Short Communication

A Comprehensive Expression Analysis of the *Arabidopsis* Proline-rich Extensin-like Receptor Kinase Gene Family using Bioinformatic and Experimental Approaches

Alina Nakhamchik [1, 3, 4], **Zhiying Zhao** [1, 3, 5], **Nicholas J. Provart** [1], **Shin-Han Shiu** [2], **Sarah K. Keatley** [1], **Robin K. Cameron** [1, 6] **and Daphne R. Goring** [1, 7]

[1] *Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2*

[2] *Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, USA*

The *Arabidopsis* proline-rich extensin-like receptor kinase (PERK) family consists of 15 predicted receptor kinases. A comprehensive expression analysis was undertaken to identify overlapping and unique expression patterns within this family relative to their phylogeny. Three different approaches were used to study *At*PERK gene family expression, and included analyses of the EST, MPSS and NASCArrays databases as well as experimental RNA blot analyses. Some of the *At*PERK members were identified as tissue-specific genes while others were more broadly expressed. While in some cases there was a good association between these different expression patterns and the position of the *At*PERK members in the kinase phylogeny, in other cases divergence of expression patterns was seen. The PERK expression data identified by the bioinformatics and experimental approaches were found generally to show similar trends and supported the use of data from large-scale expression studies for obtaining preliminary expression data. Thus, the bioinformatics survey for ESTs and microarrays is a powerful comprehensive approach for obtaining a genome-wide view of genes in a multigene family.

Receptor kinases are represented by large gene families in both *Arabidopsis* and rice (Shiu and Bleecker 2001, Shiu et al.

2004). However, biological functions are not known for many of these predicted genes. Receptor kinases clearly play important roles in signal transduction for a variety of plant processes including plant growth and development as well as plant–microbe interactions (reviewed in Dievart and Clark 2004, Haffani et al. 2004). A few ligands responsible for the activation of specific receptor kinases have been identified, and some signalling components are also known (reviewed in Dievart and Clark 2004, Haffani et al. 2004).

Receptor kinases can be grouped into several distinct classes based on motifs in their extracellular domains (Shiu and Bleecker 2001, Shiu and Bleecker 2003). The leucine-rich repeat receptor kinase family is the largest class of *Arabidopsis* receptor kinases, and there are several well-known examples that illustrate the range of functions found for receptor kinases (reviewed in Dievart and Clark 2004). For example, BRI1 and BAK1 are involved in the perception of brassinosteroids (Li and Chory 1997, Li et al. 2002, Nam and Li 2002); CLV1 in meristem differentiation (Clark et al. 1997), ERECTA in organ formation (Torii et al. 1996), HAR1 in nodulation in legumes (Krusell et al. 2002, Nishimura et al. 2002) and Xa21 in disease resistance in rice (Song et al. 1995). Within the different classes of receptor kinases, there is also extensive evidence of gene duplications which is predicted to result in functional redundancy between closely related members (Shiu and Bleecker 2003, Champion et al. 2004). Recent work on the CLV1 and ERECTA receptor kinases has clearly supported the presence of functional redundancy between closely related receptor kinases (Dievart et al. 2003, Shpak et al. 2003, Shpak et al. 2004).

The *Arabidopsis* PERK family is related to *Brassica napus* PERK1 and shares sequence similarity with plant cell wall-associated proteins (Silva and Goring 2002). Evidence is accumulating that the cell wall not only contributes to mechanical strength and cell shape, but also perceives and signals

---

[3] These authors contributed equally to this work.
[4] Present address: Sunnybrook and Women's College Health Sciences Center, Toronto, Ontario, Canada M4N 3M5.
[5] Present address: Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada S7N 0W9.
[6] Present address: Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.
[7] Corresponding author: E-mail, goring@botany.utoronto.ca; Fax: +1-416-978-5878.

A

B

C
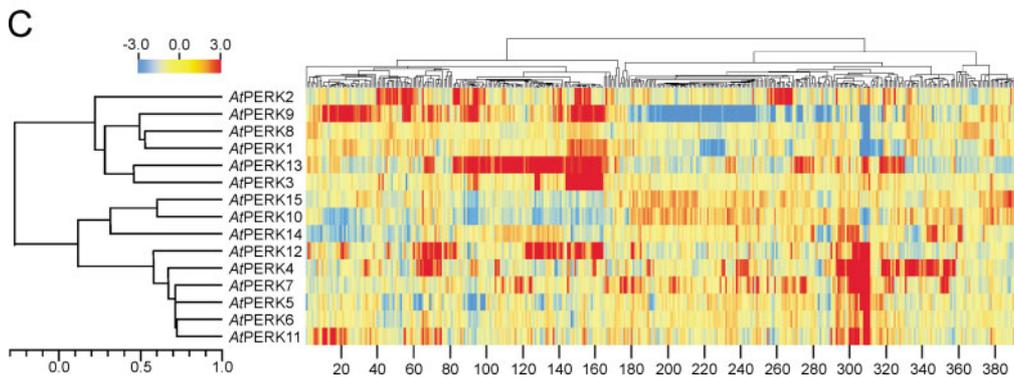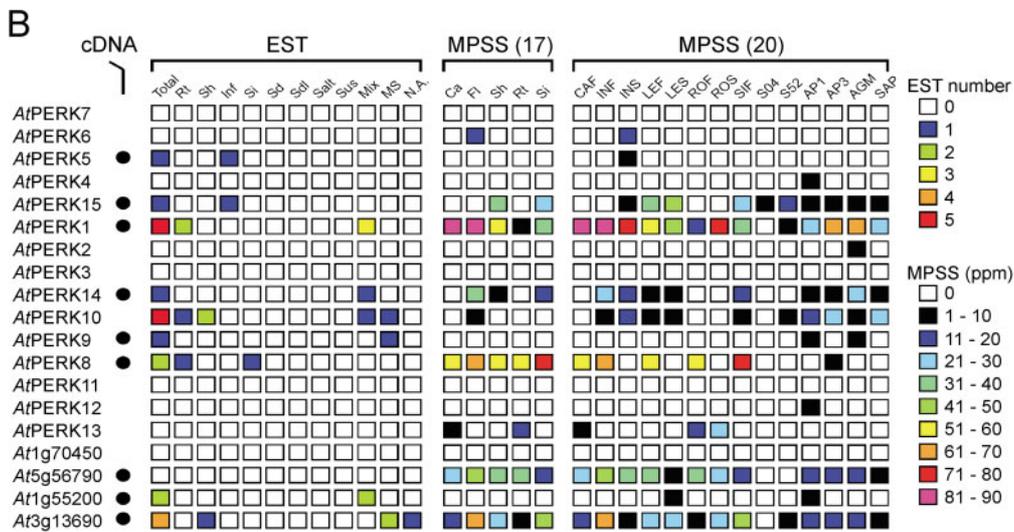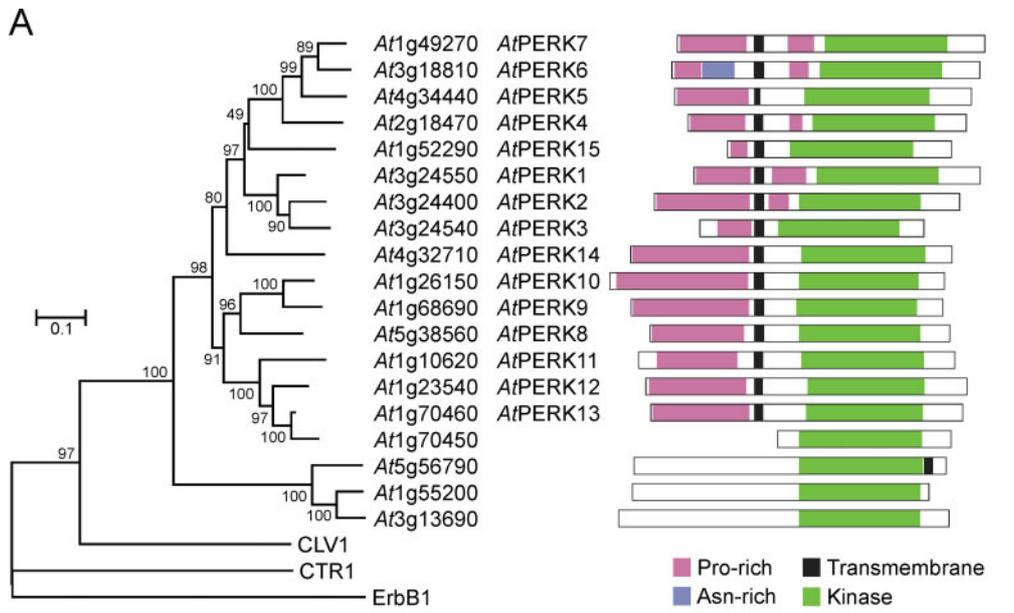
changes to the cell wall occurring in plant growth and development as well as attack by pathogens and insects (Pilling and Hofte 2003). Such a role has been demonstrated for the wall-associated kinases (WAKs), which are plasma membrane localized and strongly associated with the cell wall. WAKs have been found to be covalently linked to pectin, and antisense suppression of the WAK gene family leads to a loss of cell expansion (reviewed in Kohorn 2001, Verica and He 2002). The PERK family may represent a second group with a similar function.

The *Arabidopsis* genome contains a family of 15 PERK receptor kinases sharing the proline-rich extracellular domain, transmembrane domain and kinase domain (Silva and Goring 2002, Shiu and Bleecker 2003). The biological function of this family is largely unknown, although the antisense suppression of several *At*PERK members in transgenic *Arabidopsis* results in a number of growth defects (Y. Haffani, N. Silva, S.K. Keatley and D.R. Goring unpublished data). In this study, we have initiated a systematic analysis of the 15 *At*PERK members by performing comprehensive expression analyses on this family using both bioinformatics and experimental approaches. We have examined the utility of these different approaches in studying a whole gene family as well as correlations between *At*PERK expression patterns and their phylogenetic relationships based on kinase domain protein sequences.

In the receptor-like kinase/Pelle gene family with 625 members, >400 have a predicted transmembrane receptor domain organization (Shiu and Bleecker 2001, Shiu and Bleecker 2003). The remaining kinases in this family lack predicted extracellular domains and were termed receptor-like cytoplasmic kinases (RLCKs; Shiu and Bleecker 2001, Shiu and Bleecker 2003). Previous studies indicated that the PERK gene cluster was composed of 19 members based on the kinase domain sequences (Shiu and Bleecker 2001, Shiu and Bleecker 2003; Fig. 1A). These 19 members are shown in the phylogeny in Fig. 1A where all but one node has at least 80% support. Four of these members are RLCKs (*At*1g70450, *At*5g56790,

*At*1g55200, *At*3g13690), while the remaining 15 members are predicted receptor kinases (designated *At*PERK1-15) with a proline-rich extracellular domain similar to that found in *Bn*PERK1 (Fig. 1A; Silva and Goring 2002). Full-length cDNAs were identified from databases for several members confirming the predicted gene models (Fig. 1A, B).

The lengths of the *At*PERK extracellular domains vary considerably between different members with some having proline-rich regions in both the extracellular and cytosolic domains (Fig. 1A). AtPERK6 also contains an asparagine-rich region in the extracellular domain (Fig. 1A). All *At*PERK members, like *Bn*PERK1, lack a signal peptide at the N-terminus, and have one internal transmembrane domain followed by a stretch of positively charged amino acid residues. This organization is predicted to place the kinase domains on the cytosolic face of the plasma membrane. Consistent with predictions based on the primary sequences, we have shown that *Bn*PERK1 is localized to the plasma membrane (Silva and Goring 2002).

Our primary interest was in analyzing the 15 receptor kinases in this cluster, and we undertook an expression analysis using three different approaches to combine with the phylogenetic information. The first approach was a survey of the EST and MPSS databases to provide information on the relative expression levels and patterns of a particular gene (Fig. 1B). The second approach was to use the NASCArrays database to determine the level of co-expression between different *At*PERK members (Fig. 1C). The final approach was to conduct multiple-tissue RNA blot analyses (Fig. 2). The three RLCKs that form a separate clade (Fig. 1A; *At*5g56790, *At*1g55200, *At*3g13690) were examined based on the EST and MPSS data (Fig. 1B), but were not pursued further as they do not have the conserved configuration of the *At*PERK members. The fourth RLCK (*At*1g70450), which is clustered with *At*PERK13 on chromosome 1 may be a pseudogene as no evidence for expression could be found by RNA blot analysis, RT–PCR or through the databases (Fig. 1B, data not shown).

Fig. 1 Kinase phylogeny and expression analysis for the *Arabidopsis* PERK cluster. (A) Kinase phylogeny and domain organization. Previous phylogenies identified 19 kinases belonging to the PERK cluster (Shiu and Bleecker 2001). In addition to the *At*PERK kinases, three very closely related kinases, CLV1, CTR1 and human ErbB1 were included in the phylogeny and used as outgroup sequences. As seen by the domain organizations, 15 members are PERK receptor kinases while four are predicted to be cytoplasmic kinases (RLCKs). (B) cDNA, EST and MPSS expression data for the *At*PERK kinases. Databases (see Materials and Methods) were searched with the accession numbers for the different *At*PERK kinases. The EST abbreviations are: Total, sum of all ESTs; Rt, root; Sh, shoot (leaf and shoot apical regions); Inf, inflorescence; Si, silique; Sd, seed; Sdl, seedling; Salt, salt-treated seedling; Sus, suspension culture; Mix, whole plants; MS, whole plants, under stress. The MPSS (17) abbreviations are: Ca, callus; Fl, flower; Sh, shoot; Rt, root; Si, silique. The MPSS (20) abbreviations are: CAF, callus; INF, inflorescence set 1; INS, inflorescence set 2; LEF, leaf set 1; LES, leaf set 2; ROF, root set 1; ROS, root set 2; SIF, silique; S04, salicylic acid 4 h; S52, salicylic acid 52 h; AP1, *apetala*1 background; AP3, *apetala*3 background; AGM, *agamous* background; SAP, *sus/ap1* double mutant background. (C) Co-expression analysis of the *At*PERK members across 393 data sets from the NASCArrays database. Each column represents the results from one microarray data set, and the 393 data sets are numbered at the bottom of the figure in units of 20 (more detailed results from the 393 data sets are shown as supplementary material). The colours represent the difference from the median of the $\log_2$-transformed expression values. The gene-wide median is represented in yellow. Full red is 3 units above the median of the $\log_2$-transformed expression levels for that gene, while full blue is 3 units below the median of the $\log_2$-transformed expression levels for that gene. The scale represents the Pearson correlation coefficient (1.0 represents complete correlation of expression between genes across all experiments, 0.0 is no correlation and −1.0 is a complete anti-correlation between genes across all experiments).
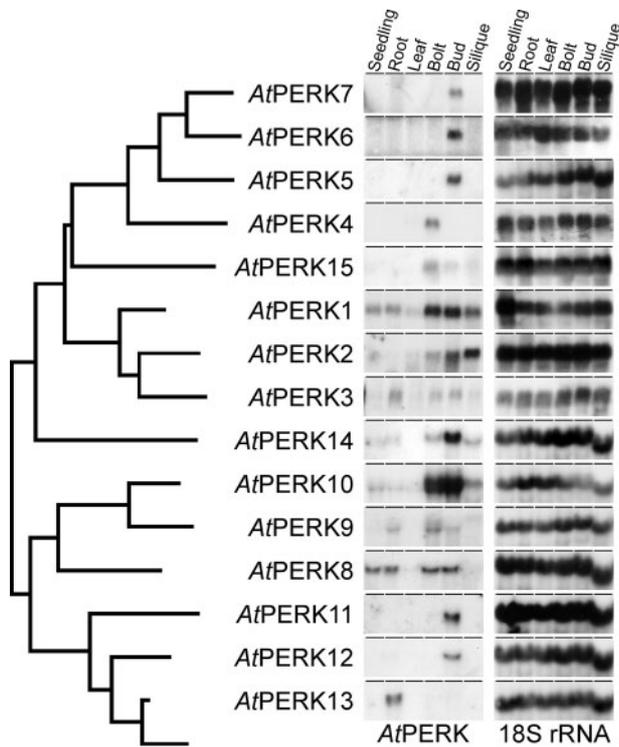
Fig. 2 RNA blot analysis for the *AtPERK1-15* genes. The kinase phylogeny from Fig. 1 is added on the left to show relationships between the different *At*PERK members. The left panels represent blots probed with *At*PERK gene-specific probes. The right panels show the same blots probed with the 18S rRNA probe as a control for loading. The tissues loaded are seedling, root, leaf, (inflorescence) bolt, (floral) buds and siliques.

The EST and MPSS data indicated that *At*PERK members have variable expression patterns. Some were more highly expressed based on the number of ESTs detected and the higher MPSS values. For example, *AtPERK1* and *8* displayed some of the highest MPSS values for the *AtPERK* family. The MPSS data also demonstrated that some *AtPERK*s were broadly expressed while others were more tissue specific (Fig. 1B). No data were obtained for *AtPERK3*, *7* and *11*, and very low values were found for *AtPERK2*, *4* and *12*. The lack of expression tags may reflect the fact that some of these PERKs tended to be expressed at relatively low levels and/or that the conditions and tissues used in generating ESTs and MPSS data were limited, and thus, may not have included the conditions under which these genes are expressed.

To overcome the potential limitations in extracting information from large-scale expression data, we conducted RNA blot analysis of all 15 *AtPERK* genes, and showed that this family contained both ubiquitous expression, and tissue-specific expression patterns (Fig. 2). The sizes of the bands detected on the RNA blots, as well as the probes used, were also consistent with the predicted gene models. Within the two major clades of the phylogeny, broadly expressed *AtPERK*

genes and tissue-specific *AtPERK* genes were observed. In the top clade (with 80% bootstrap support), *AtPERK1*, *2*, *3*, *14* and *15* were expressed in a number of different tissues with overlapping expression in bolts, buds and siliques (Fig. 2). Interestingly, *AtPERK1*, *2* and *3* are closely related to one another and are also found in a cluster on chromosome III. In contrast, *AtPERK4*, *5*, *6* and *7*, which form another sub-clade were all tissue specific, with *AtPERK4* transcripts being specific to inflorescence bolts, and *AtPERK5*, *6* and *7* being unique to floral bud tissues (Fig. 2). Interestingly, more *AtPERK*s are expressed in the influorescences than in any other tissues. In addition, although some closely related genes have similar expression patterns, they almost always show some differences in either expression profile or level. Some of these relationships were also apparent in the MPSS data. For example, *AtPERK5* and *6* were expressed in one of the inflorescence samples and *AtPERK1*, *14* and *15* were found in a number of different tissues. Consistent with our hypothesis that EST and MPSS may not be sensitive enough to pick up relatively rare transcript, all three genes without EST and MPSS data have hybridization signals on the RNA blots, and some of them are rather weakly expressed (Fig. 1, 2).

The second bioinformatics approach used was a cluster analysis of gene expression patterns across the data sets in the NASCArrays database (Craigon et al. 2004). This database, at the time of analysis, consisted of 393 *Arabidopsis* microarray data sets that surveyed a wide range of tissues, mutants and growth conditions for changes in gene expression. Data were extracted for all 15 *AtPERK* genes, and while some information about tissue-specific patterns of expression could be seen, this study was designed primarily to examine the co-regulation of these genes. Therefore, *AtPERK* genes showing a high level of co-expression across the 393 *Arabidopsis* microarray data sets were found to cluster closely together in this study (Fig. 1C). Interestingly, some of the relationships seen in the cluster analysis mimicked the phylogeny and the RNA blot results. For example, *AtPERK5*, *6* and *7* showed the highest correlation in the NASCArrays analysis (Fig. 1C), were found clustered together in the phylogeny (Fig. 1A) and showed bud-specific expression (Fig. 2). However, exceptions were also found. *AtPERK11*, another bud-specific gene (Fig. 2), grouped together with *AtPERK5*, *6* and *7* in NASCArrays analysis (Fig. 1C) although *AtPERK11* was more distant from *AtPERK5*, *6* and *7* in the kinase phylogeny (Fig. 1A). *AtPERK10* and *15* were further apart in the phylogeny (Fig. 1A), but showed good co-expression in NASCArrays analysis (Fig. 1C).

In terms of expression patterns emerging from the NASCArrays analysis, there were several trends which correlated more with the tissues used in the experiments than the treatments tested (Fig. 1C; Supplementary Fig. 1). For example, strong expression (red) was seen for *AtPERK13* in seedling and root samples (Fig. 1C; data sets #82–142); for *AtPERK3*, *9* and *13* in *Arabidopsis* cell suspension cultures (Fig. 1C; data sets #143–164); and for *AtPERK4*, *5*, *6*, *7*, *11* and *12* in flower

buds, particularly in pollen samples (Fig. 1C; data sets #302–311), fitting in with their bud-specific expression (Fig. 2). Strong expression was also seen for *AtPERK9* following control and *Pseudomonas syringae* infiltration experiments (Fig. 1C; data sets #10–58) suggesting that wounding induced the expression of *AtPERK9*. An example of strong repression (blue) was seen for *AtPERK9* in mature rosette leaves (Fig. 1C; data sets #180–248). Interestingly, the mature rosette leaf samples largely came from the Col-0 ecotype except for those in data sets # 218–231 (Fig. 1C), which used the Ws ecotype and coincidently showed repression of both *AtPERK1* and *9*, perhaps indicative of ecotype variation. Repression was also seen for *AtPERK1*, *8* and *9* in the pollen samples (Fig. 1C; data sets #306–311).

In conclusion, this study provides comprehensive expression data for the *AtPERK* family, showing both tissue-specific and ubiquitously expressed receptor kinase genes within this family. RNA blot analyses demonstrated that the majority of the *Arabidopsis* PERK family are expressed in buds (*AtPERK1-3*, *5–12*, *14*). Moreover, almost half of the *AtPERK*s were tissue specific to floral organs, perhaps indicative of their importance in this tissue. Next to floral tissues, the inflorescence bolt expressed the largest number of the *AtPERK*s. Many of the trends detected by RNA blot analysis were also reflected in the bioinformatics data extracted from the different databases, suggesting that these approaches are very useful for obtaining preliminary expression data, particularly when a large gene family is the target of interest. Nonetheless, our RNA blot analyses suggest that some transcripts may be present at a level that is not detected by large-scale expression tag analyses.

Part of the rationale for this study was our observation that single T-DNA insertion lines in the different *AtPERK* members did not show any visible phenotype (M. Aldea, S.K. Keatley and D.R. Goring, unpublished data) while the simultaneous antisense suppression of several *AtPERK* members in transgenic *Arabidopsis* resulted in a number of growth defects (Y. Haffani, N. Silva, S.K. Keatley and D.R. Goring, unpublished data). This suggests the presence of functional redundancy in the *AtPERK* gene family as has been found for other *Arabidopsis* receptor kinases (Dievart et al. 2003, Shpak et al. 2003, Shpak et al. 2004). The expression analysis presented in this study will be useful in guiding genetic cross experiments between the different *AtPERK* T-DNA insertion lines. Based on these results, genetic crosses based on relatedness in the phylogeny seem to be an appropriate direction to take. However, within a clade, there was also a switch in expression patterns from tissue-specific to a more ubiquitous pattern, and this information will need to be taken into account. Finally, the NASCArrays analysis showing good co-expression of genes such as *AtPERK10* and *15* suggests that perhaps genetic crosses between more distantly related family members may need to be considered as well when there is a strong correlation in their expression patterns.

For construction of the phylogeny, amino acid sequences corresponding to the kinase domains were used, and the kinases were chosen according to the original PERK cluster identified in Shiu and Bleecker (2001) and Shiu and Bleecker (2003). The phylogeny was generated using the neighbour-joining method (Saitou and Nei 1987) and implemented in MEGA2 (Kumar et al. 2001) with 1,000 bootstrap replicates; the percentage supports are shown on the nodes. The Poisson correction was used to account for multiple substitutions and gaps were deleted in a pairwise fashion. In addition to the PERK kinases, three kinases, CLV1, CTR1 and human ErbB1 were also included and the latter two were used as outgroups according to Shiu and Bleecker (2001).

Cognate ESTs, MPSS tags, full-length cDNAs and protein domain structures for the *At*PERK family were identified using a number of databases. The *Arabidopsis* ESTs available from GenBank as of November 25, 2003, were retrieved and the source tissues and EST counts were tabulated in Fig. 1B. The MPSS tags matching the PERK kinases were retrieved from the *Arabidopsis* MPSS database (Meyers et al. 2004; http://mpss.udel.edu/at/java.html). Only tags matching exons in the Crick strand with levels significantly different from 0 were regarded as evidence of expression. The MPSS tag counts were tabulated in Fig. 1B. The cDNA sequences released by the SIGnAL database (http://signal.salk.edu/SSP/index.html) and RIKEN (http://pfgweb.gsc.riken.go.jp/projects/raflcdna.html) were retrieved from GenBank as of May 25, 2004. The protein domain structures were predicted using SOSUI (Hirokawa et al. 1998; http://sosui.proteome.bio.tuat.ac.jp/sosui_submit.html) and PlantsP (Gribskov et al. 2001; http://plantsp.sdsc.edu/).

For the analysis of *At*PERK gene expression levels in the NASCArrays database, all 15 *At*PERK genes were represented on the ATH1 GeneChip by probe sets that are specific for each *At*PERK, as determined using a look-up table from TAIR (http://www.arabidopsis.org) called affy25k_array_elements-2004–04–05.txt. Gene expression values for each *At*PERK were retrieved across 393 data sets from NASCArrays (Craigon et al. 2004; http://ssbdjc2.nottingham.ac.uk/narrays/experimentbrowse.pl) on June 3, 2004. Data were $\log_2$ transformed and median centered, then clustered using a centered correlation metric and average linkage clustering using Michael Eisen's Cluster program (Eisen et al. 1998). Treeview was used to visualize the output (Eisen et al. 1998). More detailed results from the 393 data sets are shown as supplementary material on the journal website.

To obtain tissue for RNA blot analysis, *Arabidopsis thaliana* Col-0 seeds were sterilized in 70% ethanol, followed by treatment with Seed Sterilization Solution (20% of 6% bleach, 0.06% Tween 20) and five subsequent washes in distilled water. After vernalization for at least 1 d at 4°C in 0.1% phytagar, seeds were planted in pots and grown at 22°C, 16 h light to obtain mature tissues. Alternatively, seeds were spread on MS media (Murashige–Skoog Salt Mixture; Gibco BRL) plates for 8–10 d supplemented with sucrose (20%) (kept at 22°C, 24 h

light conditions) before being transplanted to soil. Seedling tissues were collected from plants grown on MS media for 14 d, while buds (unopened, slightly open and open), siliques (green), bolts (before senescence) and leaves (4 weeks old) were taken from potted plants. To obtain root tissues, five 8-day-old seedlings were transferred from MS plates to a flask containing liquid MS medium supplemented with 50 μg ml⁻¹ ampicillin. The flasks were subsequently shaken at 25 rpm, 22°C, 24 h light for 3–4 weeks, at which point root tissue was harvested.

Total RNA was extracted from leaves, buds, bolts, seedlings and roots using the Trizol reagent (Sigma). Silique RNA was isolated using a CTAB–LiCl (hexadecyltrimethylammonium bromide–lithium chloride)-based method, optimized for tissues high in polysaccharides and polyphenols, as described by Chang et al. (1993). Depending on the *At*PERK blot, 5 or 10 μg of total RNA were separated on a 1.2% or 1.8% formaldehyde gel, and transferred to Hybond-N+ membrane (Amersham Pharmacia), as described by Bower et al. (1996).

To obtain gene-specific hybridization, *AtPERK* probes were chosen that shared less than 70% DNA sequence identity to other *At*PERK members with the large majority displaying less than 60% DNA sequence identity. These probes corresponded predominantly to the extracellular domains, which were more divergent at the DNA sequence level. The DNA probes used were as follows (relative to the ATG): *At*PERK1, 21–1404; *At*PERK2, 31–1166; *At*PERK3, 6–1274; *At*PERK4, 75–1343; *At*PERK5, 1–535; *At*PERK6, 31–1289; *At*PERK7, 56–1474; *At*PERK8, 1–620; *At*PERK9, 1–935; *At*PERK10, 1–1561; *At*PERK11, 1–2157; *At*PERK12, 1–1232; *At*PERK13, 1–1903; *At*PERK14, 1–658; *At*PERK15, 4–1039. For *At*PERK1-4, 6, 7, and 15, the DNA probes were PCR amplified from cDNA: RNA was extracted with the RNeasy Plant Mini Kit (Qiagen), treated with DNase I (amplification grade; Gibco BRL), and cDNA was synthesized using the First Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech). The cDNA was then used in PCRs with *At*PERK-specific primers, and the PCR products were cloned into the pCR TOPO 2.1 vector (Invitrogen). For *AtPERK8-14*, full coding region cDNAs were amplified by RT–PCR (Gibco BRL or Qiagen) and cloned into the pPCRScript (+) vector (Stratagene). Probes were generated by restriction enzyme digestions. Genomic PCR was used for *At*PERK5 as its extracellular domain is contained within the first exon, and cloned into the pGEM T-Easy vector (Promega). All positive clones were sequenced to confirm their identity (York University Core Molecular Biology and DNA Sequencing Facility, Toronto, Canada).

The *At*PERK1-7 probes were labelled with [α-³²P]dATP according to Bower et al. (1996), while *At*PERK8-15 probes were labelled with [α-³²P]dCTP using the Rediprime labeling kit (Amersham). RNA blots hybridized with *At*PERK1-7 and 15 were left overnight at 42°C in 5× SSPE, 0.5% SDS, 50% formamide, 50 μg ml⁻¹ salmon sperm DNA and 10% dextran sulfate. Membranes were then washed twice in 2× SSC, 0.1%

SDS at 22°C for 15 min. This was followed by a 15 min wash at 22°C in 0.1× SSC, 0.1% SDS and two 30 min washes at 63°C in 0.1× SSC, 0.1% SDS. RNA blots hybridized with *At*PERK8-14 were hybridized overnight in Church's buffer (0.5 mM Na_2HPO_4 pH 7.2, 1 mM EDTA, 1% BSA, 7% SDS) at 65°C, followed by three 20 min washes at 65°C in 2× SSC, 0.1% SDS and one 5 min wash with 0.1 SSC, 0.1% SDS. The membranes were exposed to X-ray film for different lengths of time at –80°C using X-OMAT AR (Kodak Scientific Imaging Film) film with an intensifying screen (NEF-490). All blots were subsequently probed with the 18S rRNA probe as an internal control for loading. Each RNA blot was repeated twice.

Supplementary material

Supplementary material mentioned in the article is available to online subscribers at the journal website www.pcp.oup-journals.org.

Acknowledgments

References

Bower, M.S., Matias, D.D., Fernandes-Carvalho, E., Mazzurco, M., Gu, T., Rothstein, S.J. and Goring, D.R. (1996) Two members of the thioredoxin-*h* family interact with the kinase domain of a *Brassica S* locus receptor kinase. *Plant Cell* 8: 1641–1650.

Champion, A., Kreis, M., Mockaitis, K., Picaud, A. and Henry, Y. (2004) *Arabidopsis* kinome: after the casting. *Funct. Integr. Genomics* 4: 163–187.

Chang, S., Puryear, J. and Cairney, J. (1993) A simple and efficient method for isolating RNA from pine trees. *Plant Mol. Biol. Rep.* 11: 113–116.

Clark, S.E., Williams, R.W. and Meyerowitz, E.M. (1997) The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis. Cell* 89: 575–585.

Craigon, D.J., James, N., Okyere, J., Higgins, J., Jotham, J. and May, S. (2004) NASCArrays: a repository for microarray data generated by NASC's transcriptomics service. *Nucleic Acids Res.* 32: D575–577.

Dievart, A. and Clark, S.E. (2004) LRR-containing receptors regulating plant development and defense. *Development* 131: 251–261.

Dievart, A., Dalal, M., Tax, F.E., Lacey, A.D., Huttly, A., Li, J. and Clark, S.E. (2003) CLAVATA1 dominant-negative alleles reveal functional overlap between multiple receptor kinases that regulate meristem and organ development. *Plant Cell* 15: 1198–1211.

Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. USA* 95: 14863–14868.

Gribskov, M., Fana, F., Harper, J., Hope, D.A., Harmon, A.C., Smith, D.W., Tax, F.E. and Zhang, G. (2001) PlantsP: a functional genomics database for plant phosphorylation. *Nucleic Acids Res.* 29: 111–113.

Haffani, Y.Z., Silva, N.F. and Goring, D.R. (2004) Receptor kinase signalling in plants. *Can. J. Bot.* 82: 1–15.

Hirokawa, T., Boon-Chieng, S. and Mitaku, S. (1998) SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* 14: 378–379.

Kohorn, B.D. (2001) WAKs: cell wall associated kinases. *Curr. Opin. Cell Biol.* 13: 529–533.

Krusell, L., Madsen, L.H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N. and Stougaard, J. (2002) Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature* 420: 422–426.

Kumar, S., Tamura, K., Jakobsen, I.B. and Nei, M. (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* 17: 1244–1245.

Li, J. and Chory, J. (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* 90: 929–938.

Li, J., Wen, J., Lease, K.A., Doke, J.T., Tax, F.E. and Walker, J.C. (2002) BAK1, an *Arabidopsis* leucine-rich repeat receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* 110: 213–222.

Meyers, B.C., Lee, D.K., Vu, T.H., Tej, S.S., Edberg, S.B., Matvienko, M. and Tindell, L.D. (2004) *Arabidopsis* MPSS. An online resource for quantitative expression analysis. *Plant Physiol.* 135: 801–813.

Nam, K.H. and Li, J. (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* 110: 203–212.

Nishimura, R., Hayashi, M., Wu, G.J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y., Kawasaki, S., Akao, S., Ohmori, M., Nagasawa, M., Harada, K. and Kawaguchi, M. (2002) HAR1 mediates systemic regulation of symbiotic organ development. *Nature* 420: 426–429.

Pilling, E. and Hofte, H. (2003) Feedback from the wall. *Curr. Opin. Plant Biol.* 6: 611–616.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.

Shiu, S.H. and Bleecker, A.B. (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. *Proc. Natl Acad. Sci. USA* 98: 10763–10768.

Shiu, S.H. and Bleecker, A.B. (2003) Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in *Arabidopsis*. *Plant Physiol.* 132: 530–543.

Shiu, S.H., Karlowski, W.M., Pan, R., Tzeng, Y.H., Mayer, K.F. and Li, W.H. (2004) Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. *Plant Cell* 16: 1220–1234.

Shpak, E.D., Berthiaume, C.T., Hill, E.J. and Torii, K.U. (2004) Synergistic interaction of three ERECTA-family receptor-like kinases controls *Arabidopsis* organ growth and flower development by promoting cell proliferation. *Development* 131: 1491–1501.

Shpak, E.D., Lakeman, M.B. and Torii, K.U. (2003) Dominant-negative receptor uncovers redundancy in the *Arabidopsis* ERECTA leucine-rich repeat receptor-like kinase signaling pathway that regulates organ shape. *Plant Cell* 15: 1095–1110.

Silva, N.F. and Goring, D.R. (2002) The proline-rich, extensin-like receptor kinase-1 gene (PERK1) is rapidly induced by wounding. *Plant Mol. Biol.* 50: 667–685.

Song, W.-Y., Wang, G.-L., Chen, L.-L., Kim, H.-S., Pi, L.-Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.-X., Zhu, L.-H., Fauquet, C. and Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270: 1804–1806.

Torii, K.U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R.F. and Komeda, Y. (1996) The *Arabidopsis* ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. *Plant Cell* 8: 735–746.

Verica, J.A. and He, Z.H. (2002) The cell wall-associated kinase (WAK) and WAK-like kinase gene family. *Plant Physiol.* 129: 455–459.