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The *ltk* gene family encodes novel receptor-like kinases with temporal expression in developing maize endosperm

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Abstract

We describe the isolation and characterization of maize cDNAs that are transcribed from a small gene family and encode a novel group of receptor-like kinases (RLKs). The distinctive extracellular domain of these novel RLKs includes a unique number and arrangement of leucine-rich repeats (LRRs), a proline-rich region (PRR), a putative protein degradation target sequence (PEST), and a serine-rich region (SRR). The intracellular domain contains a putative serine/threonine protein kinase. To distinguish them from other reported RLKs, these novel RLKs were termed leucine-rich repeat transmembrane protein kinases (LTKs). Based on analysis of available deduced protein sequences, LTK1 and LTK2 were predicted to be 92.1% identical, while LTK2 and LTK3 were predicted to be 97.5% identical. Though the three LTK proteins showed high homology, the region that most distinguished LTK1 from LTK2 and LTK3 was found in the extracellular domain, in the SRR. To differentiate between expression of the individual *ltk* genes, we used the reverse transcriptase polymerase chain reaction (RT-PCR) in combination with restriction enzyme analysis. While *ltk1* transcripts were constantly present in all tissues tested, *ltk2* and *ltk3* transcripts were only detected in the endosperm. Furthermore, transcript levels for both *ltk1* and *ltk2* showed modulation during endosperm development, peaking at 20 days after pollination. These results suggest that members of the *ltk* gene family mediate signals associated with seed development and maturation.

Introduction

Cell surface receptors of higher plants transduce primary signals leading to development of reproductive organs, growth of vegetative tissue, cell differentiation, and disease resistance. In the past few years a number of genes encoding higher-plant receptor-like protein kinases (RLKs) have been discovered, but the receptor ligands or signals are largely unknown. These RLKs have a tripartite structure: an extracellular ligand-binding domain, coupled to a transmembrane motif and an intracellular serine/threonine protein kinase [36]. The higher-plant RLKs have been classified based on structural characteristics of the extracellular putative ligand-binding domain. Plant RLKs of the S type have an extracellular domain similar to that of the self-incompatibility locus glycoprotein of *Brassica*. Another major class of RLKs have extracellular domains distinguished by a variable number of leucine-rich repeats (LRRs). A third class, represented by only one member from *Arabidopsis*, has an epidermal growth factor-like extracellular domain [16]. Recently, several new RLKs have been isolated, including a TNFR (tumor necrosis factor receptor)-like receptor kinase of maize [1], a PR5 (pathogenesis-related protein 5)-like receptor kinase of *Arabidopsis* [38], a lectin-like receptor kinase of *Arabidopsis* [9], and a novel RLK of *Catharanthus roseus* [29].

Because of its multiple roles, the LRR-containing class is particularly intriguing. Among the known functions for LRR-containing RLKs are several significant physiological roles including cell differentiation [21],

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers AF023164, AF023165, AF23166 and AF023267.

plant development [5, 31] and disease resistance [30]. The LRR class of receptor kinases is found almost exclusively in plants [4, 5, 11, 21, 27, 30–32, 34, 35] with the exception of the *trk* tyrosine kinase receptors of animals which have only three LRRs in the extracellular domain [28]. The LRR class may represent an extracellular-signal transmitting system unique to higher plants which has evolved independently of the well-known tyrosine kinase signal transduction system of animals.

Members of the LRR class share a conserved intracellular serine/threonine protein kinase domain but a less conserved extracellular domain, varying in the number, length and arrangement of LRR repeats; therefore, it is possible that each member of this class transfers a different signal into the cell. These signals are likely to be polypeptides as suggested by several studies, including crystal structure analysis, implicating LRR motifs in protein-protein interaction [15, 17]. Although specific peptide ligands have yet to be found, examples of peptide signal molecules, such as ENOD40 and systemin, have recently been found in plants [26, 33]. Alternatively, LRRs may function in cell adhesion, mediating the orientation of cells during development, as is the case for the *Drosophila* proteins Toll and chaoptin [13, 17]. About ten LRR-containing RLKs have been found in higher plants, but neither the receptor ligand, nor the downstream substrate of the intracellular protein kinase domain has been identified.

Further identification and characterization of these receptor kinases are central to understanding of signal transduction in plant growth and development and environmental responses. In maize, only two RLK genes have been identified. The first RLK isolated [37] was of the S type. Recently, an RLK involved in maize epidermal differentiation was cloned by transposon tagging [1]. Here, we report the isolation and characterization of maize cDNAs encoding a novel group of receptor-like kinases. The unique extracellular domain of this group of RLKs contains an LRR region that may function as a ligand-binding domain, a proline-rich region (PRR) and serine-rich region (SRR) that might serve a structural role in the ligand-binding domain, and a novel PEST sequence that might play a role in turnover of these novel RLKs. These cDNAs, encoded by a small gene family, exhibited a unique profile of expression in developing endosperm. Therefore, a possible role of these novel RLKs in endosperm development is discussed.

Materials and methods

Plant material

Maize plants (B73 inbred line) grown in a growth chamber at 25 °C (12 h light/12 h dark) were harvested after twenty days when plants were at the two- to three-leaf stage. Harvested plants were separated into shoots and roots. For collection of endosperm and embryo samples, B73 maize was grown in our experimental field at Pelham Bay Park, Bronx, New York. Ears were harvested at 10, 15, 20 and 25 days after pollination (DAP) and the endosperm separated from the embryo. Unfertilized ovules were separated from unfertilized ears. All tissues were frozen in liquid nitrogen and stored at −80 °C.

Cloning ltk1 *cDNA by 5′ and 3′ rapid amplification of cDNA ends (RACE)*

An *ltk1* cDNA was produced by using 5′ and 3′ RACE to extend sequences present in pMPDS10, a 1.9 kb chimeric cDNA clone obtained in screening for cDNAs encoding phytoene desaturase (PDS) [19], that contained 357 bp of *ltk1* (861–1218) upstream of 1.5 kb of *pds1* (starting at nt 831, GenBank U37285). The 5′ and 3′ RACE reactions were carried out by using the 5′ RACE system for Rapid Amplification of cDNA Ends, version 2.0 and the 3′ RACE System for Rapid Amplification of cDNA Ends (Gibco-BRL, Gaithersburg, MD), respectively. For 5′ RACE, first-strand cDNA was synthesized from total leaf RNA using primer 189 as GSP1 (gene-specific primer one), 5′-CGTCCAAAAGTACCCTCT-3′, and tailing with poly(G) following the vendor's procedure. The first-round PCR amplification was carried out with primer 208 as GSP2 (5′-AAGACTCTATGTCTACTGTGG-3′) and AAP (5′ RACE Abridged Anchor Primer, 5′-GGCCACGCGTCGACTAGTACGGGIIGGGIIG-GGIIG-3′, vendor-provided) in PCR mix (20 mM Tris-HCl pH 8.4, 50 mM KCl, 0.2 mM each dNTP, 0.4 μM each primer, and 0.05 U/μl *Taq* DNA polymerase [Gibco-BRL]) containing 2.5 mM $MgCl_2$. The PCR protocol was: one cycle of 94 °C (2 min); followed by 35 cycles of 94 °C (30 s), 66 °C (30 s), 72 °C (2 min), and one cycle of 72 °C (10 min). The first-round amplification product was diluted 1:20 and then used as a template for a second-round of amplification with GSP2 and AUAP (Abridged Universal Amplification Primer, 5′-GGCCACGCGTCGACTAGTAC-3′, vendor-provided). The reaction conditions and pro-

tocol for second-round PCR were the same as for the first, except that the annealing temperature was 62 °C. The 990 bp PCR product obtained from the second-round amplification using primers GSP2 and AUAP, was treated with Klenow and subcloned into pBluescript II SK(−) at the *Sma*I site, as in Li *et al.* [19]. This clone was designated as p5R900.

For 3′ RACE, the first-strand cDNA was synthesized from total endosperm RNA using AP (Adapter Primer, 5′-GGCCACGCGTCGACTAGTACTTTTTT-TTTTTTTTTTT-3′, vendor-provided). PCR was carried out using primer 219 as GSP (5′-GGGCATTACTTGCTCAGGAT-3′) and AUAP for first- and second-round amplifications. Conditions for both PCR rounds were identical: reactions contained PCR mix with 2.0 mM $MgCl_2$. The PCR protocol was: one cycle of 94 °C (2 min); followed by 35 cycles of 94 °C (30 s), 62 °C (30 s), 72 °C (2 min); and one cycle of 72 °C (10 min). The 2.2 kb PCR product was treated with Klenow and subcloned into pBluescript II SK (−) at the *Eco*RV site, as in Li *et al.*, [19]. This clone was designed as p3R41, and contained a 2.2 kb segment (113–2310) of *ltk1*. The 990 bp 5′ RACE and 2.2 kb 3′ RACE products were sequenced and found to overlap in an 865 nt region having 100% homology.

Prior to combining the 5′ and 3′ RACE products, the 5′ RACE product was first subcloned into pMP-DS10. To extend the 5′ end of *ltk1* contained within pMPDS10, a 112 nt *Spe*I (vector site)-*Ahd* I fragment was removed and replaced by a 985 nt *Spe*I (restriction site in the AUAP adaptor sequence)-*Ahd* I fragment from p5R900 that contained nt 1–971 of *ltk1*. The resulting clone, p10F(E), contained nt 1–1218 of *ltk1* followed by the 3′ end of *pds1*.

Afterwards, the 3′ RACE product was inserted in place of the *pds* sequence. The 3′ RACE product (p3R41) and p10F(E) overlapped between nt 113 and 1218 of *ltk1*. This region also contained a unique *Bsp*MI site, thereby providing a means to replace the *pds1* sequence with the 3′ RACE product. The insert from p3R41 was released by digestion at the common *Bsp*MI site, and to create a blunt end, digested at a unique *Sma*I site within the vector. This fragment (containing nt 995–2310 of *ltk1*) was then combined with p10F(E) which had been digested by *Bsp*MI and *Eco*RV, creating a blunt end from the vector. This clone, designated as pLTK1, contained nt 1–2310 of *ltk1*.

Cloning the ltk2 *cDNA by RT-PCR*

Based on northern analysis, the *ltk1* cDNA appeared to be about 200 bp shorter than expected. The following, which was carried out to recover sequence information further upstream of *ltk1*, instead led to amplification of the *ltk2* cDNA. A maize B73 genomic DNA library (CLONTECH Laboratories, Palo Alto, CA, Catalogue No. FL 1032D), containing 7×10^6 phage clones, was screened with the insert of p5R900 and 64 positive clones were isolated. Eight clones were randomly chosen for further analysis, the purified λ DNA digested by *Eco*RI, *Sal*I, or *Bam*HI, and hybridized by a probe corresponding to the very 5′ end of the 5′ RACE product. This probe was a PCR product, ca. 300 bp, generated from clone p10F(E) using primer 227, 5′-GGGAAGATTGTACTGTACTTG-3′ and primer 231, 5′-CGCAATAACCCTCACTAAAGG-3′, derived from the vector, pBluescript II SK (−). The PCR reaction was identical to that for 3′ RACE except for using 1 ng of plasmid DNA as template. The PCR protocol was: one cycle of 94 °C (2 min); followed by 35 cycles of 94 °C (30 s), 65 °C (30 s), 72 °C (30 s); and one cycle of 72 °C (10 min). Hybridizing fragments were subcloned into pBluescript II SK(−) and sequencing was attempted with primer 226, 5′-CCCTGATCCTGACAAGTAAT-3′, which was 119 nt from the very 5′ end of the 5′ RACE product. Although several fragments hybridized to the 5′ end probe, primer 226 provided sequence from only one clone, p531, containing a 16.6 kb *Sal*I insert from the genomic clone, λ531. The *Eco*RI fragments of p531 were subcloned into pBluescript II SK(−), since the 5′ RACE product contained an *Eco*RI site 45 nt from the 5′ end, and it was expected that one of the *Eco*RI fragments would include the missing 5′ sequence. One clone, p531E2, containing a 5.07 kb *Eco*RI fragment, provided the expected sequence adjoining the *Eco*RI junction site of the 5′ RACE product. However, this *Eco*RI fragment contained an intron between the corresponding nt 15 and 16 of the 5′ RACE sequence. To gain additional exonic sequence at the 5′ end of the gene, without sequencing through the intron, this clone was then sequenced by primer 240, 5′-**AC**GATCGTTGGGGTCAA-3′, consisting of the 15 nucleotides preceding the intron combined with an additional two nucleotides (shown in bold) that could pair with the conserved 5′-GT-3′ sequence of introns. Primer 240 provided limited sequence which was then used to design primer 243, 5′-TGCACTTCACTTGTCAACAG-3′,

which allowed sequencing back into the intron. Based on this additional sequence, primer 246, 5′-GCTCTTCCTTATCGCCAT-3′, was designed, and used to obtain upstream sequence that contained a putative promoter and the first exon, as analyzed by the PCGene program [2].

To confirm the position of the promoter and the first exon encoding the 5′ end of the *ltk* mRNA, primer 247, 5′-CGGATTTGGAGGAGTCGAT-3′, was designed according to the putative 5′ exon sequence of p531E2, and used with primer 227 (see Figure 1), to amplify the 5′ end of the *ltk* mRNA by RT-PCR. One microgram total endosperm RNA was used to synthesize cDNA according to the procedure of the SuperScript Preamplification System for First Strand cDNA Synthesis kit (Gibco-BRL). One fourth of the cDNA was used as template to perform PCR with the same reaction conditions as for 3′ RACE. The PCR protocol was: one cycle of 94 °C (2 min); followed by 40 cycles of 94 °C (30 s), 58 °C (30 s), 72 °C (30 s), and one cycle of 72 °C (10 min). Based on northern analysis and available sequence, a 500 bp amplification product was expected and a 490 bp product was obtained. Although the expected size of the RT-PCR product was obtained, only the first 215 nt matched that predicted from the genomic clone; the remaining sequence slightly differed from available *ltk1* sequence, implying that the promoter contained by p531E2 did not belong to *ltk1* and that the 490 bp product represented the 5′ end of another *ltk* transcript, which we named *ltk2*.

To amplify the complete *ltk2* by RT-PCR, the genomic DNA-based primer 247 was used with primer 232 (5′-GGGAAGATTGTACTGTACTTG-3′, sequence from the 3′ RACE product). One microgram total endosperm RNA was used to synthesize cDNA according to the procedure of the SuperScript Preamplification System for First Strand cDNA Synthesis kit (Gibco-BRL). One fourth of the cDNA was used as template to perform PCR with the same reaction conditions as for 3′ RACE. The PCR protocol was: one cycle of 94 °C (2 min), followed by 35 cycles of 94 °C (30 s), 54 °C (30 s), 72 °C (2 min); and one cycle of 72 °C (10 min). The PCR product was treated with Klenow and subcloned into pBluescript II SK (−) at the *Sma*I site, as in Li *et al.* [19]. Several clones of identical size were obtained and one of them, containing a 2388 bp insert, was designated as pLTK2.

DNA sequencing

Plasmid DNA templates were isolated by the Wizard *Plus* Miniprep DNA Purification System (Promega, Madison, WI) and DNA sequencing was carried out using the Sequenase Version 2.0 DNA Sequencing Kit (United States Biochemical, Cleveland, OH). Alternatively, automatic sequencing of double stranded DNA templates was used. Both strands of *ltk1*, *ltk2*, and *ltk3* cDNAs were sequenced by primer walking.

DNA extraction and Southern analysis

Maize B73 genomic DNA was extracted from endosperm, digested, and hybridized with probes A, B, or C, as in Wurtzel *et al.* [41]. Filters were washed in 0.1% SDS and 0.1× SSC for 15 min at room temperature and twice for 15 min at 55 °C, followed by a 2–12 h exposure to a Storage Phosphor Screen, which was then scanned on a 445SI Molecular Dynamics Phosphorimager. Probe A was the 2.1 kb *ltk1* 3′ RACE product described above. Probe B was a 307 nt PCR product (nt 861–1168 of *ltk1*) amplified from pMP-DS10 using primer 231 (see cloning *ltk2*) and 229, 5′-CAAGGTCGACGAAGCTAAAGC-3′. The PCR conditions were the same as for PCR with primers 231 and 227 in *ltk2* cloning. To prepare probe C, a 490 bp RT-PCR product of *ltk2*, total endosperm RNA was used as template for first-strand cDNA synthesis and PCR amplified with primers 247 and 227 (see cloning *ltk2* and Figure 1).

RNA extraction and northern analysis

Total RNA was extracted from embryo, leaf, or roots following Logemann *et al.* [20] and from endosperm or unfertilized ovules following Wurtzel *et al.* [41]. RNA concentration was determined spectrophotometrically. Electrophoresis of total RNA on 1.4% (w/v) agarose gels containing 6% (v/v) formaldehyde, transfer to nitrocellulose, and hybridization conditions were as in Wurtzel *et al.* [41]. Filters were exposed for 24 h to a Storage Phosphor Screen which was then scanned on a 445SI Molecular Dynamics Phosphorimager.

RT-PCR and restriction enzyme diagnosis of ltk *transcript accumulation*

Total RNA was used as template for cDNA synthesis as described in the synthesis of *ltk2* cDNA. Using one fourth of the cDNA, PCR was performed with forward

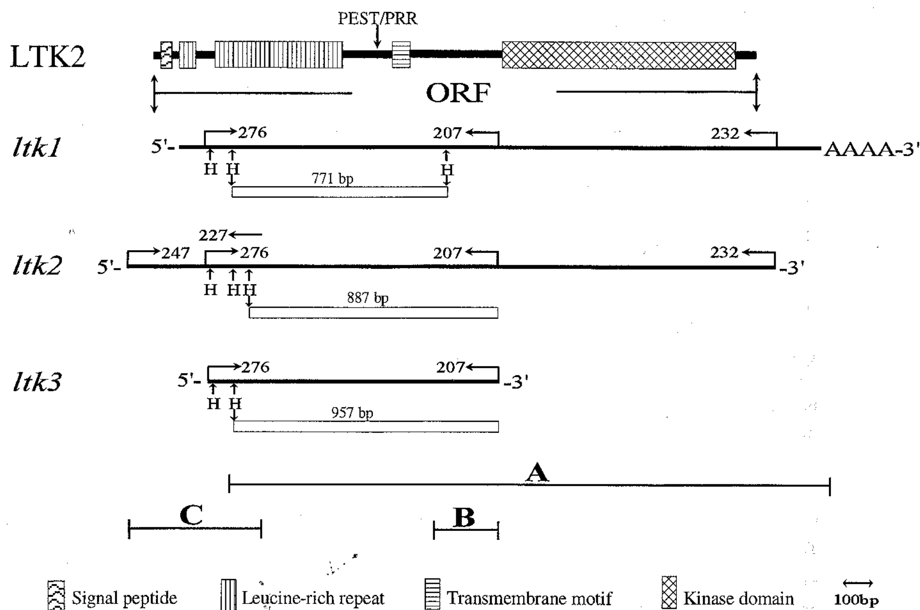

Figure 1. LTK protein and cDNA structures. ORF, open reading frame; PRR, proline-rich region; PEST, putative protein degradation site. Numbers 207, 227, 232, 247 and 276 are primers used for RT-PCR. The open boxes, above which are DNA sizes in base pairs, represent the largest fragments produced by *Hae*III (H) digestion of the RT-PCR products (generated with primers 276/207). *Hae*III sites are shown only in the region between primers 276 and 207. A, B, and C correspond to regions used as hybridization probes (further detailed in Materials and methods).

primer 276, 5′-TCACAGGTTGGCAGGCGAAT-3′ and reverse primer 207, 5′-CCATCTGTAGATCTGC-AACTGAA-3′ (see Figure 1) in a reaction containing PCR mix with 4.0 mM $MgCl_2$. The PCR protocol was: one cycle of 94 °C (2 min), followed by 40 cycles of 94 °C (30 s), 54 °C (30 s), 72 °C (1 min); and one cycle of 72 °C (10 min). To ensure linearity of the reactions, conditions were similar to those described in Li *et al.* [19]. From a 25 μl reaction, 15 μl PCR product was incubated with restriction endonuclease *Hae*III for 2 h following the vendor's specifications (Gibco-BRL) and then analyzed by electrophoresis on a 1.6% (w/v) agarose gel in comparison with 10 μl of undigested DNA remaining from the PCR reaction. Control RT-PCR reactions for amplification of *pds* and *sh* transcripts were performed as in Li *et al.* [19].

Results

Cloning of ltk1 *and* ltk2 *cDNAs*

A chimeric cDNA clone of unusual structure was found while screening a maize endosperm cDNA library for cDNAs encoding phytoene desaturase (PDS). In addition to isolating a cDNA clone encoding PDS, as described in Li *et al.* [19], one clone (pMPDS10) appeared to be chimeric; the 3′ end encoded most of PDS with the exception of an essential dinucleotide binding domain and transit sequence, in place of which was an ATP binding site and partial protein kinase sequence (data not shown). We decided to use 5′ RACE and 3′ RACE to reconstruct a cDNA, represented by the protein kinase sequence at the 5′ end of the chimeric cDNA. When leaf RNA was hybridized by the 2.3 kb reconstructed cDNA (pLTK1), a transcript of about 2560 nt was detected, suggesting that pLTK1 was missing about 200 bp at the 5′ end.

To obtain the missing 5′ end of *ltk1*, we isolated a genomic DNA clone, p531E2 (GenBank AF023267), containing the promoter region and putative first exon as shown in Figure 2. However, this sequence led to RT-PCR amplification of a longer, but slightly different cDNA, named *ltk2*, and indicated that the isolated promoter and first exon were of *ltk2* and not of *ltk1*. The full-length 2388 bp cDNA encoding the second LTK, was cloned and designated as pLTK2.

The inserts of pLTK1 and pLTK2 were sequenced (GenBank AF023164 and AF023165), and searching of GenBank revealed no obvious homologues.

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  1 AAATAAGCAAATAATGCCAGAATAATTACCGGATTTTTGCAAAGGCAGGCAAAGCAATTG
 61 ACCTCACCGCACCCGGGTTCTTGTCCCGGCGTTCTGCTGCCAGCTGCTGCTGTGCTAGCT
121 TCCAATGCCGGACGCCGCACACGCTATCCATGAATCCATCCATCCGTCTTGAAAAGCGGC
181 AGCGTGATCTGTAAAAAACAGCGGGTAAAATCAGTCGGATTTGGAGGAGTCGAATAAACA
                                   └──────────────→247
241 AGGAAACTCCTCCCGGCCCAGAAAGCACTTCACTTGCAACAGTCCCCGTCAGGGAGGGAG
                       └──────────────→243
301 CATTGCTGTGCGGGATGGCGATAAGGAAGAGCGGAGGCGGTGGCGGGGTGGGGGGTGTGG
          246←──────────────┘
361 CGCGGCGGCTGCTGCTTCTGGCCTGCTGCTGCATTTGGCCTGGTGGACGGATCTTCACTG
421 CTGCGGACACTGACCCCAACGATCGTGANTTGGAAACTTCTGCTTTCATTGTGCTTCTTC
      240←────────────────┘
481 TTCCGTGTAAATTTCTTTATTATGGGATCGCTCTTT
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Figure 2. Putative promoter sequence of *ltk2*. The boxed sequence is the putative TATA box; the putative Cap signal is underlined. The first exon, having an unidentified starting site, ends at nt 444, and contains a putative translation start codon at 315–317, shown bolded and underlined. The italicized sequence indicates the 5′ end of the first intron; the first two conserved nucleotides are shown in bold. Numbers indicate sequences used for designing RT-PCR or sequencing primers and arrows indicate direction of synthesis.

However, the predicted C termini showed similarity to protein kinases and the predicted N termini, found later to contain eight leucine-rich repeats (LRRs), showed similarity of these repeats to repeats contained within proteins having various numbers of LRRs. We also identified motifs corresponding to a signal sequence and transmembrane region (Figure 1). Since these proteins had the general structure of receptor-like kinases with LRRs in the putative extracellular domain, we named them LTK proteins, for leucine-rich repeat transmembrane protein kinase.

Comparison of ltk1 *and* ltk2 *cDNAs and their predicted gene products*

The 2388 bp *ltk2* cDNA (pLTK2) contained a complete open reading frame (ORF), preceded by a 5′-untranslated region of 99 bp and followed by a 3′-untranslated region of 114 bp. As this was an RT-PCR product, the region adjacent to and including the poly(A) tail was missing (when compared to *ltk1*, this was estimated as 159 bp, assuming a similar polyadenylation site to *ltk1* on the basis of a single band detected by northern analysis). The pLTK2 ORF encoded a polypeptide of 725 residues with a calculated molecular weight of 79.15 kDa. The 2310 bp *ltk1* cDNA (pLTK1) encoded 684 residues in the first 2054 bp followed by a 256 bp 3′-untranslated region, including the poly(A) tail. The *ltk1* and *ltk2* cDNA sequences were compared and showed 91.2% identity at the nucleotide level. A comparison of the predicted protein sequences (Figure 3) showed 92.1% identity and 94.5% similarity.

As shown in Figures 1 and 3, the structure of the predicted LTK2 polypeptide showed it to include a signal peptide and leucine-rich repeats at the amino terminus, a transmembrane region, and a protein kinase domain in the carboxyl terminus. The unique number and organization of the LRRs, combined with other peculiar sequence hallmarks, as described below, indicated that the LTK proteins were new members of the class of RLKs having LRRs in their extracellular domains. Two hydrophobic motifs were predicted by sequence analysis of LTK2 using the SOAP program of PCGene [18] (Figure 3). One region, residues 11 to 27 at the amino terminus, could serve as a signal peptide to target LTK2 to the plasma membrane. The other, residues 297 to 322, a putative transmembrane domain, separated the N-terminal LRR domain from the C-terminal protein kinase domain. Though the *ltk1* cDNA was missing the 5′ sequence needed to encode a signal peptide, it encoded, as *ltk2*, the transmembrane motif from residues 257 to 282 (Figure 3). The transmembrane motifs of both LTK1 and LTK2 were followed by positively charged residues KRRKR(H)K that could function as stop-transfer sequences [39]. During insertion of LTK proteins into the plasma membrane, the stop-transfer sequence would cause the protein kinase domain to remain inside the cell, while the LRR portion of LTK would be extracellular.

As predicted from the translated ORF, the extracellular domain of LTK proteins was found to consist of eight LRRs, the first of which was separated from the other seven tandemly repeated LRRs (Figures 3 and 4A). Each repeat ranged in length from 18 to 24 amino acids and contained a conserved core region, LXXLXLXXN, found in LRRs of other RLKs (Figure 4B). In a hydrophilic environment, this conserved core sequence is proposed to form a β-sheet structure that binds the protein ligand [12, 15]. The region

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LTK1                                           DPNDLNVLNTL11
LTK2 MAIRKSGGGGGVGGVARRLLLLACCCIWPGGRIFTAADTDPNDLNVLNTL50

LTK1 FTSLNSPGQLTGWQANGGDPCGQSWKGITCSGSGVTKIQLPNLSLTGNLA61
LTK2 FTSLNSPGQLRAWRANGGDPCGQSWQGITCSGSGVTKILLPNLSLTGNLA100
     - 1 -----                            ------- 2 -----
LTK1 YNMNNLGSLVELDMSQNNLGGGGQVQYNLPNMKLEKLNLAGNQFGGNLPY111
LTK2 YNMNNLGSLVELDLSQNNLGGGGQIQYNLPNVKLEKLNLAGNQFGGNLPY150
     - ---------- 3 ---------- ---------- 4 ----------- -
LTK1 SISTMPNLKYLNLNHNQLQGNISDVFSNLYSLSELDLSFNSLTGDLPQSF161
LTK2 SISTMPNLKYLNLNHNQLQGNITDVFSNLYSLSELDLSFNSLTGDLPQSF200
     ------ 5 -------------- --------- 6 ---------- --
LTK1 TGLSSLKKVYLQNNQFTGNINVLANLPLETLNVANNHFTGWIPSQLKKIN211
LTK2 TGLSSLKRMYLQNNQFTGYINVLANLPLETLNVGNNHFTGWIPSQLKEIN250
     ---- 7 -------------- ---------- 8 ---------
LTK1 SLQTDGNSWSTGPAPPPPPYTAPPPPPNHWNADGSS--SSSSSGGRSGIG259
LTK2 NLQTDGNSWSTGPAPPPPPYTAPPPP-NHWNGAGQNDDGSSSSGGRPGIG299
                                             ==========
LTK1 GGGVAGIIISLLVVGSVVAFLVIKRRKRKAAMKEHFEQHQPFTSFPSNEV399
LTK2 GGGVAGIIISLLIVGSVVAFFLIKRRKHKAIMEEQFEQHQPFTSFPSNEV349

LTK1 KDMKPVCEATTVDIESLASPASVNLKPPPKIERNKSFDDDDDFSNKLVAK359
LTK2 NDMKPIYESTTVDIESLASPASINLKPPPKIEQNKSFDDDDDFSNKTAAN399

LTK1 KSNITPINATVYSVADLQMATDSFSFDNLVGEGTFGRVYRAQFNDGKVLA409
LTK2 RSNITPMKATVYSVADLQMATDSFSFDNLVGEGTFGRVYRAQFNGGKVLA449
                                   --------I---------  -----
LTK1 IKKLDSTVMPFQSSDDFAELVSNISKLHHPNLNELVGYCMEHGQHLLVYD459
LTK2 IKKLDITVMPFQSSDDFAELVSNISKLHHPNLNELVGYCMEHGQHLLVYD499
     II---           ---III---  -------IV-------    -----
LTK1 FHRNGSLHDLLHLSDEYSKPLSWNTRIKIALGSARALEYLHEICSPSIIH509
LTK2 FHRNGSLHDLRHLSDEYSKALSWNSRIKFALGSARALEYLHEICSPSIIH549
     ---V--------        ------------VIa----------  ---
LTK1 KNFKSSNILLDSEFNPHLSDAGLASFIPDAEFQAAEQSAGYTAPEVDMTG559
LTK2 KNFKASNILLDSEFNPHLSDTGLASFIPGAEFQAAEQSAGYTAPEVDMTG599
     --VIb--------   ----VII----          -------VIII-----
LTK1 QYTFKSDVYSFGVVMLELLTGRRPFDSSRPRSEQSLVRWATPQLHDIDAL609
LTK2 QYTLKSDVYSFGVVMLELLTGRRPFDSSRPRSEQSLVRWAAPQLHDIDAL649
       ----------IX----------                ----------
LTK1 DRMVDPALKGLYPAKSLSRFADVLALCVQPEPEFRPPMSEVVQALVRLVQ659
LTK2 DRMVDPALKGLYPAKSLSRFADVLALCVQPEPEFRPPMSEVVQALVRLVQ699
     -X--------               ---------XI---------
LTK1 RANMTKRMLDG-DTSRRPDDLDQDFI684
LTK2 RANMTKRMLDGGDTSRGPDDQDQYFI725
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Figure 3. Protein sequence comparison of LTK1 and LTK2. The leucine-rich repeats (LRRs) are underlined and numbered 1 to 8; conserved residues are shown in bold. Residues contained within the putative signal peptide are bold and within a box; residues comprising the transmembrane domain are bold. Residues within the PEST motif are italicized, while those in the PRR are italicized and bold. A double underline indicates residues in the SRR. The subdomains of the protein kinase catalytic site are underlined and numbered from I to XI; conserved residues are shown in bold. Horizontal lines and dots indicate identical and similar residues, respectively. Dashes in the amino acid sequence are gaps inserted to produce an optimal alignment.

containing the eight LRRs showed highest similarity (about 45–64%) to various stretches of leucine-rich repeats found in the Cf-2 proteins, a pair of membrane anchored disease resistance proteins from tomato having 38 LRRs, but no kinase domain [6]. A similar comparison of the eight LRRs in the LTK proteins with the twenty LRRs of ERECTA, an *Arabidopsis* RLK involved in plant development, showed a maximum similarity of 60%. However, the number and arrangement of LRRs in LTK proteins were different from that of all known LRR-type RLKs, implying a unique ligand specificity for the LTK extracellular domains.

Unlike other LRR-type RLKs, three novel regions were found in the predicted extracellular domains of LTK1 and LTK2. One region, located between the LRR and transmembrane domain, was a putative protease target site or PEST sequence. PEST sequences are defined as hydrophilic regions of at least 12 amino acid residues, containing at least one P (proline), one E (glutamic acid) or D (aspartic acid), and one S (serine) or T (threonine), but no positively charged residues, although the region may be flanked by K (lysine), R (arginine), or H (histidine) [22]. The PEST sequences found were 'INSLQTDGNSWSTGPAPPPPPYTAPPPPPN', aa 210 to aa 239 in LTK1, and 'EINNLQTDGNSWSTGPAPPPPPYTAPPPPN', aa 248 to aa 277 in LTK2 (as analyzed by the PEST-FIND program of PCGene [25]). These sequences received PEST scores of 8.7 and 8.1, respectively, where a score greater than five indicates that the region is most likely to be degraded by proteases and that the protein will have a fast turnover rate.

The second distinct region was a proline-rich region (PRR), which actually comprised the carboxyl terminus of the PEST sequence, from aa 224 to aa 238, PAPPPPPYTAPPPPP in LTK1 and from aa 263 to aa 276, PAPPPPPYTAPPPP, in LTK2. PRRs form an extended flexible structure because of the unique structure of proline, whose side group bonds to the backbone amide position and forms a rigid structure [40]. For example, in Calcineurin A, a stretch of 11 contiguous prolines have been proposed to be involved in PRP (proline-rich protein)-protein interaction [7]. Non-repetitive PRR sequences may also mediate protein-protein interaction as seen in the Sos PRR site that mediates binding to SH3 (*src* homology 3) [24]. In the case of LTK proteins, the PRR sites were non-contiguous; in LTK1, the PRR consisted of 11 prolines in a region of 15 residues, while the PRR in LTK2 consisted of 10 prolines in a region of 14 residues. Because of its position between the LRR and transmembrane domain of LTK, the PRR may function either as a 'hinge' to increase the flexibility of the extracellular ligand binding domain, or as a 'linker' to extend the protein ligand binding site.

The third unique region noted was a serine-rich region (SRR) found immediately downstream of the proline-rich region. Surprisingly, this SRR most differentiated LTK1 from LTK2, proteins that were otherwise 94.5% similar. LTK1 (residues 243–256), contained ADGSSSSSSSGGRS, while LTK2 (residues

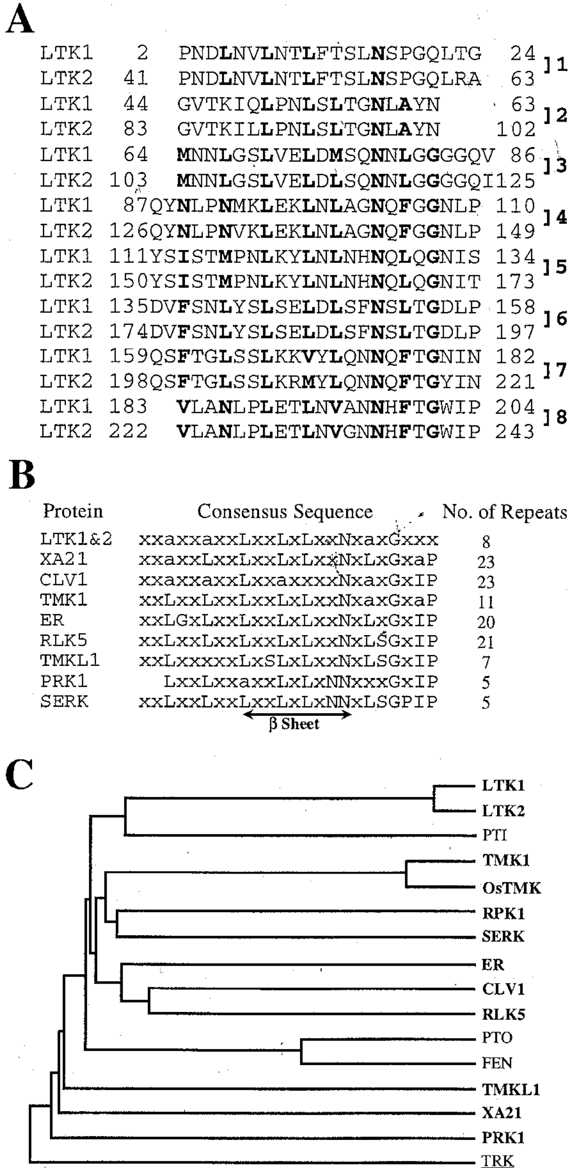

Figure 4. Comparison of LTK structural motifs with those of other RLKs. A. Comparison of the 8 LRRs in LTK1 and LTK2; conserved residues (either identical or similar) are shown in bold; sequences are flanked by residue numbers for first and last residue. B. Comparison of LRR consensus sequences for LRR type RLKs. a, hydrophobic residues; x, any residue. C. Dendrogram of alignment of protein kinase domain of LRR type RLKs (bold), three cytosolic protein kinases and TRK (underlined). GenBank accession numbers are: *Arabidopsis*, TMK1 (L00670), TMKL1 (X72863), RLK5 (M84660), ER (U47029), CLV1 (U96879), RPK1 (U55875); from carrot, SERK (U93048); from maize, LTK1 (AF023164), LTK2 (AF023165); from rice, XA21 (U37133), OsTMK (Y07748); from tomato, PTI (U28007), PTO (U02271), FEN (U13923); from *Petunia inflata*, PRK1 (L27341) and from man, TRK (M23102).

281–296) contained GAGQNDDGSSSSGGRP. The SRR sequences, which differed in number of the polar serine residues, were flanked by negatively and positively charged residues. If the SSR plays any role in ligand binding, then LTK1 and LTK2 might also differ in ligand specificity and/or affinity.

Both LTK proteins contained in their putative intracellular domains, the multiple subdomain catalytic site characteristic of protein kinases (Figure 3). Specific features of subdomains VIb and VIII marked the kinase domains of the LTK proteins as serine/threonine protein kinases, similar to all other higher plant RLKs, and distinct from the animal receptor kinases that are either tyrosine or serine/threonine kinases [8, 36]. Consensus sequences for subdomains VIb and VIII, respectively, are DLKPEN and GTPXYIAPE for serine/threonine kinases and DLAARN and FPIKWMAPE for tyrosine kinases. The observed sequence homology suggests that, like the other LRR-type RLKs, the LTKs are capable of autophosphorylation or phosphorylation of substrate polypeptides at serine and/or threonine residues [36]. In contrast to the variable extracellular LRR domain, the intracellular protein kinase domain was well conserved as compared with protein kinase domains of other LRR-type RLKs and with other cytosolic protein kinases. A comparison of protein kinase domains of all LRR protein kinases and three cytosol protein kinases was carried out using the Clustal program of PCGene [10] and the result is shown in Figure 4C. The kinase domain of TRK, the only LRR type receptor kinase in animals was also included in the analysis, but as this is a tyrosine kinase, it is not surprising that it was the least related. Interestingly, the LTK1 and LTK2 protein kinase domains showed highest homology to Pti, a defense-related cytoplasmic protein kinase from tomato [42].

The ltk *gene family of maize*

The *ltk* gene copy number was investigated by Southern analysis. When maize B73 genomic DNA was probed by an almost full length, 2.1 kb cDNA probe (probe A, Figure 1), multiple bands were observed for three different restriction enzyme digestions (Figure 5A). These probed fragments might represent multiple *ltk* genes. Alternatively, these bands might represent numerous protein kinase genes, since the C-terminal protein kinase region of *ltk* has high homology to those of other protein kinases. Therefore, an identical filter was probed by B (Figure 1), a 280 bp probe corresponding to a region upstream of the kinase domain,

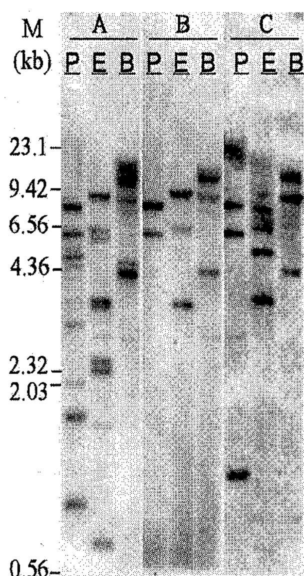

Figure 5. Southern analysis of the *ltk* gene family in maize. A, B, and C are probes shown in Figure 1. P, *Pvu*II; E, *Eco*RI; B, *Bam*HI; M, molecular weight markers.

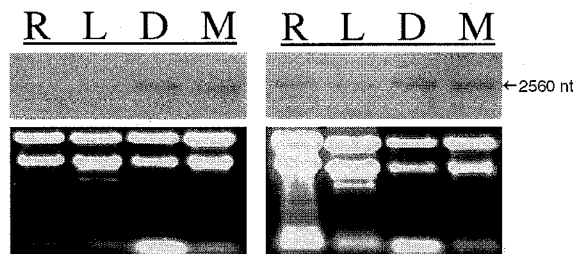

Figure 6. Northern analysis of *ltk* expression. Top panels show transcripts hybridized by probe A (Figure 1). Bottom panels show ethidium bromide stained RNA electrophoretic gels used for hybridizations. A 10 μg portion of total RNA was loaded per lane, except for R and L lanes in right panel containing 40 μg total RNA. R, roots; L, leaf; D, 20 DAP endosperm; M, 20 DAP embryo.

but downstream of the region encoding the transmembrane domain. As shown in Figure 5B, fewer bands hybridized to probe B, as compared with the number of bands that had hybridized to probe A (Figure 5A). In the *Pvu*II digestion, only two fragments, 7.8 kb and 6.0 kb, were detected by probe B, while in *Eco*RI or *Bam*HI digestions, three major bands were detected by probe B. Since the 280 bp probe B was a short probe, these results suggest that there is more than one copy of *ltk* in the maize genome.

To eliminate the possibility that multiple long introns in the region hybridized by B caused multiple bands in Southern analysis, probe B was removed and the filter hybridized by probe C, DNA encoding part of the LRR extracellular domain (Figure 1). Probe C was 490 bp and located 605 bp upstream of probe B. If the 7.8 kb and 6.0 kb *Pvu*II fragments probed by B originated from a single *ltk* gene, then probe C would have hybridized only to one but not to both bands. If the two *Pvu*II fragments probed by B were from two or more *ltk* copies, C would hybridize to both bands. As shown in Figure 5C, probe C did hybridize to both *Pvu*II fragments, demonstrating that more than one or at least two copies of *ltk* are present in the maize genome. The same deduction could be applied to the three bands generated by *Eco*RI or *Bam*HI digestion and hybridized by B. If these three fragments were from only two different genes and could be probed by B, then probe C would have hybridized to two but not all fragments. Nonetheless, as shown in Figure 5C, probe C hybridized to all three bands previously probed by B, both in the *Bam*HI and *Eco*RI digestions. This suggests that there may be three copies of *ltk* in the maize genome.

Expression of ltk *at the RNA level*

Although three *ltk* genes were suggested by Southern analysis, only a single 2560 nt transcript was detected in total RNA extracted from roots, leaves, 20 DAP endosperm, or 20 DAP embryo (Figure 6). The level of this transcript varied; four times as much RNA was required to detect comparable levels of *ltk* transcript in leaves and roots, as compared with that of endosperm and embryo samples. The role of light in modulating leaf *ltk* transcript level was also tested, but found not to have any effect (data not shown).

While one band was detected by northern analysis, it was difficult to determine which *ltk* gene was transcribed, especially if gene family members encoded transcripts of similar sizes. To differentiate among *ltk* transcripts, another approach was needed. Since *ltk1* and *ltk2* cDNAs exhibited nucleotide sequence differences, we used RT-PCR, in combination with analysis of restriction enzyme site polymorphisms, to differentiate transcripts of the *ltk* genes. First, primers 276 and 207 (shown in Figure 1) were used to amplify transcripts from leaf, root, embryo (20 DAP) and endosperm (20 DAP). Only when reverse transcriptase was added to the cDNA synthesis reaction was an approximately 1070 bp RT-PCR product obtained (Figure 7A, panels 1 and 2). Next, the RT-PCR products were digested by *Hae*III, which was expected to yield a

major fragment of 771 bp for *ltk1*, but an 887 bp fragment for *ltk2* (see Figure 1). As shown in Figure 7A (panel 3), only one 771 bp fragment resulted from *Hae*III digestion of the 1070 bp RT-PCR product amplified from leaf, root or 20 DAP embryo RNA using primers 276 and 207. Thus, *ltk1* was the only *ltk* transcript expressed in these three tissues. However, when 20 DAP endosperm RNA was used, three restriction fragments, 771 bp, 887 bp and a faint 957 bp fragment, were obtained. The 771 bp and the 887 bp fragments implied expression of *ltk1* and *ltk2*, respectively. The presence of the 957 bp fragment was unexpected. These three fragments suggested that there were three genes expressed in the endosperm, *ltk1*, *ltk2*, and a third, as yet unidentified, *ltk* gene. Alternatively, the 957 bp fragment might have been a result of partial digestion of the *ltk1* or *ltk2* RT-PCR products. If three different transcripts were indeed present, then the 1070 bp RT-PCR product obtained with primers 276 and 207 would be predicted to contain, not two different products, but three. To test the hypothesis that there were three different endosperm transcripts, the 1070 bp RT-PCR products were cloned and as predicted, three different types of clones were isolated. The insert of each clone type was sequenced; on the basis of sequence homology, two types corresponded to *ltk1* and *ltk2* were identified (data not shown). The third type, designated as *ltk3* (GenBank AF23166, Figure 1), showed 98.6% identity at the nucleotide level and 97.5% identity at the amino acid level, when compared with the corresponding sequences of the *ltk2* cDNA and deduced protein. Based on the DNA sequence of the *ltk3* RT-PCR product, it could generate a 957 bp *Hae*III fragment as observed in Figure 7A (panel 3-D).

To further investigate *ltk* expression during maize endosperm development, RT-PCR with primers 276 and 207 was performed with total RNA extracted from endosperm at various stages of development and from the maternal, unfertilized ovule tissue. As shown in the top panel of Figure 7B, *ltk* expression was relatively elevated in the maternal ovule tissue, while *ltk* transcript levels were lower at 10 DAP. From about 15 DAP, *ltk* transcript levels began to rise, reaching a peak at 20 DAP, and then decreasing as observed at 25 DAP. To determine more specifically which *ltk* transcripts were being expressed, individual *ltk* transcript levels were assayed by *Hae*III digestion of the RT-PCR product. The only detectable *ltk* transcript accumulated in unfertilized maternal ovule tissue was *ltk1*, as evidenced by the 771 bp *Hae*III fragment (Figure 7B).

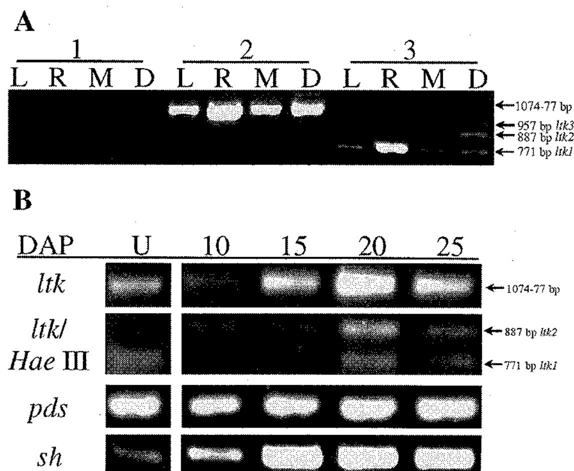

Figure 7. RT-PCR analysis of *ltk* expression. A. *ltk* transcripts amplified with primers 276 and 207; 1, without reverse transcriptase; 2, with reverse transcriptase; or 3, with reverse transcriptase but products digested by *Hae*III. L, leaf; R, roots; M, embryo (20 DAP); D, endosperm (20 DAP). B. Total RNA from unfertilized ovule (U) and developing endosperm at indicated DAP. Panels top to bottom show RT-PCR products for amplification of *ltk* transcripts; the same products digested by *Hae*III; RT-PCR products for amplification of *pds* and *sh* transcripts, respectively.

However, the *ltk* transcripts undergoing temporal modulation in endosperm development were a combination of both *ltk1* and *ltk2*, as indicated by the presence of the 771 and 887 *Hae*III fragments, respectively. The absence of the *ltk3 Hae*III product suggested that the corresponding transcript was of low abundance, as also suggested by the faint band seen in Figure 7A (panel 3-D). In comparison, RT-PCR was used to show transcripts levels for two other genes, *pds* and *sh*, which are known to be expressed in developing endosperm. As previously demonstrated, *pds* transcript levels were constant, whereas *sh* transcript levels increased from 10 to 15 DAP and remained constant thereafter [19].

Discussion

The serendipitous isolation of a chimeric *ltk-pds* cDNA led to the discovery of a new class of maize receptor-like kinases containing leucine-rich repeats in their extracellular domain. These particular LRR type RLKs were termed LTK proteins. From Southern analysis, it appeared that there were three *ltk* copies in the genome; this was later confirmed by cloning of three different RT-PCR products. Therefore, LTK proteins are encoded by a small gene family consisting of three

members, *ltk1*, *ltk2*, and *ltk3*. From sequences of the isolated *ltk* cDNAs, it was determined that *ltk1* and *ltk2* shared 91.2% nucleotide homology, while *ltk2* and *ltk3* shared 98.6% homology. However, from RFLP mapping in two different families (T232 × CM37 and CO159 × Tx303) [3], only two *ltk* loci have been detected; one locus is 0.58 cM distal to *pds1* on chromosome 1S while the other is 1.19 cM proximal to bngl619, an RFLP marker on chromosome 9L (B. Burr, personal communication). From isolation and sequencing of a 5.8 kb genomic DNA fragment containing the 3′ end of *ltk1* and *pds*, we know that *ltk1* is the *ltk* locus linked to *pds1* and transcribed in the same direction. Since available sequence for *ltk2* and *ltk3* showed 98.6% homology, these loci might represent a recent gene duplication whereby the two genes are still closely linked and cannot be differentiated by RFLP mapping. *ltk2* and/or *ltk3* might be located on chromosome 9L.

From the complete ORF available for *ltk2*, we predicted that it encodes a 79.15 kDa protein with eight leucine-rich repeats in an amino terminal extracellular domain and a serine/threonine protein kinase in a carboxyl terminal intracellular domain. The kinase catalytic domain showed homology to other serine/threonine kinases, particularly to tomato Pti1, a soluble serine/threonine protein kinase involved in plant defense [42]. Also, the putative extracellular LRR domain showed some homology to Cf-2 proteins, a pair of membrane anchored defense proteins from tomato [6]. However, the LRR domain of LTK proteins was unique in number and arrangement of LRRs as compared to any other known LRR-type RLK, implying a unique ligand specificity for the LTK extracellular domains. Despite the distinctive architecture of the LRR domain, the leucine-rich repeats do contain the consensus core sequence thought to be involved in protein-protein interaction and/or ligand binding.

In addition to the unique LRR domain, LTK proteins contained some novel motifs in the extracellular domain. A PEST sequence, thought to be involved in protein degradation, was found downstream of the LRRs. PEST sequences have been found not only in metabolic enzymes but also in proteins involved in gene expression, signal transduction and cell-cycle regulation [23]. However, it is unclear how the LTK PEST sequence, located in the extracellular domain, might function in protein turnover. Nearby the PEST sequence was a PRR, which can play a role in protein structure *vis-à-vis* ligand binding. Another novel region found in the extracellular domain was an SRR. Though the function of this serine-rich region is unclear, its proximity to the LRR and PRR may suggest some role in ligand binding. Moreover, this region most distinguished LTK1 from LTK2, suggesting that not only are LTK proteins very different from all other LRR-type RLKs, but may differ from each other regarding ligand specificity and/or affinity. In addition to variation in the SRR domain, other minor differences were observed in the predicted intracellular regions external to the kinase catalytic domain. Intracellular segments external to the putative protein kinase catalytic domain have been proposed to affect substrate specificity [36]. Minor residue differences between LTK1 and LTK2 intracellular domains may be interpreted to influence substrate specificity and/or affinity of the corresponding kinases. Subtle amino acid sequence differences between LTK types can have profound effects on extracellular ligand binding and/or intracellular signal transduction.

From northern analysis, only one size transcript could be detected; however, use of RT-PCR combined with restriction enzyme digestion allowed monitoring of *ltk* gene-specific expression. Although *ltk* transcripts were found to be of low abundance, transcript levels were higher in endosperm and embryo when collected at 20 DAP, as compared to leaves and roots of young seedlings. Of the three *ltk* transcripts, *ltk1* was the only one expressed in all tissues examined, including roots, leaves, endosperm, embryo, and unfertilized ovules; *ltk2* and *ltk3* were only expressed in the endosperm. However, *ltk3* was difficult to detect routinely, although an *ltk3* cDNA could be cloned. While *ltk2* was undetectable in unfertilized ovules, it was detected in developing endosperm, even at 10 DAP. This suggests that fertilization triggers increased *ltk2* transcript levels in developing endosperm cells. Furthermore, endosperm development was also accompanied by modulation of the *ltk1* transcript level. However, at 20 DAP, when both *ltk1* and *ltk2* were expressed in the endosperm, only *ltk1* was expressed in the embryo.

Although the LRR and kinase domains of LTK showed similarity to corresponding regions of defense-related proteins, the observation that *ltk1* and *ltk2* transcript levels were modulated during endosperm development suggests that LTK proteins may play a role in endosperm development. Other LRR type RLKs have been implicated in control of plant development; in Arabidopsis, ERECTA (ER) is involved in determination of organ shape and vegetative growth, although the biochemical mechanism is unknown [31] and Clavata1 (CLA1), expressed in the inflorescence, may regulate differentiation of apical meristem cells [5].

In maize, the process of double fertilization establishes initiation of two parallel developmental programs, one leading to a mature embryo and the other leading to the endosperm. As described by Kiesselbach [14], immediately after fertilization, endosperm nuclei divide mitotically without formation of cell walls; after about two days, the free nuclei continue dividing while forming cell walls only on one side until the endosperm is completely cellular at about 4 DAP; at 5 DAP, cell division occurs throughout the endosperm; at about 6 DAP the endosperm is solidifying and surrounding nucellar tissue is being digested away; also, the basal endosperm cells differentiate to form placental tissue; between 10–20 DAP, most cell division is confined to the outer region of the endosperm, away from the embryo; after about 20 DAP, cell divisions are limited to the periphery several cells deep, where surface cells eventually differentiate into the aleurone layer. It is unclear what signals control the repression of cell division in the endosperm after 10 DAP. Interestingly, the change in *ltk* gene expression correlated with this temporal and regional control of cell division in endosperm development. When endosperm cell division becomes more restricted between 10–20 DAP, *ltk2* and *ltk1* transcript levels are steadily increasing. Since the LRR domain may interact with polypeptide ligands or cell surface proteins, LTK proteins could transduce signals involved in regulation of cell division. Similarly, LTK1 may have some role in control of cell division in other parts of the plant, as its transcripts are constantly present.

Alternatively, the increased transcript levels of *ltk1* and *ltk2* might be significant in regulating later stages of endosperm development associated with establishment of dormancy. After 20 DAP, the kernel proceeds towards the maturation phase and dormancy where only the embryo and aleurone layer of the endosperm develop tolerance to desiccation associated with this stage. More defined analysis of *ltk* transcript localization in specific cells of developing endosperm and in other tissues, combined with isolation of *ltk*-specific mutations, will help elucidate the role of each LTK type in maize growth and development. The LTK proteins and their corresponding genes represent new tools for probing signal transduction associated with the intriguing process of endosperm and seed development.

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