EXPRESSION OF α-TUBULIN GENES DURING COTTON FIBER DEVELOPMENT

David J. Whittaker and Barbara A. Triplett
USDA-ARS Southern Regional Research Center
New Orleans, LA

Abstract

Cotton fibers are single cell trichomes that elongate rapidly and synchronously by a diffuse growing mechanism (Seagull, 1990; Tiwari and Wilkins, 1995). Turgor-driven cell elongation is confined to a major axis of growth by highly organized arrays of cellulose microfibrils, (Giddings and Staehelin, 1991; Delmer and Amor, 1995). In expanding cotton fibers, patterns of microtubule deposition correlate precisely with the wall microfibril arrays (Seagull, 1986, 1992). The cortical microtubules are thought to provide spatial information necessary for alignment of cellulose microfibrils (Williamson, 1991; Cyr and Palevitz, 1995).

The major structural component of microtubules is tubulin, a heterodimeric protein composed of two highly conserved subunits, α and β. Both α- and β-tubulins are encoded by multigene families in eukaryotes (Cleveland and Sullivan, 1985: Silflow *et al*., 1987). In cotton fiber cells, nine α-tubulin and seven β-tubulin isotypes have been identified by immunoblot analyses of two-dimensional gels (Dixon *et al*., 1994). Two α-tubulin and seven β-tubulin isotypes showed preferential accumulation in fibers, and appeared to be temporally regulated. Here we present the isolation of partial α-tubulin cDNAs from cotton fiber, and the results of an investigation of α-tubulin transcript levels during fiber elongation. This investigation focuses on the developmental transition from rapid elongation to onset of secondary wall synthesis that occurs at 14-18 days post anthesis (DPA).

Five partial cDNAs (GhTua1-5) encoding 3' fragments of distinct α-tubulin genes were amplified by PCR using RNA isolated from 10 DPA cotton fibers, and cloned in plasmid vectors. The amplification of distinct α-tubulin cDNA fragments indicates that the expression of multiple genes contributes to the diversity of α-tubulin proteins found in fiber. A de-tyrosinated subset of α-tubulins has been identified in fiber (Dixon and Triplett, unpublished), indicating post-translational modifications also contribute to isotype diversity.

The open reading frames of clones GhTua1-5 show high nucleotide sequence identity to each other, and to α-tubulin genes isolated from other plant species, including *Arabidopsis thaliana* (Kopczak *et al*., 1992) and maize (Villemur *et al*., 1992). Whereas GhTua2 and GhTua3 share high nucleotide identity across the 3' untranslated region, the 3' untranslated regions of clones GhTua1, Ghtua2, GhTua4 and GhTua5 are highly divergent. Antisense transcripts of these divergent 3' untranslated regions were used as gene-specific probes in Northern analyses of α-tubulin transcripts. Transcript levels were examined in a series of elongating fiber samples, hypocotyl, root, cotyledon and mature pollen. Total α-tubulin transcript levels were assayed with an antisense probe to the partial open reading frame of clone GhTua2.

Levels of total α-tubulin transcripts in fiber were much higher than levels in the other tissues assayed, reflecting the rapid cell elongation occurring in developing fiber. None of the tubulin genes we investigated was expressed exclusively in fiber tissues, and transcripts of GhTua1, GhTua2/3, GhTua4 and GhTua5 were all abundant in fibers from 10 through 14 DPA. However, two discrete patterns of transcript accumulation were observed following the onset of secondary wall synthesis, which occurred at approximately 16 DPA in our samples. Whereas GhTua2/3 and GhTua4 transcripts remained abundant through to 20 DPA, GhTua1 and GhTua5 transcripts dropped to lower levels by 16 DPA.

The differences in transcript accumulation we observed indicate differential transcription of distinct genes contributes to the greater isotype diversity observed in elongating fibers prior to secondary wall synthesis (Dixon *et al*., 1994). During fiber development, microtubules exhibit specific changes in orientation, organization, number, length, and proximity to the plasmalemma. These changes are most apparent in the developmental transition from rapid elongation and primary wall synthesis to the onset of secondary cell wall synthesis and a slowing of elongation (Seagull, 1992). The patterns of transcript abundance and isotype diversity seen in elongating fiber cells suggest that transcriptional control of specific tubulin genes may influence mictotubule architecture, and consequently microfibril deposition and cell form.

Since distinct tubulin genes are preferentially expressed at different stages of fiber development, tubulin genes appear to be good targets for manipulation, in order to generate transgenic cotton with modified fiber properties. Current work focuses on the isolation and characterization of α-tubulin promoters. We wish to investigate the differences between the promoters of α-tubulin genes which show distinct expression patterns in fiber, and explore the utility of these promoters in transgene constructs.

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