

**ANALYSIS OF PROMOTER ACTIVITY
OF COTTON LIPID TRANSFER PROTEIN
GENE LTP6 IN TRANSGENIC
TOBACCO PLANTS**

Chuan-Yu Hsu and Din-Pow Ma

**Department of Biochemistry and Molecular Biology
Mississippi State University
Mississippi State, MS**

Abstract

A cotton (*Gossypium hirsutum*) genomic clone (1.7 kb DNA insert) harboring the lipid transfer protein gene *Ltp6* had been previously isolated and characterized. The *Ltp6* contains an open reading frame of 360 bp, which is interrupted by a single intron of 136 bp. The *Ltp6* gene is specifically expressed in cotton fiber cells (seed hairs) and is developmentally regulated. By using PCR amplification, the *Ltp6* promoter and a series of 5' deletions of the promoter were generated and then cloned into the pBI101 plasmid upstream of the GUS (β -glucuronidase) reporter gene. These constructs were introduced into *Agrobacterium tumefaciens* LBA4404 by using a freeze-thaw method. Leaf disks of tobacco (*Nicotiana tabacum* L.) were transformed with *Agrobacterium tumefaciens* LBA4404 carrying the various promoter-GUS pBI101 plasmids. The GUS expression in transgenic tobacco plants was determined by the histochemical localization method and the fluorometric assay. Histochemical analyses of the transgenic tobacco seedlings indicated that the *Ltp6* promoter directed the GUS expression only in trichomes (hair cells). Fluorometric GUS assays showed that the promoter activity of the undelimited *Ltp6* promoter (nt -447 to -1) was at least 1,000 times weaker than that of the 35S promoter of cauliflower mosaic virus (CaMV). Sequential deletions of the promoter gradually decreased the expression level of the GUS gene. No GUS activity was observed when the 5' deletion of the *Ltp6* promoter reached to nt -86, which removed the CAAT and TATA putative promoter elements.