Mol Gen Genomics (2005) 273: 217–224
DOI 10.1007/s00438-005-1115-z

ORIGINAL PAPER

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Cloning and characterization of a gene for an LRR receptor-like protein kinase associated with cotton fiber development

Received: 18 September 2004 / Accepted: 14 December 2004 / Published online: 7 April 2005

Abstract Cotton fiber is an ideal model for studying plant cell elongation and cell wall biogenesis, but the genes that are critical for the regulation of fiber development are largely unknown. We report here the cloning and characterization of a receptor-like kinase gene (designated *GhRLK1*), expression of which is induced during the period of active secondary wall synthesis in the cotton fiber cells. We demonstrate that GhRLK1 is located in the plasma membrane and shows dual specificity as both a serine/threonine kinase and a tyrosine kinase. Our results suggest a possible role of *GhRLK1* in the signal transduction pathway that is involved in the induction and maintenance of active secondary wall formation during fiber development.

Keywords Cotton fiber · Development · Receptor-like kinase · Signal transduction

Introduction

The cotton fiber is a single, elongated epidermal cell of the seed coat. The development of cotton fiber can be divided into four distinct yet overlapping stages: initiation, elongation, secondary wall synthesis and maturation (Basra and Malik 1984). Fiber elongation begins on the day of anthesis and lasts for 20–30 days. In some cotton cultivars the fiber cells can reach a length of 3–6 cm. The synthesis of the secondary cell wall, which is composed almost exclusively of cellulose, begins slightly before the cessation of elongation and continues for several weeks, with the most active cellulose synthesis occurring at about 24 DPA (days post-anthesis). During the transition period between the termination of fiber elongation and the onset of secondary wall formation, the rate of fiber elongation decreases rapidly, whereas that of the secondary wall synthesis increases tremendously (Meinert and Delmer 1977; Wilkins and Jernstedt 1999). Due to its exceptional cell length and high cellulose content, cotton fiber is considered as an excellent model for the study of plant cell elongation and cellulose biosynthesis (Kim et al. 2001). Although much progress has been made in identifying genes that are expressed predominantly at the stages of cell elongation, secondary wall formation and the transition between the two phases, little is known about the molecular mechanisms that regulate these cellular events in the developing fibers.

Plant receptor-like kinases (RLKs) play crucial roles in various signaling processes during growth and development, hormone perception, self-incompatibility, symbiont recognition and pathogen responses (Morris and Walker 2003). Like animal RTKs, plant RLKs usually consist of an extracellular domain, a single-pass transmembrane region, and a cytoplasmic kinase domain. In contrast to animal receptor kinases, which mainly phosphorylate tyrosine residues, plant RLKs exhibit serine/threonine kinase activity (Becraft 2002), with only two reported exceptions that show dual specificity, i.e. phosphorylation of both serine/threonine and tyrosine residues (Mu et al. 1994; Shah et al. 2001a).

Based on the amino acid sequences of their extracellular domains, RLKs are categorized into several major subclasses (Becraft 2002), the largest of which is the subfamily of the leucine-rich repeat (LRR) receptor-like

Communicated by G. Jürgens

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kinases. LRR-RLKs contain in their extracellular domains different numbers and arrangements of LRR, which are involved in protein-protein interaction and in targeting of the receptors (Kobe and Deisenhofer 1994; Shah et al. 2001b). In recent years, many advances have been made in the study of LRR-RLKs, and it was found that this group of proteins plays important roles in diverse processes of plant development (Dièvart and Clark 2004; Torri 2004). *Arabidopsis*, for example, contains 216 *LRR-RLK* genes (Shiu and Bleecker 2001). Among these, the *CLAVATA1* (*CLV1*) gene is known to be involved in meristem differentiation (Clark et al. 1997), the *HAESA* gene controls the floral organ abscission (Jinn et al. 2000), the *SERK1* (somatic embryogenesis RLK) gene functions in ovule development and embryogenesis (Hecht et al. 2001), the *EMS1* (excess microsporocytes) gene controls somatic and reproductive cell fates (Zhao et al. 2002), the *ERECTA(ER)* gene regulates the shape of various shoot organs (Torri et al. 1996), and the *Brassinosteroid Insensitive1* (*BRI1*) gene plays a critical role in BR signal transduction (Li and Chory 1997; Li et al. 2002; Nam and Li 2002). In light of the results obtained in *Arabidopsis*, as well as of other plants, LRR-RLKs appear to be involved in almost all aspects of plant development.

In this study, we have identified an *RLK* gene from *Gossypium hirsutum* (named *GhRLK1*) that is a member of the LRR subfamily. In vitro kinase assays demonstrated that GhRLK1 is a dual-specificity kinase that can phosphorylate both serine/threonine and tyrosine residues. Expression of *GhRLK1* was fiber-specific and developmentally regulated, with the highest expression occurring at the time when synthesis of the cellulose of the secondary wall is most active. Subcellular localization studies indicated that GhRLK1 was targeted to the plasma membrane. These results suggest that GhRLK1 may be an important component of the signal transduction pathway that controls the development of the cotton fiber.

Materials and methods

Plant material and tissue isolation

Seeds of the *G. hirsutum* variety TM-1 (the genetic standard) were treated with H_2SO_4 and sterilized with 0.1% $HgCl_2$ for 10 min, then sown on agar plates containing MS medium and grown under tissue culture conditions for 2 weeks. Roots, hypocotyls, and leaves were then collected from the seedlings. Flowers and fibers were harvested from field-grown cotton plants. For the collection of fibers, the flowers were labeled using plastic tags on the day of anthesis and bolls were harvested at 6, 9, 12, 15, 18, 21, 24, and 27 DPA, respectively. Fibers removed from the ovules and the other tissues collected were immediately frozen in liquid nitrogen and stored at −80°C.

Total RNA extraction

Total RNAs were prepared from the cotton roots, leaves, hypocotyls, flowers, ovules and fibers using the method of ultracentrifugation described by John and Crow (1992).

Fluorescence differential display (FDD)

The FDD was performed according to the instructions provided with the Hieroglyth kit for mRNA profiling by differential display analysis (Beckman). Total RNAs isolated from fibers at 9, 21, and 27 DPA were used for FDD analysis. The anchor primers were AP3 (5′-T7dT_{12}GG-3′) and AP7 (5′-T7dT_{12}AC-3′), the random primers were APR17 (5′-M13r-CTGCTAGGTA-3′), APR18 (5′-M13r-TGATGCTACC-3′), APR19 (5′-M13r-TTTTGGCTCC-3′) and APR20 (5′-M13r-TCG-ATACAGG-3′). The PCR products were separated by electrophoresis on 5.6% polyacrylamide gels.

The amplified cDNA fragments that displayed differences in expression levels at the three developmental stages were subjected to reverse Northern blot analysis using ^{32}P-labeled first-strand cDNA probes prepared from total RNAs of 9-DPA, 21-DPA, and 27-DPA fibers, respectively. Two rounds of reverse Northern blot analysis were conducted to validate the differential expression of the identified cDNAs.

DNA sequencing and protein analysis

The DNA sequences were determined using an automated DNA sequencer (Genecore, Shanghai). The ORF analysis, protein sequence alignment and motif analysis were performed with NCBI Blast, DNAMAN, Motif-Scan and PESTfind (ExPASy server) programs.

Northern analysis and semi-quantitative RT-PCR

Total RNAs (15-μg per sample) were denatured and fractionated by electrophoresis on 1.2% (w/v) formaldehyde gels. The RNAs were transferred onto nylon membranes (Hybond-N+, Amersham, UK) by capillary transfer. After pre-hybridization for 2 h at 65°C in Church buffer (7% SDS, 1% BSA, 1 mM EDTA, 250 mM phosphate buffer), the blots were hybridized for 16 h with a ^{32}P-labeled probe made from the 3′UTR of the *GhRLK1* cDNA, then washed several times at 65°C and exposed to X-ray films for autoradiography. 18S rRNA was used to quantity differences in RNA loading.

Reverse transcription for first-strand cDNA synthesis was performed using 1 μg of total RNAs from 6-DPA, 9-DPA, 12-DPA, 15-DPA, 18-DPA, 21-DPA, 24-DPA and 27-DPA cotton fibers, respectively. Aliquots (1 μl) of the RT products were then used for PCR. *GhRLK1* cDNA was amplified by PCR with the oligonucleotide

primers sense (5′-GCTCTAGACGGACGAAGAAG-CCCAAACT-3′) and antisense (5′-CGAGCTCAAG-CAGCAGCAATCAAGGC-3′). After initial denaturation at 94°C for 3 min, PCR was carried out for 25 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 3 min, followed by a final extension at 72°C for 10 min. The products of PCR were separated by electrophoresis on 0.8% agarose gels. The *histone* gene was used as an internal control.

DNA gel-blot analysis

Genomic DNA was extracted from leaves of the cotton (*G. hirsutum*) variety TM-1 using the Plant DNA Extraction kit (Amersham); 10-μg aliquots of genomic DNA were digested with the restriction enzymes *Eco*RI, *Eco*RV and *Xba*I at 37°C overnight and separated by electrophoresis on 1% agarose gels. The DNA was then transferred onto nylon membranes (Hybond-N+, Amersham) by capillary transfer, and the membranes were prehybridized and hybridized with radiolabeled cDNA probe as described above for Northern blot analysis.

Expression and purification of recombinant protein GhRLK1-K

The ~1-kb cDNA fragment encoding the kinase domain of GhRLK1 was amplified by PCR using the high-fidelity enzyme Platinum Pfx DNA Polymerase (Invitrogen, UK) and the product was cloned into the pGEM-T easy vector. Subsequently, the fragment was excised by digestion with *Sph*I and *Hind*III, and subcloned into the expression vector pQE30. The plasmid construct was transformed into *Escherichia coli* (strain M15) cells that had been grown in LB culture with vigorous shaking at 37°C to a density equivalent to an OD_{600} of 0.6. IPTG (isopropyl-β-D-thiogalactopyranoside) was added to the culture to a final concentration of 1 mM to induce expression of the recombinant protein (named GhRLK1-K), the cells were incubated for a further 5 h and then harvested. The fusion protein was purified as described by Nishiguchi et al. (2002); the supernatant was concentrated using a Centricon Centrifugal Filter Device equipped with a YM-3 membrane (Millipore, MA, USA) and stored at −20°C.

In vitro kinase activity assay

Aliquots (10 μg) of the purified GhRLK1-K were used for kinase assays. Reactions (25-μl) containing 50 mM Tris–HCl (pH 7.6), 50 mM KCl, 5 mM Mn^{2+}, 2 mM DTT, 10% (v/v) glycerol, and 15 μCi [$\gamma-^{32}$ P]ATP were incubated at 22°C for 1 h, and terminated by the addition of 10 mM EDTA. Then, 5× sample buffer was added to the reaction tube and the mixture was incubated for 1–2 min at 98°C. The phosphorylated protein was visualized by autoradiography after electrophoresis on a 12% SDS-polyacrylamide gel. For phosphoamino acid analysis, fusion protein labeled with [$\gamma-^{32}$ P]ATP was electrophoretically transferred to PVDF membrane (Gelman) and autoradiographed. The radiolabeled protein band corresponding to GhRLK1-K was then excised and partially hydrolyzed in 6 N HCl at 110°C for 1 h (Kamps and Sefton 1989). The supernatant was evaporated to dryness, and resuspended in 6 μl of the high voltage electrophoresis buffer (pH 1.9) containing 2 μg each of phosphoserine and phosphothreonine and 4 μg of phosphotyrosine as standards. Samples were spotted onto TLC (thin-layer chromatography) plates (Merck) and two-dimensional thin-layer electrophoresis was performed according to Van der Geer and Hunter (1994). The first dimensional analysis was carried out at 1.5 kV for 20 min at pH 1.9 [in 2.2% (v/v) formic acid, 7.8% (v/v) acetic acid] and the second dimensional analysis was done at 1.6 kV for 13 min at pH 3.5 [in 0.5% (v/v) pyridine, 5% (v/v) acetic acid, 0.5 mM EDTA]. Ninhydrin (0.25% in acetone) was used to visualize the standards and the labeled spots were detected by autoradiography.

Subcellular localization of GhRLK1

The cDNA fragment containing the region coding for the putative extracellular and transmembrane domains of GhRLK1 (nucleotides 1–377) was fused to the 5′ end of the GFP coding region (Tanaka et al. 2002), and the fragment was subcloned into the pPZP111 (Hajdukiewicz et al. 1994) expression vector under control of the CMV 35S promoter. Two micrograms of the plasmid construct was used to coat gold particles, and the plasmid containing GFP alone was used as control. Onion bulbs were cut open and the inner epidermal layers were peeled off and carefully placed on a plate containing MS in 3% agar. The peels were bombarded with the plasmid-coated gold particles using a gene gun (PDS-1000/He; Bio-Rad, ON, Canada), and then incubated at 25°C overnight on a fresh plate (Zhao et al. 2002). Plasmolysis of the cells was resulted by placing each sample in 0.8 M mannitol for 5–10 min. Cells were observed using a laser scanning confocal microscope (Leica). GFP fluorescence was induced by excitation at 488 nm with an argon laser.

Results

Cloning of the *GhRLK1* cDNA and analysis of its predicted product

To identify genes that are specifically expressed at a given stage of cotton fiber development, mRNAs from 9-DPA, 21-DPA, and 27-DPA fibers, which represent

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MYGLWGFERLKLRMMVVMLLMLVLSLFEQNMSFSSPLNREGLALLRFKQRVGSDPFGALS     60
NWKEIDGEIDPCSWFGVECSDEKVVILNLKDLCLVGNLGPEFGKLENLKSILLRNNSLSG    120
SIPQEIGELKELEVLDLGFNNFSGPFPSDFGNNLSLTTLLLDNNEFLGNLAPEIYEVKML    180
SEFQVDENQLTDAATIPSCKSSGFPWNIAQPGDIAYGRRLQQVFVPSKTANERVSQLSPS    240
PSPSESSFSPSMSPSPSSLSPSESPSSIFLTPAPSPLPSPSPELAPAPALQPPVDPPVSI    300
SEPPQSHNAPANSPASTPRQISNENSDSKHHIFPILIASIGGSLIVLVSVLSIILFRNSK    360
VVSVKPWATGLSGQLQKAFVTGVPKLKRSELEAACEDFSNVIGTFSDGTVYKGTLSSGVE    420
IAVTSTAISSREDWSKNLETQFRNKIDSLSKVNHKNFVNLIGYCEENTPFTRMMVFEYVP    480
NGSLYEHLHIQEAEHLDWGMRLRIAMGITYCLEHMHQLTPPIAHRNLQSCSVYLTEDYAA    540
KISDFSFLNNATAAKVGSATMELLESPSADAESNVYSFGVILFEMITGRIPYSIDNSSLA    600
DWASDYLKRDQPLKEMVDPTLKFFQGDDLEKLFEVVKTCVNPDPKERPTMREVAAKLKEI    660
TAMGPDGATPKLSPLWWAELEILSTEPS*                                    688
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Fig. 1 Predicted amino acid sequence of GhRLK1. The *dotted* lines indicate the leucine-rich repeats (LRR), the putative signal peptide is *underlined*, the residues comprising the transmembrane domain are *shaded*, and the PEST sequence is shown in *bold face*. The *broken lines* mark the protein kinase domain (the conserved residues required for the kinase activity of plant RLKs are *boxed*, and the *asterisks* denote those found in animal RTKs). The cDNA sequence encoding the kinase domain of GhRLK1 has been deposited in the EMBL database under Accession No. AY212968

the phases of fast-elongation, the transition between cessation of cell elongation and onset of secondary wall formation, and rapid-synthesis of the secondary wall cellulose, respectively, were compared by the method of FDD. A cDNA fragment of 607 bp with sequence homology to plant *LRR- RLK* genes was found to be abundant among the PCR products of mRNAs from 21-DPA fibers, and the corresponding gene was designated as *GhRLK1*. To obtain the full-length cDNA, the fragment was used as a probe to screen a cDNA library of 18–21-DPA cotton fibers, and a 2.3-kb cDNA clone of *GhRLK1* was obtained. Since this cDNA was also incomplete at the 5′ end, a 5′RACE (Rapid Amplification of cDNA Ends) experiment was performed, and the full-length 2539-bp *GhRLK1* cDNA (Fig. 1) encoding a polypeptide of 688 amino acids with 165 bp of 5′UTR and 285 bp of 3′UTR was acquired. Protein structure analysis indicated that GhRLK1 consisted of an extracellular domain, a transmembrane region and a C-terminal cytoplasmic kinase domain. Several features were observed within the extracellular domain of GhRLK1, including an N-terminal signal peptide of 26 amino acids, four tandem copies of LRR, and a putative PEST sequence which is within a serine/proline-rich domain (Fig. 1). The PEST is a proteolytic signal found in highly regulated proteins (Rechateuber 1990), and a score of 5 or greater with the PESTfind program (EMBnet Austria) is regarded as significant. GhRLK1 has a PEST score of 14.4, suggesting that this protein is subjected to stringent regulation in cotton fiber cells. Among plant RLKs, PEST sequences have so far only been reported in LTKs, the novel receptor kinases found to be expressed temporally in maize endosperm (Li and Wurtzel 1998).

A BLAST search with the complete amino acid sequence of GhRLK1 indicated that it shared a high degree of homology with a number of other proteins; a putative Ser–Thr protein kinase (AAD25942) from *Arabidopsis* and a predicted LRR-RPK (BAC75564) from rice showed the highest homology, with 52 and 57% identity and 68 and 76% similarity, respectively.

Tissue-specific and developmentally regulated expression of *GhRLK1*

The steady-state levels of *GhRLK1* mRNA in various organs of cotton were analyzed using its 3′UTR as a gene-specific probe. As shown by Northern analysis, expression of *GhRLK1* occurred predominantly in cotton fibers; no transcription was detected in other organs, except for a trace signal in the hypocotyls (Fig. 2a). During fiber development, the transcription level of *GhRLK1* was low at the elongation stage (Fig. 2b, lanes 1–4), started to increase at the onset of secondary wall synthesis (Fig. 2b, lanes 5–6), and reached a peak at the time when the secondary wall cellulose was being actively synthesized and then declined (Fig. 2b, lanes 6–8). This expression pattern of *GhRLK1* is similar to that of the *CelA1* gene, which codes for the catalytic subunit of cellulose synthase (Pear et al. 1996). Together, this result

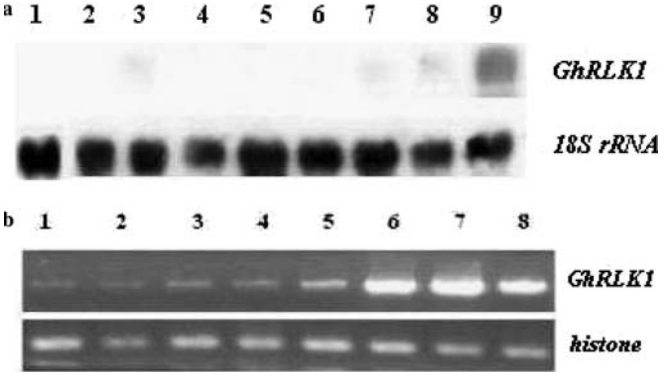

Fig. 2 The expression pattern of *GhRLK1*. **a** Northern analysis of the expression pattern of *GhRLK1* in various organs. The *upper panel* shows the transcripts detected by a gene-specific probe for *GhRLK1*. 18S rRNA was used to quantity differences in RNA loading. Fiber age is shown in DPA. *Lane 1* 24 DPA ovules; *Lane 2* roots; *Lane 3* hypocotyls; *Lane 4* leaves; *Lane 5* flowers; *Lanes 6–9*: 6-, 12-, 18-, 24-DPA fibers, respectively. **b** RT-PCR analysis of *GhRLK1* expression in developing cotton fibers. *Lanes 1–8*: 6, 9-, 12-, 15-, 18-, 21-, 24- and 27-DPA fibers, respectively. A *histone* gene was used as an internal control

showed a clear correlation between *GhRLK1* expression and active synthesis of the secondary wall cellulose in developing fiber cells.

Southern analysis of the *GhRLK1* gene

The copy number of *GhRLK1* in the cotton genome was analyzed by Southern analysis using the gene-specific 3′UTR as probe. The presence of three bands in three different restriction digests indicates that *GhRLK1* is a gene with a low copy number in the allotetraploid cotton genome (Fig. 3).

Biochemical characterization of the GhRLK1 kinase

To assess the kinase activity of GhRLK1, the cytoplasmic kinase domain (named GhRLK1-K) of GhRLK1 was expressed as a His-tagged polypeptide in *E. coli*, and purified by passage over nickel resin (Fig. 4, lanes 2–4). The native GhRLK1-K protein was tested for autophosphorylation activity, and the appearance of a single radioactive band (Fig. 4, lane 5) demonstrated that GhRLK1 was indeed able to phosphorylate itself. To further determine which amino acids of GhRLK1 were autophosphorylated, the $\gamma-^{32}$ P-labeled proteins were subjected to partial hydrolysis and the resulting products were analyzed by two-dimensional thin-layer electrophoresis. As shown in Fig. 5, labeled spots corresponded not only to the positions of phosphoserine and phosphothreonine, but also to that of phosphotyrosine—although to a lesser extent. These results indicate that GhRLK1 is a functional and dual-specificity protein kinase.

GhRLK1 possesses consensus motifs that are characteristic for plant serine/threonine kinases and the conserved sequence associated with animal tyrosine specific protein kinases (Fig. 1). The results of our kinase activity assays are therefore in good agreement with the predicted biochemical function of GhRLK1.

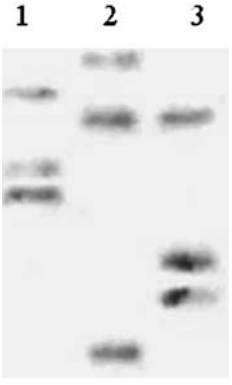

Fig. 3 Southern analysis of the *GhRLK1* gene in the cotton genome. Genomic DNA was digested with various restriction enzymes, fractionated by electrophoresis on 1% agarose gel, blotted onto a nylon membrane, and hybridized with gene-specific probe. *Lane 1 Eco*RI; *Lane 2 Eco*RV; *Lane 3 Xba*I

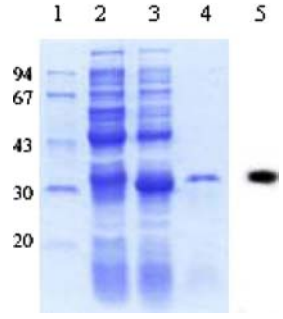

Fig. 4 Autophosphorylation of GhRLK1-K. *Lane 1* molecular mass marker (kDa); *Lane 2* total protein before IPTG induction of *GhRLK1-K* expression; *Lane 3* total protein after IPTG induction of *GhRLK1-K* expression; *Lane 4* purified GhRLK1-K; *Lane 5* [γ-32P]-labeled GhRLK1-K after kinase activity assay. *Lanes 1–4* show the stained gel; *lane 5* shows an autoradiograph. See Materials and methods for details of the assay

Subcelluar localization of GhRLK1

To examine if GhRLK1 is a transmembrane receptor kinase, as expected from its amino acid sequence, the subcellular localization of GhRLK1 was determined. As onion epidermis consists of large, living, transparent cells in a monolayer and has been used as a model system for the visualization of GFP (Scott et al. 1999), we chose this system to investigate the subcellular localization of GhRLK1 in a transient expression assay. The cDNA fragment containing the coding region for the putative extracellular domain and transmembrane domain of GhRLK1 was fused immediately upstream of

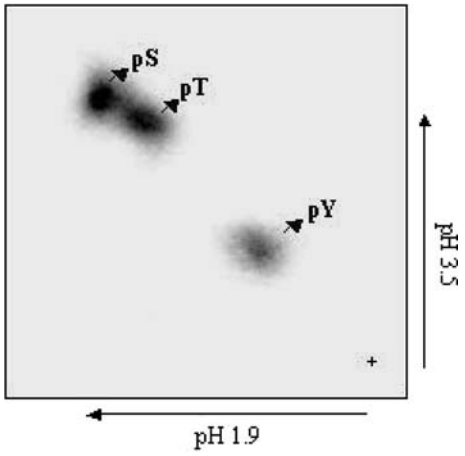

Fig. 5 Analysis of phosphoamino acids obtained by hydrolysis of autophosphorylated GhRLK1-K. [γ-32P]-labeled GhRLK1-K was hydrolyzed with HCl and subjected to two-dimensional thin-layer electrophoresis. The plate was then exposed to X-ray film, and the resulting autoradiograph is shown. *pS* phosphoserine; *pT* phosphothreonine; *pY* phosphotyrosine; the *plus* sign indicates the origin where the sample was applied before electrophoresis

the GFP coding region, and cloned into the plant expression vector pPZP111 under the control of the CMV 35S promoter. The constructs encoding GFP alone and the GhRLK1-GFP were separately introduced into onion epidermal cells by particle bombardment. As revealed by confocal microscopy (Fig. 6), while the free GFP protein was distributed throughout the cell—in the nucleus, in the cytoplasm, and on the surface (Fig. 6a)—the GhRLK1-GFP fusion protein was observed only at the cell periphery (Fig. 6b). To distinguish between the cell wall and the plasma membrane, a plasmolysis experiment was conducted. Exposure of the bombarded onion epidermal cells to 0.8 M mannitol resulted in plasmolysis. Since GhRLK1-GFP fluorescence was perceptible only at the internalized plasma membrane and not at the cell wall (Fig. 6c), the localization of GhRLK1 at the plasma membrane was confirmed. The same result was obtained when the analysis was done with tobacco BY-2 cells transformed with the GhRLK1-GFP construct by *Agrobacterium*-mediated transformation (date not shown).

Discussion

GhRLK1 is an active receptor-like protein kinase with dual specificity

In this work, we have identified an LRR-RLK protein kinase from developing cotton fibers. Based on its protein structure, subcellular localization and kinase activity, it is evident that GhRLK1 is a dual specificity-type receptor kinase. So far, tyrosine kinase activity has been reported for only two plant RLKs. One is PRK1 (pollen receptor-like kinase 1) from *Petunia inflata*, which is expressed specifically in pollen and pollen tubes (Mu et al. 1994); the other is SERK1 from *Arabidopsis* (*AtSERK1*), which is expressed mainly during early stages of embryogenesis (Shah et al. 2001a). Although the majority of plant RLKs identified to date showed Ser/Thr activity, it is believed that more plant RLKs should also have tyrosine activity (Becraft 2002) since dual specificity would be expected to broaden their spectrum of downstream substrates (Shah et al. 2001a).

The cloning and biochemical characterization of *GhRLK1* and its product thus adds a new member to the group of dual specificity kinases known in plants.

GhRLK1 may be an important modulator of cotton fiber development

Cotton fiber is a highly specialized single cell. Elongation and secondary cell wall synthesis are two important phases in fiber development. At the transition between these two stages, complex biochemical and structural changes (e.g., in the orientation of deposition of cellulose microfibrils) occur. Although efforts have been devoted to characterizing the cellular functions of genes that are specifically expressed at a certain stage of fiber development, very few of them have been proven to play an essential role except that a recent study has demonstrated that a sucrose synthase gene (*Sus*) plays a critical role in fiber initiation and elongation (Ruan et al. 2003). Hence, many aspects of fiber development remain mysterious. Unresolved questions include the following: what is the mechanism that regulates the termination of fiber elongation? What is the signal that triggers the onset of the secondary cell wall formation? Are these two processes modulated by a coupled system? etc. Answering these questions will lead not only to the elucidation of the molecular mechanisms regulating fiber development, but also to strategies for manipulating the progression of fiber development and possibly affecting the properties of the fibers (Delmer 1999). *GhRLK1* expression starts to increase at the beginning of secondary cell wall synthesis, and remains at a high level throughout the period when secondary wall formation is most active. Furthermore, there is an evident correlation between the expression profiles of *GhRLK1* and *CelA1* (Pear et al. 1996) in *G. hirsutum*. These features of *GhRLK1* suggest that it may play a role in the signal transduction pathway involved in the induction and maintenance of active synthesis of the secondary wall cellulose. Apart from *GhRLK1*, a *Rac* gene (*Rac13*) has also been found to be expressed highly at the onset of secondary wall synthesis (Delmer et al. 1995). It is postulated that this gene is involved in the signal

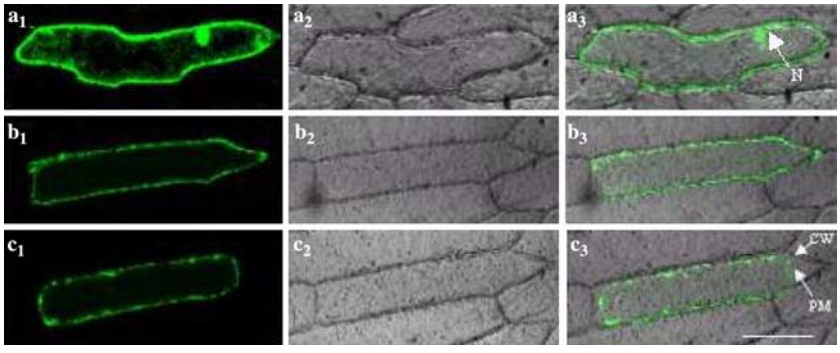

Fig. 6 Subcellular localization of the GhRLK1-GFP fusion protein. Onion epidermal cells containing free GFP (**a**) or GhRLK1-GFP before (**b**) and after plasmolysis (**c**). *Panel 1* are fluorescence images; *panel 2* bright-field images, and *panel 3* show the merged image. *N* nucleus; *CW* cell wall; *PM* plasma membrane. The *scale bar* indicates 200 μm

transduction pathway controlling the cytoskeletal reorganization that determines the patterns of cellulose deposition at the time of transition. The signaling pathways downstream of plant RLKs are quite diverse (Tichtinsky et al. 2003; Diévart and Clark 2004; Torri 2004). Among the downstream target proteins of RLKs identified so far is a Rho-GTPase-related protein (Rop) which may interact with CLV1 and may be responsible for signaling downstream of CLV1 in *Arabidopsis* (Trotochaud et al. 1999). Because of the similarity between the expression patterns of *GhRLK1* and *Rac13*, it would be intriguing to see if they are involved in the same signaling pathway and, if so, whether Rac13 is a downstream target of GhRLK1.

The regulation of cotton fiber development is an attractive area to study because of its theoretical and practical importance. Although the in vivo function of GhRLK1 remains to be determined, our results represent a first step towards understanding the regulatory mechanisms that control cotton fiber development. We are currently, generating transgenic cotton plants to observe the impact of increased or decreased expression levels of *GhRLK1* (by overexpression and RNAi approaches) on the development of cotton fiber cells. Apart from this, identification of potential ligands and downstream substrates of GhRLK1 is another focus of our research directed towards the elucidation of the signal transduction pathway involving GhRLK1.

Acknowledgments This work was supported by the "863" High Technology Development Program (Grant No. 2004AA222090) and the National Special Program for Research and Industrialization of Transgenic Plants (Grant No. JY03-A-04-02). We thank Mr. Guang-Jun Dong for help with confocal microscopic analysis, and Prof. Lucien Caro for reading and polishing the manuscript.

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