EXPRESSION AND REGULATION OF THE COTTON FIBER GENE LTP3 ENCODING A LIPID TRANSFER PROTEIN

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Abstract

A cotton (Gossypium hirsutum L. cv DES119) fiber cDNA library was constructed in lambda gt10 using a PCR-based method. Through differential screening, one full-length cDNA clone (GH3) was isolated and subsequently sequenced. The nucleotide and derived amino acid sequences indicated that GH3 encodes a lipid transfer protein (LTP3) of 120 amino acids. Southern analysis of cotton genomic DNA suggested that Ltp3 is a member of a small multigene family. Two homologous genes (Ltp6 and Ltp12) were retrieved from a cotton genomic library using ^{32}P-labeled GH3 as a hybridization probe. All three Ltp genes were found to be expressed in fiber cells as detected by RT-PCRs. Northern analysis, however, indicated that the transcript level of Ltp3 is much higher than those of Ltp6 and Ltp12. LTP3 was expressed in E. coli as a maltose binding protein (MBP)-fused construct, and the fusion protein was purified for raising anti MBP-LTP3 serum used in the immunodetection of the LTP3 protein level during fiber development. The 5' and 3' flanking regions of Ltp3 were cloned with a genomic DNA walking method. The Ltp3 promoter was systematically analyzed by Agrobacterium-mediated tobacco transformation employing the GUS gene as a reporter. GUS expression assays on the tobacco transformants suggested that cis-elements which confer the fiber/trichome specific activity of Ltp3 promoter are located within a 315-bp DNA fragment (-614~-300 bp relative to the translational start codon ATG of Ltp3). The characterized Ltp genes and their promoter/regulatory elements will be valuable in genetic engineering for fiber modification.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:617-617 (1999)
National Cotton Council, Memphis TN