

# CLONING AND CHARACTERIZATION OF COTTON FIBER GENES ENCODING LIPID TRANSFER PROTEIN

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## Abstract

A fiber-specific cDNA clone (GH3) encoding lipid transfer protein (LTP) was used as a hybridization probe to retrieve corresponding genomic clones from a cotton genomic library. A LTP genomic clone (GH6) was isolated and its open reading frame (ORF) is homologous to that of GH3, but is interrupted by a single intron (136 bp) located in the region corresponding to the C-terminal of the protein. A long distance PCR method was used for 5' genomic DNA walking to clone promoter and upstream regulatory elements of the GH3 Ltp gene.

## Introduction

A fiber-specific cDNA clone (GH3) encoding lipid transfer protein (LTP) was isolated using a differential screening method (Ma et al., 1995). To understand the expression and regulation of LTP, the corresponding Ltp gene and its flanking regions (promoters and regulatory elements) were isolated and subsequently characterized.

## Materials and Methods

Cotton genomic libraries were either purchased from Clontech or prepared by *Eco*RI digestion of genomic DNA and then cloning into the lambda vector gt10. Gene sequences were determined by the dideoxy chain termination method and analyzed using the DNASIS program (Hitachi Corp.). Genomic DNA walking was carried out using the procedure of Siebert et al. (1995).

## Results and Discussion

Several positive genomic clones were isolated using the hybridization method. One of the clone, GH6, containing a 1.7 kb DNA insert, was characterized.

## Nucleotide and Derived Amino Acid Sequences of GH6

GH6 Ltp consists of an ORF of 360 bp and is interrupted by a single intron of 136 bp located in the region corresponding to the C-terminal of the protein. Its derived amino acid sequence has 64% identity with that of GH3. The GH6 LTP also contains a hydrophobic signal peptide

of 26 amino acid residues at the N-terminal, which is characteristic of exported proteins.

## Northern Blot Analysis

Northern analysis indicated that the GH6 gene is differentially expressed in fiber cells in a temporal manner. However, its expression level is lower than that of the GH3 Ltp gene.

## Acknowledgements

This research is supported by the Cotton Research Enhancement Program, Mississippi Agricultural and Forestry Experiment Station (MAFES), and a NRICGP grant from USDA (USDA 95373112459).

## References

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