs tested have been shown to possess calcium-stimulated protein kinase activity, with the exception of a member of Subfamily VII [28]. The members of this group have no consensus EF hands.

The identification of seven distinct CDPK subfamilies with potentially different biochemical properties may explain why plant genomes encode a multitude of CDPK genes.

Function

Eight classical *Arabidopsis* CDPK isoforms and a soybean CDPK have been expressed in *E. coli* [40, 39, 35, 34]. All of these possess calcium-stimulated protein kinase activity, with as much as 100-fold activation by exogenous calcium [35]. CDPKs are the only plant protein kinases that have been shown to be directly activated by calcium.

A soybean CDPK has been shown to autophosphorylate serine and threonine residues [78], and CDPKs possess serine/threonine kinase signature sequences. Some CDPK isoforms, such as an oat plasma membrane CDPK, have both calcium-stimulated and lipid-stimulated protein kinase activity [86]. An *Arabidopsis* CDPK isoform is stimulated by lysophosphatidylcholine and lysophosphatidylinositol [6]. This lipid stimulation is reminiscent of the synergistic stimulation of protein kinase C by calcium and diacylglycerol.

With regard to the subcellular residence of CDPKs, oat roots have a calcium and lipid-activated CDPK that is associated with the plasma membrane [86]. At least a part of the soybean CDPK pool appears to colocalize with actin filaments as seen by immunofluorescence microscopy using monoclonal antibodies [77]. CDPK activity has been localized to the cytoplasm and symbiosome membranes as well [80], and it is possible that myristoylation plays a role in CDPK membrane association.

Although plants have many CDPK isoforms within their genomes, the biological role of CDPKs is unknown. No phenotypic mutants have been ascribed to a lesion in a CDPK gene. Recent work, using maize leaf protoplasts containing transgenes encoding constitutively ac-

tive CDPK isoforms, indicates that CDPKs may be involved in activating stress-inducible promoters [90].

Identification of CDPK substrates suggests a diversity of functions for these kinases. A membrane-associated CDPK can phosphorylate the H^+-ATPase of oat roots [88]. The tonoplast intrinsic protein, α-TIP, is a putative water channel that is phosphorylated by an *Arabidopsis* CDPK [44]. NADH: Nitrate Reductase activity, which is important in nitrogen assimilation, appears to be modulated by reversible phosphorylation at Ser-543, and at least one of the protein kinases involved has the properties of a CDPK [3]. A constitutively active CDPK has been shown to activate a guard cell vacuolar chloride channel [72]. A soybean CDPK phosphorylates nodulin-26, an integral membrane transporter of unknown specificity which is involved in the formation of nodules containing nitrogen-fixing bacteria [116]. Site-directed mutagenesis of serine-262 indicates that phosphorylation of this residue alters the transport properties of Nod26 by increasing voltage-dependent gating [53]. This is currently the best characterized case of CDPK regulation of a protein activity via phosphorylation. Most of the putative CDPK substrates described are membrane proteins, and the association of CDPKs with membranes may mean that CDPKs modulate plant membrane proteins in response to different calcium signals.

Although identification of in vivo substrates will shed light on CDPK function, genetic analysis of CDPK function via gene disruption may prove to be a much more powerful technique for placing a given CDPK in a particular signal transduction pathway. Recently Sussman and coworkers have found plants harboring T-DNA insertion alleles of the *Arabidopsis* CPK4, CPK6, CPK9, CPK10 and CPK11 genes [52], (J.S. Satterlee, R.D. Green, and M.R. Sussman, *unpublished observations*). Phenotypic characterization of plants containing homozygous insertion alleles of individual or multiple CDPK genes will help to identify the biological function of specific CDPK isoforms.

Histidine Protein Kinase Homologues

Structure

Histidine kinases have been well studied in prokaryotic systems, and often participate in signal transduction pathways involving responses to extracellular cues [68, 99]. These kinases are capable of histidine autophosphorylation, in which the γ-phosphate of ATP is transferred to a specific histidine residue of the kinase. This phosphate is then transferred from the histidine to an aspartate residue in the ''response regulator'' [68]. Figure 4 shows the modular structure of several histidine kinase/response regulator proteins. Because a histidine kinase is paired with its cognate response regulator in a signal transduction pathway, these proteins have been referred to as ''two-component'' sensor-regulators or transmitter-receivers. As shown in Fig. 4, a single polypeptide may contain a histidine kinase domain (B), or a response regulator domain (E), or both the histidine kinase and response regulator domains may occur within the same polypeptide (A, C, D).

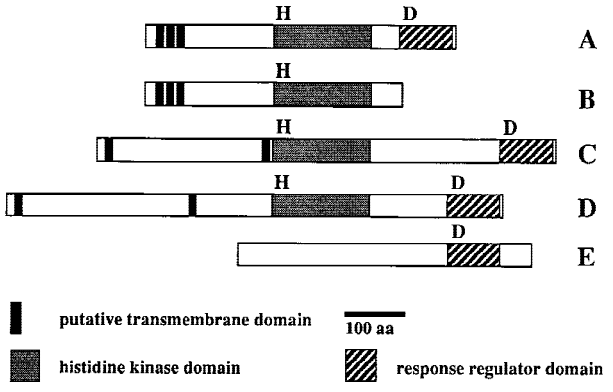

Fig. 4. Modular structure of plant and yeast histidine kinases. Schematic diagrams of the following histidine kinase homologues are shown: (*A*) ETR1, an *Arabidopsis* histidine kinase homologue with a histidine kinase domain and a response regulator domain [13]; (*B*) Never-ripe, a tomato histidine kinase homologue lacking a response regulator domain [117]; (*C*) CKI1, an *Arabidopsis* histidine kinase homologue with a histidine kinase domain and a response regulator domain [45]; (*D*) SLN1, a *Saccharomyces cerevisiae* histidine kinase homologue with a histidine kinase domain and a response regulator domain [67]; and (*E*) SSK1, a *Saccharomyces cerevisiae* protein with a response regulator domain, but lacking a histidine kinase domain [58]. H indicates the approximate position of the putative autophosphorylated histidine residue, and D indicates the position of the aspartate phosphoacceptor in the response regulator domain. All of the proteins possessing histidine kinase domains have 2 to 3 putative transmembrane domains, although the membrane-spanning topology of these proteins is unknown.

Eukaryotic histidine kinase homologues were identified concurrently in both budding yeast [67] and in *Arabidopsis thaliana* [13]. These kinases have been implicated in osmoregulation, as well as signal transduction pathways involving the plant hormones ethylene and cytokinin.

Several histidine kinase homologues which appear to be important in ethylene signal transduction have been identified. The *Arabidopsis ETR1* gene was found to be a histidine kinase homologue [13]. As shown in Fig. 4, this gene encodes a protein containing three membrane spanning domains, a histidine kinase domain, and a C-terminal response regulator domain [13]. The tomato eTAE1 protein is very similar in structure to ETR1 [119]. The *Arabidopsis* ERS protein contains three putative membrane spanning domains and a domain with histidine kinase homology, but has no response regulator

gene [41]. The tomato Never-ripe (NR) protein is structurally similar to ERS [117].

Another plant histidine kinase homologue, *CKI1,* seems to play a role in signal transduction in response to the plant hormone cytokinin. The deduced protein contains two putative transmembrane domains as well as the histidine kinase and response regulator domains [45]. Many prokaryotic histidine kinases have two transmembrane domains with the histidine kinase domain on the cytoplasmic side of the membrane [68].

Function

How do histidine kinases transduce signals? A well-characterized example is the oxygen-sensing system of the prokaryote *Rhizobium,* which is of particular relevance since oxygen, like ethylene, is a gas. In this system, the FixL protein contains an N-terminal oxygen-binding heme cofactor [31]. When no oxygen-bound heme is present, autophosphorylation of the C-terminal histidine kinase domain is stimulated [30], although in other systems histidine kinase activity may be inhibited by a given environmental signal. The FixJ response regulator then transfers the phosphate from the histidine residue to an aspartate residue. The FixJ protein has homology to transcriptional activators and may then function directly or indirectly to stimulate expression of the *nifA* and *fixN* genes [18].

Missense mutations in the *ETR1* gene make *Arabidopsis* plants insensitive to the gaseous plant hormone ethylene [13, 8, 12]. Application of exogenous ethylene to dark grown seedlings inhibits root and shoot elongation, enhances radial hypocotyl swelling, and maintains the structure of the apical hook (for reviews *see* [23, 27, 8]). *ETR1* mutant plants lack these responses [7], as do mutants in the *ETR1* tomato homologue *Never-ripe* [117] indicating that these genes play a crucial role in ethylene signal transduction.

All of the alleles of *ETR1* and *Never-ripe* characterized thus far are dominant, and transgenic expression of a mutant version of *ERS,* an *Arabidopsis ETR1* homologue lacking the response regulator domain, also causes a dominant ethylene-insensitive phenotype [41]. It may be that only dominant-negative or gain-of-function mutations can be isolated, if *ETR1*-like genes have redundant *in planta* function [8].

Heterologous expression of the ETR1 protein generates ethylene binding sites in *Saccharomyces cerevisiae* [85], while mutations in the three putative transmembrane domains of ETR1 which correspond to the gain-of-function or dominant-negative ETR1 alleles, interfere with ethylene binding. These data, in conjunction with the mutant phenotype, indicate that ETR1 is an ethylene receptor. ETR1 has been shown to form homodimers covalently linked via a disulfide bond [85], and could potentially form heterodimers with other *Arabidopsis* ETR homologues. Recently ETR1 has been shown to have histidine kinase activity (G.E. Schaller, *personal communication).*

The *CKI1* (cytokinin-independent) gene encodes another *Arabidopsis* histidine kinase homologue [45] which appears to be involved in cytokinin signal transduction. Cytokinins are plant hormones involved in a variety of processes including cell division, chloroplast development, shoot initiation, and delay of leaf senescence [19]. Tatsuo Kakimoto performed a genetic activation screen in which calli transformed with a constitutive promoter were selected for the ability to regenerate shoots in the absence of exogenous cytokinin. Overexpression of *CKI1* results in plants which phenotypically resemble *Arabidopsis* that have been treated with exogenous cytokinin. It is unclear whether the CKI1 protein is a cytokinin receptor, a regulator of cytokinin biosynthesis, or participates in cytokinin signal transduction in some other fashion.

Histidine kinase homologues have also been identified in eukaryotes such as *Dictyostelium,* in which *DhkA* plays a role in *Dictyostelium* development [111], while *DokA* is involved in osmoregulation [89]. *Nik1* is a histidine kinase homologue from *Neurospora crassa.* Deletion of this gene causes defects in morphology of the hyphae which are exacerbated under high osmoticum [1]. Rat genes encoding a pyruvate dehydrogenase kinase [73] and a mitochondrial branched-chain α-ketoacid dehydrogenase kinase (BCKDH kinase) [74] have been cloned. The deduced proteins have homology to histidine kinases, but surprisingly the BCKDH kinase appears to phosphorylate two BCKDH serine residues.

Eukaryotic histidine kinase signal transduction has been best characterized in *Saccharomyces cerevisiae* in which the *SLN1* and *YPD1* genes transduce signals in response to changes in osmoticum. In this case, *SLN1* and YPD1 participate in sequential phosphotransfers [75]. Interestingly, this yeast signal transduction pathway modulates a downstream MAP kinase cascade [58], while the *Arabidopsis* ethylene signaling pathway involves *ctr1,* a Raf-like, MAPKKK homologue [47]. There is genetic evidence that *ctr1* acts downstream of *ETR1* in ethylene signal transduction.

Conclusions

As we learn more about the machinery of signal transduction we find both similarities and differences with respect to how organisms solve problems in signaling. Histidine kinase homologues have now been identified in prokaryotes, plants, animals, and fungi, while receptor protein kinases are important in both plant and animal signal transduction. Most animal receptor kinases have tyrosine-specific signature sequences, but no kinases

with conventional tyrosine-specific signature sequences are encoded by the yeast genome [43], and none have yet been cloned from plants. In animal systems many membrane-associated receptors are of the seven transmembrane type [21], but no plant homologues of these receptors have been definitively identified. The only biochemically characterized plant hormone receptor is ETR1, a histidine kinase homologue. Protein kinase A, protein kinase G, protein kinase C, and Ca^{2+}/calmodulin-dependent protein kinase are important second messenger-modulated kinases in animal systems, but no unequivocal homologues of these kinases have been found in plants. Plants do have protein kinases with calmodulin-like domains that are directly stimulated by calcium.

While it is evident that plant and animal signal transduction mechanisms have many common themes, plants are not merely sessile animals with chloroplasts. There are clear and significant differences between plant and animal signal transduction. Studying unusual plant signal transduction molecules such as serine/threonine receptor protein kinases, calmodulin-like domain protein kinases, and plant histidine kinases will reveal much about how plants transduce signals and may provide important insight into signaling pathways which are, as yet, undiscovered in fungi and animals.

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