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Changes in the Accumulation of α- and β-Tubulin Isotypes during Cotton Fiber Development

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The expression of α- and β-tubulin proteins in developing fibers and several other tissues of cotton (*Gossypium hirsutum*, cv Texas Marker 1) have been analyzed by immunoblots of one- and two-dimensional gels utilizing anti-tubulin antibodies as probes. As a percentage of total protein, fibers had greater amounts of tubulin than did hypocotyls, roots, leaves, or cotyledons. Both α- and β-tubulin, having apparent molecular masses of approximately 50 kD and isoelectric points between pH 5 and pH 6, were resolved on a single two-dimensional gel. Under the conditions used, α-tubulin was less acidic in the isoelectric focusing dimension and migrated slightly faster in the sodium dodecyl sulfate dimension than did β-tubulin. Nine α-tubulin isotypes that formed two distinct groups were identified on immunoblots of two-dimensional gels. The three most abundant α-tubulin isotypes were common to all tissues examined. Seven distinct β-tubulin isotypes were also identified. Although their level of accumulation differed, four of the β-tubulin isotypes were common to all tissues. Preferential accumulation of isotypes was more apparent in fibers than in the other tissues examined. Two α-tubulin isotypes and two β-tubulin isotypes showed preferential accumulation in 10- and 20-d postanthesis fibers, respectively.

Microtubules in association with other components of the cytoskeleton have a central function in many important processes in eukaryotic cells, including cell division, intracellular transport, cell motility, and cell morphogenesis. In plants, microtubules have a unique role in morphogenesis. Microtubules appear to have a direct influence on the morphology of individual cells as well as an indirect influence on the morphology of the entire plant, since overall plant morphology is collectively determined by the shape of individual cells (Lloyd, 1991; Shibaoka, 1991). This shaping influence of microtubules in plants is a result of their involvement in establishing cellular division planes and in determining the axes of cellular elongation. The influence of microtubules in determining axes of cellular elongation appears to be associated with the deposition of cellulose microfibrils in the cell wall. For example, in plant cells that are expanding isodiametrically, microtubules and wall microfibrils are oriented randomly and are believed to allow expansion of the cell in all directions. In contrast, during plant cell elongation, both microtubules and wall microfibrils are oriented transversely to the long axis of the cell and are thought to limit turgor-driven expansion of the cell to a single dimension (reviewed by Giddings and Staehelin, 1991).

In many higher plant systems, microtubules appear to direct the pattern and orientation of cellulose microfibrils within plant cell walls (for review, see Giddings and Staehelin, 1991; Williamson, 1991). Changes in microtubule patterns resulting from developmental cues, environmental signals, treatment with plant growth regulators, or microtubule-disrupting agents are often mirrored by equivalent changes in the pattern of wall microfibrils. Very little is known, however, about the mechanism responsible for changes in microtubule patterns and how these changes influence the deposition of cell wall microfibrils and cell elongation.

Developing cotton (*Gossypium hirsutum*) fibers are well suited for examining the changes in microtubule organization that influence wall microfibril deposition and cell elongation. Cotton fibers are single-celled trichomes that grow nearly synchronously from the ovule epidermis. Analysis of changing microtubule patterns during fiber development shows a precise correlation between the patterns of microtubules and wall microfibrils (Seagull, 1986, 1992). Furthermore, changes in these patterns coincide with changes in elongation rates of developing fibers. During fiber development, microtubules exhibit specific developmental changes in orientation, organization, number, length, and proximity to the plasmalemma (Seagull, 1992). These changes are most apparent in cotton fibers during the transition from a developmental stage distinguished by rapid cell elongation and the synthesis of primary cell wall, to a stage characterized by the slowing of elongation and the onset of secondary cell wall synthesis. This transition period occurs between 16 and 18 DPA in the fiber cells that were used in this study. Consistent with increases in microtubule length and number that occur during fiber development, the amount of α- and β-tubulin (the major component of microtubules) increases during this transition period in cotton fiber development (Kloth, 1989).

In all higher plants analyzed to date, multiple tubulin isotypes have been detected (reviewed by Hussey et al., 1991). Tubulin isotypes may result from the expression of individual genes or the posttranslational modification of a single gene product (reviewed by Fosket and Morejohn, 1992). Furthermore, the expression of tubulin isotypes may

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Abbreviations: 2-D, two-dimensional; DPA, days postanthesis; TBS-T, Tris-buffered saline with Tween.

be tissue specific or may be influenced by developmental or environmental signals (Kerr and Carter, 1990; Carpenter et al., 1992; Joyce et al., 1992). To determine whether the developmental changes in microtubule orientation, number, and length that are seen during cotton fiber development are associated with changes in the proteins that make up microtubules, we have examined the accumulation of α- and β-tubulin proteins during cotton fiber development. A comparison of cotton fibers with other cotton plant tissues indicated that most of the tubulin isotypes do not show tissue-specific patterns of expression. Only a few isotypes show preferential accumulation in specific tissues. Significantly, changes in both α- and β-tubulin isotypes were detected during fiber development.

MATERIALS AND METHODS

Plant Material

All plant materials used in this study were harvested from the *Gossypium hirsutum* var Texas Marker-1. Roots, hypocotyls, and cotyledons were harvested from young seedlings when they reached a height of approximately 5 cm. Leaves were harvested from the upper branches of mature plants and were approximately 50% expanded. Developing cotton fibers were harvested at 10 and 20 DPA. After excising the developing seeds from the boll (carpel), we carefully removed the immature fibers from the seeds by hand, ensuring that the fiber samples were not contaminated with other cell types.

Chemicals

All chemicals were purchased from Sigma.[2]

Protein Extraction

Proteins were extracted from all tissues according to the method of Barent and Elthon (1992). Briefly, plant tissue (0.5–1 g) was ground in liquid nitrogen with a mortar and pestle. The powdered tissues were transferred to a centrifuge tube and extracted with 5 mL of extraction buffer (700 mM Suc, 50 mM Tris [pH 8.0], 2 mM DTT, 100 mM KCl, 5 mM EDTA) and 6 mL of water-saturated phenol. To minimize proteolytic degradation, the following were added to the extraction buffer: 20 μg/mL leupeptin, 10 μg/mL aprotinin, 20 μg/mL pepstatin, 2 mM PMSF, 2 mM benzamidine, 2 mM benzamide, and 20 mM diethyl dithiocarbamate. After the sample was centrifuged for 10 min at 6000*g*, the phenol layer was transferred to a clean centrifuge tube; 25 mL of 100 mM ammonium acetate in methanol were added and the proteins were allowed to precipitate for 2 h at −20°C. After the sample was centrifuged for 10 min at 6000*g* the pellet was washed with methanol to remove excess phenol and then briefly dried under vacuum. The pellet was resuspended in a minimum volume of 1% SDS and 100 mM DTT and warmed to 37°C. Resuspended samples were microfuged briefly to remove insoluble material. Protein concentrations were determined by the Bradford method (Bio-Rad Laboratories) using bovine γ-globulin as a standard (Bio-Rad) and under conditions in which SDS did not interfere.

2-D Electrophoresis

Protein samples for first-dimension IEF were prepared according to the method of Holloway and Arundel (1988). Depending on the tissue and the antibody to be used, 20 to 900 μg of protein extract in a total volume of 30 μL were mixed with 45 mg of urea and 15 μL of lysis buffer (6% ampholine [pH 4–6], 50% 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid, 100 mM DTT). Typically, 25 μL of this protein sample solution were loaded onto tube gels for IEF. Protein samples were loaded at the cathode end of 19-cm × 1.5-mm tube gels and overlaid with a solution of 4 M urea. The first-dimension tube gel mixture consisted of 9 M urea, 4% ampholine (pH 4–6), 3.3% acrylamide stock (30% acrylamide, 1.8% bisacrylamide), and 4% 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid. The upper buffer chamber contained 0.1 M NaOH (degassed) with 0.01 M phosphoric acid in the lower chamber. Electrophoresis was done at 500 V for 18 h and then increased to 2500 V for an additional 4 h. During electrophoresis the temperature of the apparatus was maintained at 30°C with a circulating water bath. Under these optimized IEF conditions, tubulin focuses in the central region of the tube gel.

After IEF, the gels were extruded from the tubes and equilibrated for 10 min at room temperature in equilibration buffer (125 mM Tris-HCl [pH 6.8], 5 mM DTT, 5% SDS, 10% glycerol, bromphenol blue "trace"). Following equilibration, the central 7 cm were cut from each tube gel and layered on top of a 12.5% SDS polyacrylamide gel for electrophoresis in the second dimension, according to the method of Laemmli (1970). SDS-PAGE was run with a Hoefer minigel apparatus at 20 mA/gel constant current until the bromphenol dye front migrated off the gel. Proteins were transferred to nitrocellulose in an electrophoretic blotting apparatus according to the manufacturer's recommendations (Idea Scientific, Minneapolis, MN).

Immunodetection of Tubulin Isotypes

Immunodetection of tubulin isotypes was accomplished using commercially available anti-tubulin antibodies. A rat monoclonal anti-α-tubulin antibody prepared against yeast α-tubulin (YOL1/34) was purchased from Sera-Lab, Ltd. (Crawley Down, UK), and a mouse monoclonal anti-β-tubulin antibody prepared against chick brain β-tubulin (DM1B) was obtained from Amersham. Antibodies bound to tubulin on immunoblots were detected with an enhanced chemiluminescent detection system (Amersham) with the following modifications to the manufacturer's suggested protocol. All incubations, antibody dilutions, and washes were done in TBS-T and milk (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.1% Tween-20, 5% nonfat dry milk [Difco Laboratories, Detroit, MI]). After a 1-h blocking incubation at room temperature, the membranes were incubated overnight at 4°C

[2] Names of companies or commercial products are given solely for the purpose of providing specific information: their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

with anti-α-tubulin (YOL1/34) diluted 1:2500 or anti-β-tubulin (DM1B) diluted 1:10,000. After incubation with the primary antibody, the membranes were washed according to the manufacturer's recommendations but in TBS-T with milk. Incubation with the secondary antibody was also done overnight at 4°C. The secondary antibody, sheep anti-mouse or sheep anti-rat, conjugated to horseradish peroxidase (Amersham), was diluted 1:20,000 or 1:5,000, respectively. After incubation with the secondary antibody, the membranes were washed as above. Before the membranes were placed into the enhanced chemiluminescent detection reagent, they were washed briefly in TBS-T to remove residual milk. Multiple images of each blot were recorded on Kodak X-AR film with exposure times ranging from 15 s to 10 min.

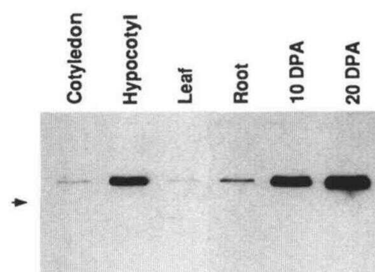

Figure 1. Immunoblot of an SDS-PAGE gel probed with anti-β-tubulin antibody (DM1B). Each lane was loaded as indicated with 50 μg of total protein from that tissue. Arrow indicates the position of the 46-kD marker.

RESULTS

The principal objective of this study was to characterize the α- and β-tubulin isotypes expressed during cotton fiber development. For this reason, 10 and 20 DPA fibers were selected because they represent two defined stages in fiber development. At 10 DPA, fibers are characterized by rapid cell elongation and synthesis of primary cell wall, and at 20 DPA, cell elongation has slowed and secondary wall synthesis has begun. Moreover, these two times delimit a transition period when microtubules are changing in their orientation, number, length, and proximity to the plasma membrane. Also important was the comparison of α- and β-tubulin isotypes in fibers with isotypes expressed in other tissues of the cotton plant.

A preliminary analysis by SDS-PAGE of β-tubulin levels in 10- and 20-DPA fibers, hypocotyls, roots, leaves, and cotyledons indicated that the amount of β-tubulin as a percentage of the total extracted protein differed dramatically (Fig. 1). Protein extracts from fibers and hypocotyls had a greater proportion of β-tubulin than the other tissues examined. Overall, cotton fibers had the greatest amount of β-tubulin with the highest levels appearing at 20 DPA. Similar results were obtained when α-tubulin levels were compared in these tissues (data not shown). For 2-D gel analysis of α- and β-tubulin isotypes, we compensated for the disparity in the amount of tubulin in the tissues by loading nearly equivalent amounts of tubulin. Therefore, the presence of an isotype on the 2-D gels represents its level of abundance as a proportion of total tubulin and not its level of accumulation in the tissue.

The extraction procedure and electrophoretic conditions optimized in this study permitted the analysis of both α- and β-tubulin on a single, 2-D gel (Fig. 2). Proteins extracted from 10-DPA fibers were resolved on a 2-D gel and blotted to nitrocellulose. Blots probed with both anti-α- and anti-β-tubulin antibodies were used to produce this composite showing the relative positions of α- and β-tubulin isotypes. The α- and β-tubulin subunits, from all of the cotton tissues analyzed, resolved on 2-D gels with isoelectric points between pH 5 and 6 and with an average apparent molecular mass of approximately 50 kD. The α-tubulin isotypes migrated slightly faster in the SDS dimension and were less acidic in the IEF dimension than the β-tubulin isotypes. In addition, the α-tubulin isotypes focused into two distinct clusters in the IEF dimension (isoelectric point approximately 5.8–6 and 5.5–5.7), whereas β-tubulin isotypes formed only one cluster (isoelectric point approximately 5.3–5.6).

A total of nine α-tubulin isotypes were detected in the tissues examined (Fig. 3). These isotypes are numbered 1 through 9 according to their frequency and relative abundance. For example, isotype 1 occurred in all of the tissues analyzed, and in comparison to the other isotypes, it appeared to represent a greater proportion of tubulin within the samples. Isotype 9, in contrast, was found only in 10-DPA cotton fibers and represented only a small fraction of the total tubulin present in 10-DPA fibers. The three most common α-tubulin isotypes 1, 2, and 3 were found in all tissues. These three isotypes, along with isotype 6 (found in 20-DPA fiber, root, and leaf), formed the more acidic cluster of α-tubulins. The remaining isotypes, 4, 5, 7, 8, and 9, formed a cluster of less acidic α-tubulins.

In a comparison of similar tissues, differential patterns of α-tubulin accumulation ranged from obvious to subtle. This differential accumulation was most evident in a comparison of 20- and 10-DPA cotton fibers where six differences in the pattern of α-tubulin isotypes were observed (Fig. 3, A and B, respectively). Isotypes 1, 2, and 3 were present during both developmental stages and appeared to represent similar proportions of the total tubulin in both samples. In contrast, isotypes 4, 5, 7, 8, and 9 were present at 10 DPA and were either absent or present only at very low levels in 20-DPA fibers. Although less apparent, the low level occurrence of isotype 6 in 20-DPA fibers and its absence in 10-DPA fibers provided another contrast between these two stages of cotton fiber development.

The α-tubulin isotypes in hypocotyls and roots (Fig. 3, C and D, respectively) also had similar proportions of isotypes 1, 2, and 3. Isotypes 4, 5, and 7 appeared to be more abundant in hypocotyls and were either absent or greatly diminished in roots. Isotype 6, present at low levels in roots, was absent in hypocotyls.

When leaves and cotyledons were compared (Fig. 3, E and F), isotypes 1, 2, and 3 again appeared at similar proportions. But, in contrast to the other tissues discussed previously, isotype 2 appeared to be slightly more abundant compared with isotype 1. Low levels of isotypes 5 and 6 showed differential accumulation, with 5 being present in cotyledons and 6 being present in leaves.

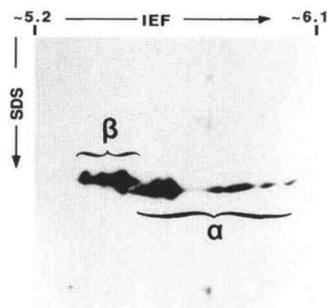

Figure 2. Immunoblots of 2-D gels probed with anti α- and β-tubulin antibodies were used to make this composite showing the relative position of α- and β-tubulin.

When tissues with less similarity were compared, e.g. roots and leaves (Fig. 3, D and E, respectively), it was apparent that the more abundant α-tubulin isotypes that were present remained very similar. Two very distinct tissues, 10-DPA cotton fibers and hypocotyls (Fig. 3, B and C, respectively) showed remarkable similarity in the pattern of α-tubulin isotype accumulation. The less acidic cluster of α-tubulins (isotypes 4, 5, 7, 8, and 9) reached their highest levels of accumulation in these two tissues. When all of the tissues are considered, there were only two isotypes that appeared to have tissue-specific accumulation: isotypes 8 and 9, which were found in 10-DPA cotton fibers. In addition to being tissue specific, these isotypes also appeared to be specific to the elongation phase of fiber development.

A total of seven β-tubulin isotypes were detected in the tissues surveyed (Fig. 4). As with the α-tubulin isotypes, they are numbered (1–7) depending on their frequency and relative abundance as a fraction of the total tubulin in the tissue. A comparison of β-tubulin isotypes in 20- and 10-DPA fibers (Fig. 4, A and B, respectively) indicates that β-tubulin proteins were also differentially expressed during cotton fiber development. Isotypes 1, 2, 3, and 4 were present in both 10- and 20-DPA fibers and appeared to accumulate to approximately the same relative levels of abundance. Isotype 5, present in only minor amounts at 10 DPA, was absent in 20-DPA fibers. In a more striking contrast, isotypes 6 and 7 were present at easily detectable levels in 20-DPA fibers and appeared to be completely absent at 10 DPA.

The expression of β-tubulin isotypes in hypocotyls and roots (Fig. 4, C and D, respectively) was very similar. Isotypes 1 through 5 were expressed in both of these tissues with only small variations in their levels of abundance. Isotype 3 was maintained at similar proportions in both hypocotyls and roots. Isotypes 1 and 2 represented a slightly larger fraction of the total tubulin in hypocotyls, whereas isotypes 4 and 5 were present at somewhat higher proportions in roots.

Leaves and cotyledons (Fig. 4, E and F, respectively) showed some differences in the pattern of β-tubulin accumulation. Isotype 4 represented a greater proportion of the total tubulin that was present in leaves than in cotyledons and may obscure a possible low level presence of isotype 5 in leaf tissue. Although less dramatic, isotype 3 was also present in greater amounts in leaf tissue. In contrast, isotypes 1 and 2 may have been slightly more abundant in cotyledon tissue.

Of the seven β-tubulin isotypes detected, four of the isotypes, 1, 2, 3, and 4, were present in all of the tissues examined. The proportion of isotypes 1 and 3 appeared to be nearly equivalent in all of the tissues analyzed. Isotype 2, although common to all tissues, appeared to be less abundant in hypocotyls and roots (Fig. 4, C and D). Minor accumulations of isotype 4 were seen in most tissues; however, in roots and leaves (Fig. 4, D and E) this isotype appeared to be preferentially expressed. Isotype 5 was present only at very low levels in 10-DPA fibers, hypocotyls, roots, and cotyledons. Because it was present in only minor amounts in these other tissues, isotype 5 may be present, but below the limit of detection, in 20-DPA fibers (Fig. 4 A) and may be obscured in leaves by the heavy accumulation of isotype 4 (Fig. 4E). In the figures shown, the accumulation of isotypes 6 and 7 appeared to be limited to 20-DPA fibers. However, overexposure of the immunoblots to the film indicated that isotype 7 may have been present at extremely low levels in hypocotyls and cotyledons (data not shown). The low level presence of β-isotype 7 in these two tissues was the only instance in which deliberate overexposure of immunoblots to the film

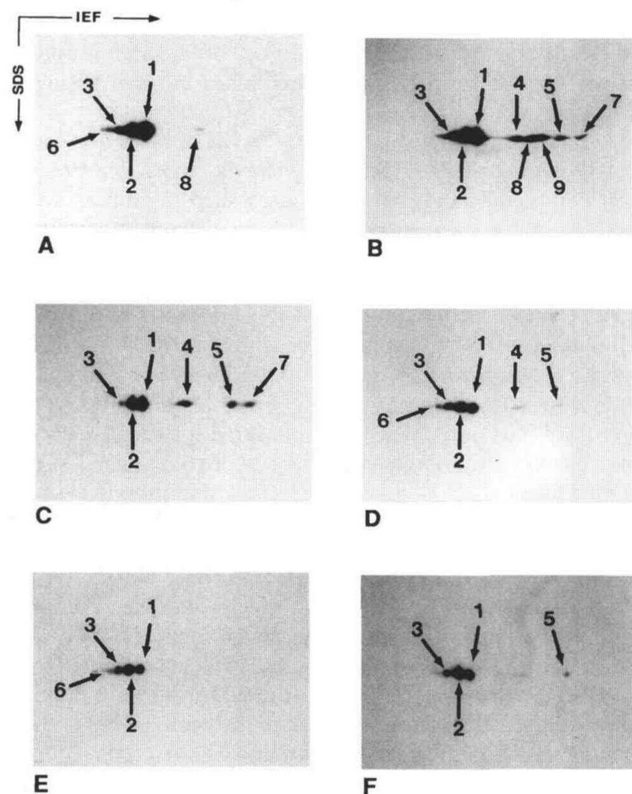

Figure 3. Immunoblots of 2-D gels probed with anti-α-tubulin antibody (YOL 1/34). Because the amount of α-tubulin, as a percentage of total extractable protein, differs in each tissue, the amount of total protein loaded on each gel was adjusted empirically so that approximately equal amounts of α-tubulin were loaded. A, 20-DPA fiber; B, 10-DPA fiber; C, hypocotyls; D, root; E, leaf; F, cotyledon.

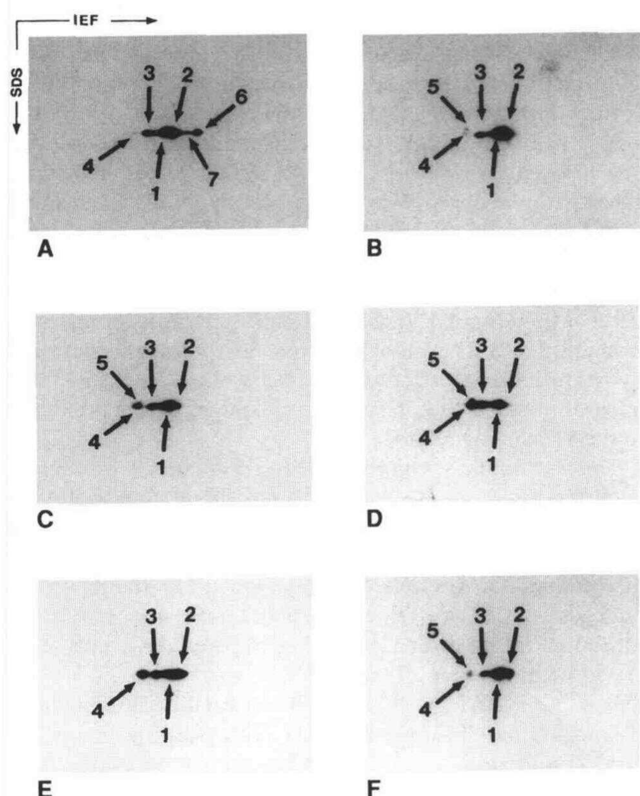

Figure 4. Immunoblots of 2-D gels probed with anti-β-tubulin antibody (DM1B). The amount of β-tubulin, as a percentage of total extractable protein, differs in each tissue. Therefore, the amount of extract loaded on these gels was adjusted empirically so that approximately equal amounts of β-tubulin were loaded on each gel. A, 20-DPA fiber; B, 10-DPA fiber; C, hypocotyls; D, root; E, leaf; F, cotyledon.

revealed the presence of an additional isotype. This observation suggests that, if additional tubulin isotypes existed in the tissues analyzed, they were extremely rare. The distribution and the relative abundance of both α- and β-tubulin isotypes in each of the cotton tissues analyzed are summarized in Table I.

DISCUSSION

The amount of both α- and β-tubulin as a percentage of extracted protein differed dramatically in the tissues analyzed. Disparity in the amount of β-tubulin detected in the tissues with the anti-β-tubulin antibody (Fig. 1) could perhaps be accounted for by the existence of other abundant β-tubulin isotypes in these tissues that the antibody does not recognize. This possibility does not seem likely, however, since the monoclonal antibody (DM1B) used for this study binds within a region of the β-tubulin protein that is evolutionarily conserved in fungi, algae, birds, mammals, and higher plants (Hussey et al., 1988). The DM1B antibody has been used successfully to characterize β-tubulin isotypes in a variety of plant species (Hussey and Gull, 1985; Hussey et al., 1988; Kerr and Carter, 1990; Waldin et al., 1992). Moreover, two of these studies used a second anti-β-tubulin antibody as a probe to verify their results and failed to detect the presence of other β-tubulin isotypes that were not recognized by the DM1B antibody. Similarly, the antibody used to detect α-tubulin (YOL1/34) has a binding site within a conserved region on the α-tubulin subunit and has been shown to react with α-tubulins from diverse sources (Fosket and Morejohn, 1992). The usefulness of the YOL1/34 antibody in this study was confirmed by using another anti-α-tubulin antibody, DM1A (Amersham). The DM1A antibody that also binds within a highly conserved region of the α-tubulin subunit did not detect additional α-tubulin isotypes on immunoblots of 2-D gels (data not shown).

Another factor that could account for the disparity in the

Table I. *The distribution and the relative abundance of α- and β-tubulin isotypes in cotton tissues*

The most abundant isotype within each tissue is indicated as +++, moderate abundance is ++, low abundance is +, and very low abundance is +/−. Since the amount of protein extract loaded on the 2-D gels was adjusted to equalize the amount of tubulin, these values represent only the relative proportion of each isotype to the other isotypes within the same tissue and do not represent the amount of isotype present within a tissue.

	Isotypes								
	1	2	3	4	5	6	7	8	9
α-Tubulin									
20-DPA fibers	+++	+++	++			+		+/−	
10-DPA fibers	+++	+++	++	+	+		+	+	+
Hypocotyls	+++	+++	+	+	+		+		
Roots	+++	+++	++	+/−	+/−	+			
Leaves	++	+++	++			+/−			
Cotyledons	++	+++	++		+/−				
β-Tubulin									
20-DPA fibers	+++	+++	++	+/−		++	+		
10-DPA fibers	+++	+++	++	+/−	+/−				
Hypocotyls	+++	++	+	+	+/−				
Roots	+++	+	+++	+++	+				
Leaves	+++	+++	+++	+++					
Cotyledons	++	+++	+	+/−	+/−				

amount of tubulin present in the tissue extracts is protein degradation. In preliminary trials there was obvious protein degradation (particularly with α-tubulin) that resulted in a complete loss of signal with antibody probes or the detection of bands/spots having dramatic shifts in their mol wts and/or their isoelectric points. As sample preparation techniques were modified these obvious indications of protein degradation disappeared and the patterns for α- and β-tubulin became reproducible. Therefore, the dramatic differences in the amount of tubulin present on SDS gels as well as changes in the patterns of tubulin isotypes seen on 2-D gels are accurate indicators of tubulins present within the tissues.

Tubulin levels were highest in 20- and 10-DPA fibers and hypocotyls. The high level of tubulin seen in 20-DPA fibers is not surprising, since it coincides with biochemical and cytological studies of tubulin and microtubule changes in developing cotton fibers (Kloth, 1989; Seagull, 1992). High levels of tubulin in 10-DPA fibers and hypocotyls are also consistent with the observations of others who have shown that tissues made up of cells undergoing rapid elongation have higher levels of tubulin protein and tubulin mRNA than tissues in which cells are not elongating (Cyr et al., 1987; Bustos et al., 1989; Han et al., 1991; Mendu and Silflow, 1993). It is interesting that tissues in which rapid cell elongation is occurring (10-DPA fibers and hypocotyls, Fig. 3, B and C, respectively) also contain the highest levels of isotypes composing the less acidic cluster of α-tubulins (isotypes 4, 5, 7, 8, and 9). Furthermore, a comparison of 10-DPA fibers with 20-DPA fibers (elongation has slowed substantially by 20 DPA) showed a nearly complete disappearance of this cluster of α-tubulin isotypes in 20-DPA fibers (Fig. 3, A and B). More experiments are needed, however, to establish a link between elongation and the presence of particular tubulin isotypes. For example, comparisons of other elongating and nonelongating tissues, such as careful dissection of the hypocotyl or root into elongating and nonelongating regions, may reveal preferential expression of tubulin isotypes associated with elongation.

Of the nine α-tubulin and seven β-tubulin isotypes characterized in these tissues, only five isotypes appear to accumulate preferentially in specific tissues. One of these, β-tubulin isotype 4 is specific to leaves and roots. The other four isotypes are all preferentially expressed in cotton fibers: α-tubulin isotypes 8 and 9 in 10-DPA fibers and β-tubulin isotypes 6 and 7 in 20-DPA fibers. Cotton fibers are highly specialized, single cells growing from the epidermal surface of ovules. As such, fiber cells differ dramatically from the other tissues examined in this study. It is not surprising, therefore, that differences in tubulin isotype accumulation are seen in comparisons between fibers and the other tissues. What is surprising, however, is that the tubulin isotypes that appear to accumulate preferentially in fibers also appear to have preferential accumulation during specific stages of fiber development.

The differential accumulation of both α- and β-tubulin isotypes in 10- and 20-DPA fibers may be correlated with the dramatic changes in cortical microtubule arrays that have been observed during cotton fiber development. Cotton fibers initiate from ovule epidermal cells on or near the day of anthesis. Soon after initiation, as the fiber begins to elongate, the cortical microtubules in the developing fibers orient transversely to the long axis of the cell. Cellulose microfibrils in the primary walls of elongating fibers are also oriented transversely during this stage. Elongation is very rapid, with fiber length increasing 1000- to 3000-fold during this period of development. Approximately 16 to 18 DPA, fiber elongation slows with the onset of secondary wall synthesis. Coincident with the slowing of elongation and the beginning of secondary wall synthesis, cortical microtubules begin changing orientation from transverse to steeply pitched helices that nearly parallel the long axis of the cell as the fiber nears maturity (Seagull, 1992). This reorientation of microtubules is mirrored by microfibrils in the secondary cell wall. Also at this time, microtubules increase in number, in length, and in proximity to the plasma membrane.

This is the first report of differential usage of tubulin isotypes during the development of a single plant cell type. Furthermore, because cotton fibers are terminally differentiated, the observed changes in α- and β-tubulin patterns in 10- and 20-DPA fibers must be linked to the observed changes in the cortical microtubule array and cannot be attributed to the presence of other microtubule arrays associated with dividing cells.

It is not known whether the differential accumulation of α- and β- tubulin isotypes directly influences the changes in cortical microtubules that have been observed during each stage of fiber development. Formerly, it was hypothesized that different tubulin isotypes are functionally specialized for specific roles within microtubule arrays (Fulton and Simpson, 1976). In contrast, more recent evidence suggests that most tubulin isotypes are functionally interchangeable and do not appear to have specialized functions. As a result, other proposals have been advanced to explain why multiple isotypes exist (reviewed by Fosket and Morejohn, 1992; Gaertig et al., 1993; Ludueña, 1993). The most intriguing of these new proposals is that, although individual isotypes may not confer specific function to microtubule arrays, they may act to alter the dynamic behavior of microtubules with regard to assembly kinetics, stability, and ligand-binding properties (Ludueña, 1993). Consequently, a cell may regulate the characteristics of its microtubule arrays by changing relative amounts of tubulin isoforms. Observations of microtubule dynamics in developing cotton fibers are consistent with this proposal for isotype function. Pharmacological studies using microtubule-disrupting agents indicated that the protein composition of individual microtubules varies during fiber development and that there is a heterogeneous population of microtubules present in cotton fibers during both primary and secondary cell wall synthesis (Seagull, 1990).

Further analysis of both α- and β-tubulin isotypes is needed to determine which isotypes arise from posttranslational modification and which are the result of differential gene expression. With this information, isotype-specific probes may be generated and used to gain further understanding of the relationships among tubulin isotype accumulation, microtubule dynamics, cell wall deposition, and cell elongation.

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