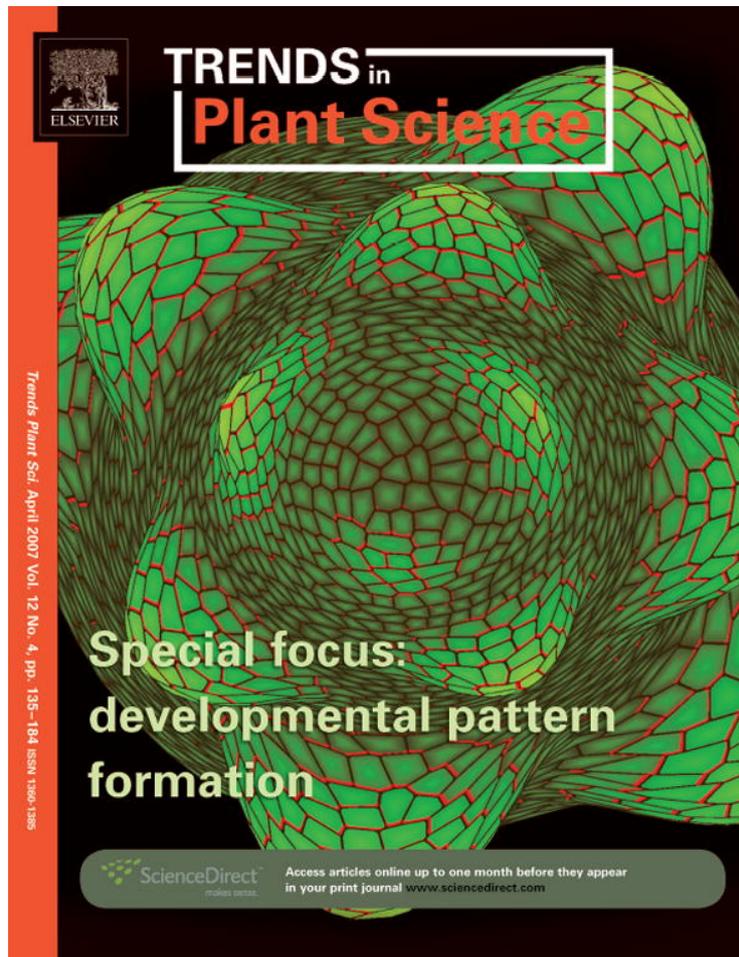


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Arabidopsis PPP family of serine/threonine phosphatases

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Serine/threonine-specific phosphoprotein phosphatases (PPPs) are ubiquitous enzymes in all eukaryotes, but their regulatory functions are largely unknown in higher plants. The Arabidopsis genome encodes 26 PPP catalytic subunits related to type 1, type 2A and so-called novel phosphatases, including four plant-specific enzymes carrying large N-terminal kelch-domains, but no apparent homologue of the PP2B family. The catalytic subunits of PPPs associate with regulatory protein partners that target them to well defined cellular locations and modulate their activity. Recent studies of phosphatase partners and their interactions have directed attention again to functional dissection of plant PPP families, and highlight their intriguing roles in the regulation of metabolism, cell cycle and development, as well as their roles in light, stress and hormonal signalling.

Protein phosphatase families

Protein phosphorylation and dephosphorylation represent a major form of reversible post-translational modification that controls many regulatory circuits in eukaryotes by modulating the conformation, activity, localization and stability of substrate proteins [1]. Whereas eukaryotic protein kinases appear to be derived from a single ancestor [2], protein phosphatases are thought to be recruited from various origins [3,4]. According to their substrate specificity, protein phosphatases are classified into families of Ser/Thr [serine/threonine-specific phosphoprotein phosphatase (PPP) and metal ion-dependent protein phosphatase (PPM)], Tyr [phosphotyrosine phosphatase (PTP)], and dual specificity phosphatase (DSP) Ser/Thr- and Tyr-specific enzymes (Figure 1). DSPs and PTPs use an active Cys residue to catalyse analogous hydrolytic reactions, and their catalytic pockets share common structural features. Ser and Thr side-chains are dephosphorylated in a bicentral metal ion-assisted reaction by PPPs (which include subfamilies of PP1, PP2A and PP2B, and novel phosphatases) and by PPMs (also known as PP2Cs). Except for PTPs that play crucial roles in animal cell differentiation, plants seem to use members of all known phosphatase families [5,6].

This review focuses on the regulatory functions of the Arabidopsis PPP family of Ser/Thr phosphatases, including recent advances in functional studies of novel enzymes (PP4 to PP7 and PPP kelch phosphatases).

Catalytic subunits of Arabidopsis PPPs are encoded by 26 genes (Figure 2). PP2B (also known as calcineurin or PPP3), which is an important Ca²⁺-regulated PPP in other eukaryotes, is absent in plants. PPPs occur in various complexes with regulatory and targeting subunits, and modulate the activity and specificity of catalytic subunits, and target the enzymes to specific locations [7,8]. Recent progress in functional identification of phosphatase subunits and interacting factors has highlighted the central roles of plant PPPs in cellular signalling.

Protein phosphatase 1

Mammalian PP1s preferentially dephosphorylate the β subunit of phosphorylase kinase and are specifically inhibited by the heat-stable inhibitors I-1 and I-2. By contrast, PP2As dephosphorylate the α subunit of phosphorylase kinase and are insensitive to I-1 and I-2. Okadaic acid is a more potent inhibitor of PP2A than of PP1. Based on these distinctive features, PP1 phosphatases were identified in several plant species [9]. Arabidopsis PP1 isolated by microcystin affinity chromatography is inhibited by okadaic acid and inhibitor I-2, but shows greater sensitivity to microcystin-LR, nodularin, and calyculin A compared with the mammalian PP1s. Mass spectrometry analysis of purified Arabidopsis PP1s revealed between four and six catalytic subunit isoforms [10], whereas heterologous probes identified eight TOPP (Type One Protein Phosphatase) genes [11,12]. TOPP7/AtPP1bg has now been designated TOPP6 and At5g43380 has been renamed TOPP7. Sequencing the Arabidopsis genome revealed a ninth gene for a TOPP8 isoenzyme (PP1 iso8), which is also expressed [13]. Plant TOPP and animal PP1 sequences show significant phylogenetic divergence [14]. Because of the heterogeneity of their N- and C-terminal domains, TOPPs can also be classified into distinct subfamilies (Figure 2). All TOPPs are likely to be active enzymes because they carry all the conserved residues that make up the active site of rabbit PP1 α [15].

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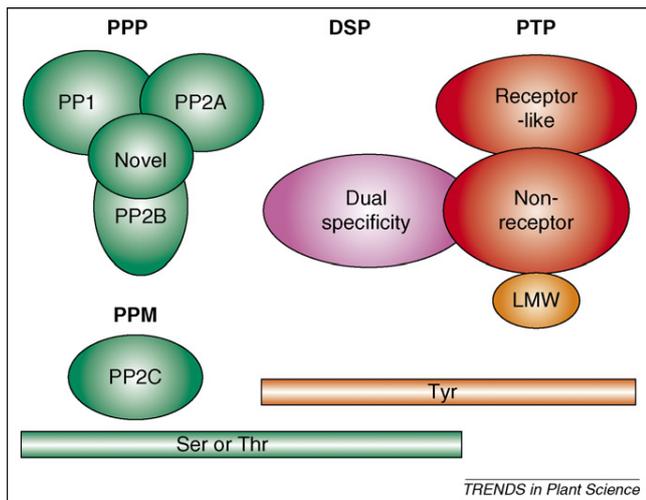


Figure 1. Classification of protein phosphatases according to their substrate specificities. Ser/Thr phosphatases include the phosphoprotein phosphatase PPP and metal ion-dependent PPM families. PPMs are often referred to as PP2Cs. The PPP group includes subfamilies of PP1 (known as TOPP in plants), PP2A, PP2B, and a novel class of phosphatases. PP2Bs are not present in plants. The phosphotyrosine phosphatase (PTP) family contains receptor-like, non-receptor, and low molecular weight (LMW) phosphatases. The catalytic mechanisms of PTPs are closely related to the dual specificity phosphatases (DSPs), which mediate the dephosphorylation of serine, threonine and tyrosine residues.

Several TOPPs are predicted to function in cell cycle regulation. TOPP3 and TOPP6 contain putative CDK2 recognition sites [16], but it is unknown whether they are inhibited by CDK2 kinases. TOPP2 suppresses the budding yeast *glc7* mutation, which is not complemented by TOPP1 and TOPP3 [17]. TOPP1 is a suppressor of the fission yeast *cdc25^{ts}/wee1⁻* mutation, which arrests the cell cycle before mitosis but, unlike TOPP2, it cannot suppress the defect of sister chromatid separation in the *dis2-11* mutant [18,19]. TOPP6/*AtPpbg* complements the *Aspergillus nidulans bimG11* mutation that, like *dis2-11*, causes a temperature-sensitive defect in anaphase [12].

TOPP isoforms are detected in large protein complexes and, hence, are likely to be able to bind several interacting proteins [10]. TOPP1 has been reported to show two-hybrid interaction with AXR3, a member of the AUX/IAA family of transcription factors, which suggests an involvement in auxin signalling (J. Jowett *et al.*, unpublished)*. *Arabidopsis* encodes potential orthologues of some ancient PP1 regulatory subunits, including the inhibitor-2, inhibitor-3 and nuclear SDS22 proteins (Table 1), which form multiple cooperative contacts with the catalytic subunit without the involvement of the conserved PP1-binding RVxF signature [20]. In addition, a putative orthologue of PP1 regulator NIPP-1 (At5g47790), which contains a PP1-binding motif, has also been identified [21]. We found that DDL (At3g20550) corresponds to another NIPP-1 orthologue that carries a forkhead-associated domain [22]. However, whether these NIPP-1-like proteins function as phosphatase regulators is an open question. A database report indicates that the *ddl* mutant has delayed growth, curly leaves, and abnormal flowers, suggesting a

* J. Jowett *et al.* (2003) Identification and characterisation of AXR3-interacting proteins (Poster 343), 14th International Conference on *Arabidopsis* Research, 20–24 June 2003, Madison, WI, USA.

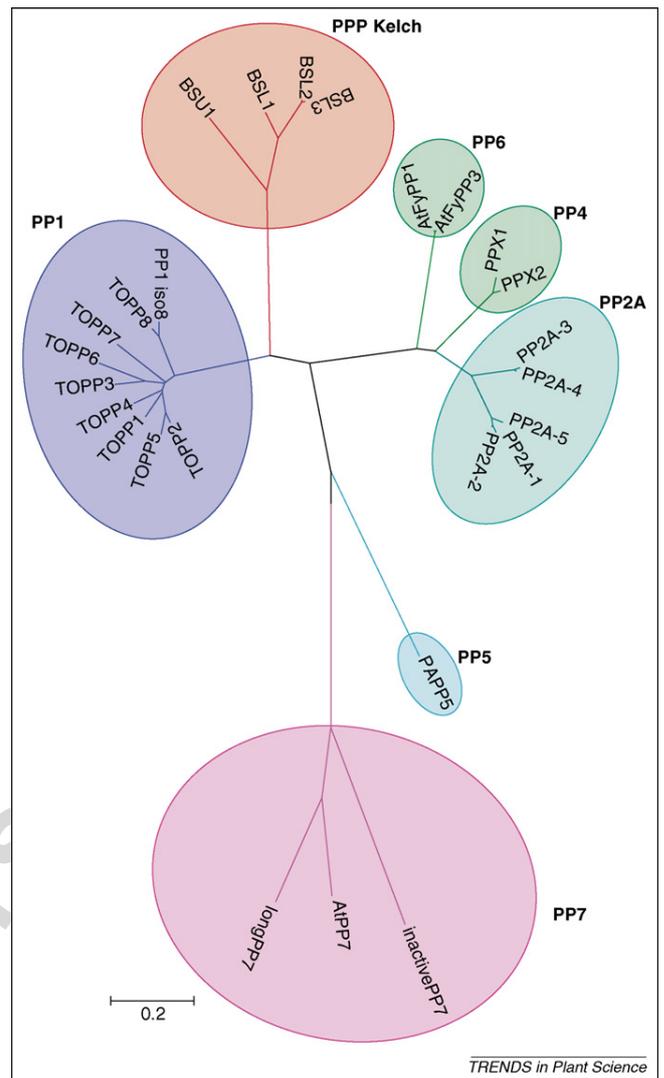


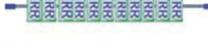
Figure 2. Family tree of *Arabidopsis thaliana* phosphoprotein phosphatase (PPP) catalytic subunits. Phylogenetic and molecular evolutionary analyses of amino acid sequences of PPP catalytic subunits were conducted using the MEGA software version 3.1 [64]. Subfamilies are circled and labelled with abbreviations explained in the text. The bar represents 0.2 amino acid substitutions per site in the primary structure. The AGI numbers of phosphatases are: TOPP1 = At2g29400, TOPP2 = At5g59160, TOPP3 = At1g64040, TOPP4 = At2g39840, TOPP5 = At3g46820, TOPP6 = At4g11240, TOPP7 = At5g43380, TOPP8 = At5g27840, PP1 iso8 = At3g05580, PP2A-1 = At1g59830, PP2A-2 = At1g10430, PP2A-3 = At3g58500, PP2A-4 = At2g42500, PP2A-5 = At1g69960, PPX1 = At4g26720, PPX2 = At5g55260, PAPP5 = At2g42810, AtFyPP1 = At1g50370, AtFyPP3 = At3g19980, AtPP7 = At5g63870, longPP7 = At1g48120, inactivePP7 = At5g10900, BSU1 = At1g03445, BSL1 = At4g03080, BSL2 = At1g08420, BSL3 = At2g27210.

role for DDL in regulating plant development (<http://arabidopsis.org>).

Protein phosphatase 2A

Catalytic (36 kDa) subunits of PP2A phosphatases occur either in association with a 65-kDa regulatory A subunit, or together with a third variable B-subunit in heterotrimeric complexes [7]. The B subunits, which determine the substrate specificity and subcellular localization of PP2As, are classified into 55-kDa B, 54–74-kDa B', and 72–130-kDa B'' subunit families. PP2As have been reported to regulate the activity of key plant metabolic enzymes (e.g. sucrose phosphate synthase, 3-hydroxy-3-methylglutaryl-CoA reductase-1 and nitrate reductase) *in vitro*, but

Table 1. Putative *Arabidopsis thaliana* PP1 regulator proteins

AGI number	Name	1 100 200	Domain ^a
At5g47790	NIPP-1 homologue		FHA
At3g20550	DDL (DAWDLE)		FHA
At5g19680	SDS22 homologue		LRR RI
At5g52200	Inhibitor-2 homologue		IPP-2
At2g31305	Inhibitor-3 homologue		PPI_Ypi1

^aDomains were identified using the Smart search program (<http://smart.embl-heidelberg.de>). Blue boxes represent unstructured elements, pink box shows a region of low complexity.

FHA: forkhead-associated domain, LRR: leucine rich repeat, IPP-2: protein phosphatase inhibitor-2 domain, PPI_Ypi1: *Saccharomyces cerevisiae* protein phosphatase 1 inhibitor Ypi1 domain. The scale shows the number of amino acid residues.

most of these data have yet to be verified *in vivo* [23,24]. *Arabidopsis* has five PP2A catalytic subunits, which are classified into two groups (Figure 2). All PP2A catalytic subunit genes are expressed in all organs, albeit at different levels [25]. Overexpression of putative G-protein coupled receptor *GCR1* was found to stimulate expression of an unidentified PP2A catalytic subunit gene coinciding with the upregulation of gibberellin signalling [26]. The silencing of a subfamily of tomato PP2A catalytic subunit genes enhanced the response to bacterial and fungal avirulence proteins, increased PR gene expression, and localized cell death in stems and leaves, which suggests a potential role for PP2A in defence signalling [27].

Arabidopsis PP2A subunit A isoforms PDF1, PDF2 and RCN1 (Table 2) are composed of tandem HEAT repeats that form a hook-like architecture for binding the catalytic and regulatory B subunits [28]. RCN1 (roots curl in NPA) was first defined as a regulator of auxin transport and gravitropism [29]. In addition to increasing sensitivity to naphthylphthalamic acid (NPA), the *rcn1* mutation results in abscisic acid (ABA) insensitivity at the levels of seed germination, guard cell responses and gene expression, reduces PP2A activity, and leads to defects in apical hook formation, as well as in root and hypocotyl elongation [30,31]. Remarkably, the *eer1* (enhanced ethylene response 1) mutant carries a deletion in the *RCN1* gene. RCN1 interacts *in vitro* with the PP2A-1 catalytic subunit that can bind the Raf-like kinase CTR1, a negative regulator of ethylene signalling [32]. Although RCN1 and PP2A-1 are not phosphorylated by CTR1, it is possible that CTR1 is a substrate of PP2A. RCN1 was also identified as a two-hybrid binding partner of cyclophilin ROC7. However, silencing *ROC7*, unlike the *rcn1* mutation, stimulates root growth without affecting wild-type NPA response [33]. Plants carrying mutations in the PP2A subunit A genes *PDF1* and *PDF2* show only slight phenotypic changes [34]. However, double mutants carrying *rcn1* and either *pdf1* or *pdf2* exhibit severe deficiencies, including abnormal embryogenesis, defective radial cell expansion, dwarfing and sterility. This indicates that RCN1 plays a fundamental role in the regulation of PP2A activity. Because PP2A

subunit A isoforms perform partially overlapping functions, the effects of *pdf1* and *pdf2* mutations are unmasked only when RCN1 is absent.

Little is known about the functions of the two *Arabidopsis* PP2A 55-kDa B subunit isoforms that carry five degenerate WD-40 repeats (Table 2) and show 43–48% amino acid sequence identity with yeast and animal B subunits [35]. *Arabidopsis* has nine PP2A B' subunits, of which only AtB'α and AtB'β contain putative nuclear targeting sequences. The *AtB'γ* gene produces alternatively spliced transcripts. One of these, carrying a 5'-intron, accumulates under heat stress, and the 5'-intron serves as a translational enhancer under normal and heat stress conditions [36]. *Arabidopsis* encodes five PP2B'' subunits (Table 2) that carry putative calcium-binding EF-hand motifs. Furthermore, TON2 (TONNEAU2) represents a B''-like subunit, which shows similarity to the human B''-subunit PR72 and interacts with the PP2A catalytic subunit in two-hybrid assays. The *ton2* mutation affects seedling body organization and causes abnormalities in microtubule assembly and in the formation of the preprophase band before mitosis [37].

In addition to conserved subunits, PP2A enzymes interact with various cellular and viral proteins. For example, yeast Tap42 can associate with PP2A and with Sit4, a closely related phosphatase and key enzyme of the TOR signalling pathway [38]. *Arabidopsis* TAP46 (46 kDa) represents a TAP42 orthologue that shows co-immunoprecipitation with a PP2A catalytic subunit. Transcription of *TAP46* is induced by chilling, which suggests a role for a TAP46–PP2A complex in cold stress signalling [39].

Protein phosphatase 4

PP4 (also known as PPX) phosphatases are structurally related to the catalytic subunits of PP2A and are implicated in the regulation of microtubule-centrosome interactions in animals [40]. *Arabidopsis* encodes two PP4 enzymes, PPX-1 and PPX-2 (Figure 2), which are expressed at low levels in various organs. PPX-1 shows co-localization with ferredoxin in root epidermal cells, and immunolocalization indicates that PPX-1 is a luminal protein in root plastids [41]. However, it is still unclear

Table 2. *Arabidopsis thaliana* PP2A regulatory subunits

AGI number	Alias(es)	Subunit type	1 100 200	Domain ^a
At1g13320	PR65 PDF2	A-subunit		HEAT
At1g25490	ATB β EER1 REGA RCN1	A-subunit		HEAT
At3g25800	PR65 PDF1	A-subunit		HEAT
At1g17720	ATB β	B-subunit		WD40
At1g51690	ATB α	B-subunit		WD40
At1g13460	ATB'θ	B'-subunit		B56
At3g09880	ATB'β	B'-subunit		B56
At3g21650	ATB'ζ	B'-subunit		B56
At3g26020	ATB'η	B'-subunit		B56
At3g26030	ATB'δ	B'-subunit		B56
At3g54930	ATB'ε	B'-subunit		B56
At4g15415	ATB'γ	B'-subunit		B56
At5g03470	ATB'α	B'-subunit		B56
At5g25510	ATB'κ	B'-subunit		B56
At1g03960	ATB''β	B''-subunit		EF-hand
At1g54450	ATB''γ	B''-subunit		EF-hand
At5g18580	EMB40 FASS1 GDO (GORDO) TON2 (TONNEAU 2)	B''-subunit		EF-hand
At5g28850	ATB''ε	B''-subunit		EF-hand
At5g28900	ATB''δ	B''-subunit		EF-hand
At5g44090	ATB''α	B''-subunit		EF-hand
At5g53000	MNB8.6	TAP46		TAP42

^aDomains were identified with the Smart program (<http://smart.embl-heidelberg.de>). Blue boxes represent unstructured elements, pink boxes show regions of low complexity. BLAST means that the given domain was found only in a blast search. PFAM indicates that the PFAM collection of domains was used for identification. The scale shows the number of amino acid residues.

whether PPX-1 acts in the regulation of plastid-specific protein kinases and substrates.

Protein phosphatase 5

Although present in all eukaryotes, PP5s have not been extensively studied because they show low activity *in vitro* and little similarity to other phosphatases (Figure 2). PP5s carry three N-terminal tetratricopeptide (TPR) motifs that, together with their C-terminal sequences, act as

autoinhibitory domains. PP5s are activated by arachidonic acid and polyunsaturated fatty acids that bind to the TPR domains, and are inhibited by okadaic acid and microcystin. Mammalian PP5s are components of glucocorticoid receptor/Hsp90 and cryptochrome (CRY1 and CRY2) complexes and involved in the regulation of ion channels, hormone receptor signalling, cell cycle and apoptosis. Arachidonic acid and the microtubule depolymerizing agent nocodazol stimulate dissociation of PP5 from

heat-shock protein complexes and its subsequent proteolytic activation [42,43].

Like animal PP5s, plant PP5 enzymes are activated by arachidonic acid, unsaturated fatty acids and the removal of their TPR domains, and inhibited by okadaic acid [44]. In tomato and *Arabidopsis*, PP5 is encoded by single genes that produce two alternatively spliced transcripts encoding PP5 isoforms [45]. The 62-kDa isoform, which carries two N-terminal trans-membrane domains, is localized in the endoplasmic reticulum and its function is as yet unknown. The 55-kDa PP5 isoform, PAPP5 (Phytochrome-Associated Protein Phosphatase 5; Figure 2), interacts with phytochrome PhyA in the two-hybrid system and preferentially dephosphorylates the biologically active far-red light-absorbing Pfr form of oat PhyA *in vitro* [46]. The specificity of PAPP5 is probably due to preferential binding of its TPR

domain to the Pfr form of PhyA. PAPP5 also binds PhyB; conversion of Pr to Pfr by red light-pulse enhances PAPP5 association with PhyB. Interaction of PAPP5 with PhyA is also spectral form dependent. In the dark, PAPP5 and PhyB are localized in the cytoplasm – upon illumination, they are translocated into the nucleus, where PAPP5 co-localizes with PhyB into speckles. Experiments with *papp5* mutants and overexpressing lines show that increasing PAPP5 levels correlate with greater photoresponsiveness mediated by both PhyA and PhyB, which leads to increased expression of light-inducible genes. In the case of PhyA, dephosphorylation increases PhyA stability and affinity for a downstream signal transducer, the nucleoside diphosphate kinase 2 (NDPK2) [46–48]. PP5 also shows two-hybrid interaction with the tomato I-2 disease resistance protein and two members of the Hsp90 family. Silencing of

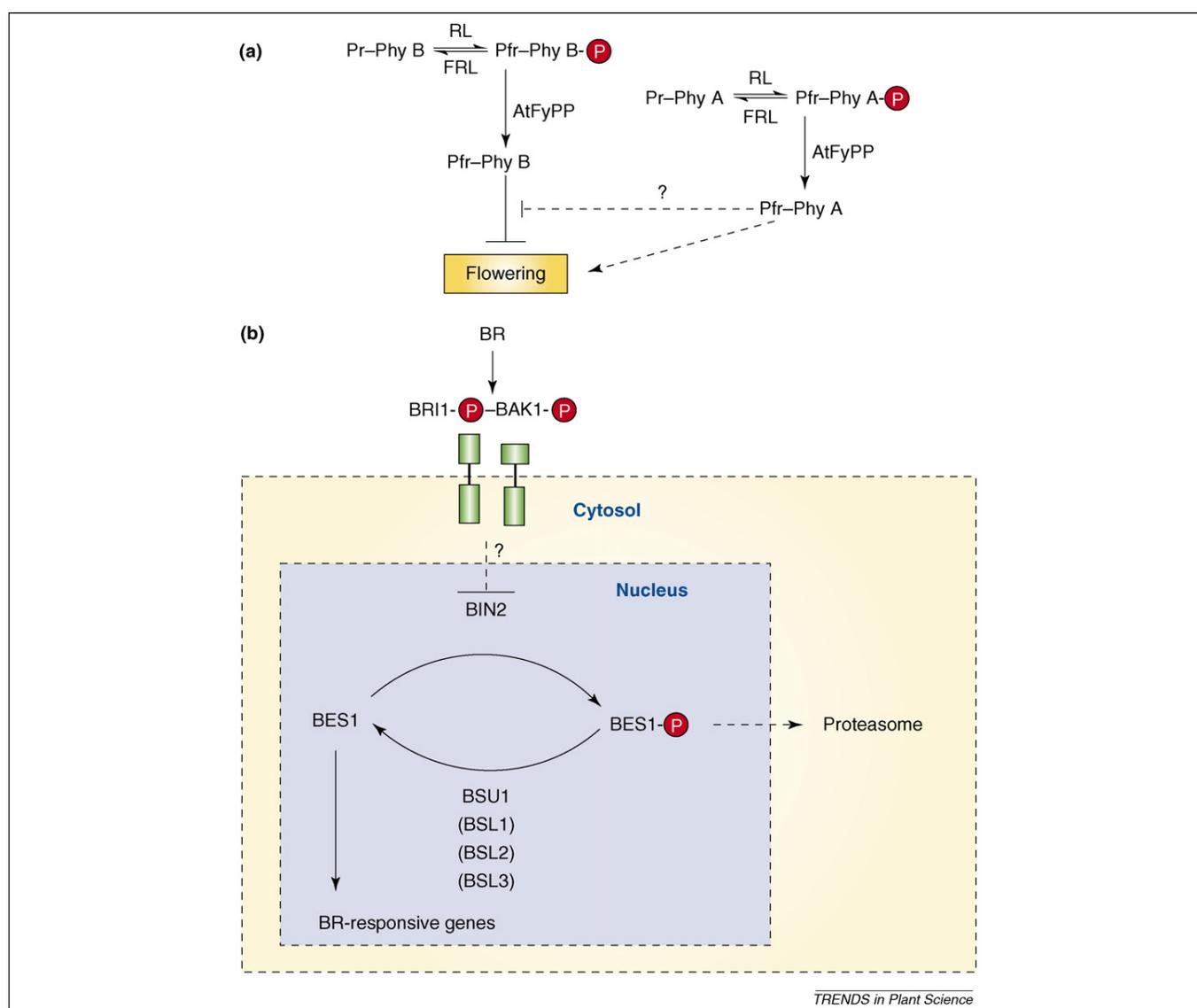


Figure 3. Physiological roles of plant PP6 and kelch-repeat containing phosphoprotein phosphatases. **(a)** PP6 phosphatases (AtFyPP1 and/or AtFyPP3) regulate flowering time by dephosphorylating phytochrome A and B. Abbreviations: AtFyPP, *Arabidopsis thaliana* phytochrome-associated protein phosphatase; FRL, far-red light; Pfr, active far-red absorbing form of phytochrome; Phy, phytochrome; Pr, inactive red-light absorbing form of phytochrome; RL, red light. P indicates the phosphorylated forms of the proteins. **(b)** Kelch-repeat-containing phosphatases are positive regulators of brassinosteroid signal transduction. Hypophosphorylated BES1 promotes the transcription of a set of brassinosteroid-responsive genes. Abbreviations: BR, brassinosteroids; BRI1 (BRASSINOSTEROID INSENSITIVE 1) and BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1), plasma-membrane localized receptor kinases; BIN2 (BR-INSENSITIVE 2), glycogen synthase kinase-3; BES1 (bri1-EMS-suppressor 1), transcription factor.

Hsp90, but not PP5, blocks cell death triggered by I-2, indicating that proper folding of I-2 is controlled by an Hsp90–PP5 complex [49].

Protein phosphatase 6

PP6 phosphatases, including *Saccharomyces cerevisiae* Sit4, *Schizosaccharomyces pombe* Ppe1, *Drosophila melanogaster* PPV and human PP6, are involved in the regulation of transcription, translation, morphogenesis and cell cycle [3]. Pea PP6, named FyPP, was found to interact with oat phytochrome PhyA and *Arabidopsis* PhyB in yeast. *In vitro* pull-down assays indicate that FyPP preferentially associates to the phosphorylated form of PhyA and Pfr forms of PhyA and PhyB [50]. Whereas PhyA and PhyB are imported to the nucleus upon red-light illumination, FyPP shows constitutive cytoplasmic localization. *Arabidopsis* has two PP6 orthologues, AtFyPP3 and AtFyPP1 (Figure 2), which differ at only two amino acid residues. Pea FyPP and AtFyPPs dephosphorylate autophosphorylated oat PhyA *in vitro* but, unlike PP2As, only in the presence of Fe²⁺ or Zn²⁺. Overexpression of pea FyPP in *Arabidopsis* results in delayed flowering, whereas down-regulation of AtFyPP causes early flowering, suggesting phenotypic analogies with *phyA* and *phyB* mutants [50]. FyPP-mediated dephosphorylation of PhyA and PhyB has been proposed to have opposite effects on flowering time regulation (Figure 3a). The photoperiod-dependent flowering time regulators *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) show altered regulation in FyPP antisense and *atfyp3* mutant lines. However, in the FyPP overexpressing lines, the AtFyPP mRNA levels were unaffected and the *atfyp3* mutation alone also caused early flowering. This discrepancy was explained by the fact that AtFyPP3 is expressed at higher levels under long-day conditions than under short days, whereas AtFyPP1 does not show such differential regulation [50]. Hence, the available data suggest that the *Arabidopsis* PP6 genes play distinct roles in PhyA and PhyB regulation and possibly in flowering time control.

Protein phosphatase 7

Plant PP7s have no close homologues in other kingdoms. In addition to AtPP7, *Arabidopsis* has two PP7-like genes (Figure 2). At1g48120 encodes a long PP7, whereas the product of At5g10900 is presumably inactive given that it lacks essential amino acids in the catalytic centre [15]. Unlike other PPPs, AtPP7 carries three inserts in the C-terminal half of its catalytic domain. AtPP7 can be activated by proteolytic cleavage, indicating that the first insert functions as an autoinhibitory region. AtPP7 is resistant to okadaic acid and calyculin but is inhibited by inorganic phosphate. Therefore, AtPP7 could possess a phosphate-binding site for allosteric regulation that could function as a phosphate sensory module [51]. AtPP7 also carries putative Ca²⁺-binding EF hand motifs and interacts with calmodulin in a Ca²⁺-dependent manner analogously to PP2B/calcineurin-like enzymes. However, unlike calcineurins, AtPP7 is inhibited by calmodulin. The activity of recombinant AtPP7 is reversibly regulated by redox-agents, but the biological significance of this observation is not yet clear [52].

Silencing of *Arabidopsis* AtPP7 was found to cause a defect in cryptochrome (CRY)-mediated blue-light signalling leading to a loss of hypocotyl growth inhibition by blue light and attenuation of blue-light-regulated gene expression [53,54]. AtPP7 appeared to be a potential candidate for a CRY phosphatase because cryptochromes are localized in the nucleus in darkness and AtPP7 also shows predominant nuclear localization [55]. However, no direct interaction was detected between AtPP7 and CRY1 [53]. Therefore, it was proposed that AtPP7 acts downstream of cryptochrome. Cryptochromes regulate the expression of chloroplast genes, such as *psbD*, through blue-light-regulated nuclear σ factors such as SIG5 [56]. AtPP7 could act through such a regulatory circuit given that SIG5 transcription is reduced in AtPP7 antisense plants but induced in wild type upon blue-light irradiation. Nonetheless, wild type and AtPP7-deficient plants show no significant difference in CRY1 and CRY2 transcription in blue or white light, and AtPP7 overexpression does not lead to blue-light hypersensitivity. Thus, further studies are required to define the precise function of AtPP7 in blue-light signalling.

Protein phosphatases with kelch-repeat domains

Biochemical and genetic studies have identified four novel phosphatases in *Arabidopsis* that are distantly related to PP1 and carry tandem N-terminal kelch-repeats that form β -propeller structures for protein–protein interactions [57,58] (Figure 2). A genetic screen for dominant suppressors of the brassinosteroid receptor *bri1* mutation led to the identification of the *bsu1-1D* mutant, which overexpresses BSU1 (At1g03445), one of these kelch-repeat phosphatases [58]. The three other BSU1-like genes were designated BSL1 (At4g03080), BSL2 (At1g08420) and BSL3 (At2g27210).

BSU1 expressed in *E. coli* functions as an active phosphatase. Like PP1, BSU1 is inhibited only by okadaic acid but is insensitive to the PP1 inhibitor protein I-2. BSU1 was localized in the nucleus. BSU1 overexpression resulting from the dominant *bsu1-1D* mutation partially suppresses the dwarf phenotype of the *bin2* mutant, which overexpresses a glycogen synthase kinase-3 homologue that acts as a negative regulator of brassinosteroid (BR) signalling [58]. Perception of the steroid hormone brassinolide by the LRR (leucine-rich receptor) kinase BRI1 triggers dephosphorylation and subsequent accumulation of transcription factors BES1 and BZR1 that function as positive regulators of BR-induced genes [59]. BIN2 acts downstream of BRI1 and by phosphorylation triggers ubiquitination and proteasomal degradation of BES1 [60–62] (Figure 3b). Dephosphorylated BES1 is more abundant in the *bsu1-1D* mutant, and BIN2-phosphorylated BES1 is dephosphorylated by recombinant BSU1 *in vitro*. Thus, BSU1 opposes the effect of BIN2 by promoting dephosphorylation and activation of BES1. In BR-stimulated cells, BIN2 is inhibited and BSU1 is activated by an unknown mechanism [63]. Combinations of *bsu1* and *bsl1* knockouts confer no BR-related phenotypic change, which indicates a functional overlap between the BSU1 and BSL phosphatases. However, *bsl2 bsl3* double RNAi knock-down plants have a dwarf phenotype that resembles

the phenotype of plants with weak *bri1* alleles. Because the *BSL* genes are expressed at higher levels than *BSU1*, *BSL2* and *BSL3* probably account for most of the *BES1* dephosphorylating activity [58].

Concluding remarks

Despite of some unexplored fields, recent advances in the functional analysis of PPPs in *Arabidopsis* and other plants show that nearly all conserved families of protein phosphatases play pivotal roles in plant cell signalling. Studies of PPPs have shed light on numerous novel mechanisms, but many more regulatory interactions are probably still hidden. Without uncovering these, we shall not be able to determine how PPPs balance the action of >1000 protein kinases. The 'golden age' of plant PPPs is still to come, possibly as part of systems biology.

Acknowledgements

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