

Plant Gene Register

Promoter of a Lipid Transfer Protein Gene Expressed in Barley Aleurone Cells Contains Similar *myb* and *myc* Recognition Sites as the Maize *Bz-McC* Allele¹

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Using the cDNA clone B12A (1) as a probe, we isolated the corresponding genomic sequence from a λEMBL3-library (Table I, Fig. 1). The B12A transcript, which is homologous to the A1f cDNA (2), is differentially expressed in the aleurone layer during seed maturation and germination and encodes a protein with lipid transfer activity (3). In view of a transcript recently identified to encode a novel LTP² (R. Kalla, unpublished results), we propose to denominate the gene encoding the B12A/A1f transcript *Ltp1*. Here we present the sequence of a 1487-bp *SacI*-*NsiI* fragment of the EMBL3 clone that contains the structural part of *Ltp1* including a 133-bp intron and 787 bp of the upstream region (Fig. 1). The 5' end of the *SacI*-*NsiI* fragment is referred to as nucleotide No. 1 and the 3' end is No. 1487. The sequence of the coding region of *Ltp1* is identical with those of the B12A and A1f cDNAs. Also, B12A is identical with *Ltp1* outside the coding region, whereas for A1f differences are found that probably are ascribable to allelic variation between cv Bomi (B12A) and cv Himalaya (A1f).

Analysis of the promoter region of *Ltp1* identified several putative regulatory motifs that may be involved in controlling *Ltp1* transcription in aleurone cells (Fig. 1). Of these, a well-documented *cis*-acting sequence element, the *myb* protein recognition site TAACTG (4), is found at position 512, 175 bases upstream of the putative TATA box. At position 530, *Ltp1* contains the sequence GCACCAGC, which is very similar to the *myc* recognition site (5). An AT block of 13 bp is located starting at 495. A similar organization of the same three elements is present in the *Bz-McC* allele of maize *Bz1* (6). The expression of this maize gene in aleurone cells is believed to be regulated by the interaction of the *myb*-like regulatory *C1* protein (7) and the *myc*-like *R* protein(s) (8). Furthermore, a sequence found in several promoters of genes expressed in aleurone layers was identified in *Ltp1* at position 406. This sequence, CATGTAA, resembles the CATGTAA motif (9) present in the promoters of both B22E and (1–3;1–4)-β-glucanase in barley, and *C1* in maize.

Based on these findings, strong indications point to the possibility that the spatial regulation of *Ltp1* in aleurone layers

Table I. Characteristics of *Ltp1*

Organism:	<i>Hordeum vulgare</i> , cv Bomi.
Location on Chromosome:	Unknown; according to Southern data, gene copy number is low (2).
Gene; Function:	<i>Ltp1</i> ; LTP activity <i>in vitro</i> (3).
Techniques:	Screening of library, restriction mapping, subcloning, dideoxy sequencing.
Clone type:	<i>SacI</i> - <i>NsiI</i> restriction fragment of genomic clone.
Source:	Genomic EMBL3 library made from DNA of etiolated seedlings, 7 d old.
Method of Identification:	Sequence comparison to published cDNAs (1, 2).
Sequencing Strategy:	M13 ssDNA templates; complete dideoxy sequencing of both strands.
Features of Promoter Region:	Several putative regulatory motifs are found (Fig. 1).
Features of Encoded Protein:	Open reading frame of 117 amino acids consisting of a 26-amino acid signal sequence and a 91-amino acid mature protein in which eight cysteine residues are conserved (2).
Antibodies:	Available (2).
EMBL Accession No.:	X59253.

results from the binding of one or more transcriptional activator protein(s) at the above-mentioned sites. The search for cDNA clones encoding such factors is underway. Particle gun delivery of different promoter constructs into aleurone tissue of developing and mature barley and maize grain tissues is presently being done to test the functionality of the putative regulatory elements.

LITERATURE CITED

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² Abbreviations: LTP, lipid transfer protein; bp, base pair(s).

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1  GAGCTCCAAGGCATCACCAAGCTTCTATGACGCCAAAACATCCAAGAAAGATATGTACTAGGATACCAAGCACCC
76  AAGAGTAAACGGAGGAAGTATAATATAAGGCCCTGTTTGATAACAAAGTAGTAAAAAACTAAAGTATTAAAAAC
151  TGCAGTAATTTTACGTGTAGATAGAAAATACCATGGTTTAAATATAATAATATTTTTTGCAGTATTCACAATGTA
226  GAGAAACTGTTTGATTACGCCACATATTACTGCAGTTTAGATCGAGCAAGTACACGGGAAGAAGATAACGACGTC
301  CCACCCCTTCTTTTCGCCTTCTCTGTTTTTTAAAAAGAGGTCTGGGGTTAGTTTTTTCAATACTGCAGTTTTAAA
376  ATCACAATTCTTAGAGGCAACCAACACCTCATTGTAATAAACTATGATAATCTCCAAACTGCAGTATTCTA
451  AAAATACTACAAAATTCTTTGTTATCAACAGGGCCTAAGGAGTTAAAAAAATTTAGCCGTAAGTGAAGTCTCGG
526  CGAGGCACCAGCAGCTAGCAGTCATCAACACTTGATGGTTGGCAAAGCCGAGTCGACGTGTCGCGGGGCTCGGCC
601  TGAGCGGGAGATCAATCTGTTCTCCAGTAACCCCGTCGATTGGCCCGCCGACTAAAGCATCCAGGCATCTCTC
676  GCTCGAACCCCTATTAAAGCCCTCCATTCTCCCAACATTCTCCACACCTCCAGAGTTGCTCATCACTAGCTA
751  GTACGTTGTACTGTTAGCTACAGATTAAGAAGTGATC ATG GCC CGC GCT CAG GTA CTG CTC ATG
                                     M  A  R  A  Q  V  L  L  M
815  GCC GCC GCC TTG GTG CTG ATG CTC ACG GCG GCC CCG CGC GCT GCC GTG GCC CTC AAC
      A  A  A  L  V  L  M  L  T  A  A  P  R  A  A  V  A  L  N
872  TGC GGC CAG GTT GAC AGC AAG ATG AAA CCT TGC CTG ACC TAC GTT CAG GGC GGC CCC
      C  G  Q  V  D  S  K  M  K  P  C  L  T  Y  V  Q  G  G  P
929  GGC CCG TCC GGC GAA TGC TGC AAC GGC GTC AGG GAT CTC CAT AAC CAG GCG CAA TCC
      G  P  S  G  E  C  C  N  G  V  R  D  L  H  N  Q  A  Q  S
986  TCG GGC GAC CGC CAA ACC GTT TGC AAC TGC CTG AAG GGG ATC GCT CGC GGC ATC CAC
      S  G  D  R  Q  T  V  C  N  C  L  K  G  I  A  R  G  I  H
1043 AAT CTC AAC CTC AAC AAC GCC GCC AGC ATC CCC TCC AAG TGC AAT GTC AAC GTC CCA
      N  L  N  L  N  N  A  A  S  I  P  S  K  C  N  V  N  V  P
1100 TAC ACC ATC AGC CCC GAC ATC GAC TGC TCC AG gtgattaaatttacactcatccagagtgaat
      Y  T  I  S  P  D  I  D  C  S  R
1164 ctttaaaaagaactatatttacgaacggagtgagtagataggaacattcatccacgtaaaatttggtgatattaa
1240 cattaacacgcatgattgacctgcag G ATT TAC TGAGCGACGATCCGTCAAGCTGGTGCTCAGCTCATCGA
                                     I  Y  *
1310 TCCACGTGGAGCTGAAGCGCGCAGCCTCTGTCCCTATGTAGTATGGCTACCAGTTATGCCGAGTTTATGCTGAAT
1385 AAGAACTCTCTCCTGTACTCCTTTGGAGGAGATCAGTATCTATGTACGTGAGAGTTGAGAGTTTGTACCATCGGC
1460 ACTCCAGTGTTTATGGACTATATGCAT

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Figure 1. Nucleotide sequence and deduced amino acid sequence of *Ltp1*. Intron is indicated by lowercase letters, TGA stop codon by an asterisk, and putative CAAT and TATA by boxes. A 21-bp inverted repeat is indicated by arrows, and in between, four 8-bp palindromic sequences are overlined. The motif indicated by thick underlining resembles the CATGTAAA motif present in the promoters of several genes expressed in aleurone cells (9). An AT block followed by a *myb* consensus recognition site and a *myc*-like binding motif are indicated by double underlining. A similar organization of these three elements is present in the *Bz-McC* allele of maize *Bz1* (6).

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