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PROTEIN PHOSPHATASES IN PLANTS

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■ **Abstract** Phosphorylation and dephosphorylation of a protein often serve as an "on-and-off" switch in the regulation of cellular activities. Recent studies demonstrate the involvement of protein phosphorylation in almost all signaling pathways in plants. A significant portion of the sequenced *Arabidopsis* genome encodes protein kinases and protein phosphatases that catalyze reversible phosphorylation. For optimal regulation, kinases and phosphatases must strike a balance in any given cell. Only a very small fraction of the thousands of protein kinases and phosphatases in plants has been studied experimentally. Nevertheless, the available results have demonstrated critical functions for these enzymes in plant growth and development. While serine/threonine phosphorylation is widely accepted as a predominant modification of plant proteins, the function of tyrosine phosphorylation, despite its overwhelming importance in animal systems, had been largely neglected until recently when tyrosine phosphatases (PTPs) were characterized from plants. This review focuses on the structure, regulation, and function of protein phosphatases in higher plants.

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INTRODUCTION

One Nobel prize in physiology honors the discovery of protein phosphorylation that regulates virtually all cellular activities in eukarytic systems (69). The attachment to or removal of a phosphate group from a protein often has profound effects on the structure and thereby the functional properties of the protein. For example, phosphorylation can regulate an emzyme activity by initiation of allosteric conformational changes, which may directly block the access to the active site (61, 62). Phosphorylation can regulate the interaction among protein partners that must form complexes in order to function (100). Some proteins need to be phosphorylated in order to target the destination site in the cell where they function. As a result, nearly all aspects of cell function involve reversible phosphorylation. These include metabolism, cell cycle progression, ion transport, developmental control, and stress responses. This diverse spectrum of cellular functions is reflected by the large number of intracellular proteins that are subject to reversible phosphorylation and the number of protein kinases and phosphatases that catalyze the reactions.

A typical protein kinase catalyzes the phosphorylation of the hydroxyl group on amino acid residues including serine, threonine, and tyrosine. According to the substrate specificity, protein kinases and phosphatases are classified into two major groups, serine/threonine (Ser/Thr) and tyrosine kinases (or phosphatases). Although all protein kinases are structurally related to one another (56, 60), protein phosphatases are defined by at least three distinct families (8, 21, 95, 118). The PPP and PPM families consist of Ser/Thr phosphatases, and the protein tyrosine phosphatase (PTP) family includes both tyrosine-specific and dual-specificity phosphatases (26, 126). Even within the same family, significant structural diversity can be generated by the presence of unique regulatory and targeting domains or by the attachment of regulatory subunits to the catalytic subunits. These regulatory domains or regulatory subunits may localize the protein complexes to a specific subcellular compartment, modulate the substrate specificity, or alter catalytic activity.

Both protein kinases and phosphatases were originally discovered in animal systems. More recent studies in plants have uncovered the unique structure and function of these enzymes in plant-specific developmental and physiological processes. While the large number of protein kinases and their functions have been recognized for many years, the number, diversity, and function of protein phosphatases have only been appreciated more recently. Even less is known regarding protein phosphatases in higher plants. In each section of this Chapter, I first describe major paradigms established in animal (and yeast) systems, followed by findings on protein phosphatases that are unique in higher plants.

SER/THR PHOSPHATASES

Nomenclature

The earliest biochemical studies in animal systems (reviewed in 21a) defined two major types of Ser/Thr phosphatases based on their substrate specificity and

pharmacological properties: Type 1 and Type 2. Type 1 phosphatase (PP1) prefers the beta-subunit of phosphorylase kinase as substrate and is inhibited by nanomolar concentrations of two small peptide inhibitors, inhibitor 1 and 2. Type 2 phosphatases (PP2) preferentially dephosphorylate the alpha-subunit of phosphorylase kinase and are insensitive to inhibitor 1 and 2. PP2 enzymes can be further divided into PP2A, 2B, and 2C, simply by their dependence on divalent cations. Whereas PP2B and PP2C are regulated by Ca^{2+} and Mg^{2+}, respectively, PP2A, like PP1, does not require divalent cations for activity. A group of drugs including okadaic acid, calyculin A, and cantharidin have also been useful in distinguishing members of PP1- and PP2-type enzymes. For example, both okadaic acid and calyculin A potently inhibit the activity of PP1 and PP2A but are not effective to PP2B and PP2C. Cantharidin inhibits only PP2A but not others (77). These reagents have been used as effective tools in defining cellular functions of these enzymes in various systems ranging from mammalian to plant cells (see below).

This simple classification system was implemented about three decades ago and is still in use today, although a more systematic nomenclature has been suggested after a large number of genes encoding these phosphatases were identified from eukaryotic organisms. Sequence and structural analyses of these gene products demonstrate that PP1, PP2A, and PP2B are more closely related and defined as PPP family, whereas PP2C, pyruvate dehydrogenase phosphatase, and several other Mg^{2+} -dependent Ser/Thr phosphatases are more similar to each other, and hence are referred to as the PPM family (9, 21).

General Properties of Ser/Thr Phosphatases

Although the PPP family enzymes share a common catalytic region of approximately 280 amino acids, they become divergent when comparing their N- and C-terminal noncatalytic domains. These enzymes are further distinguished by their associated regulatory subunits that form a diverse array of holoenzymes. In animals, PP1 is involved in controlling multiple cellular functions including glycogen metabolism, muscle contraction, cell cycle progression, neuronal activities, and splicing of RNA. These processes are regulated by different holoenzymes in which the same catalytic subunit (PP1c) is complexed to distinct regulatory and targeting subunits (36, 55). Similar to PP1, the diverse functions of PP2A are attributed to the presence of a large number of B regulatory subunits that individually assemble with each core heterodimer of PP2Ac and a 65-kDa A-subunit (134). PP2B is a Ca^{2+}-, calmodulin-dependent protein phosphatase (68). The holoenzyme contains the catalytic A and regulatory B subunits. Besides the core catalytic region, the A subunit contains several unique structural domains including the B-interaction, calmodulin-binding, and an autoinhibitory domain. The B subunit is a Ca^{2+} sensor protein that, like calmodulin, contains four Ca^{2+}-binding EF-hand motifs. When the cytosolic Ca^{2+} concentration reaches micromolar levels, calmodulin assembles into the A-B heterodimer and activates the phosphatase activity (68). Besides the classical PPP enzymes included in the PP1/2A/2B subgroups, other phosphatases with high homology to the PPP family have been identified that contain unique

domains or play distinct functions. These more distantly related enzymes are referred to PP4, PP5, PP6, and PP7 (21) (Figure 1).

The crystal structures of PP1 and PP2B have been solved and provide models for the structure and catalysis of PPP enzymes (33, 45, 47, 67). Based on the primary sequence, it is not surprising that PP1c and PP2B share a common catalytic domain structure. The catalytic domain of PP1c and PP2B consists of a central "sandwich" formed by two subdomains of a "helix-sheet" mixture. The crystal structure of the two enzymes also reveals the importance of metal ions in the catalytic reaction of PPP family phosphatases. These metal ions include Mn^{2+} and Fe^{2+}/Fe^{3+} for

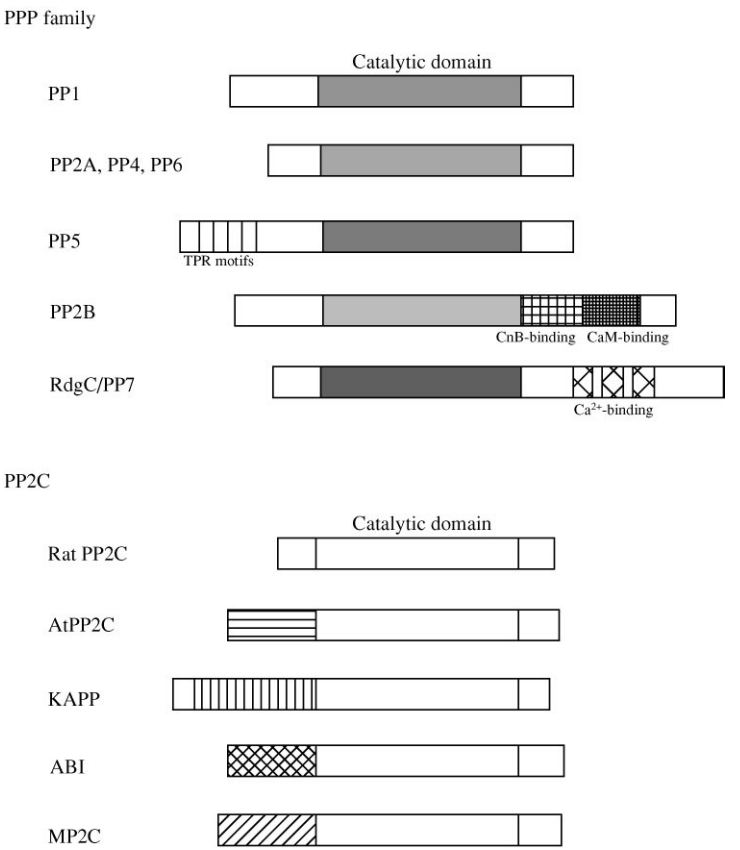

Figure 1 Structural domains of Ser/Thr phosphatases. The PPP family members all have a catalytic domain of about 280 amino acids. The sequence of this domain is highly conserved among the members (40–60% identity). The PP2C family members have a catalytic domain that is less conserved (20–35% identity). While the PPP family enzymes from plants are similar to animal homologues, the PP2C members are highly diversified.

PP1c (33) and Zn^{2+} and Fe^{2+}/Fe^{3+} for PP2B (141), although both enzymes should, based on structure, contain the same metal ions. This point is still debatable. A number of studies suggest that PPPs catalyze dephosphorylation in a single step with a metal-activated water molecule or hydroxide ion (10). This mechanism of catalysis would not involve the formation of a phosphoryl-enzyme intermediate, in sharp contrast to the mechanism for tyrosine phosphatases described in later sections.

PP2C is the representative group of the PPM family. Although the primary sequence of PP2C and other PPM family members share no homology with the PPP enzymes, the structural folds of these two families are strikingly similar (24). Mammalian PP2C consists of two domains: The N-terminal catalytic region forms a central sandwich structure by two antiparallel sheets; the C-terminal region is a unique antiparallel helix structure remotely attached to the catalytic domain by a loose strand, suggesting a role for defining substrates rather than activity. Also in the catalytic domain are two Mn^{2+} ions in a binuclear metal center coordinated by four invariant Asp residues and one nonconserved Glu residue. These residues are located on the top of the sandwich channel. Dephosphorylation may be catalyzed by a metal-activated water molecule that serves as a nucleophile, as proposed for the PPP family (10).

Ser/Thr Phosphatases in Plants

Studies on plant protein phosphatases started with biochemical analyses of protein extract from plant tissues reviewed earlier (78, 78a, 113a). Using substrates for mammalian phosphatases and the same pharmacological agents (e.g., okadaic acid), PPP family enzymes such as PP1 and PP2A have been detected in plants such as *Brassica napus* (83), pea, carrot, and maize (82). In addition to substrate specificity and pharmacological properties, the primary structure of plant phosphatases is also highly similar to that of the mammalian enzymes. This became obvious after genes encoding members of PP1-, PP2A-, and PP2C-type phosphatases were cloned from a number of plant species. Nevertheless, different structural domains and unique functions have been identified through studies on plant enzymes.

Early identification of genes encoding PP1 and PP2A began in the early 1990s. A multigene family in maize encodes PP1 (113a) and recombinant maize PP1 is inhibited by peptide inhibitor 2 and by okadaic acid with a similar sensitivity to animal enzymes as measured by IC50. Recombinant maize PP1 requires Mn^{2+} for activity, consistent with the fact that Mn^{2+} is an essential metal ion in the catalytic domain of the PPP family enzymes, as discussed above (33). Although PP1 activity is associated with large protein complexes in plant extract (82), other proteins (presumably the regulatory subunits) in the complexes and their corresponding genes have not been identified, except for the catalytic subunit (PP1c).

Native PP2A in mammalian cells is found as either a heterodimer consisting of a 36-kDa catalytic subunit and a 65-kDa regulatory A subunit, or as a heterotrimer

that is assembled by the heterodimer core and one of the regulatory B subunits (134) (Figure 2). Genes for both catalytic and regulatory A and B subunits have been identified from plants. Following the initial cloning of a PP2A catalytic subunit from *B. napus* (84), similar genes have been found in other plants such as alfalfa (103) and *Arabidopsis* (5, 16). High sequence similarity (over 80%) has been found between plant enzymes and animal homologues. Genes for the 65-kDa A regulatory subunit and for B subunits have also been cloned from *Arabidopsis* (43, 71, 107, 113, 135). The deduced peptide sequences of plant A and B regulatory subunits share approximately 70% similarity with animal homologues.

Despite strong evidence for the function of PP2B-like activity (2, 14, 80, 99), no plant gene has been identified for a typical catalytic subunit that resembles animal PP2B. A multigene family encoding calcineurin B–like (CBL) proteins has been identified (70), but further studies identified a family of novel protein kinases (referred to as CIPKs) as targets for CBLs (79, 111). A surprising finding of a

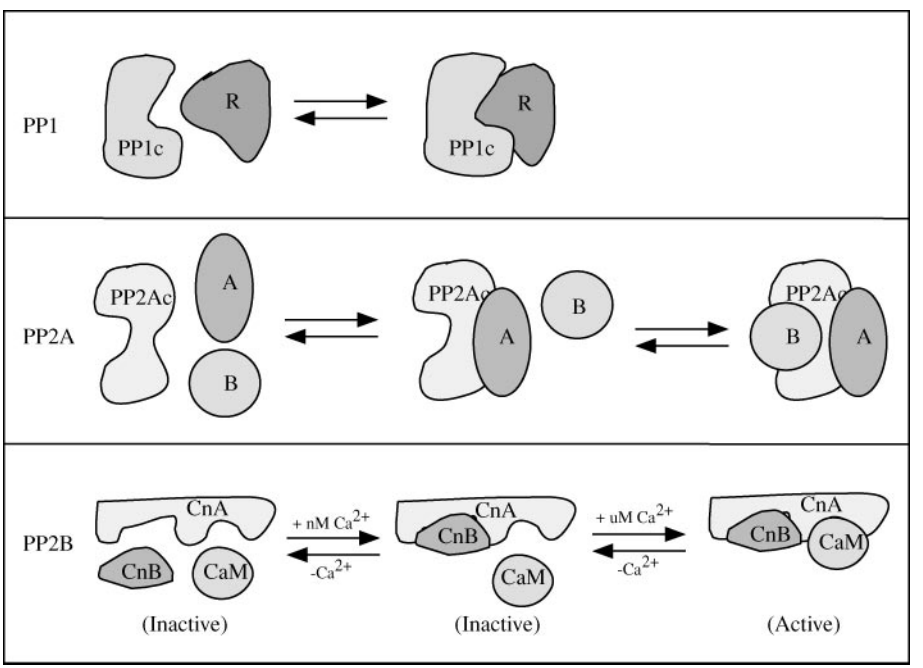

Figure 2 Subunit composition and regulation of PPP family enzymes. PP1 holoenzymes are heterodimers composed of catalytic PP1c and regulatory R subunit. PP2A holoenzymes exist in either heterodimer or heterotrimer form. PP2B is often present in a heterodimer form with low activity (catalytic CnA and regulatory CnB) under "resting" levels of calcium. Elevated levels of calcium recruit calmodulin (CaM) into the heterodimer to form highly active trimeric enzymes.

CBL-CIPK paradigm in plants but not in animals or fungi indicates a change in paradigm on the regulatory enzymes involved in calcium-dependent signal transduction in plants versus animals and fungi (79).

Unlike animals that produce only a few isoforms of PP2C, higher plants produce a large and diverse family of PP2C-like enzymes. Earlier cloning work has identified several PP2C-like genes from *Arabidopsis* and alfalfa. Unlike PPP family enzymes that have been largely conserved in primary strucuture among plant and animal enzymes, plant PP2C members share low homology to animal enzymes (only 20–35% identity at the amino acid level). In addition, each plant enzyme contains unique N-terminal extensions (Figure 1). These structural domains are not found in any animal or fungal enzyme. An *Arabidopsis* PP2C homologue, PP2C-At, was identified by its ability to complement a defect in yeast strains with mutations in cAMP-dependent signaling pathways (70a), although its role in *Arabidopsis* is unknown. PP2C-At contains a C-terminal region with 35% identity to rat PP2C and a unique N-terminal region with no significant homology to any protein in the database. Another *Arabidopsis* PP2C homologue, ABI1, was identified as a component in a signaling pathway in response to the plant hormone abscisic acid (ABA) (73, 92). The C-terminal region of ABI1 protein shares 35% identity with rat PP2C; again the N-terminal region is unique. The recombinant enzyme is a typical PP2C with Mg^{2+}-dependent phosphatase activity (13). The phosphatase activity of ABI1 is not sensitive to okadaic acid nor is it regulated by Ca^{2+}. A recently identified ABI1 homologue, ABI2, is 80% identical to ABI1 and is also involved in ABA signaling (74, 105). The *Arabidopsis* KAPP (kinase-associated protein phosphatase) is a PP2C member that was isolated as an interacting partner protein of RLK5, a receptor-like kinase from maize (115). Based on the deduced peptide sequence, KAPP protein contains three domains, an N-terminal membrane anchor, a kinase-interacting domain, and a C-terminal PP2C catalytic domain. Consistent with this structural prediction, recombinant KAPP displays an Mg^{2+}-dependent activity that dephosphorylates phosphocasein (115). In addition, the kinase-interacting domain is necessary and sufficient for interacting with RLK5. An alfalfa PP2C, MP2C, has been isolated for its activity in regulating a stress response pathway in yeast (90). In addition to the C-terminal PP2C catalytic domain, MP2C also has a unique N-terminal region that shows no homology to any known protein. The N-terminal extension of plant PP2Cs may be responsible for interaction with other proteins (H. Hirt, personal communications).

The recent completion of the *Arabidopsis* genome sequencing projects has made it possible to identify all the genes encoding putative Ser/Thr phosphatases. The annotation effort associated with the genome sequencing projects and some additional genome-wide surveys (e.g., 66a) have revealed a large number of genes for proteins related to Ser/Thr phosphatases. These include at least 7 genes for PP1 catalytic subunits, 6 genes for PP2A catalytic subunits, 70 genes for PP2C, and about a dozen for other types of PPP enzymes related to PP4, PP5, PP6, and PP7 (4, 66a). A unique feature of plant Ser/Thr phosphatases is the abundance and diversity of PP2C-type enzymes that are not comparable to PP2Cs in other organisms. The

functional significance of PP2C enzymes in plants is being explored through a number of approaches (see below). Another interesting finding during the genome sequence analysis is the presence of several PPP type enzymes that contain large N-terminal extensions (66a; S. Luan, unpublished results). This group of PPP enzymes appears to be unique in higher plants and may function in plant-specific processes.

Regulation of Ser/Thr Phosphatases

Precise regulation of a cell's phosphorylation status requires the activity of both kinases and phosphatases to be precisely regulated. Protein phosphatases are regulated in the cell at the level of expression, protein localization, substrate specificity, and activity of these enzymes.

In higher plants, expression of some Ser/Thr phosphatases appears to be highly regulated by spatial and temporal cues, although only a few studies have been dedicated to this topic. For example, genes for PP2A catalytic subunits are coexpressed in most tissues but the expression levels are developmentally regulated (5, 16). Expression of a regulatory subunit of PP2A is strongly induced under heat shock (71). The transcript level of a PP2C member, MP2C, increases dramatically upon wounding (90). The stress-induced expression of MP2C is transient, consistent with a possible role of these gene products in the early steps of stress signaling pathways. Two related PP2Cs, ABI1 and AtPP2CA, are induced by low temperature, drought, high salt, and ABA. The cold- and drought-induced expression of these genes was ABA dependent (121). More recent studies on gene expression using microarray profiling have identified protein phosphatase genes that are regulated by wounding and other stress signals (e.g., 18a). Such genome-wide approaches to gene expression will eventually generate a complete expression pattern of all genes, including those for phosphatases. Such information will be crucial for understanding the in vivo function of each phosphatase in plants.

Phosphorylation is a common posttranslational modification of many protein molecules. As enzymes involved in reversible phosphorylation, protein phosphatases are themselves regulated by phosphorylation (12, 17). However, little is known about this aspect of regulation in plant Ser/Thr phosphatases.

Different members often contain distinct noncatalytic regions that regulate various aspects of the phosphatase. Perhaps the most common function for these domains is to direct the localization of the catalytic activity in a cell. A novel PPP family enzyme, PP5, contains a tetratricopeptide repeat TPR domain for interaction with the cytoplasmic anchor chaperone HSP90 (18). The PP2B catalytic subunit contains a region that interacts with the regulatory subunit and calmodulin, respectively. Such interaction can direct the enzyme to cell membranes because the regulatory subunit can be modified by myristoylation (68). The plant PP2C member KAPP contains a membrane anchor and a domain for interacting with receptor-like kinases. Both these domains facilitate KAPP localization to the plasma membrane (76). Indeed, recent studies on the receptor-like protein kinase

CLAVATA 1 (CLV1) have shown that KAPP is part of the CLV1-CLV2-CLV3 complex (127).

The noncatalytic regions also have other functions including the regulation of catalytic activity. For example, the human PP7 contains multiple Ca^{2+}-binding motifs for activation by high levels of Ca^{2+} in the cell (53). PP2B has a calmodulin-binding domain for activation by interaction with calmodulin (68). The C-terminal region of PP2B is an autoinhibitory domain that inhibits the phophatase activity in the absence of calmodulin binding. Whether any plant protein phosphatase is subject to this type of regulation is not yet determined.

Interaction with regulatory proteins may be the most common mechanism for regulation of protein phosphatases (Figure 2). In mammalian systems, one catalytic subunit of PP1 dephosphorylates a number of physiological substrates. This seemingly paradoxical situation was resolved by the discovery of multiple regulatory subunits that target the same catalytic subunit to various subcellular locations and, therefore, distinct substrates. In addition, these regulatory subunits often change the specific activity of a protein phosphatase toward different substrates. For example, the M110 subunit, responsible for the association of PP1c with the myofibrils of the muscle cells, enhances the activity of PP1c against myosin light chain and suppresses activity toward glycogen phosphorylase (58, 59). Besides defining substrate specificity, some regulatory subunits allow the catalytic subunit to be modified by specific events. For instance, the GM regulatory subunit of PP1 is phosphorylated by PKA, resulting in dissociation of the PP1c and GM and hence the substrate glycogen (25). Because the binding of distinct regulatory subunits to PP1c is mutually exclusive, the binding sites must be the same or overlapping region of PP1c protein. Indeed, structural analysis has defined a region of PP1c and similar motifs in different regulatory subunits as important for interaction (34). To date, more than a dozen regulatory subunits of PP1 have been characterized from mammalian cells. Approximately 100 more novel PP1c-binding proteins have recently been reported (22). In contrast to this vigorous research effort, study on PP1 regulatory subunits in higher plants has yet to be initiated.

Much like PP1, PP2A is also highly regulated by its regulatory subunits. Although PP2Ac is anchored by a 65-kDa A subunit, PP2Ac activity and substrate specificity are largely controlled by a number of regulatory B subunits. At least 20 B regulatory subunits (54–130 kDa in molecular weight) have been identified from mammalian systems. These B subunits are further divided into three groups, the 55-kDa B, 54–74-kDa B′, and 72–130-kDa B″ subunits (88, 134). The best-known function of these subunits is to target the PP2Ac to distinct subcellular locations. For example, the 55-kDa B subunit targets PP2Ac to the microtubule (114) and B′ subunits facilitate nuclear location of PP2Ac (87). As discussed earlier, several studies showed presence of PP2A regulatory subunits in higher plants (43, 107, 113, 135). Some studies have begun to provide information on the specific function of these subunits in plants (see below).

Protein phosphatases, like many other signaling molecules, can be inhibited or activated by small molecules that occur naturally in the cell. The discovery of

endogenous inhibitors for PP1 established this paradigm (21a). Two heat-stable proteins, inhibitor 1 and 2 (I-1 and I-2), were first purified from liver and muscle extract for their ability to inhibit PP1 activity. The function of these peptide inhibitors is highly regulated by phosphorylation. The I-1 (18 kDa) is effective only after it is phosphorylated at a specific threonine by PKA (35). Although I-2 (22 kDa) does not require phosphorylation to be an active inhibitor, the potency of inhibition increases 106-fold after phosphorylation (29). Secondary structure prediction provides a model for the mechanism of PP1c inhibition by I-2 (45). PP1 is also potently inhibited by a natural toxin, microcystin LR, a complex cyclic heptapeptide. The crystal structure of PP1c complexed with microcystin LR shows several contacting sites on the PP1c surface (45). One involves a carboxylate and carbonyl group of the toxin that interacts with two of the metal-bound water molecules, thus directly blocking substrate binding. Other sites involve the formation of covalent bonds between PP1c and the toxin side chain.

PP2A holoenzymes are activated by ceramide, a lipid second messenger in mammalian systems (30, 41). Ceramide is produced from the sphingomycin cycle and specifically activates two of the regulatory subunit-containing holoenzymes of PP2A. This indicates that these PP2A holoenzymes may be modulated by the extracellular signals that trigger sphingosine hydrolysis. As ceramide is found to be a regulatory molecule in plant cells (98), PP2A regulation may count for part of its function. PP2C (MP2C) is activated by unsaturated fatty acids that are products of membrane lipid degradation by lipases (11).

Small molecule pharmacological agents have been crucial in the study of cellular function of specific phosphatases. Okadaic acid, calyculin A, and microcystin-LR are potent inhibitors of both PP1 and PP2A. Although in vitro studies indicate that okadaic acid inhibits PP2A with higher potency as compared to its potency to PP1, in vivo application of okadaic acid to distinguish PP1 and PP2A has proved to be difficult (110). More specific inhibitors include peptide inhibitors I-1 and I-2 for PP1 and cantharidin for PP2A (77). Because PP2C and PP2B require divalent cations Mg^{2+} and Ca^{2+}, respectively, EDTA and EGTA have been used successfully to inhibit these phosphatases by chelating the divalent cations. However, to effectively distinguish one phophatase from the others, a combination of these inhibitors is often required.

Function of Ser/Thr Phosphatases

As reversible phosphorylation regulates almost all cellular activities in eukaryotes, functions of protein phosphatases encompass the whole spectrum of cell and developmental biology.

PP1/2A

When associated with different regulatory subunits, the same phosphatase can perform different functions. This is best illustrated by the function of PP1 in animal

cells. Glycogen metabolism may be the first pathway in which a regulatory role of a protein phosphatase was recognized (21a). Neuronal and hormonal control of glycogen metabolism is mediated by changes in the phosphorylation state of glycogen phosphorylase, phosphorylase kinase, and glycogen synthase. The glycogen synthase is activated, and enzymes for glycogen hydrolysis are inhibited, by phosphatases. PP1 is the major phosphatase in the regulation of glycogen metabolic enzymes. For this purpose, a specific regulatory subunit RG1 targets the catalytic subunit PP1c to the glycogen particles and stimulates the activity of PP1c toward glycogen-bound enzymes such as glycogen synthase and glycogen phosphorylase, increasing glycogen accumulation. The M110 subunit recruits PP1c to the myofibrils and increases the activity of PP1 to dephosphorylate myosin light chain, thereby inducing muscle relaxation (59). Another putative regulatory subunit, PNUTS (phosphatase 1 nuclear targeting subunit), is implicated in targeting PP1c into the nucleus where PP1 dephosphorylates a number of substrates, including histone H1 (3), retinoblastoma protein (96, 97), and nuclear lamin (124), which all affect cell cycle progression. Nuclear localization of PP1 may also regulate RNA splicing because PP1 regulates the distribution of pre-mRNA splicing factors (93). In addition, PP1 plays a critical role in neuronal activity by regulating ion channel activities (140).

Like PP1, PP2A catalytic subunits are also located in various compartments in a cell and play diverse functions ranging from metabolism, to cell cycle control, to telomerase activity. A specific isoform of PP2Ac is essential for early embryonic development (44). A mutation in the 65-kDa A subunit of PP2A is found in numerous cancers, implicating PP2A as a tumor suppressor (131). In fission yeast, two catalytic subunit genes encode PP2A activity. Deletion of both genes is lethal, and one gene is responsible for negative regulation of mitosis (66). Further evidence for this notion came from studies in *Xenopus* oocytes where PP2A inhibits histone H1 kinase and hence cell cycle progression (72). At least one example shows inhibition of telomerase activity by PP2A. Incubation of cell nuclear telomerase extracts with protein phosphatase 2A (PP2A) abolished telomerase activity. In contrast, cytoplasmic telomerase activity was unaffected, and protein phosphatases 1 and 2B were ineffective (75). A number of studies have shown that PP2A regulates a variety of ion channels in animal cells (48, 108, 145).

Our knowledge of the function of PP1 and PP2A in higher plants comes mostly from studies that use pharmacological approaches. The application of specific inhibitors such as okadaic acid and others in a specific cellular or developmental process provides much of the information regarding the importance of a subclass of phosphatases in plants. Because it is difficult to distinguish PP1 from PP2A by using okadaic acid, most studies defined the okadaic acid–sensitive process as involving PP1/PP2A. For example, PP1/2A is required for activation of metabolic enzymes including nitrate reductase (NR) and sucrose phosphate synthase (SPS), which play a key role in carbon and nitrogen metabolism. The current view on the mechanism and significance of reversible phosphorylation in regulation of these enzymes was recently reviewed (126a). Inhibitor analysis also indicates that

PP1/2A is involved in ion channel regulation, gene expression, and developmental processes that are reviewed above (78, 78a, 113a).

Studies using genetics approaches have shown that PP2A and its regulatory subunits are crucial for plant developmental processes and hormonal signal transduction. The *RCN1* gene encodes a 65-kDa A subunit of PP2A (42). Mutation in the *RCN1* gene causes a defect in root curling and hypocotyl hook formation in *Arabidopsis*. In addition, auxin transport in the *rcn1* mutant is more sensitive to the polar transport inhibitor naphthylphthalamic acid (42). Further analysis has linked the mutation in the *RCN1* gene with a reduction in PP2A activity in the protein extract of the *rcn1* mutant and a defect in differential cell elongation (28). In addition, the gravity response and basipetal auxin transport appear to be altered in the *rcn1* mutant (104). These findings show that PP2A plays a role in the regulation of auxin transport. The *Arabidopsis ton2* mutants display abnormalities in cell shape that result in changes in overall plant morphology (15). Cellular analysis reveals abnormalities of the cortical microtubular cytoskeleton; these include disorganization of the interphase microtubule array and lack of the preprophase band before mitosis. The *TON2* gene encodes a protein with a C-terminal domain that is highly related to the B″ subunit of PP2A in higher plants. The TON2 protein interacts with an *Arabidopsis* type A subunit of PP2A in the yeast two-hybrid system, implicating TON2 and other subunits of PP2A in the control of cytoskeletal organization in plants (15).

PP2C

Several studies in yeast and mammalian systems suggest that PP2C is involved in stress signal transduction but the mechanisms are different. In mammalian cells, PP2C regulates stress signaling by modulating p38 and the JNK MAPK pathway (122), whereas in fission yeast, PP2C regulates stress response independently of the stress-activated kinase cascade (40). PP2C also plays a role in regulation of the cystic fibrosis transmembrane conductance regulator, a chloride channel (81). A novel form of PP2C has recently been isolated from *Dictyostelium* that is essential for cell type differentiation (7). One PP2C isoform from *Caenorhabditis elegans* has a unique N-terminal extension required for the process of sex determination (52).

As described above, plants contain a much larger and more diverse family of PP2C than animal and yeast systems. One PP2C subfamily, ABI1 and ABI2, was identified as a component in the ABA signal transduction pathway (73, 74, 92, 105). The identified mutations in *ABI* genes result in dramatically decreased sensitivity of the mutant plants to ABA in several aspects including stomatal regulation. The *abi* mutants display enlarged stomatal apertures in the presence of ABA that normally causes stomatal closure. Because stomatal movement is controlled by ion channels in the plasma membrane and tonoplast of guard cells (132), studies were directed to ion channel regulation by ABA in both wild-type and *abi* mutants. In the wild- type *Arabidopsis* plants, an anion channel involved in stomatal closure is activated by ABA, whereas in mutant plants this anion channel becomes insensitive

to ABA, thereby blocking ABA-induced stomatal closure (102). In tobacco plants, however, expression of the same *abi* mutant protein has a different effect on ion channels in guard cells: The anion channel is not affected by abi mutant protein, but both outward and inward K^+ channels become less sensitive to ABA application (6). These studies suggest that different plants may utilize distinct molecular components to respond to ABA. Although ABI1 and ABI2 both encode a PP2C, they appear to function differently in guard cells (102). Note that both *abi* mutations are dominant, suggesting that they may be gain-of-function mutations. As a result, it has been difficult to define the mechanism of ABI1/2 action in ABA signaling using the original mutants. In a transient system, ABI1 appears to serve as a negative regulator of ABA-induced gene expression in maize mesophyll cells (109). A recent genetic analysis confirmed that ABI1 functions as a negative regulator in ABA signal transduction (46, 89). An ABI1/2-related PP2C in *Arabidopsis* (AtPP2CA) has been shown to be regulated by stress signals including drought, cold, high salt, and ABA (121). Transgenic antisense plants exhibited enhanced freezing tolerance and increased sensitivity to ABA during the development of frost tolerance and seed germination. Expression of cold- and ABA-induced genes is also enhanced in the antisense plants, indicating that AtPP2CA is a negative regulator of ABA responses during cold acclimation. A PP2C member has been shown to interact with an inward potassium channel, implicating PP2C in the regulation of ion channels (130).

Another PP2C member, KAPP, was identified as a partner protein for a receptor-like protein kinase (116). Recent studies suggest that KAPP regulates the function of at least some receptor-like kinases (RLKs). For example, KAPP interacts with CLV1, an RLK required for normal development of shoot meristem in *Arabidopsis* (20). Biochemical studies show that KAPP can dephosphorylate autophosphorylated CLV1 (117, 136). In addition, overexpression of KAPP in transgenic *Arabidopsis* mimics the phenotype of the *clv1*mutant (136). Reduction of KAPP levels suppressed the *clv1* phenotype (117). Both studies suggest that KAPP serves as a negative regulator of CLV1 signaling pathway, possibly by acting directly on the receptor-like kinase. Biochemical analysis of CLV1 complexes in the wild-type and mutant plants shows that CLV1, CLV2, CLV3, KAPP, Rop (a small GTP-binding protein), and possibly other proteins are present in the same complex (127, 128). Because KAPP interaction with RLKs depends on the autophosphorylation of RLKs, KAPP interaction may occur after activation of the RLK by its ligand, and dephosphorylation of RLK by KAPP may serve as an "off" switch in the RLK signaling process. To date, several hundred receptor-like kinases have been identified from higher plants. KAPP and KAPP-like protein phosphatases may be an important component of receptor kinase-mediated signaling pathways.

As described above, PP2C plays a role in MAPK regulation in animals. Using the yeast strain with a defect in the MAPK pathway for pheromone response, an alfalfa cDNA clone was identified by its ability to suppress the MAPK pathway (90). The gene encodes a PP2C member, MP2C, that acts at the level of MAPK kinase kinase (Ste11) in yeast. This PP2C inactivates the stress-activated MAPK

(SAMK) in vitro. The MP2C mRNA levels are induced by wounding that activates the SAMK pathway (90). It is possible that MP2C also regulates the SAMK pathway in vivo by dephosphorylating and inactivating SAMK and/or its regulators, although experiments have yet to be performed to confirm this hypothesis.

PROTEIN TYROSINE PHOSPHATASES

Nomenclature and General Properties of PTPs

Tyrosine phosphorylation is a well-established mechanism for cellular regulation in animals. In particular, protein-tyrosine phosphorylation serves as a common mechanism by which growth factors and cytokines regulate cellular proliferation and differentiation in animals (23, 57). The level of tyrosine phosphorylation in normal cells is determined by the balanced activity of protein tyrosine kinases (PTKs) and PTPs. Even the slightest tipping of this balance may result in cancer or abnormal cell death. As a result, a typical animal cell expresses a large number of PTKs and PTPs to fine-tune cellular proliferation/differentiation. A recent estimate from the nearly completed human genome sequence revealed more than 100 PTPs (S. Luan, unpublished information). Based on phosphoamino acid specificity, PTPs can be further divided into two large groups: tyrosine-specific PTPs and dual-specificity PTP (DsPTPs). Tyrosine-specific PTP dephosphorylate phosphotyrosine but not phosphoserine/threonine, whereas the DsPTPs dephosphorylate both (118, 126). The tyrosine-specific PTPs can be further categorized into receptor-like and intracellular groups. The receptor-like PTPs generally have an extracellular putative ligand-binding domain, a single transmembrane region, and one or two cytoplasmic PTP domains. The intracellular PTPs, exemplified by PTP1B and SHP1, contain a single catalytic domain and various amino or carboxyl terminal extensions including SH2 domains that have targeting or regulatory functions. Examples of dual-specificity phosphatases include the MAP kinase phosphatases, the cell cycle regulator cdc25 phosphatases (32), and the tumor suppressor PTEN (85) (Figure 3). All PTPs are characterized by their sensitivity to vanadate, ability to hydrolyze p-nitrophenyl phosphate, insensitivity to okadaic acid, and lack of metal ion requirement for catalysis.

Although the overall protein sequences of tyrosine-specific PTPs and DsPTPs share little homology, they all contain a signature motif in the catalytic core: (V/I)HCXAGXGR(S/T)G that harbors an essential cysteinyl residue required for formation of the phosphoenzyme intermediate. In addition, the secondary and tertiary structures of all PTPs bear high similarity in the catalytic region. The substrate specificity is determined by sequences outside the catalytic domain (26, 126). More recently, the DsPTPs are often referred to as DSPs (dual-specificity phosphatases) for convenience and to reflect the fact that these enzymes work on both phosphotyrosine and phosphoserine/threonine. DsPTPs/DSPs share the same signature motif in the catalytic core and a very similar structural fold with all PTPs, but they do not share any sequence or structural similarity with Ser/Thr phosphatases. For

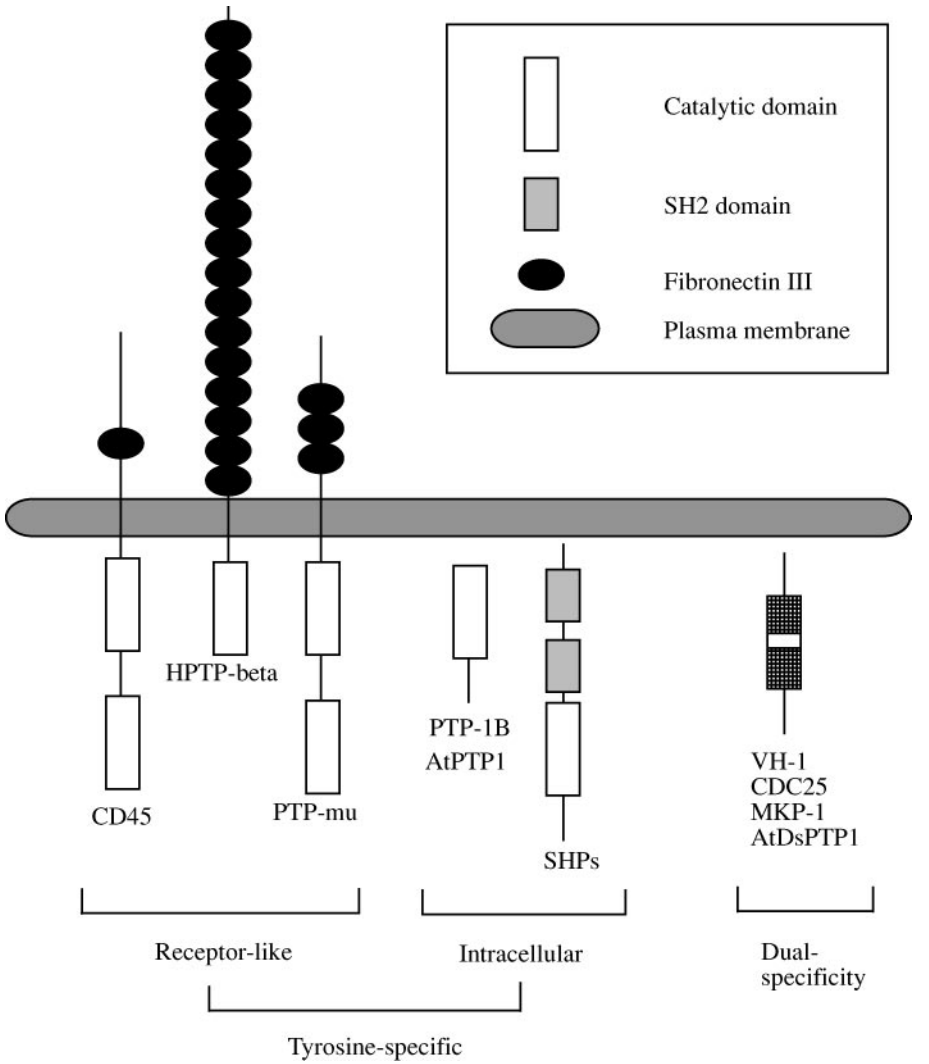

Figure 3 Structural domains of some representative protein tyrosine phosphatases. Receptor-like PTPs often have two catalytic domains, whereas intracellular PTPs have only one. The DsPTPs are different in overall sequence but always contain the catalytic domain signature motif (as indicated by an open window in the center of the molecule). Plant intracellular (AtPTP1) and dual-specificity PTP (AtDsPTP1) are listed.

consistency with earlier literature in plant PTP studies, we refer to the dual-specificity phosphatases as DsPTPs in this review.

Crystal structures of both tyrosine-specific and dual-specificity PTPs have been solved and provide insights into the structural basis for catalysis and substrate specificity (10). Despite the sequence divergence between these two subfamilies of PTPs, all crystal structures solved so far display essentially the same core structural features: the central four-stranded parallel beta-sheet surrounded on both sides by one and four alpha-helices. The DsPTPs appear to be truncated versions of tyrosine-specific PTPs. The signature motif residues are found within a single loop, nestled at the base of a cleft on the surface of the protein. The essential cysteine is in the position for nucleophilic attack on an incoming phosphotyrosyl residue. The remaining residues of the core motif function to increase the nucleophilicity of the catalytic cysteine and to bind to and position the phosphate group. The arginyl residue in the signature motif is particularly important for this function. The depth of the catalytic cleft determines the substrate specificity and is set by an invariant tyrosyl residue in the tyrosine-specific PTPs. Therefore, only phosphotyrosine (not phosphoserine/threonine) is long enough to access the catalytic cysteine (26, 126).

Several factors regulate the activity of PTPs in animals. Many PTPs in animals are expressed in a tissue-specific or developmental stage–specific manner, and their expression levels are often modulated by environmental conditions. These expression patterns are closely related to their specific functions (95). For example, CD45 is expressed preferentially in the lymphocytic tissues in mammalian systems and is important in signal transduction in T and B cells. Several receptor-like PTPs are selectively expressed in the neuronal tissues in Drosophila and are involved in neural cell adhesion. A cytoplasmic SH2-containing PTP, SHP-1, is expressed at a high level in the hematopoietic cells and plays a critical role in lymphoid cell differentiation (95). DsPTPs play a role in the regulation of MAPK pathways, and their expression is often induced by mitogenic and stress signals (63, 65, 119). This is also true in yeast where tyrosine-specific PTPs are involved in MAPK regulation and are responsive to stress signals (137). In addition, Msg5, a dual-specificity PTP involved in pheromone signal transduction, is also regulated by pheromone application (31).

All PTPs contain a catalytic cysteine that must be in a reduced form for phosphatase activity. Oxidation of cysteine under oxidative stress reversibly inactivates the PTP (27). Perhaps the most common mechanism of regulation is to direct the localization of the catalytic activity in a cell. The receptor-like PTPs in animal cells all contain a transmembrane domain for plasma membrane targeting (95, 118). Some DsPTPs in mammalian cells such as MKP1 and MKP2 are strictly localized to the nucleus (49, 106).

Plant PTPs and Their Function

Although it is well established that tyrosine phosphatases play critical roles in animals, demonstrating that tyrosine phosphatases exist in higher plants has been

accomplished only recently. The first bona fide tyrosine phosphatase (AtPTP1) was characterized in *Arabidopsis* in 1998 (139), 10 years after PTPs were identified in animal cells (125). In the same year, the first dual-specificity phosphatase (AtDsPTP1) was also identified from *Arabidopsis* (51). Other plant species clearly contain PTPs similar to AtPTP1 (38). After the *Arabidopsis* genome sequence became available, sequence searches identified at least a dozen more genes that encode proteins with a typical catalytic core motif of PTPs (66a; L. Sokolov, R. Gupta, G. Pandey, and S. Luan, unpublished results), although the enzymatic properties of their protein products must still be determined. The tyrosine-specific PTP from *Arabidopsis*, AtPTP1, appears to be a member of cytoplasmic PTPs. It contains the catalytic core motif at the C-terminal region and an N-terminal extension of unknown identity. The recombinant AtPTP1 protein specifically dephosphorylates phosphotyrosine but not phosphoserine/threonine in protein substrate. Like other PTPs, the cysteinyl residue in the signature motif is essential for catalytic activity of recombinant AtPTP1. Specific inhibitors for PTPs such as vanadate inhibit AtPTP1 activity (139). The dual-specificity PTP from *Arabidopsis*, AtDsPTP1, is similar to the animal MKPs and is capable of dephosphorylating both phospho-tyrosine and phospho-serine/threonine (51). Other than the signature motif in the catalytic core (of about 10 amino acids), AtPTP1 and AtDsPTP1 share little sequence homology. This is also true with other PTP-like genes recently identified from the *Arabidopsis* genome (66a; L. Sokolov, R. Gupta, G. Pandey, and S. Luan, unpublished results). For example, a tyrosine phosphatase recently identified from *Arabidopsis* and tomato (37) represents a subgroup of PTPs with similarity to laforin in mammals. This laforin-like PTP interacts with an SNF1-related protein kinase, although the functional significance of this interaction remains to be determined (37). Identification of these PTPs provides a stepping stone to better understanding of the functional significance of tyrosine phosphorylation in higher plants.

A diverse array of functions have been identified for PTPs in animal cells (95). The receptor-like PTPs play crucial roles in cell-cell interaction and adhesion. The intracellular tyrosine-specific PTPs are critical "partners" for tyrosine-specific kinases in regulating the tyrosine phosphorylation status of many proteins (95). To date, a typical tyrosine kinase has not been identified from plants or fungi, consistent with the finding that these organisms have fewer tyrosine phosphatases. Although at least 100 PTPs are encoded by the human genome, there are only about half a dozen in yeast and approximately 20 putative PTPs in *Arabidopsis*. Among these 20 or so genes, only one encodes a tyrosine-specific PTP and other genes may encode DsPTPs (66a; L. Sokolov, R. Gupta, G. Pandey, and S. Luan, unpublished results). Perhaps the common theme in PTP function in all eukaryotes concerns the function of DsPTPs.

A family of DsPTPs plays a critical role in the regulation of MAPK pathways. MAPK family members are essential components in many signaling pathways, including the growth factor receptor signaling pathways and the stress response pathways in mammalian cells. For activation, MAPK requires phosphorylation of both a threonine and a tyrosine residue in the kinase activation domains (1).

A significant body of literature has demonstrated the function of DsPTPs in the dephosphorylation and inactivation of MAPKs (63, 64). Studies in yeast indicate that both tyrosine-specific and DsPTPs play a role in regulating MAPKs (138, 142). In mammalian cells, a variety of DsPTPs exhibit activity toward activated forms of MAPKs both in vivo and in vitro. In each case, dephosphorylation of a MAPK isoform by a DsPTP results in loss of MAPK activity. Dual-specificity PTPs have rather strict substrate specificity toward different isoforms of MAPKs (63, 64). As a result, multiple DsPTPs are often found in a single cell type that harbors multiple MAPK isoforms. Therefore various combinations of MAPK and DsPTP isoforms may constitute modules in distinct signaling pathways in a single cell type (63, 64). Studies in both budding and fission yeast demonstrate that tyrosine-specific PTPs are also important for MAPK regulation. For example, the osmosensing pathway in budding yeast is initiated by a two-component histidine kinase, Sln1, that activates a kinase cascade involving a MAPK member, Hog1. Both biochemical and genetic analyses indicate that Hog1 is inactivated by PTP2 (137). A similar MAPK-mediated pathway links environmental changes to the regulation of mitosis in fission yeast. In this pathway, Spc/Sty1 MAPK is inactivated by tyrosine-specific PTPs including both Pyp1 and Pyp2 (112).

Because a typical tyrosine kinase is not found in higher plants, the importance of tyrosine phosphorylation in plant cells was controversial until the finding of tyrosine phosphatases and MAPKs. The MAPK cascade is a critical component in numerous signal transduction pathways in higher plants [for reviews, see (91, 94, 123, 144)]. Consistent with the diversity of MAPK functions, a large number of genes in the *Arabidopsis* genome have been identified that encode MAPKs (about 20 genes), MAPK kinases (MAPKKs) (10 genes), and MAPKK kinases (MAPKKKs) (about 60 genes) (123, 144). While different MAPKs may play distinct functions, at least in some cases, many distinct signals appear to converge at the same MAPK. For example, SIPK in tobacco or MPK6 in *Arabidopsis* is activated by a number of different pathogenic stimuli and abiotic stress signals (123, 144). These studies demonstrate that MAPK activation is a common early step in the response to stress and hormonal and developmental signals.

As discussed above, MAPK activation in animal and yeast requires dual phosphorylation at a closely spaced pair of threonine and tyrosine residues. Early studies show that activation of plant MAPKs is associated with phosphorylation of tyrosine residue(s) in the protein (e.g., 120). A detailed biochemical analysis was recently performed using AtMPK4 (an Arabidopsis MAPK) and AtPTP1 to show that tyrosine phosphorylation of AtMPK4 protein is required for its activation (54). In this study, AtMPK4 was shown to be phosphorylated at a tyrosine residue during the activation process. Dephosphorylation of this tyrosine by AtPTP1 resulted in loss of kinase activity (54). Because tyrosine phosphorylation of MAPK protein is required for activation of the kinase, dephosphorylation of the tyrosine residue often occurs to bring the enzyme back to an inactive state. A signal activates a

MPAK rapidly (often reaching peak activity within a few minutes) and the MAPK activity subsequently returns to the basal level to quench the signaling process. Such transient on-and-off switching is very important for the physiological process regulated by MAPKs. Prolonged or constant activation of a MAPK cascade can have detrimental consequences for the cell, as best illustrated by tumorigenesis in mammalian cells where MAPK is constantly on (86). In budding yeast, the Hog1 pathway is required for osmotic stress tolerance yet a constant MAPK activity of Hog1 actually renders yeast hypersensitive to the stress (137). The protein phosphatases, especially PTPs and DSPs, play a critical role in turning off the activity of MAPKs and are essential for maintaining the precise kinetic pattern of MAPK activation and inactivation. This paradigm also applies to regulation of MAPKs in plants and is consistent with the presence of a number of PTPs in higher plants.

Following in vitro biochemical analysis of AtPTP1 and AtDsPTP1 in the regulation of MAPK activities (51, 54), evidence from genetic studies also began to accumulate to demonstrate a physiological link between MAPK activation and PTP activity *in planta*. In one such study (129), screens for UV-sensitive mutants in *Arabidopsis* led to identification of a putative DsPTP, named AtMKP1, as essential for UV resistance. The mutant plants appear to have higher MAPK activity under UV illumination, implicating AtMKP1 as a negative regulator of MAPK(s) in UV response (129). Consistent with the in vitro studies, AtPTP1 function has been linked to inactivation of MAPKs in vivo. The activation of several MAPK isoforms is significantly more robust in the *Atptp1* knockout mutant than in the wild-type plants, whereas overexpression of AtPTP1 results in delayed activation of these kinases in the transgenic plants (R. Gupta & S. Luan, unpublished data). Although it remains to be determined whether AtDsPTP1 and other putative DsPTPs play a role in MAPK activity control in vivo, MAPK regulation clearly serves as a major target for PTP function in plants as well as in animals and fungi.

Using several specific PTP inhibitors, MacRobbie (84a) demonstrated that a PTP activity is essential for stomatal closure induced by four different factors including ABA, external calcium, darkness, and H_2O_2. Clearly, each of these factors may cause changes in intracellular calcium homeostasis that, in turn, alter the K^+ channel activity in both plasma membrane and tonoplast (132). ABA signaling in guard cells involves a calcium-dependent pathway. H_2O_2 serves as a downstream messenger for ABA and activates an inward calcium channel in the plasma membrane of guard cells (101). Darkness elicits calcium fluxes across chloroplast membranes that may also affect calcium concentration in the cytosol (107a). It is therefore possible that the PTP component lies downstream from the calcium channel and upstream of K^+ channel activity in the plasma membrane and tonoplast. The study by MacRobbie identified efflux from the vacuole, but not fluxes across the plasma membrane, as the target process for the PTP inhibitors, suggesting that a protein specifically related to tonoplast K^+ channels is

regulated by tyrosine phosphorylation and dephosphorylation. As discussed above, the major targets for PTPs in plants are MAPKs that participate in numerous signaling processes. However, MAPK does not appear to regulate the ABA-induced K^+ efflux from the vacuole, as a MAPK kinase inhibitor did not interfere with the process (84a).

Other non-MAPK targets of tyrosine phosphorylation include actin and profilin (50, 62a). MAPKs are autophosphorylated and phosphorylated by MAPKK at the tyrosine residue. One intriguing question concerns the molecular nature of the kinase(s) that phosphorylates actin and profilin at the tyrosine residues. Are they tyrosine-specific kinases or dual-specificity kinases? Identification of these kinases will open a new avenue to the understanding of tyrosine phosphorylation in plant cells.

An important subclass of DsPTP is CDC25 protein phosphatase that is required in cell cycle progression. As its name indicates, CDC25 was initially identified as a *cdc* gene in which a loss-of-function mutation halts cell cycle in yeast. Homologues of CDC25 have subsequently been found in all animal systems examined. Biochemical and genetic analyses have defined a role for CDC25 in the activation of CDC2 protein kinase (32). Inactivation of CDC2 involves phosphorylation of a specific tyrosine residue in the protein. The phosphatase activity of CDC25 is responsible for dephosphorylating the inhibitory tyrosine residue and activating CDC2 kinase. Despite evidence that yeast CDC25 activates cell division in plant cells (143), a CDC25 homologue has not been identified in any plant examined so far. Other DsPTP(s) may substitute for the function of CDC25 in the regulation of plant CDC2 kinase.

A subgroup of DsPTPs has recently been identified for its function as a tumor suppressor. This subclass, represented by PTEN/MMC1, contains the catalytic core of PTPs and has a structural domain with high similarity to a cytoskeleton protein, tensin (85). Biochemical studies show that PTEN has a low dual-specificity phosphatase activity toward protein substrates but a high phosphatase activity against phosphotidylinositol 3,4,5-triphosphate (PIP3). The phosphatase activity specifically dephosphorylates the 3′ phosphate group in PIP3. The finding of PTEN as a tumor suppressor is thus consistent with the fact that production of PIP3 by PI3 kinase (PI3K) is a key step in transforming the cell into tumor (39). Further studies have confirmed that PTEN functions in the PI3K pathway to balance the activity of PI3K (85). Several genes identified from *Arabidopsis* encode proteins with high homology to PTEN/MMAC1 (51a). One PTEN-like gene, AtPTEN1, is specifically expressed in pollen grains and is essential for pollen development (51a). In contrast to the major function of PTEN in cell division in animals, AtPTEN1 appears to be required for pollen maturation after cell divisions (51a). Nevertheless, PTEN in animals also has other functions other than tumor suppression (85). Further study to identify the physiological substrate of plant PTENs will shed light on the mechanism underlying PTEN function in plant cells. In addition to AtPTEN1, mutants of other PTEN-like genes have also been isolated in

Arabidopsis and should provide information on their function in plant growth and development.

FUTURE PERSPECTIVES

The biochemical properties of protein phosphatases have been highly conserved among eukaryotic organisms. Future effort will be directed to functional analysis of these enzymes in the context of plant physiology and development. A combination of genetic, cell biological, and biochemical approaches will be required to elucidate the function of protein phosphatases and the molecular mechanism(s) underlying their action in plant cells. A good example is given by the study of ABI1 function. Genetic screens identified the *abi* mutants that exhibit ABA insensitivity. Molecular cloning and biochemical analysis have demonstrated the molecular nature of ABI1 as PP2C type protein phosphatase (13, 73, 92). A transient cellular assay complementing the genetics approach points to a possible role for ABI1 as a negative regulator of ABA signaling (109), a finding further confirmed by genetic studies (46). Cell biological approaches linked ABI1 to ABA regulation of ion channels in guard cells, a further step in delineating the molecular mechanism of ABI1 function in ABA signaling pathways (6, 102). Since plant protein phosphatases were last reviewed in this series (113a), the most significant progress in the field has been the molecular characterization and functional analyses of tyrosine phosphatases in plants. This advance has helped us to reconsider the function of tyrosine phosphorylation/dephosphorylation in plant cell signaling (79a). Nevertheless, a significant difference is noted between animals and plants regarding the function of protein phosphorylation in signal transduction. Figure 4 (see color insert) compares two predominant pathways in animals and plants. Receptor tyrosine kinases serve as receptors for peptide growth factors in animals whereas Ser/Thr protein kinases (RLKs) serve as receptors for peptide regulators in plants. Corresponding phosphatases (PTP versus Ser/Thr PP) regulate the activity of receptor kinases. Following such a major difference, an overlapping paradigm utilizes the MAPK cascade to further transmit the signal to the unique cellular responses in animal and plant cells. Similarly, PTP and DsPTP are utilized in both animals and plants to regulate MAPK activities. Since the completion of sequencing of the *Arabidopsis* genome and identification of all the genes encoding putative protein phosphatases, a number of laboratories are trying to make the connection between the sequence information and the functional significance in plants. Reverse genetics procedures such as T-DNA insertional mutants and RNA interference (RNAi) techniques will be especially important for such an endeavor (19, 51b, 133). Despite the structural similarities in all eukaryotes, protein phosphatases, like their partner kinases, perform distinct functions in various organisms that have different developmental programs. Plant biologists will be responsible for dissecting the unique functions of protein phosphatases/kinases in plant growth and development.

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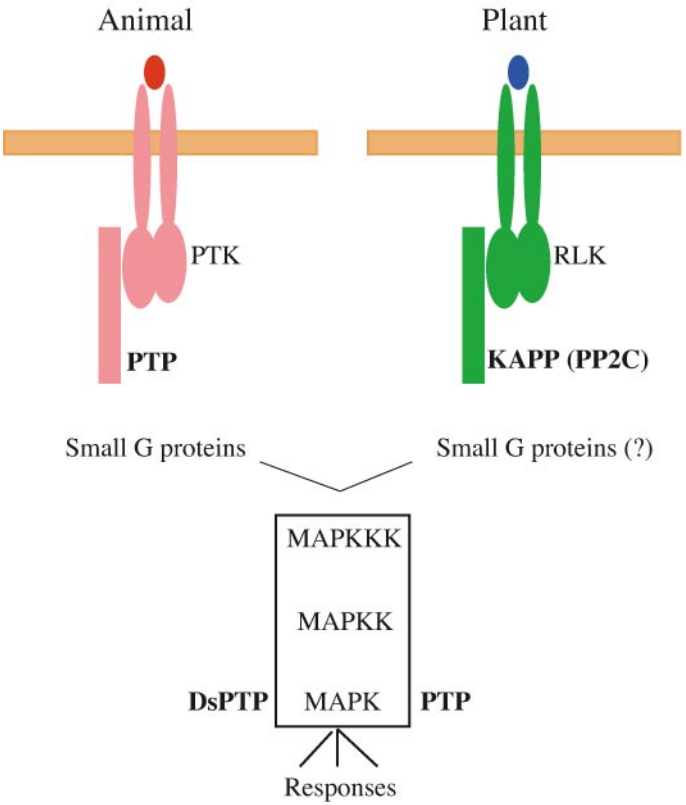

Figure 4 Comparison of animal growth factor pathway with plant peptide regulator pathway. The growth factor receptors are protein tyrosine kinases (PTK) and plant peptide receptors are Ser/Thr kinases (RLK). Dimerization and autophosphorylation may present a common mechanism for activation by their ligands and dephosphorylation by PTP or Ser/Thr phosphatase for inactivation. The MAPK cascade follows either PTK or RLK activation to transmit signals to cellular responses. Tyrosine phosphatases (PTP and DsPTP) are involved in inactivation of the MAPK cascade in plants as well as animals.