Molecular Characterization of Two Highly Homologous Receptor-like Kinase Genes, RLK902 and RKL1, in Arabidopsis thaliana

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Receptor-like kinases (RLKs) constitute a large family of signal perception molecules. We characterized two highly homologous RLK genes, RLK902 and RKL1, in Arabidopsis. RLK902 and RKL1 showed a 75% amino acid sequence identity over their entire regions. In the RLK902 pro::GUS transgenic lines, GUS activity was strong in the root tips, lateral root primordia, stipules, and floral organ abscission zones, while the RKL1 promoter activity was dominant in the stomata cells, hydathodes and trichomes of young rosette leaves, and floral organ abscission zones. Neither the rlk902 mutant line, rkl1 mutant line nor rlk902/rkl1 double-knockout mutant line showed any significant phenotypes under normal growth conditions. These results suggest that RLK902 and RKL1 might mediate the signal transduction pathway in which at least one other complementary signaling pathway to these two RLKs might exist.

Key words: receptor-like kinase; Arabidopsis; root tip; lateral root primordial; stomata cell

Plants perceive and respond to numerous endogenous and exogenous stimuli such as phytohormones, pathogens, nutrients, light and stress to suit different developmental conditions. The results of recent studies suggest that receptor-like kinases (RLKs) play important roles in the detection and transduction of extracellular signals.1,2) RLKs are a family of transmembrane proteins that contain an extracellular domain, a transmembrane domain, and a cytoplasmic kinase domain.3,4) It is generally believed that RLKs activated in the presence of extracellular ligands transduce the signals by phosphorylating intracellular target proteins. RLKs are classified into several groups based on their extracellular domain structure: a leucine-rich repeat (LRR) domain,5) an S domain (homologous to the self-incompatibility locus glycoprotein),6) an epidermal growth factor (EGF) domain,6) and a lectin domain.7) There are more than 600 genes in Arabidopsis that have been predicted to encode RLKs,3,4) about 200 of which belong to the LRR group. LRR-RLKs play important roles in such cellular processes and stress responses as the maintenance of meristem by CLV1,5) organ elongation by ER,5) floral organ abscission by HAEESA,10) brassinosteroid signaling by BRI1 and BAK1,11–13) cell proliferation and differentiation by PSKR,13) and flagellin perception by FLS2.15) Except for these limited numbers of RLKs, however, little is known about the biological functions of other LRR-RLKs.

To investigate the functions of LRR-RLKs, we characterized two RLK genes, RLK902 and RKL1.16) RLK902 and RKL1 showed 75% amino acid sequence identity over their entire regions and 82% in the kinase domains. We present in this report the expression analyses of RLK902 and RKL1 and discuss the signal transduction pathways mediated by these two RLKs.

Materials and Methods

Plant materials. Arabidopsis thaliana ecotype Columbia was used for all the experiments described in this report. The plants were grown under continuous light at 23°C. The aerial parts were obtained from plants grown on rock wool and roots from plants grown on an MS-agar (0.8%) medium for RNA preparation and β-glucuronidase (GUS) staining. The rlk902 mutant line was obtained by screening the activation tagging line stock prepared by Weigel et al.17) rkl1 was a T-DNA inserted line prepared by Salk Institute (SALK_099094.22.10). The T-DNA flanking sequence is available on the http://signal.salk.edu/ web site,18) and the insertion site was confirmed by us. Both the activation tagging line stock and rkl1 seeds were obtained from Arabidopsis Biological Resource Centre. The homozygous T-DNA inserted line of each mutant was cross-pollinated, and its progeny were screened for
double mutant lines (rlk902/rlkl) by PCR.

**RNA preparation and northern blotting analysis.** A northern blotting analysis was carried out to examine the tissue specificity. The total RNA was isolated from different tissues or whole plants (about 100 mg fresh weight) by using an RNeasy plant mini kit (Qiagen). Ten μg of total RNA was separated on 1% agarose gel containing 1% formaldehyde, before being transferred to a nylon membrane (Hybond-N+, Amersham Pharmacia). The full-length cDNAs of RLK902 and RKL1 were amplified by PCR with the following primers: sense for RLK902, 5'-GGCGGCGCGG(Smal)ATTCGATCTTCTTCTACAC-3'; antisense for RLK902, 5'-GGCTTTTCGAC(Sal)-CCCACCCGATCGTGC-3'; sense for RKL1, 5'-GGACCAGCCGGCCGCCGGCCCGG(Smal)TTAATCTAGCTCGTTCCAC-3'; antisense for RKL1, 5'-GGGCAGGCG(Smal)TGATCCTGTCAC-3'; the 2.75-kb RLK902:GFP fusion gene fragment was amplified by PCR with the respective sense primers for RLK902 and RKL1. The amplified cDNA fragments were cloned into the EcoRI site of CaMV35S-sGFP(S65T)-NOS3 (generously presented by Y. Niwa21)). The resulting plasmid was partially digested with SalI and then fully with EcoRI, and the 2.75-kb RLK902:GFP fusion gene fragment was replaced with the GUS gene of pBI902GUS by cloning into the Smal/EcoRI sites. Fluorescence microscopy was performed with a BX60 microscope (Olympus) with a GFP filter (Olympus).

**Construction of the GFP reporter gene and fluorescence microscopy.** Full-length cDNA of RLK902 was excised with SalI from pBS902 and cloned into the SalI site of CaMV35S-sGFP(S65T)-NOS3 (pMP90) by triparental mating23). Arabidopsis was transformed by the method of Bechtold and Pelletier20) and transformants were selected on an MS-agar (0.8%) medium containing kanamycin (100 μg/ml). GUS staining was performed in a 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc), 0.5% (v/v) Tween-20, 0.5 mM K3[Fe(CN)6], 0.5 mM K4[Fe(CN)6], and 20% methanol.

**Construction of the GUS reporter gene, plant transformation and histochemical GUS staining.** The respective 2000-bp and 2548-bp genomic DNA fragments upstream of the coding region of RLK902 and RKL1 were amplified by PCR with the following primers: sense for RLK902, 5'-GCAAGCTTT(HindIII)AACTAATAATTTTCAATACAAATG-3'; antisense for RLK902, 5'-GC-GGATCC(BamHI)GTGTAAGAAAACAAGAGAGAAGCC-3'; sense for RKL1, 5'-GACCGGC(Gmal)GCTTTAGACTTTTCTCTTGTGTTTCTTG-3'; antisense for RKL1, 5'-GACCGGGG(Smal)TGTCGTAATTCTAGAGAAGAGAGAAGG-3'. The amplified fragments were cloned into pGEM T-easy (Promega). After selecting the clones with no sequence error, the promoter fragments of RLK902 were excised with HindIII and BamHI, and then cloned into the HindIII/BamHI sites of the pBlII21GUS expression binary vector (Clontech), giving the pBI902GUS plasmid. The RKL1 promoter fragment was excised with Smal and then cloned into the HindIII/Smal sites of pBlII121 after blunting the HindIII site, giving the pBlI001GUS plasmid. The resulting vectors were introduced into Agrobacterium GV3010.
In the RKL1 pro::GUS transgenic lines, GUS activity was detected in the vascular tissues of roots, but not in the root tips (Fig. 4A), contrary to the RLK902 pro::GUS line. GUS activity was detected in the trichomes of young rosette leaves and hydathodes (Fig. 4B). In the floral tissues, GUS activity was detected in the floral organ abscission zones (Fig. 4C), filament apex (Fig. 4D), and in the stomata cells of anthers (Fig. 4D arrows), inflorescence stems (Fig. 4E) and sepals (Fig. 4F). Apart from the root vascular tissues and floral abscission zones, RLK902 and RKL1 did not show common expression localization.

**Subcellular localization of RLK902**

To determine the subcellular localization of RLK902, a green fluorescent protein (GFP) was fused with the C terminus of full-length RLK902 cDNA. This fusion gene was expressed under the control of the same promoter region of RLK902 as that for the above-mentioned promoter-GUS experiment. RLK902:GFP fluorescence localized at the cell surface was observed in the root tips and lateral root primordia (Figs. 5A-C). In the floral tissues, RLK902:GFP fluorescence was detected in all cell layers in the root tips (Fig. 5D). To confirm the localization of RLK902:GFP to the plasma membrane, root tissues were treated with (Fig. 4D), and in the stomata cells of anthers (Fig. 4D arrows), inflorescence stems (Fig. 4E) and sepals (Fig. 4F). Apart from the root vascular tissues and floral abscission zones, RLK902 and RKL1 did not show common expression localization.

![Fig. 2](image-url)  
**Expression Patterns of the RLK902 and RKL1 Transcripts in Different Tissues.** Ten microgram of total RNA isolated from different tissues was hybridized with a RNA probe generated from full-length cDNA of RLK902 or RKL1. F, flowers; S, stems; L, rosette leaves; R, roots.

![Fig. 1](image-url)  
**Sequence Comparison between RLK902 and RKL1.** Gaps, which were introduced to provide maximum alignment, are indicated by dashes. Identical amino acids are shaded in black and similar amino acids are shaded in light gray. LRR1–LRR5, leucine-rich repeats 1–5; I–XI, protein kinase domain I–XI; SP, signal peptide; TM, transmembrane domain.
0.8 M mannitol to induce plasmolysis. Fluorescence was localized on the round-shaped plasma membranes separated from the cell walls (Fig. 5F).

Characterization of rlk902 and rkl1

The T-DNA insertion lines of RLK902 and RKL1 were respectively obtained by screening mutant stock from ABRC or ordering the T-DNA tagging line whose T-DNA flanking sequence had been determined. Northern hybridization showed that RLK902 and RKL1 were not respectively expressed in rlk902 and rkl1 (data not shown). Neither rkl1 nor rlk902 showed significant phenotypes under normal growth conditions, so an rlk902/rkl1 double-knockout mutant was prepared. The homozygous T-DNA insertion lines for both RLK902 and RKL1 were screened out by PCR. The rlk902/rkl1 mutants again showed no obvious phenotype when compared to the wild-type plants, suggesting that there were additional RLKs or other signaling pathways complementing the defect of RLK902 and/or RKL1 functions.
Discussion

RLKs in plants have been proposed to play important roles in detecting and transducing extracellular signals in various processes. However, little is known about the specific role of each RLK. One possible reason for this is that the mutation of a single RLK gene might not give any clear phenotype, it thus being difficult to elucidate the biological function by a genetic approach. This situation is true for a large gene family with many members such as Myb-class transcription factors. To expand our knowledge on the roles of RLKs, we focused on two, RLK902 and RKL1, which showed 75% identity at the amino acid sequence level.

Under normal growth conditions, the promoter activity of RLK902 was localized in the root tips, lateral root primordia, root vascular tissues, stipules and floral organ abscission zones; however, the rlk902 mutant did not show any differences from the wild-type plant in root elongation, lateral root initiation, morphology of stipules, or floral organ abscission. This indicates that there might exist a functionally redundant signaling pathway(s), possibly mediated by RLKs. Previous reports have shown that RLK4 and ARK3 were expressed in the lateral root primordium, IRK in the root apex, and HAESA in the floral organ abscission zone. Genetic analyses using mutants of these RLKs and rkl902 would be needed to elucidate their cooperative function.

The expression pattern in root tissues suggests that RLK902 was involved in cell proliferation. In contrast, the expression in the aerial parts suggests senescence, since the promoter activity was localized in the floral organ abscission zones and also in the stipules which are not supposed to develop any further. It is difficult at the moment to interpret this apparent contradiction. It is known that RLKs function as hetero dimers to perceive and transduce signals, as in the case of CLV1/CLV2 and BRI1/BAK1. RLK902 might be involved in multiple signaling events by forming dimers with different counterpart molecules, thus interpreting its complicated localized expression.

GUS activity driven by the RKL1 promoter was also detected in different types of cells: root vascular tissues, floral organ abscission zone, filament apex, stomata cells of anthers, sepals and stems, trichomes of young rosette leaves, and hydathodes. The expression in stomata cells and hydathodes implies that RKL1 may have acted to control the water status, this implication being supported by the result that the transcript level of RKL1 was decreased by a drought treatment and recovered after replenishing the water. This result suggests that RKL1 might act for stomata opening. In this case, RKL1 should be upstream of ABA on the signaling pathway for regulating stomata opening, because RKL1 did not respond to an ABA treatment. We have so far obtained results that the rkl1 mutant is neither sensitive nor tolerant to drought conditions (data not shown). Again, the presence of other molecules complementing the disruption of the RKL1 function must be presumed.

We analyzed in this study the most homologous two
RLKs among a large number of RLK members. Not all the results supported the notion that RLK902 and RKL1 shared the same biological function, consequently providing an example of the reverse-genetic approach using genes with high homology not always producing a fruitful result. Nevertheless, our results provide the detailed expression profiles of two RLKs in terms of their spatial localization which must give fundamental information that is valuable for the comprehensive understanding of a large RLK gene family. Moreover, it still remains possible that RLK902 and RKL1 may have common biochemical functions, especially in their downstream signal transduction, since the highest homology resides in their kinase domains. Indeed, we have identified some cDNA clones whose products interacted commonly with the kinase domains of these two RLKs in a yeast two-hybrid system (the results will be published later). Since we have seen that the gene expression of both RLKs was responsive to such stress conditions as wounding, salicylic acid and pathogen attack (data not shown), a detailed analysis of those interacting clones, including stress responses, would help to elucidate the complex signaling pathways mediated by RLK902 and RKL1. Based on the expression analysis, there being the possibility that RLK902 and/or RKL1 under stress conditions, it would also be necessary to analyze the rkl902 mutant, rkl1 mutant and rkl902/rkl1 double-knockout mutant under stress conditions.

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References


