

**MOLECULAR CLONING OF A COTTON
FIBER SPECIFIC CDNA ENCODING
CELL WALL PROTEIN**

H. Tan and D.P. Ma

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

Abstract

A fiber-specific cDNA clone (GCW1) was isolated from a cotton fiber cDNA library using a differential screening method. Nucleotide and derived amino acid sequence analyses indicated that GCW1 encodes a proline-rich cell wall protein. Northern blot analysis showed that the cell wall protein gene is developmentally regulated and is preferentially expressed in fibers. The promoter region of the GCW1 gene was obtained by a long distance PCR amplification method.

Introduction

Cotton (*Gossypium hirsutum*) fiber is a differentiated epidermal single cell from a developing seed. Of the many genes involved in the control of fiber synthesis and development, only a few have been isolated and characterized. Molecular cloning of fiber cDNAs and their corresponding genes will be the first step to understand the molecular mechanism of fiber development.

Materials and Methods

A PCR-based method (Belyavsky et al., 1989) was used to construct a cotton fiber cDNA library in lambda gt10. The fiber specific cDNA clone was isolated using a differential screening method (Sargent, 1987). DNA sequences were determined by the standard dideoxy chain termination method. DNA walking of the 5' and 3' regions of the GCW1 gene was performed as described by Siebert et al. (1995).

Results and Discussion

The GCW1 Encodes A Proline-Rich Cell Wall Protein

A fiber specific cDNA clone (GCW1) containing a 0.43 kb insert, was isolated and sequenced. The deduced amino acid sequence of GCW1 indicated that it encodes a proline-rich cell wall protein. Similar to cotton fiber lipid transfer proteins (LTPs), the GCW1 protein contains a hydrophobic signal peptide sequence. It also consists of repeat units of pentapeptide Pro-Pro-Val-Tyr-Thr/Glu. The complete GCW1 gene sequence including the 5' (Promoter) and 3'

regions was obtained by a long distance PCR using the GCW1 specific primers and adaptor primers.

Developmental Regulation of the GCW1 Gene

Northern blot analysis showed that the GCW1 gene is developmentally regulated. The GCW1 is only expressed in the fiber cells, and the GCW1 mRNA levels steadily increase after 5 DPA and continue to increase at 20 DPA by the stage of the secondary cell wall synthesis.

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References

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