Rhizobiales-like Phosphatase 2 from Arabidopsis thaliana Is a Novel Phospho-tyrosine-specific Phospho-protein Phosphatase (PPP) Family Protein Phosphatase*

Received for publication, August 10, 2015, and in revised form, December 21, 2015. Published, JBC Papers in Press, January 7, 2016, DOI 10.1074/jbc.M115.683656

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Reversible protein phosphorylation, mediated by protein kinases and phosphatases, is a regulatory mechanism key to the functioning of all cell types. With an estimated minimum of 75% of all human proteins controlled by this mechanism (1), connecting the biochemical characteristics of protein kinases and phosphatases to their cellular function is of central importance. Reversible protein phosphorylation can occur on a number of residues, but largely occurs in both plants and animals on serine, threonine, and tyrosine residues at approximate percentages of 86, 12, and 2, respectively (2–6).

Protein kinases exist as one large superfamily with over 1050 members encoded in the genome of the model organism Arabidopsis thaliana (6). Conversely, A. thaliana maintains only 150 protein phosphatases, which are categorized into four distinct families conserved across eukaryotes. These include the phospho-protein phosphatases (PPPs), phospho-protein metallo-phosphatases (PPM), phospho-tyrosine phosphatases (PTPs), and the Asp-based catalysis phosphatases (7). Unlike protein kinases, which employ a single catalytic mechanism, each of the four protein phosphatase families employs differing catalytic mechanisms (7). The PPP, PPM, and Asp-based protein phosphatases coordinate metal ions in their active sites to assist in catalysis, and each has been shown to specifically target phosphorylated serine (pSer) and threonine (pThr) residues on protein substrates (7). The PPM catalytic domains have N- and C-terminal extensions that confer substrate specificity and regulation, whereas the PPP family enzymes all have additional subunits that define function (6). The PTP family protein phosphatases operate independently of metal ion co-factors (7, 8), and alternatively employ a cysteine from the conserved CX_3R motif to catalyze the removal of phosphate from substrates. The PTP family was defined by their ability to dephosphorylate phospho-tyrosine (pTyr) residues. In humans, this group is now referred to as the classic PTPs and is described as specifically dephosphorylating only pTyr on proteins. Other PTP family members exist including a large subset of proteins called the dual specificity phosphatases (8–10). These phosphatases maintain a variety of catalytic site structures capable of dephosphorylating both pTyr, pSer, and pThr residues in addition to other molecules such as phospho-inositides, glycogen/starch, and mRNA (8, 10). A. thaliana genome analysis has identified many homologues of the human dual specificity phosphatases, but only a single classic PTP family phosphatase (11, 12), whereas humans maintain 38 of the classic tyrosine-specific enzymes (9). Despite the presence of only a single pTyr-specific classic PTP family phosphatase in plants (AtPTP1), phospho-proteomic studies have consistently found levels of protein tyrosine phosphorylation in A. thaliana and Oryza sativa (2, 13) that parallel in abundance to humans.

Recently, two new groups of PPP family protein phosphatases were identified in plants that possess all the defining PPP enzyme motifs and domains (14–16), but overall appear to be

* This work was supported by the Natural Sciences and Engineering Research Council of Canada. The authors declare that they have no conflicts of interest with the contents of this article.

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The abbreviations used are: PPP, phospho-protein phosphatase(s); PPM, phospho-protein metallo-phosphatase; PTP, phospho-tyrosine phosphatase; RLPH, Rhizobiales/Rhodobacterales/Rhodospirillaceae-like phosphatase; pTyr, phospho-tyrosine; pSer, phospho-serine; pThr, phospho-threonine; TAP, tandem affinity purification; cTAP, C-terminal TAP; Ni-NTA, nickel-nitrilotriacetic acid; pNPP, para-nitrophenylphosphate; IP, immunoprecipitation; PIS, pre-immune serum; rSAPK3, rat SAPK3.
more related to bacterial protein phosphatases. The first group, the *Shewanella*-like phosphatases (SLP1 and SLP2), form two highly conserved and phylogenetically distinct sub-groups across all plants (15, 16). The second group of bacteria-like protein phosphatases is the Rhizobiales/Rhodobacterales/Rhodospirillaceae-like phosphatases (RLPHs), which were named due to their phylogenetic relatedness to phosphatases encoded by these bacterial groups (14, 16). It is the RLPHs that are the focus of this study. We present a comprehensive analysis of *A. thaliana* RLPH2 (AtRLPH2), a novel phospho-tyrosine-specific PPP family protein phosphatase.

**Experimental Procedures**

Protein concentration was determined by Bradford reagent using BSA as a standard.

**Plant and Cell Culture Growth—** *A. thaliana* wild type seeds were surface-sterilized for 4 h, placed in either magenta boxes containing liquid Murashige and Skoog medium or 0.6% agar plates containing 0.5× Murashige and Skoog medium, and stratified at the dark at 4 °C for 2 days. Magenta boxes were placed under constant light for 10 days with one medium change. Seedlings from plates were transferred to soil and grown in 12-h light/12-h dark conditions for 45 days. Roots from magenta boxes and each part of the plant (rosette, stem, flower, and silique) were harvested, frozen with liquid N2, and placed under constant light for 10 days in a 1:10 ratio, harvested, and stored as in Ref. 17.

**Cloning of AtRLPH2 and AtSLP1—** *Atg009970* (AtRLPH2) and *Atg07010* (AtSLP1) clones were obtained from The Arabidopsis Information Resource. Each was propagated by PCR (AtRLPH2-fwd ATGGCGCAAAGGACGGAACCGGTG; AtRLPH2-rev ACTAGACAAATTATCGGGTCAGGAT; AtSLP1-fwd ATTGCTCCCTTACCTCAAATCC; AtSLP1-rev ATAGTAATCTGCAACCTGAAGTTC) with primers that additionally contained forward and reverse Gateway homologous recombination adaptor sites (Invitrogen). Homozygosity of the insertion and absence of background. Homozygosity of the insertion and absence of background. Homozygosity of the insertion and absence of background.

**AtRLPH2 T-DNA Insertion Lines—** T-DNA insertion mutant lines for *atrlph2-1* (RATM-13-2130-1_G) and *atrlph2-2* (RATM-13-3204-1_G) were obtained from RIKEN and are in a Nössen background.

**Immunoprecipitation—** Either anti-AtRLPH2 IgG or rabbit pre-immune serum (PIS) IgG (40 μg) was incubated with protein A-Sepharose 4B (Life Technologies) and covalently coupled to the bead as in Ref. 22. Dark-grown *A. thaliana* cell culture protein extract (50–70 mg) (23) was mixed with the antibody-coupled beads end-over-end for 4 h at 4 °C. Beads were washed 5 times with 1 ml of 50 mM HEPES, pH 7.5, 150 mM NaCl and 0.1% (v/v) Tween 20.

**Immunoblotting—** Protein was extracted from different plant tissues as described in Ref. 23, and 5 μg were separated by 14% SDS-PAGE, transferred to a nitrocellulose membrane, blocked, and probed with 2 μg/ml anti-AtRLPH2 antibody, followed by secondary antibody diluted 1:5000 and developed with ECL (PerkinElmer).

**Enzymatic Analysis—** AtRLPH2 activity was measured either by the small molecule phosphatase substrate para-nitrophenyl-

The GST-Fer construct, obtained from Dr. Nicholas Tonks (Cold Spring Harbor Laboratory), was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL (18), expression was induced with 0.4 mM isopropyl-1-thio-β-d-galactopyranoside at 37 °C for 2 h, and the construct was purified on glutathione-Sepharose 4B (GE Healthcare).

**Tandem Affinity Purification (TAP) of AtSLP1 and AtRLPH2—** AtSLP1 and AtRLPH2 TAP-tagged constructs were transformed into Agrobacterium GV3101 and used to transfect *A. thaliana* via the floral dip method (19). AtSLP1- and AtRLPH2-TAP-expressing *A. thaliana* plants were grown in soil under a 12-h light/12-h dark cycle. AtSLP1- and AtRLPH2-TAP proteins were isolated in parallel, each from 20 g of rosette tissue, as described previously (17), and was employed in enzyme assays immediately after the final Ni-NTA purification step, where the matrix was washed but not eluted.

**Anti-AtRLPH2 Antibodies—** Pure bacterial expressed AtRLPH2-V5-H6 was used as an antigen to generate polyclonal antibodies in a rabbit (20). Anti-AtRLPH2 IgG was affinity-purified from crude immune serum by a membrane method as described previously (21).

**Protein Expression and Purification—** AtRLPH2-V5-H6 was expressed in BL21 (DE3) CodonPlus-RIL *Escherichia coli* at 22 °C, with 0.1 mM isopropyl-1-thio-β-d-galactopyranoside for 18 h, and purified in two steps using Ni-NTA followed by Mono-Q chromatography as described in Ref. 15. The unbound Mono-Q fraction contained >95% pure AtRLPH2-V5-H6 (data not shown) and was confirmed to be AtRLPH2 by mass spectrometry. AtSLP1-H6 was purified using Ni-NTA as described previously (15).
phosphate (24) (pNPP; Sigma) or by measuring phosphatase-catalyzed phosphate release from metabolites or phospho-peptide substrates using malachite green reagent (24, 25). Standard pNPP assay conditions included pre-incubation of 150 ng of AtRLPH2-V5-H6 with small molecules in 1× dilution buffer consisting of 100 mM HEPES-NaOH, pH 7.5, 150 mM NaCl (totaling a volume of 20 μl) for 10 min at 30 °C. Each assay was then supplemented with 130 μl of buffer containing 5 mM pNPP followed by an additional 10-min incubation at 30 °C.

Malachite green (Sigma-Aldrich) assays were performed using 1 μg of recombinant phosphatase protein incubated for 1 h at 30 °C in 160 μl of buffer containing each substrate with control assays lacking protein phosphatase. To determine kinetic parameters, assays were performed for 30 min using the sAPK3 peptide (see Table 1) with pTyr, pSer, or pThr at the phospho-position. $K_v$ and $k_{cat}$ were determined using the Michaelis-Menten and Lineweaver-Burk plots.

Assays performed using TAP-purified AtSLP1- and AtRLPH2-cTAP employed 65 μl of protein phosphatase-bound Ni-NTA isolated from 20 g of rosette tissue. Each aliquot of coupled Ni-NTA matrix was resuspended in 235 μl of buffer containing phosphorylated peptide and was incubated for 1 h at 30 °C. Ni-NTA matrix was pelleted, and 160 μl of reaction mix were removed and quenched with malachite green solution. Beads with immunoprecipitated endogenous AtRLPH2 (and control beads) from one-half of an IP experiment were resuspended in 160 μl of 1× dilution buffer containing the phosphorylated peptide. Controls consisting of a blank without enzyme and a PIS IgG IP were performed in parallel. Each assay was incubated for 1.25 h at 30 °C. Beads were pelleted, and supernatant was quenched with malachite green solution. IP assays employed phospho-peptides derived from the proteins: DAPP1 (PRKVEEPSpYESVVRH), SPAK (CTRNKVRLpTFVGTTP), and TSC2 (QLHRSVpSWADSAK), with phospho-residues as indicated.

For GST-Fer dephosphorylation assays, immunoprecipitated endogenous AtRLPH2 beads (and control beads) from one-half of an IP experiment were resuspended in 1× dilution buffer containing 0.5 μg of phospho-GST-Fer and incubated at 30 °C for up to 24 min. Every 3 min, one tube was boiled with 5× SDS mixture. GST-Fer tyrosine phosphorylation state was tested by immunoblotting with anti-phospho-tyrosine antibody as described above. For loading control, the membrane was stripped with 2 M MgCl2 and 0.1% acetic acid for 10 min at room temperature and then probed with anti-GST antibody (Immunology Consultants Laboratory, RGST-45A-Z). This experiment was performed in triplicate, and the representative blot is shown. Bands were quantified with ImageJ.

### Results

**AtRLPH2 is a Novel Plant Protein Tyrosine Phosphatase**

**AtRLPH2 is a widely expressed cytosolic enzyme.** A, affinity-purified anti-AtRLPH2 IgG (2 μg/ml) detected down to 0.25 ng of purified AtRLPH2-V5-H6 by Western blotting (WB). B, AtRLPH2 antibody (2 μg/ml) was used to probe either a WT or two separate AtRLPH2 T-DNA insertion line crude extracts (atrlph2-1, atrlph2-2). Prior to Western blotting, the membrane was Ponceau 5 (PS)-stained for equal loading (lower panel). AtRLPH2 is detected as an immunoreactive band at 36 kDa. C, extracts from various *A. thaliana* plant tissues probed with anti-AtRLPH2 IgG show broad tissue expression of AtRLPH2. D, crude lysate, isolated nuclei, and cytosolic fractions from *A. thaliana* rosettes (left panels) and cell culture (right panels) were Western blotted (WB) for AtRLPH2. Known cytosolic marker UDP-glucose pyrophosphorylase (UGPase) and nuclear marker histone H3 were used to verify the subcellular localization of AtRLPH2 and purity of each fraction. All mass markers are shown in kDa.

**AtRLPH2 is a Broadly Expressed Cytosolic Phosphatase**—Previous bioinformatic analysis determined RLPH2 to be found widely across photosynthetic eukaryotes, where it was predicted to have a cytosolic localization (16). To confirm its subcellular localization and tissue expression, we generated and affinity-purified antibodies to recombinant AtRLPH2 (see “Experimental Procedures” and Fig. 1). This antibody could detect as little as 0.25 ng of tagged AtRLPH2 (Fig. 1A) and detected a protein in crude extracts at the predicted mass (~36 kDa) of the endogenous AtRLPH2 (Fig. 1B). This band was confirmed to be AtRLPH2 by demonstrating its disappearance in two separate knock-out lines (Fig. 1B), neither of which displayed any obvious growth phenotype. Western blotting of *A. thaliana* tissues demonstrated AtRLPH2 protein expression in all tissues examined (Fig. 1C).
To explore AtRLPH2 subcellular localization, nuclear and cytosolic fractions were prepared from both rosettes and cell culture. Blotting each fraction with cytosolic and nuclear marker proteins (Fig. 1D) revealed a cytosolic localization for AtRLPH2. Consistent with cell fractionation and bioinformatics, immunolocalization imaging of cultured A. thaliana cells revealed that AtRLPH2 is cytosolic and excluded from the nucleus (Fig. 2, A–H). Identical results were found in A. thaliana roots (Fig. 2, M–P). No signal was observed when the knock-out line atrlph2-1 was used (Fig. 2, I–L), supporting the notion that the anti-AtRLPH2 signal observed in Fig. 2, A and M is derived solely from antibody binding AtRLPH2. The immunoreactive band of ~50 kDa observed during Western blotting (Fig. 1B) is likely an epitope only exposed once proteins are denatured in SDS. Blotting with PIS IgG gave no signal for roots or cell culture (data not shown).

Enzymatic Characterization of AtRLPH2—Poly-His-tagged AtRLPH2 (AtRHLPH2-V5-H6) was expressed and purified from E. coli. Size exclusion chromatography revealed a monomer of 49.1 kDa, despite a denatured SDS-PAGE mass of ~40 kDa (data not shown). AtRLPH2 was found to have a pH optimum of 7.0–7.8 (Fig. 3A) with a specific activity of 20 nmol Pi min⁻¹ mg⁻¹ toward the artificial phosphatase substrate pNPP (Fig. 3A). Screening of known metal cation co-factors of various protein and non-protein phosphatases showed no stimulation of phosphatase activity, with highest activity observed in the control condition containing 5 mM EDTA (Fig. 3B). Interestingly, Zn²⁺ inhibited the phosphatase activity of AtRLPH2, just as Zn²⁺ is the most potent metal ion inhibitor of human classic tyrosine phosphatases (27).

Several phosphate-containing compounds and classic protein phosphatase inhibitors were evaluated for their inhibitory effect on AtRLPH2 (Fig. 4). Phosphate analogs NaF and Na₃VO₄ are PPP and PTP family protein phosphatase-specific small molecule inhibitors, respectively (27). Both NaF and Na₃VO₄ substantially reduced AtRLPH2 activity with IC₅₀ values of 8.9 mM and 2.1 μM, respectively (Fig. 4, A and B). Additionally, the naturally occurring PPP family protein phosphatase-specific small molecule inhibitors okadaic acid and microcystin-LR were also examined for their inhibitory effect on AtRLPH2 (Fig. 4, C and D). Concentrations that would completely inhibit the PPP family member PP2A failed to have any inhibitory effect on AtRLPH2. AtRLPH2 also exhibited nanomolar PP²⁺ sensitivity (IC₅₀ 350 nM), in addition to micromolar

FIGURE 2. Immunofluorescence imaging of AtRLPH2 in A. thaliana cell culture and roots. A–C, affinity-purified antibody was used to localize AtRLPH2 in cultured cells. A, fixed cells were probed with anti-AtRLPH2 IgG (5 μg/ml) and visualized with Alexa Fluor 488 (Alexa 488)-conjugated secondary antibody, whereas DNA was stained and visualized with propidium iodide (B), and the images were merged (C). White arrows indicate nuclei where AtRLPH2 is excluded. Controls with antigen-blocked anti-AtRLPH2 IgG are shown in panels E–G. In panels I–K, A. thaliana roots from the T-DNA insertion line atrlph2-1 were probed with anti-AtRLPH2 IgG and visualized with Alexa Fluor 488 (I), DNA was marked with propidium iodide (J), and the signals were merged (K). The same is shown for WT roots in panels M–O. Panels D, H, L, and P are the differential interference contrast (DIC)/Nomarski images of the left-hand panel of cells. Scale bar = 25 μm.
to millimolar sensitivity to ADP (IC$_{50}$ 356 µM), P$_i$ (IC$_{50}$ 1.1 mM), and AMP (IC$_{50}$ 5.2 mM), as well as remarkable sensitivity to ATP (IC$_{50}$ 2.2 µM) (Fig. 4, E and F). Glucose 6-phosphate and glycerol 3-phosphate had no notable inhibitory effect on AtRLPH2 activity up to a 5 mM concentration (Fig. 4F).

**AtRLPH2 Is a Novel Plant Protein Tyrosine Phosphatase**—Utilizing Ser, Thr, and Tyr phospho-peptides to decipher the substrate preferences of PPP family members (PP1, PP2A, PP2B, PP2C) and classic PTPs has previously been employed successfully (28–32). Therefore, using a panel of phosphorylated peptides consisting of pSer, pThr, and pTyr, the substrate specificity of AtRLPH2 was evaluated (Table 1). AtRLPH2 exhibited low activity toward pSer- and pThr-only peptides, but displayed typically 50-fold greater activity against pTyr-containing peptides. No measurable activity was detected when employing the non-phospho-peptide substrates phospho-enolpyruvate, AMP, ADP, ATP, GTP, dATP, dihydroxyacetone phosphate, PP$_i$, glucose-6-P, and glycerol-3-P. Among the *A. thaliana* PPP family enzymes, we recently characterized the other bacteria-like phosphatases, *Shewanella*-like protein phosphatases AtSLP1 and AtSLP2 (15). For comparison, bacterially expressed AtSLP1 was assayed in parallel with AtRLPH2.
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FIGURE 5. Phospho-peptide substrate preferences of purified A. thaliana bacteria-like PPP family protein phosphatases. A. thaliana SLP1 (AtSLP1, black bars) and RLP2 (AtRLPH2, gray bars) were expressed and purified as tagged fusion proteins in E. coli. A, 1 μg of each purified phosphatase was incubated with 1 μM designated phosphorylated peptide at 30 °C for 1 h to determine their phospho-substrate preference. The phospho-residue is indicated below the peptide name. pTyr, pThr; pS; pSer; pY. B, bacterially expressed tagged AtRLPH2 kinetic constants were determined by performing assays with increasing amounts of rSAPK3 peptide (pTyr; black), or rSAPK3 peptide with a pSer (light gray) or pThr (dark gray) substituted for the pTyr. Only the pTyr peptide yielded adequate activity to calculate $K_m$, $k_{cat}$, and $k_{cat}/K_m$ values, which are presented in the inset. C, constitutively expressed AtSLP1-cTAP (black bars) and AtRLPH2-cTAP (gray bars) were isolated from A. thaliana rosette leaf tissue as described under “Experimental Procedures.” Incubation of each TAP purified protein with either 500 μM BRCA1 (pTyr) or 500 μM hSAPK4 (pTyr) phospho-peptides revealed each protein phosphatase substrate preference. The inset shows a Western blot (WB) of the Myc epitope of the TAP tag on each protein to demonstrate that equal amounts of phosphatase were used in each assay. Phosphatase activity was determined using a stop time malachite green assay. Error bars represent S.E. (n = 3).

to assess its preference for pSer, pThr, and pTyr peptides (Fig. 5A). Unlike AtRLPH2, AtSLP1 exhibited a broad specificity profile for phospho-peptide substrates, effectively dephosphorylating pSer, pThr, and pTyr peptides. Next we performed assays to determine AtRLPH2 kinetic parameters toward a pSer, pThr, and pTyr peptide (Fig. 5B). To allow a direct comparison, we selected the rSAPK3 peptide and also synthesized the same peptide with pSer or pThr in place of the pTyr residue. Due to the extremely low activity with the pSer and pThr peptides, no reliable $K_m$ or $k_{cat}$ values could be determined. However, using the pTyr-containing rSAPK3 peptide, we determined a $k_{cat}$ value of 0.053 s$^{-1}$ and a $K_m$ of $\sim 390 \mu M$.

Next we employed TAP of overexpressed, in planta constructed AtSLP1 and AtRLPH2 to verify these phospho-peptide substrate preferences (Fig. 5C). Using equal amounts of enzyme, AtRLPH2-cTAP displayed little to no detectable activity toward a pThr-containing peptide, but still robustly dephosphorylated a pTyr peptide, whereas AtSLP1-cTAP still maintained measurable phosphatase activity toward the pTyr peptide, but with a marked preference for the pThr-containing peptide. To further support this observation, we next immunoprecipitated the endogenous AtRLPH2 from wild type plant cell culture for in vitro assays, and again the enzyme displayed a remarkable preference for pTyr peptides (Fig. 6A).

The human tyrosine kinase Fer (Fps/Fes-related) autophosphorylates on tyrosine residues when expressed in bacteria. This makes it an excellent substrate for human classic PTPs (33). We purified the bacterially expressed auto-phosphorylated GST–Fer kinase, (Fig. 6E) and used it in an assay with immunoprecipitated AtRLPH2 (Fig. 6B). As shown in Fig. 6, C and D, AtRLPH2 readily dephosphorylated the pTyr-protein, again supporting the notion that in vivo, AtRLPH2 is a tyrosine phosphatase.

Discussion

In human cells, ~2% of protein phosphorylation events occur on tyrosine. It is well established that this ~2% is a fundamental component of cellular signaling (5). Despite an equivalent degree of tyrosine phosphorylation in humans and plants, genome analysis of plants has revealed a lack of dedicated tyrosine kinases. Select members of the large receptor-like protein kinase, calcium-dependent protein kinase (CDPKs), and MAPKK (MEK) families in A. thaliana have shown an ability to phosphorylate proteins on tyrosine, exhibiting dual specificity for the phosphorylation of Ser, Thr, and Tyr (33). Additionally, the glycogen synthase kinase 3 (GSK3) and calcium-dependent protein kinase-related enzymes (CRKs) autophosphorylate on tyrosine (34). There are two main subfamilies of protein phosphatases capable of dephosphorylating pTyr: the classic and dual specificity PTPs. The classic PTP subfamily enzymes are dedicated tyrosine phosphatases, whereas several dual specificity PTP subfamily enzymes dephosphorylate pTyr, but most target other specific phospho-substrates, including pSer and pThr (8). Plants encode for a single tyrosine phosphatase equivalent to the human enzymes (PTP1) (11), and it has the ability to dephosphorylate the activation loop pTyr of AtMPK4 (35). Plants also encode for many equivalents of the human dual specificity subfamily of PTPs (9,
As a whole, this body of evidence supports the idea that protein tyrosine phosphorylation is a key aspect of plant signaling, yet only a single tyrosine-specific protein phosphatase has been characterized in plants.

A number of plant protein phosphatases resemble bacterial phosphatases (14–16), which, based on sequence, clearly belong to the Ser/Thr-specific PPP family enzymes. In this study, we show that one of these enzymes, AtRLPH2, is cytoso-
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described in *Shewanella* (38), SHLP2 has been shown to preferentially dephosphorylate phospho-tyrosine.

With the abundance of plant tyrosine phosphorylation, and a paucity of classic tyrosine-specific phosphatases, we demonstrate that AtRLPH2 is one of the missing components in this area of plant signaling. We also show for the first time that a member of the plant PPP family of phosphatases has the capability to dedicate its activity solely toward phospho-tyrosine. All other PPP family enzymes have rigid active sites that are Ser/Thr-specific (7). A structure for AtRLPH2 may reveal a better understanding of how this enzyme evolved to have the unique capacity to specifically dephosphorylate phospho-tyrosine.

**Author Contributions**—R. G. U. cloned AtRLPH2-V5-HIS6 and AtRLPH2-cTAP, and in addition performed enzymatic assays involving these proteins. R. G. U. and A. M. L. created anti-AtRLPH2 IgG, with A. M. L. responsible for a research related to the anti-AtRLPH2 IgG thereafter. A. M. L. created and performed experiments related to AtRLPH2 T-DNA knockout lines and determined kinetic parameters. J. M. and S. assisted A. M. L. in visualizing the subcellular localization of AtRLPH2. R. G. U., A. M. L., and G. B. M. were responsible for manuscript assembly.

**Acknowledgments**—We thank Nick Tonks (Cold Spring Harbor Laboratory) for the GST-Fer construct. We also thank Dr. D Alessi of the Medical Research Council Protein Phosphorylation and Ubiquitination Unit (Dundee) for several phospho-peptides used in assays.

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