Plant Protein Kinase Families and Signal Transduction

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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 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, MAPKKK 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, Suc nonfermenting; SRK, S-receptor kinase; Tsl, TOUSLED.
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 111 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 denoting 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.
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 a tp k1 and a tp k2 (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-a, 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 RKN1 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. PKA B1 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 Schizo-
saccharomyces 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 resi-
dues. The enzyme responsible for this dual phosphoryla-
tion, MAPKK, represents an unusual class of eukaryotic
protein kinases that will phosphorylate on Ser, Thr, and
Tyr residues. Activating phosphorylations occur in the con-
served 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 to-
ward 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 en-
hanced MBP kinase activity upon addition of purified Xe-
nopus MAPKK. Moreover, auxin treatment leads to accu-
mulation 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 mam-
mals, Drosophila, yeast, and plants. Mammalian GSK-3,
implicated in hormonal responses, is functionally homo-
gous to a Drosophila gene, shaggy/zeste-white 3, which
is required for specifying cell fate and polarity during em-
bryo development. The plant members of this family are
encoded by small multigene families. Three alfalfa ho-
logos, MsKs, are differentially expressed during develop-
ment (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 phos-
phorylation 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 pat-
terns 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 pro-
tein kinase subunits (Padmanabha et al., 1990). CKII from
yeast and mammals exists as a tetrameric complex consist-
ing of two α catalytic subunits and two regulatory β sub-
units, α₂β₂. In plants, however, both monomeric and oli-
gomeric forms of CKII have been purified. Counterparts of
both the catalytic protein kinase α and regulatory β sub-
units have been cloned from plants (Dobrowsolska et al.,
1991; Mizoguchi et al., 1993; Collinge and Walker, 1994).
Studies using the recombinant Arabidopsis subunits have
demonstrated that a tetrameric α₂β₂ complex can be
formed and that the regulatory β subunit stimulates cata-
lytic activity. Both native and recombinant CKII phos-
phorylate 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 are 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 phos-
phorylation in other eukaryotes, may also be regulated by
Tyr phosphorylation in plants. Although the protein ki-

nases 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 pro-
tein 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 autophos-
phorylation on the cytoplasmic kinase domain, a require-
ament 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 cyto-kinine 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
been identified in plants, including a MAPKK and a MAP-
been cloned from plants (see "CMGC Group"). Moreover, a MAPKKK. The final element of this pathway, MAPK, has
the bifunctional MAPKK, which in turn is activated by (c)
activated by dual phosphorylation on Thr and Tyr by (b)
ple physiological processes, including response to mating
ment (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 MAPKKK, which in turn is activated by (c)
a MAPKKKK. 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 MAP-
KKK homolog from tobacco. Southern blot analysis of
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 eukary-
otic 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 characteriza-
tion of plant protein kinases and their interactions will lead
to insights into the mechanisms controlling plant growth
and development. Genetic approaches, which were instrumen-
tal 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 combina-
tion of genetic, biochemical, and molecular approaches to
place protein phosphorylation in a physiological context.

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