Supplemental Protocol S1. Cryo-FE-SEM:

A cryo field emission SEM (Hitachi S4300SE/N; Hitachi High Technologies Americas, Inc, Pleasanton, CA) coupled with a cryogenic preparation unit (Emitech K1250X; EmiTECH Inc., Fall River, MA) was used to observe hydrated, frozen, fibers *in situ*. The following steps optimized observation of life-like fibers. (1) The cryo-preparation unit and the cryo-stage inside the SEM specimen chamber were pre-cooled to -155°C. The protective cold shroud for the cryo-stage was cooled to -180°C. (2) Bolls of known DPA were excised from plants in a nearby greenhouse, and the pedicel was placed in water. (3) Liquid nitrogen was converted to slush in the cryo-system's freezing chamber (following the manufacturer's instructions). (4) A rectangular window was cut with a scalpel through the boll wall, avoiding damage to underlying fibers. (5) The piece of boll wall was lifted away. (6) At 6 DPA and later when the boll diameter exceeded the height of the preparation chamber, the back half of the boll was cut off with a double-edge razor blade. (7) The back of the boll was adhered to the cryo-system's sample mounting sled, which had been pre-attached to the specimen exchange rod. The adhering agent was pre-mixed carbon paint/OCT solution (1:1, v/v). Products used were PELCO® Conductive Graphite (#16051, Ted Pella Inc., Redding, CA) and Tissue-Tek® OCT Compound for Cryostat Sectioning (#27050, Ted Pella Inc.). (8) The mounted boll was frozen by plunging into pre-slushed nitrogen. Only one boll was excised from the plant at a time, and less than 10 min elapsed before freezing. After beginning to cut the boll wall, the sample was frozen within 1 min. For subsequent manipulations, the vacuum system was controlled so that the frozen boll was never exposed to air during transfer of the sample mounting sled to the preparation chamber. (9) The stereomicroscope integral to the preparation chamber was used to inspect the fiber surface for ice crystals that formed during freezing and transfer. (10) Any surface ice that had formed during specimen transfer was sublimed away by heating the stage to -90°C, typically from 20 to 45 min. The time varied according to the age of the boll, since the larger bolls required longer times for the fiber surface to reach sublimation temperature. (11) While the boll was still in the preparation chamber, the sample mounting sled was re-cooled to -155°C prior to light sputter coating (5 nm of gold-palladium under argon, 20 sec). (12) The still-frozen boll was transferred to the pre-cooled SEM stage. (13) Micrographs were taken under 1 to 2 kV accelerating voltage at 40 μamps beam current, with working distance adjusted appropriately for magnification. The stage was tilted and rotated to optimize the viewing angle of different fiber attributes.

Supplemental Protocol S2. Structural analyses and immunolabeling:

All chemical fixatives were EM grade from either Ladd Research (Hatfield, PA) or Electron Microscopy Sciences (Williston, VT).

Cryo-fixation and freeze substitution: Using previously described methods (Salnikov et al., 2003), minimally disturbed fibers still attached to seeds were frozen by plunging in liquid nitrogen-cooled propane within a few minutes of removal through a window cut in the wall of the boll, which was still attached to the plant.

Chemical fixation methods: For ultrastructural analysis of cell walls and CFML, a lab microwave (Biowave Model 34700; Pelco Redding, CA) was used with temperature restricted to $\leq$ 36.5°C during fixation and $\leq$ 40°C during infiltration. At some steps as specified below, 50.8 cm (20 in) Hg vacuum was applied. As described above, a window was cut in the boll wall, and seeds with attached fiber were carefully lifted from the center of the locule and immersed in 50 mM sodium phosphate buffer (pH 7.2) with 2% (w/v) glutaraldehyde (2 days, 4°C). Using a microwave, samples were rinsed in the same buffer (250 W, no vacuum, 3 x 1 min), exchanged to aqueous reduced osmium [made just before use by mixing equal volumes of 2% (w/v) OsO_4 and 3% potassium ferricyanide (w/v)]; microwave-fixed (150W, under vacuum, 1 min), and then kept on ice 20 min. Samples were dehydrated in an acetone series (50, 70, 90, 100%; 250W, no vacuum, 45 sec each step), and infiltrated in Spurr's resin:acetone (1:1, 3:1) followed by 2 x 100% resin (250W with vacuum, 3 min each step). After 3 h rotation in 100% resin (RT), samples were flat-embedded (Salnikov et al., 2003) and polymerized (60°C, overnight).

For TEM immunolabeling similar to published methods (Ruan et al., 2004), seeds with attached fiber were fixed in 3% (w/v) glutaraldehyde in 25 mM sodium phosphate buffer (pH 7.0) under vacuum for 2 h. After transfer to new fixative, samples were stored overnight at 4°C, then rinsed in the same buffer (3 times, 15 min each). Fibers were secondarily fixed in 1% (w/v) OsO_4 in the same buffer, and then dehydrated in an EtOH series (30, 50, 70, 90, and 2 x 100%, 1 h each step, 4°C). Infiltration occurred in two changes of LR White resin:EtOH (1:1, 4 h each) then 3 x 100% resin (first overnight at 4°C, then 4 h at RT, followed by exchange to new resin immediately before flat-embedding). Polymerization occurred under 360 nm UV light (overnight, 4°C). Because carbohydrate epitopes were the labeling targets, use of OsO_4 could be tolerated while also providing better preservation of the cytoplasm. Results with and without OsO_4 were equivalent, and all negative results were confirmed without OsO_4 (data not shown).

Sectioning, immunolabeling, and staining prior to TEM observation: To obtain cross-sections of flat-embedded fibers, a ~ 2 mm^2 resin area was cut out with a razor blade and glued vertically onto a blank resin block. Thick sections were made and stained with 1% (w/v, aqueous) Toluidine Blue for preliminary observation in the light microscope. Ultrathin sections for TEM were cut with a diamond knife on an ultramicrotome, collected on Formvar-coated grids, and immunolabeled (if applicable). For immunolabeling, basic solutions used were: 20 mM Tris buffer, pH 8.2, plus 0.02% (w/v) azide (TB); Tris buffered saline (TBS; TB plus 150 mM NaCl, pH 7.5). TB was used in later steps to minimize gold aggregation and non-specific labeling. Thin sections collected on nickel grids were: (a) blocked (30 min, RT, high humidity chamber) in TBS plus 0.06% (w/v) bovine serum albumin (TBS/BSA); (b) incubated (overnight, 4°C) in TBS/BSA with primary antibody (1:3 dilution); (c) washed 3x by dipping grids in TBS; and (d) incubated in TB plus 0.06% (w/v) BSA and 1:30 secondary antibody made in goats (1 h, RT). For JIM5, an anti-rat secondary antibody coupled to 15 nm colloidal gold was used (Electron Microscopy Sciences), and for CCRC-M1 an anti-mouse secondary antibody coupled to 10 nm colloidal gold was used (Amersham Bioscience/GE Healthcare, Piscataway, New Jersey). Grids were then washed in TB and distilled water before staining with 2% (w/v, aqueous) uranyl acetate (15 min) and lead citrate (4 min). Specimens were photographed in the TEM (FEI/Philips EM 208S), and the negatives were scanned at high resolution (Epson 4870 scanner) for preparation of digital plates.

For the antibody 2F4, labeling was performed in a modified buffer, 20 mM Tris-HCl, pH 8.2, containing 0.5 mM $CaCl_2$ and 150 mM NaCl according the instructions of Plant Probes (Leeds, UK). Negative results were confirmed in TEM analysis and fluorescence analysis (see below).

Sectioning and immunolabeling prior to fluorescence observation: The same results were obtained when semi-thin sections were labeled within 1 to 24 h after sectioning and on samples fixed with (Fig. 4, A to I) and without OsO_4 (data not shown). Semi-thin (250 nm) sections were cut with an ultramicrotome (Reichert-Jung Ultracut E, Wien, Austria) and mounted on glass slides that had been coated with adhesive (chrome-alum gelatin: 0.5% (w/v) gelatin and 0.05% chromium potassium sulfate). Optionally, sections were pre-treated 60 min with 0.1 M Na_2CO_3. Immunolabeling was performed at room temperature by applying and removing a series of 10 µl droplets as follows. Sections were blocked for 1h in KPBS (0.5 M NaCl in 0.01 M potassium

phosphate, pH 7.1) with 3% (w/v) non-fat dry milk, followed by washing in KPBS (5 min). Primary antibodies (1:5 in blocking solution) were applied and incubated 2 to 2.5 h, followed by washing with KPBS (3 x 5 min each). Secondary antibody (1:100 dilution in KPBS of goat anti-rat IgG conjugated to Alexa Fluor 488, Invitrogen, Carlsbad, CA) was applied and incubated 60 min followed by washing (1 x KPBS, 1 x H_2O, 5 min each). Final mounting was in an antifade agent (CITIFLUOR AF1; Electron Microscopy Sciences, Hatfield PA).

Sections were photographed at the same exposure time with a digital camera attached to an epi-fluorescence microscope (Eclipse 80i microscope with DS-Ri1 camera head and NIS-Elements Basic Research Software; Nikon, Melville, NY). With an ineffective antibody (2F4) the frames were entirely black, demonstrating that positive signals did not arise from autofluorescence. In order to allow comparison of relative intensity levels between samples, no image processing was performed for Figure 4 A to I. For Figure 4J, image processing to align and superimpose two images was accomplished in Adobe Photoshop CS3. For JIM5 untreated (Fig. 4A), the signal was changed to red and opacity was set to 92%. For JIM5 after Na_2CO_3 treatment (Fig. 4D), the signal was retained as green and the opacity was retained at 100%. The levels function in Adobe Photoshop was used on the composite image to adjust the brightness for optimum viewing. The composite image was compared to the original to verify that red (or orange or yellow) marked the locations of the original dim signal for JIM5 on untreated sections. An excerpt of the composite image (boxed area in A and D) is shown at high magnification in Figure 4J.

Supplemental Protocol S3. Histology of the seed and attached fiber using cyrogenic methods:

Processing in paraffin allowed the sectioning and subsequent observation of entire seeds and attached fibers. Initial freeze substitution allowed the orderly packing of fiber around a 10 DPA seed to be observed, whereas this organization was destroyed in traditional paraffin processing. First, a homemade device for freeze substitution of bulk samples (Grimson and Blanton, 2006) containing fixative [4% (w/v) glutaraldehyde in anhydrous acetone] was pre-cooled to -80°C. Each sample chamber contained 10 mL fixative over molecular sieves that had been pre-dried overnight at 400°C. A window (~1 cm^2) was cut in the boll wall across the suture line to reveal a locule. The seed and associated fibers were lifted from underneath with a spatula and frozen in melting liquid nitrogen slush (produced by applying a vacuum to a dewar containing liquid nitrogen). Without thawing, the sample was transferred into the -80°C fixative and left at -80°C for 14 d. Then fresh fixative (pre-cooled to -80°C) was added, followed by another 14 d incubation. After 28 d at -80°C, the freeze-substitution module was transferred successively to -40°C (7 d), -20°C (3 d), 4°C (2 d), and RT (1 d). The seed/fiber packet, which retained its original shape and size, was carefully removed, placed in a covered beaker, and rinsed in 2 x 100% acetone at RT (8 h), followed by acetone:xylene (1:1, v/v; 8 h), and 3 x pure xylene (each 4 h). The sample in xylene was transferred to 58°C oven and allowed to equilibrate. Solid paraffin chips were added progressively to the covered beaker over a 10 d period so that xylene slowly exchanged with paraffin. It was observed that the slow infiltration by the addition of paraffin chips disturbed the delicate spatial integrity fibers much less than decanting one solution and replacing it with the next. After 10 d, the remaining xylene was evaporated over 2 d from the uncovered beaker. The paraffin was then carefully decanted and replaced with fresh liquid paraffin for 1 d (58°C), and this process was repeated. A solid paraffin base was created in a peel-away mold, and the seed/fibers were then carefully placed into the mold with a spatula and covered with liquid paraffin. The mold was floated on an ice-water bath, and then plunged under the surface to rapidly cool the sample to prevent the paraffin from cracking.

For microtomy and staining by standard methods (Ruzin 1999), the paraffin block was mounted on a 2.54 cm microtome chuck, end-on, and 12 μm thick sections were taken on a rotary microtome (American Optical Model 820; Tissue-Tek disposable microtome blade, Miles

Inc., Elkhart IN). Ribbons of sections were applied to a layer of 3% formalin on slides pre-coated with Haupt's adhesive and warmed to 52°C to spread the sections. After drying at 52°C overnight, the sections were deparaffinized and stained in a standard histological stain series of 1% (w/v) aqueous Safranin O (12 h) followed by 1% (w/v) Fast Green in 95% EtOH (2 min). After re-exchange to 100% xylene, permanent slides were made by: (a) putting several drops of Preservaslide (MCB Reagents, Cincinnati, OH) on top of the sections; (b) covering with 22 x 60 mm coverslips applied carefully to avoid bubbles; and (c) drying overnight at 52°C with a fishing weight on top.

Supplemental Protocol S4. Analysis of cell wall cellulose and sugars:

Frozen fiber at 10, 17, 19, 21, and 30 DPA was pulverized under liquid nitrogen and lyophilized. As expected for cotton fiber, starch content was confirmed to be negative by use of the following procedure. Approximately 40 mg dried fiber in pre-weighed 1.5 mL snap-top tubes was extracted 5 x 55 min with 1 mL sterile hot water (65°), with centrifugation to separate supernatant and pellet. The combined hot-water-soluble material was filtered, lyophilized, weighed, resuspended in 1 mL sterile water, and stored at -20ºC until further analysis. For starch analysis, 100 µL of fiber extract was diluted in 400 µL water, followed by addition of 20 µL KI solution (0.5% w/v I_2 and 1% w/v KI). No purple color formed from the cotton sample, whereas the reaction was immediate when amylopectin (2mg/mL) was used as a positive control. The insoluble pellet was further extracted with a series of solvents (1 mL each; 2 extractions at each step): cold 80% EtOH, cold 100% EtOH, chloroform: methanol (1:1, v/v), and acetone, followed by centrifugal evaporation. The cell wall pellet was stored at -20℃ prior to further analysis.

Crystalline cellulose content was determined by a modification of the Updegraff assay (Updegraff 1969). After organic extraction, 1 to 2 mg dry fiber or 1 to 6 mg Avicel as a standard was boiled 1 h with acetic-nitric reagent. Acid insoluble material was collected by centrifugation, washed (3 x 3 mL in water, 1x in acetone), dried overnight, and hydrolyzed in 1 mL concentrated H_2SO_4 (1 h, RT, gentle shaking). Released glucose was quantified with anthrone reagent (0.2% w/v anthrone in H_2SO_4). Anthrone reagent (750 µL) was added to 400 µL water plus 10 µL cellulose hydrolysate, boiled 5 min, and OD at 620 nm was determined.

Sugars were analyzed as TMS-methylglycoside derivatives, which quantifies both neutral and acidic glycosyl residues in non-cellulosic cell wall matrix polymers (York et al., 1985). Each dried pellet and resuspended hot water soluble extract was aliquoted (~1-4 mg) into glass reaction tubes containing 20 µg inositol as an internal standard. After lyophilization, the sample was hydrolyzed in ~0.5 mL 1 M HCl in anhydrous MeOH (17 h, 80ºC) followed by drying in a gentle dry-air stream. Residual HCl was removed by duplicate washing with MeOH and air-drying. TMS-methyl glycosides were created by incubation (80ºC, 20 min) with 200 µL Tri-Sil reagent (Pierce/Thermo Fisher Scientific, Inc., Rockford, IL). The derivatized samples were dried under air, resuspended in hexane, filtered through glass wool-packed Pasteur pipettes, air dried, and finally resupended in 100 µL hexane. Sugar content was analyzed by injecting 2 µL onto a GC-MS system.

Supplemental Protocol S5. Quantitative real-time reverse-transcription PCR (qPCR):

To generate a large family of target genes, we looked for cotton homologs of Arabidopsis genes that had been classified into cell wall related gene families (http://cellwall.genomics.purdue.edu/families/index.html). All the corresponding cotton sequences from the G.h.fbr-sw sequence set (Haigler et al., 2005) were selected as targets, as well as few additional cell-wall-related genes sequenced from other cotton fiber cDNA libraries. Any corresponding longer cotton contigs for target genes were retrieved from a cotton comprehensive gene assembly (http://agcol.arizona.edu/pave/cotton/).

In the absence of a fully sequenced *Gossypium* genome, primers were designed using homologous Arabidopsis sequences as a guide in order to minimize the possibility of amplification of closely homologous genes. This strategy was particularly useful because of the close relationship between *Arabidopsis* (Brassicales) and *Gossypium* (Malvales) within Rosids II of the plant phylogenetic tree. The cotton unigenes and their translated sequences were thoroughly compared with the Arabidopsis sequence database to select the unique primer design sites for the closest Arabidopsis homolog that were also shared by the known cotton sequence. Primer Express 3.0 software (Applied Biosystems) was used to design forward and reverse primers (Table S4). The uniqueness of each primer was further confirmed by local Blast against the cotton comprehensive gene assembly. To promote equal amplification, the amplicon size was kept between 50 to 150 base pairs. Although the results show that primers designed in this way can demonstrate developmentally regulated expression corresponding to other data (e.g. for *GhRAC13* and *GhCTL1*, Fig. 5B), it cannot be excluded that, arising from *Gossypium hirsutum* allotetraploidy, more than one closely related gene was amplified.

Total RNA was isolated (Spectrum Plant Total RNA Kit, Sigma-Aldrich, St. Louis, MO) from $\leq$100 mg (fresh weight) liquid-nitrogen-pulverized fiber at 6, 10, 15, 19, 20, 21, 22, 23, 26, and 30 DPA. The yield from two extractions at each DPA was combined and then purified of contaminating DNA (On Column DNase Digestion, Sigma-Aldrich). RNA was reverse transcribed by RT-PCR in 20 μL "TaqMan Reverse Transcription Reagents" (#N8080234, Applied Biosystems, Foster City, CA). Briefly, 1.0 μL oligo $(dT)_{16}$ mixture (50 μM) was added to 400 ng of total RNA in 0.2 mL PCR strip tubes. The volume was adjusted to 8.7 μL with nuclease free water, and then incubated (70ºC, 5 min). PCR reactions were set up on ice by addition of 4.0 μL dNTP, 2 μL buffer (10x), 4.4 μL $MgCl_2$ (20 mM), 0.4 μL RNase inhibitor,

and 0.50 μL of reverse transcriptase, followed by mixing and centrifugation. For RT-PCR, the reaction mixtures were incubated (25ºC, 10 min), reverse transcribed (48ºC, 1 h), inactivated (95ºC, 5 min), and cDNA was stored at -20ºC.

In qPCR, *Gheif5* (eukaryotic translation initiation factor-5) was used as an endogenous normalizing gene; it had nearly equal expression in 6 to 30 DPA fiber (data not shown). PCR reaction mixtures [12.5 μL of SYBR green, 150 nM forward and reverse primers (Table S4), 10 ng of cDNA in a total volume of 25 μL adjusted with RNase free water] in 96 well optical plates were briefly vortexed, centrifuged, and loaded into a real time PCR machine (Model 7300; Applied Biosystems). PCR conditions were 50ºC, 2 min; 95ºC, 10 min; followed by 40 cycles of (95ºC, 20 sec and 60ºC, 1 min). Dissociation curves were analyzed to verify the absence of non-specific amplification and primer-dimer formation. Generally, the data were analyzed automatically with the SDS software, but, when necessary, manual adjustments for the baseline and Threshold Cycle (Ct) were made. The Ct values were used to calculate the ΔCt and ΔΔCt to determine each gene's expression pattern relative to a time of high rate elongation at 10 DPA ($2^{-\Delta\Delta Ct}$).

Supplemental Protocol S6. Enzyme activity assays:

β-galactosidase acivity was measured by hydrolysis of β-D-galactopyranoside (Pressey, 1983). Fiber (~0.08-0.12 g fresh weight) was vortexed in 1.0 mL of 100 mM sodium acetate buffer, pH 4.0, containing 0.5 M NaCl, extracted (15 min, 4°C), centrifuged to clarify (15,000 x *g*, 5 then 10 min), and stored on ice prior to assay. Enzyme reactions (400 µL total volume) were incubated for 0.5 h at 37°C in 62.5 mM sodium acetate buffer containing 0.025% (w/v) bovine serum albumin, 50 µL fiber extract, and 3.25 mM β-D-galactopyranoside. Reactions were terminated by addition of 0.2 M sodium carbonate (0.5 mL). The OD arising from the release of p-nitrophenol was measured at 415 nm. A standard curve was prepared from p-nitrophenol (10-50 nmol), and enzyme activity was expressed as µmole p-nitrophenol released h^{-1} mg $protein^{-1}$.

Activity of XG endo-hydrolase (XEH) was assayed as described with minor modification (Kallas et al., 2005). The reaction mixtures (200 µL total volume) contained sodium acetate buffer (75 mM, pH 6.0), 100 µL fiber extract (prepared as above), and 100 µg of tamarind XG (P-XYGLN, Megazyme International Ireland Ltd, Wicklow, Ireland). After incubation (30°C, 24 h), reactions were terminated by addition of 100 µL 1 M HCl. Color development occurred (30 min, RT, dark) after addition of 800 µL 20% (w/v) Na_2SO_4 and 200 µL potassium tri-iodide reagent [1.0% KI (w/v) and 0.5% iodine (w/v) in water]. A standard curve was prepared using 10 to 100 µg XG, and decrease in OD at 620 nm indicated XEH activity. Results were expressed as ng XG degraded h^{-1} mg $protein^{-1}$.

Pectin methylesterase (PME) activity in cotton fibers was quantified as described (Jiang et al., 2005). Fiber (40-50 mg fresh weight) was vortexed in 600 µL buffer (0.1 M citric acid, 0.2 M NaH_2PO_4, pH 5.0). Reaction mixtures (800 µL total volume) contained 750 µg esterified pectin (P-9561, Sigma-Aldrich) and 50 µL fiber extract in 0.2 M sodium phosphate buffer (pH 7.0). After 18 h incubation (37°C), color development occurred for 15 min after addition of 200 µL 0.5% (w/v) ruthenium red (RT). Each reaction mixture was then mixed with 400 µL 0.6 M $CaCl_2$, clarified by centrifugation (15,000 x *g*, 10 min), and PME activity was expressed as (change in OD at 534 nm)/(mg protein).

Supplemental Table S1. Probes used in CoMPP or TEM immunolabeling and qualitative results on immunolabeling intensity.

The qualitative results for intensity of TEM immunolabeling are indicated on a scale of 5 (high) to 0 (not detected) in the last column, with results for 10 and 17 DPA separated by a comma. The notation 'n.d.' means that immunolabeling was not performed in this study. Bold type for the antibody name signifies that the antibody also labeled the inner primary wall of cotton fiber. Positive results for JIM7 were demonstrated in the fluorescence microscope (see Figure 4) when semi-thin sections were labeled within 24 h after sectioning. Xyloglucan epitopes (e.g. XLLG) are explained at: http://cell.ccrc.uga.edu/~mao/ xyloglc/Xtext.htm# xyloglucan%20-%20composition%20and%20glycosyl%20sequence.

Typical polymer recognized	**Probe Name**	**Epitope recognized and/or other information about binding**	**Source**	**CFML immuno-labeling at 10 and 17 DPA**
cellulose	CBM3a	crystalline cellulose	gift of P. Knox	0
callose	BS 400-2	1,3-β-glucan	BioSupplies	n.d. (see Salnikov et al. 2003)
Homogalacturonan (HG)	**JIM5**	GalA oligomers, preferring some esters on HG; recognizes DP>7	CarboSource	2, 2
	JIM7	GalA oligomers, preferring more esters on HG	CarboSource	strongly positive (see immuno-fluorescence results, Fig. 4)
	CCRC-M38	able to bind fully de-esterified HG; recognizes DP>4	CarboSource	3, 3
	PAM1	blockwise de-	Plant Probes	0

		esterified HG		
	LM7	non-blockwise de-esterified HG	Plant Probes	0
	2F4 (see note below)	Ca^{++}-cross-linked, low esterified HG	Plant Probes	0
Xyloglucan (XG)	**anti-XG**	binds non-fucosylated XG, likely the backbone	gift of L. A. Staehelin (Lynch and Staehelin 1992)	3, 4
	CCRC-M1	XXFG; (alpha-Fuc-(1,2)-beta-Gal)	CarboSource	5, 5
	CCRC-M106	XXFG	CarboSource	3, 3
	CCRC-M58	XLLG	CarboSource	2, 2
	CCRC-M100	XXXG	CarboSource	0
	CCRC-M103	XXXG	CarboSource	0
	CCRC-M87	XLLG, XLXG, XXFG (strong); XXLG (weak)	CarboSource	4, 3
	CCRC-M88	same as M87	CarboSource	4, 4
	CCRC-M93	XLLG (strong); XLXG & XXFG (moderate); XXLG (weak)	CarboSource	3, 2
	CCRC-M95	same as M93	CarboSource	3, 2
	CCRC-M101	same as M93	CarboSource	3, 3
	CCRC-M104	same as M93	CarboSource	4, 3
Rhamnogalacturonan I (RG-1)	CCRC-M14	unbranched regions of RG-1 backbone (no DP dependence,	CarboSource	0

		binds to chain-internal residues)		
	CCRC-M69	same as CCRC-M14	CarboSource	0
	CCRC-M36	unbranched regions of RG-1 backbone (DP>7), may bind to a free chain end	CarboSource	0
	CCRC-M72	unbranched regions of RG-1 backbone (DP>5), may bind to a free chain end	CarboSource	0
RG-1 side chains	LM5	1,4-ß-D-galactan	PlantProbes	0
	LM6	1,5-α-L-arabinan	PlantProbes	0
	LM8	xylogalacturonan	PlantProbes	0
	LM9	feruloylated-(1,4)-β-D-galactan	PlantProbes	n.d.
	CCRC-M85	side-chains of RG-1, details not yet determined	Carbosource	0
mannans/galacto-mannans	BS 400-4	1,4-β-mannan	BioSupplies	n.d.
xylans	LM10	1,4-ß-D-Xyl disaccharide	PlantProbes	n.d.
	LM11	1,4-ß-D-Xyl tetramer	PlantProbes	n.d.
extensin	LM3		PlantProbes	n.d.
arabinogalactan-protein	LM2	β-linked glucuronic acid	PlantProbes	n.d.

Notes on Supplemental Table S1

All probes are monoclonal antibodies, except CBM3a, which is a carbohydrate binding module, and anti-XG, which is a polyclonal antibody. Information about probe specificity was obtained

from: http://cell.ccrc.uga.edu/~mao/wallmab/Antibodies/antib.htm; http://www.plantprobes.net/; the article by Lynch and Staehelin (1992); or for probes still being characterized, by personal communication from Michael Hahn (hahn@ccrc.uga.edu).

Labeling for 2F4 was performed in a modified buffer, 20 mM Tris-HCl, pH 8.2 containing 0.5 mM $CaCl_2$ and 150 mM NaCl according the instructions of Plant Probes (Leeds, UK). Negative results were confirmed with both TEM and fluorescence microscopy with and without pre-treatment in sodium carbonate.

Sources of Probes Listed in Supplemental Table S1:

BioSupplies, Australia (http://www.biosupplies.com.au/)

CarboSource, Athens, GA (http://www.ccrc.uga.edu/~carbosource/CSS_home.html)

PlantProbes, Leeds, UK (http://www.plantprobes.net/)

Paul Knox (Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT, UK; j.p.knox@leeds.ac.uk)

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Supplemental Table S2. Sugar analysis of the water insoluble fiber wall matrix fraction

Data for 10 to 30 DPA are expressed as average nmol sugar/mg cotton fiber cell wall. Glucose from fibrillar cellulose was not detected by the methods used.

DPA	**10**	**17**	**19**	**21**	**30**
Arabinose	684.33	894.87	245.56*	296.36*	58.16*
SD	*418.87*	*46.48*	*92.96*	*106.85*	*28.76*
Fucose	32.18	63.54	23.44*	25.89*	12.88*
SD	*16.66*	*10.84*	*9.57*	*3.06*	*4.99*
Galacturonic acid	299.37	429.82	229.41	217.94*	98.45*
SD	*136.18*	*25.77*	*124.69*	*14.98*	*38.35*
Galactose	185.91	279.56	123.89	131.45*	42.52*
SD	*97.26*	*18.72*	*57.48*	*26.58*	*17.33*
Glucuronic acid	38.22	64.97	23.06	24.48	7.43
SD	*25.66*	*25.29*	*15.79*	*13.22*	*0.60*
Glucose	236.12	287.14	240.52	298.28	324.46
SD	*219.59*	*76.38*	*137.85*	*175.53*	*77.92*
Mannose	20.97	26.60	27.35	57.94	4.66*
SD	*8.09*	*1.46*	*26.46*	*60.92*	*1.83*
Rhamnose	164.19	241.72	98.81*	103.29*	33.85*
SD	*90.84*	*14.82*	*39.12*	*7.68*	*15.01*
Xylose	233.62	422.35	166.76	219.64	108.37*
SD	*150.11*	*97.97*	*140.76*	*60.12*	*69.52*
Total Insoluble Sugars	1894.91	2710.57	1178.80	1375.27	690.78

Notes on Supplemental Table S2: SD = standard deviation for means of two independent biological samples (pooled fiber as described in Materials and Methods). Means are based on 2-3 technical replicates for each of two biological samples. Means that were significantly lower compared to 17 DPA ($p \leq 0.05$) are highlighted by an asterisk.

Supplemental Table S3. Sugar analysis of the water soluble fiber wall matrix fraction

Data for 10 to 30 DPA are expressed as average nmol sugar/mg cotton fiber cell wall.

DPA	**10**	**17**	**19**	**21**	**30**
Arabinose	56.91	50.70	56.36	62.11	114.43*
SD	*1.93*	*4.71*	*15.51*	*12.00*	*10.67*
Fucose	4.24	0.00	1.21	3.28	4.77
SD	*2.62*	*0.00*	*1.71*	*1.36*	*3.09*
Galacturonic acid	1.59	0.68	3.95	1.41	12.63
SD	*0.10*	*0.96*	*0.18*	*2.00*	*3.61*
Galactose	43.19	40.65	53.45	49.52	81.18
SD	*0.03*	*4.12*	*12.38*	*8.10*	*23.02*
Glucuronic acid	7.65	5.16	7.59	7.73	10.60
SD	*2.54*	*0.79*	*3.35*	*2.09*	*4.25*
Glucose	1124.55	1296.66	1213.25	1232.65	1221.47
SD	*20.92*	*102.69*	*149.53*	*53.23*	*28.90*
Mannose	13.77	11.16	11.66	10.97	18.05*
SD	*0.52*	*0.54*	*3.26*	*0.39*	*1.40*
Rhamnose	16.09	9.54	10.07	12.30	33.16*
SD	*0.29*	*2.62*	*1.58*	*3.46*	*6.46*
Xylose	13.95	9.11	9.03	10.26	15.21
SD	*1.57*	*4.93*	*0.47*	*0.80*	*0.27*
Total Soluble Sugars	1281.94	1423.66	1366.57	1390.23	1511.50

Notes on Supplemental Table S3: SD = standard deviation for means of two independent biological samples (pooled fiber as described in Materials and Methods). Means are based on 1 assay of the two biological samples. Means that were significantly higher compared to 17 DPA ($p \leq 0.05$) are highlighted by an asterisk. (For galacturonic acid, the mean values at 17 and 30 DPA were different with $p = 0.058$.)

Supplemental Table S4. Primers used to amplify cotton genes in quantitative real-time reverse-transcription PCR

Cotton Gene Name	Database Accession ID	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
Gheif5	CO492947	GGTTGCCATTGTGCAAGGA	CCGTAGGTGAGCGTTAATCAGA
GhCTL1	AY291285	CCGACCAAGAACGACACGTT	ACCTCGCCCACAAACTTGAT
GhRAC13	S79308	GTGAAGGCTGTTTTCGATGCT	TCTCCTTTTGCAAGGCTTTCTC
GhGH17-C	Cotton12_04225_01	CAAAGGCCGGTATTTCTGATG	ATGCTACGGTGTTCGGTTCAA
GhGH17-B	Cotton12_00340_02	GATCTCTCTGAAGTGGCAAATCAC	TCCTTTTGCTCCAATTGCATT
GhGH17-D	Cotton12_23061_01	CAGCACGGATACTTGTCAATGC	CAGGAATAATCGACGAAGCAAGA
Ghbglu46	Cotton12_18238_01	TGCTGTTGACAGTGCATCTAAGTG	GAAAACGGATACGGTGATGTGA
GhGH3-a/ GhGH3-b	Cotton12_00002_02	AACGGATTACTCGGCATCCA	CGGTCACAAGATCGTGATTAGC
Ghbgal1	Cotton12_07638_01	GCTAATCGCAGGAAGAATCAGTAGA	CCTTCACGGAAGTTTCCACAA
Ghbgal12	Cotton12_34622_01	CCGGTGTCCCATGGATTATG	ATTGGGAGTGAAGTTTTCGCAGTA
Ghbgal8	Cotton12_15125_01	AGTTGCCCATCTCCGAATCA	ACACCTCCCATGGCTAAAACTG
Ghbgal9	Cotton12_10595_01	TACGTGTGCCTCGAATTCTCTGT	TCTGTGGTTTCATGTCACTTATGGA
Ghexpa4	Cotton12_13766_01	ATATCGTGAAGGTGAGCGTGAA	GATTGACCAACCAAAACTGCATT
Ghexpa6	Cotton12_11024_01	GAATATCGTGAAGGCTAGTGTGAAAG	ACTGCCTGTGACCCTAAATGAGA
GhEXP1, expansin	AF512539, AY189969	CCGTGACAGCCACCAACTTT	TTCTGCTATCCGCAAGAATGC
Ghexpla2	DR463856	ATAAATAACCCCAATTTTCCAATCAG	GTCCCAAATGGTGCCTTACAA
Ghpae-a	Cotton12_16483_01	GGATGCTGTTGATCGAGCTAATAA	GCATTTCTCATTCCTTTTGCTAGTAGA
Ghpae-b	Cotton12_00006_01	CCCAGTGCTGCTGACAAAAG	TGTTCAGGGCACTTAAGAATTGC
Ghpae-c	Cotton12_00013_10	GCTCGATGCCGTAAAAATATTCTC	CTGCAATTCCCTTGTTTCCAA
Ghpe-E	CO495780	ACCGACCCAAACCAGAACAC	TCCATGGCCTCCCTAGATATGT
Ghpe-D1	DR460940	TTGGTGACGCCTCCGTAGTAT	GGGTCTTTCCGGCCTTGT
Ghpe-D2	Cotton12_00620_02	CGGTTTCCGGTATGGTTCAA	TGAAATTCCCACTCCCATCTG
Ghpe-D3	DR461911	GGGAGGGATTCATAGCGAAAG	AGAGGTCTGACCCACTCCTTAAAG
Ghpg-A1	Cotton12_36589_01	TGCTACATATGTGTTGGCGATGA	GCACTGACCATAGAACGGACAA
Ghpg-A2	Cotton12_41244_01	ACGTAACGGCGATCAATACAGA	CTTCATCGTATTCAACGTCATGTTC
Ghpg-A3	CO492632	CCGCAGAGAGCTTTACGAGTCT	ATTCCAGTGTGCCTTCGTTGA
Ghpl-A1	Cotton12_00006_05	GGTCCGAAGGAGACTTGTTACTGA	AGCACCAGCATTGGAAGTCAT
Ghpl-A2	Cotton12_13918_01	TGCACCAGAGAGTGACTGGAA	GCTCGAAGCCTTGGAATAGCT
Ghrhgl	Cotton12_28336_01	GAAGAATTAATGCAGGCGCAAT	ACGGCCAGTGTTGTTTGTTTC
Ghxth28	Cotton12_00034_02	TTTTCGTCGAAGAAGTGTGATACC	CTGGTCTGGTCATAGCAGTAGGAA
Ghxth29	Cotton12_27571_01	CTCTTTCCATCCTCGCTTGCT	CCCGAGTGCGCAGTTGAT
Ghxth30	Cotton12_37078_01	GCGATGGCAATCTTGTTCGT	GCACTGAAAAATCCATGTTGATACA
Ghxth6	Cotton12_26335_01	GGAACCATCATCATATTGCGTTT	TTGGGTATGGGATGTTTTTTGC
Ghxth7	Cotton12_06611_01	ACAAGGACTTCGACATTGAAGGA	TGGCATTAAGGGCTTGATAAGC

Notes for Supplemental Table S4

Accession IDs beginning with Cotton_12 derive from http://agcol.arizona.edu/cgibin/pave/adv_search.cgi?AssemblyID=Cotton12.
Others derive from http://www.ncbi.nlm.nih.gov/.

Abbreviations:
Gh--*Gossypium hirsutum*, GH--glycosyl hydrolase, with the following number indicating the family

Cotton Gene Name System:
Upper case indicates an official name for a cotton gene.
Lower case gene name with number: adopted from the closest Arabidopsis homolog with that name, as shown at TAIR (www.arabidopsis.org) and/or http://cellwall.genomics.purdue.edu.
Lower case gene name with letter or letter plus number: gene shows homology to an unnamed Arabidopsis gene of that (putative) type.

Supplementary Table S5: Expression patterns for cotton genes in fiber, 6 to 30 DPA, determined by quantitative real-time reverse-transcription PCR (qPCR).

Cotton Gene Name	Database Accession ID for cotton sequence	Closest Arabidopsis Homologue	E value, cotton vs. Arabidopsis	Description for Arabidopsis at www.arabidopsis.org and/or http://cellwall.genomics.purdue.edu
GhCTL1	AY291285	At3g16920	1.00E-153	chitinase, similar to chitinase-like protein
GhRAC13	S79308	At5g45970	3.00E-91	ARAC2/ROP7, Rac-like (ROP GTPase family)
GhGH17-C	Cotton12_04225_01	At2g16230	0	glycosyl hydrolase family 17 (group C)
GhGH17-B	Cotton12_00340_02	At4g17180	1.00E-115	glycosyl hydrolase family 17 (group B)
GhGH17-D	Cotton12_23061_01	At1g66250	8.00E-41	glycosyl hydrolase family 17 (group D)
Ghbglu46	Cotton12_18238_01	At1g61820	9.00E-91	BGLU46, beta-glucosidase
GhGH3-a/ GhGH3-b	Cotton12_00002_02	At5g20940 At5g20950	0	glycosyl hydrolase family 3
Ghbgal1	Cotton12_07638_01	At3g13750	0	BGAL1, beta-galactosidase / lactase
Ghbgal12	Cotton12_34622_01	At4g26140	1.00E-131	BGAL12, beta-galactosidase / lactase
Ghbgal8	Cotton12_15125_01	At2g28470	0	BGAL8, beta-galactosidase / lactase
Ghbgal9	Cotton12_10595_01	At2g32810	1.00E-55	BGAL9, beta-galactosidase / lactase
Ghexpa4	Cotton12_13766_01	At2g39700	8.00E-84	EXPA4, expansin (alpha group)
Ghexpa6	Cotton12_11024_01	At2g28950	1.00E-134	EXPA6, expansin (alpha group)
GhEXP1, expansin	AF512539 AY189969	At2g40610	1.00E-104 1.00E-111	EXPA8, expansin (alpha group)
Ghexpla2	DR463856	At4g38400	6.00E-69	EXPLA2, expansin family
Ghpae-a	Cotton12_16483_01	At5g45280	4.00E-68	pectinacetylesterase
Ghpae-b	Cotton12_00006_01	At4g19410	1.00E-154	pectinacetylesterase
Ghpae-c	Cotton12_00013_10	At2g46930	1.00E-170	pectinacetylesterase
Ghpe-E	CO495780	At2g45220	8.00E-87	pectinesterase family (group E)
Ghpe-D1	DR460940	At4g33220	3.00E-48	pectinesterase family (group D)
Ghpe-D2	Cotton12_00620_02	At3g43270	0	pectinesterase family (group D)
Ghpe-D3	DR461911	At4g02300	1.00E-56	pectinesterase family (group D)
Ghpg-A1	Cotton12_36589_01	At1g19170	1.00E-101	polygalacturonase (pectinase) family (group A)
Ghpg-A2	Cotton12_41244_01	At4g23500	5.00E-74	polygalacturonase (pectinase) family (group A)
Ghpg-A3	CO492632	At3g42950	4.00E-33	polygalacturonase (pectinase) family (group A)
Ghpl-A1	Cotton12_00006_05	At1g04680	0	pectate lyase family (group A)
Ghpl-A2	Cotton12_13918_01	At1g67750	0	pectate lyase family (group A)
Ghrhgl	Cotton12_28336_01	At2g22620	9.00E-49	rhamnogalacturonan 1 lyase
Ghxth28	Cotton12_00034_02	At1g14720	1.00E-136	XTH28, xyloglucan endotransglycosylase
Ghxth29	Cotton12_27571_01	At4g18990	2.00E-14	XTH29, xyloglucan endotransglycosylase
Ghxth30	Cotton12_37078_01	At1g32170	3.00E-71	XTH30/XTR4; xyloglucan endotransglycosylase
Ghxth6	Cotton12_26335_01	At5g65730	1.00E-101	XTH6, xyloglucan endotransglycosylase
Ghxth7	Cotton12_06611_01	At4g37800	1.00E-135	XTH7, xyloglucan endotransglycosylase

Repeat Cotton Gene Name	Gene expression level at 6 to 30 DPA relative to 10 DPA (linear scale)									
	6	10	15	19	20	21	22	23	26	30
GhCTL1	0.45	1	1.22	53.43	28.00	385.13	2510.08	3803.63	3373.26	**4269.46**
GhRAC13	0.38	1	3.10	4.69	6.24	**9.83**	9.19	4.67	3.41	2.17
GhGH17-C	0.38	**1**	0.44	0.20	0.26	0.31	0.12	0.11	0.06	0.01
GhGH17-B	0.63	1	1.15	1.19	1.37	2.06	3.26	3.97	3.52	**4.13**
GhGH17-D	0.88	1	0.94	1.02	**2.00**	1.39	0.34	0.22	0.19	0.11
Ghbglu46	0.41	1	3.79	7.13	10.94	20.22	**22.28**	12.17	5.93	6.41
GhGH3-a/ GhGH3-b	0.82	**1**	0.34	0.10	0.06	0.06	0.03	0.01	0.01	0.01
Ghbgal1	0.33	1	1.57	1.65	2.18	**2.31**	1.26	1.17	0.85	0.77
Ghbgal12	0.69	1	1.65	2.00	4.16	**4.22**	1.72	1.09	1.22	0.98
Ghbgal8	0.96	1	0.53	0.73	1.84	2.91	3.80	**4.48**	3.61	3.95
Ghbgal9	0.57	1	0.88	1.11	1.60	**2.04**	1.67	1.90	1.30	1.07
Ghexpa4	0.41	1	**1.54**	0.38	0.28	0.11	0.02	0.01	0.00	0.00
Ghexpa6	0.06	1	2.04	2.81	**2.91**	1.69	0.15	0.04	0.04	0.00
GhEXP1, expansin	0.49	1	**1.07**	0.83	1.03	1.00	0.45	0.30	0.10	0.03
Ghexpla2	0.25	**1**	0.08	0.07	0.12	0.17	0.08	0.10	0.05	0.05
Ghpae-a	0.31	1	1.61	1.05	1.97	**2.64**	1.24	0.63	0.56	0.23
Ghpae-b	0.30	1	1.42	0.86	1.37	**2.06**	1.18	0.65	0.49	0.22
Ghpae-c	0.47	1	1.19	4.94	11.88	**16.45**	16.26	11.77	5.83	4.91
Ghpe-E	0.34	1	0.95	272.42	4228.91	11961.14	30071.09	**40983.89**	23268.25	16644.55
Ghpe-D1	0.78	**1**	0.45	0.07	0.06	0.07	0.02	0.02	0.01	0.01
Ghpe-D2	0.31	1	**3.10**	1.53	1.39	0.97	0.08	0.03	0.02	0.00
Ghpe-D3	0.52	**1**	0.74	0.29	0.29	0.35	0.05	0.00	0.02	0.00
Ghpg-A1	0.79	1	0.89	3.05	8.48	**13.36**	10.15	6.16	3.78	4.02
Ghpg-A2	0.27	1	0.95	0.62	**1.58**	1.43	0.34	0.14	0.06	0.02
Ghpg-A3	0.78	1	1.09	2.73	12.04	30.41	**51.39**	38.06	24.76	13.70
Ghpl-A1	0.32	1	**1.33**	0.89	1.02	0.99	0.17	0.05	0.01	0.00
Ghpl-A2	0.33	1	1.04	1.43	2.36	**2.37**	0.84	0.18	0.05	0.00
Ghrhgl	0.62	**1**	0.52	0.13	0.23	0.29	0.11	0.03	0.06	0.13
Ghxth28	0.23	1	2.93	10.98	21.86	**30.56**	17.27	23.75	25.16	34.46
Ghxth29	0.19	1	3.15	12.10	23.48	**24.14**	10.70	8.73	6.12	5.06
Ghxth30	0.15	1	4.09	14.19	36.34	**47.62**	16.68	11.52	9.19	5.19
Ghxth6	0.13	1	4.32	14.98	15.51	**28.61**	19.12	17.63	10.31	5.48
Ghxth7	0.51	1	1.05	1.43	1.16	**2.06**	1.51	1.42	0.95	0.55

Notes for Supplementary Table S5

Putative classes for translated cotton sequences were assigned by homology to Arabidopsis sequences classified the same way (http://www.arabidopsis.org and/or http://cellwall.genomics.purdue.edu).

Data were normalized to the endogenous control, *G. hirsutum eif5* (eukaryotic translation initiation factor-5, GenBank Accession: CO492947). *Gheif5* had nearly constant expression in 6 - 30 DPA fiber.

Bold gene name indicates gene expression profiles shown graphically in the manuscript

Accession IDs beginning with Cotton_12 derive from http://agcol.arizona.edu/cgi-bin/pave/adv_search.cgi?AssemblyID=Cotton12. Others derive from http://www.ncbi.nlm.nih.gov/.

Two Arabidopsis IDs associated with one cotton sequence indicates equal homology to both.

Bold indicates maximum expression ratio for each gene relative to its own value at 10 DPA.

Abbreviations:

Gh: *Gossypium hirsutum*

GH: glycosyl hydrolase, with the following number indicating the family

Cotton Gene Name System:

Upper case indicates an official name for a cotton gene

Lower case gene name with number: adopted from the closest Arabidopsis homolog with that name, as shown at TAIR (www.arabidopsis.org) and/or http://cellwall.genomics.purdue.edu

Lower case gene name with letter or letter plus number: gene shows homology to an unnamed Arabidopsis gene of that (putative) type

Supplemental Table S6. Activities of cell wall modifying enzymes in cotton fiber between 10 to 30 DPA

The enzymes assayed were galactosidase, xyloglucan endo-hydrolase (XEH), and pectin methylesterase (PME).

DPA	galacto-sidase[1]	*SE*	XEH[2]	*SE*	PME[3]	*SE*
10	23.26	*1.59*	62.22	*3.77*	8.17	*1.03*
15	21.29	*0.88*	100.93	*6.69*	13.98	*0.60*
19	24.53	*1.15*	127.98	*4.93*	21.72	*1.54*
20					18.41	*0.96*
21	31.20	*0.64*	87.16	*6.43*		
23	34.36	*1.33*	12.79	*2.57*	69.58	*4.21*
26	28.64	*1.23*	3.57	*1.25*		
30	28.95	*0.57*	0.00	*0.00*	85.52	*7.16*

Notes for Supplemental Table S6

Units of activity are indicated by superscripts:

[1] p-nitrophenol released (μ mol hr^{-1} mg^{-1} soluble protein)

[2] XG degradation (ng h^{-1} mg^{-1} soluble protein)

[3] Pectin demethylation ($\Delta OD_{534\ nm}$/mg soluble protein)

Means are the average of 4 biological replications; SE = standard error.

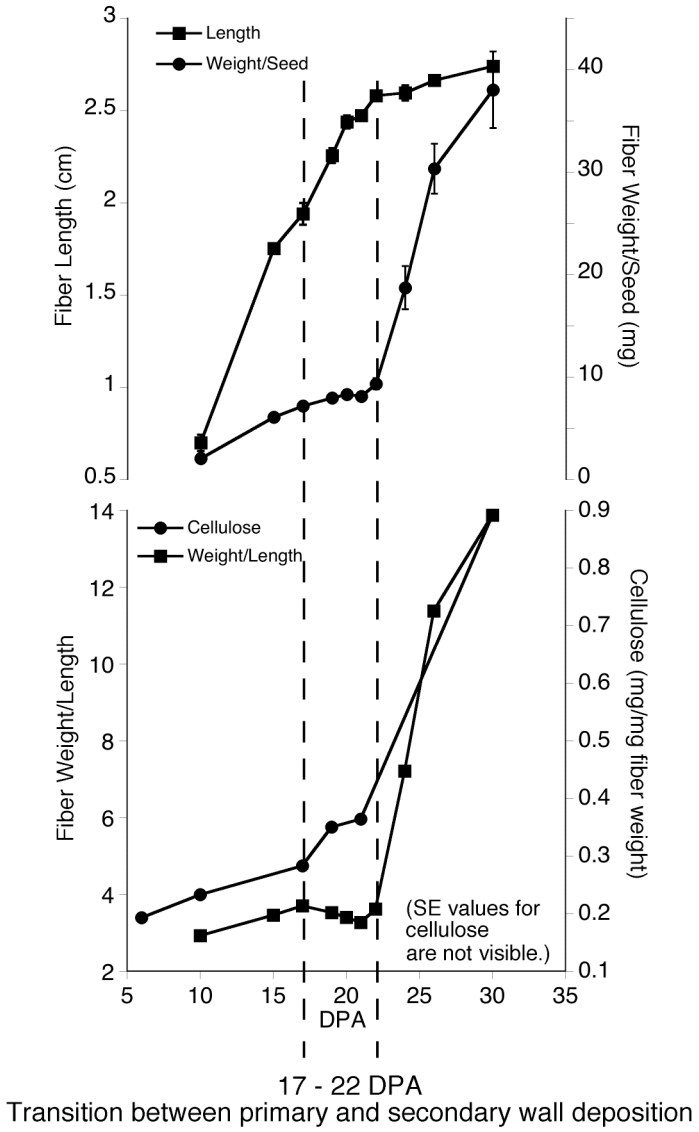

Figure S1. Fiber growth and cellulose accumulation from 6 to 30 DPA for *G. hirsutum* cv. Deltapine 90 grown in July a tightly controlled greenhouse with a 26°C/22°C day/night temperature cycle. The top graph shows fiber length (cm) and fiber dry weight/seed (mg). The bottom graph shows cellulose content (mg/mg fiber dry weight) and fiber weight/length. The sharp increase in fiber weight/length after 22 DPA is due to deposition of secondary wall cellulose. Under these conditions, the transition between primary and secondary wall deposition occurs between 17 to 22 DPA.

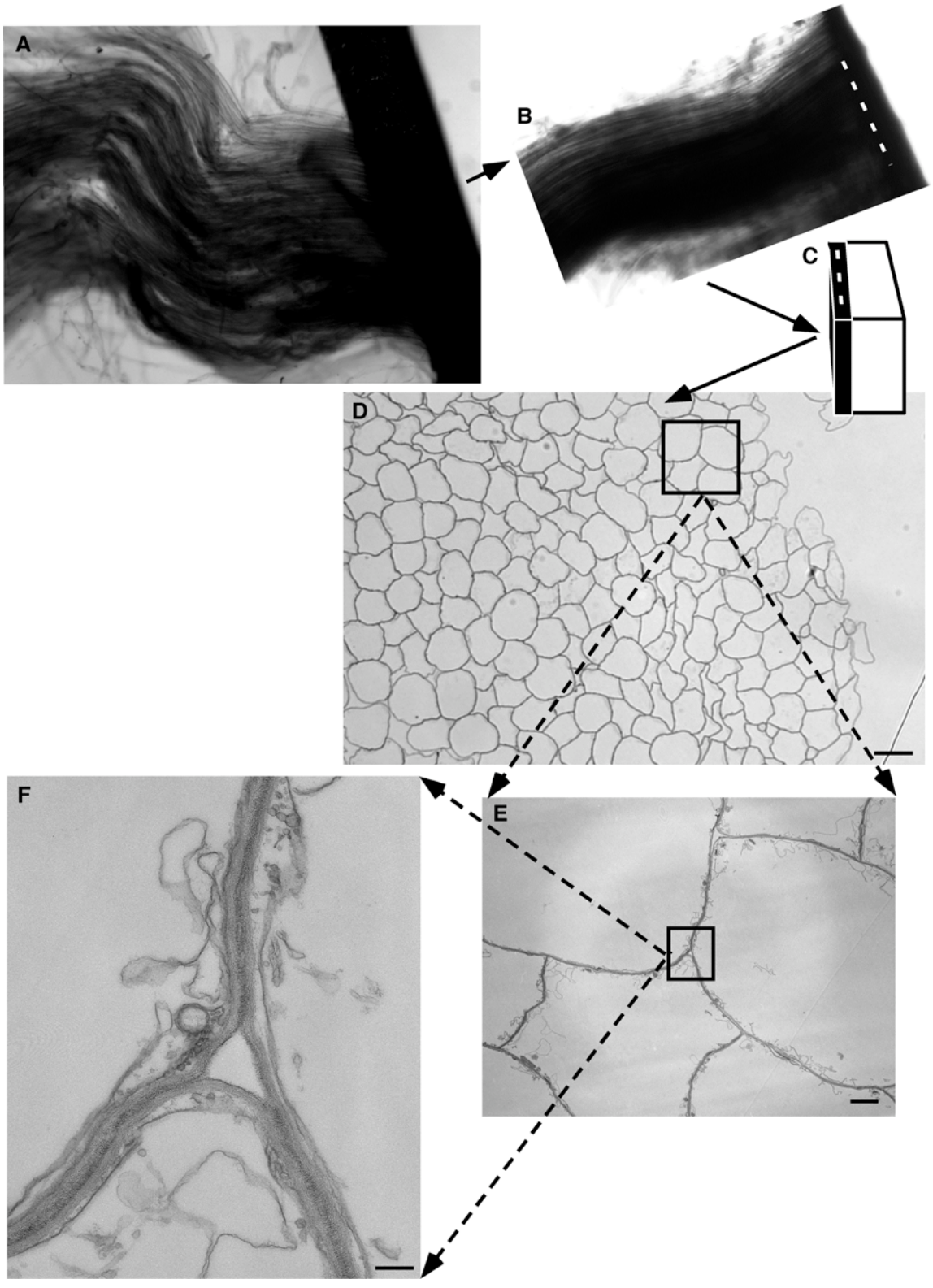
A
B
C
D
E
F

Figure S2. Micrographs of 15 DPA fiber showing how fiber bundle cross-sections were generated. Light micrographs are shown in (A, B, D), (C) shows a diagram of one of the technical steps, and (E and F) show TEM micrographs. The micrograph in (A) shows a flat-embedded fiber bundle containing many fibers running in parallel to the plane of the embedding resin. From such a flat-embedded sample, a piece of resin plus fiber (B) was cut out with a razor blade. (The thick black line on the right side in (A) indicates the position of a razor cut.) The rectangular piece of resin in (B), containing the fiber bundle lying in the plane of the resin (black mass), was glued vertically to the side of a blank resin block (symbolized by the white rectangle) as diagrammed in (C). By this action, the area marked by the dashed line in (B) became oriented parallel to the block face (see dashed line in C), so that fibers in the bundle could be cross-sectioned. A thick section of a cross-sectioned bundle of fiber viewed in the light microscope after staining with Toluidine Blue is shown in (D). Next, thin sections were taken for TEM observation, with the boxed areas shown at successively higher magnification in (E) and (F). (E) shows parts of eight joined fibers, whereas (F) shows three joined fibers within the larger group. In (F), the corner between three fibers is empty of CFML material, but fibers are more frequently completely joined at corners as in Figure 1B. Bars in: (D) = 25 μm; (E) = 3 μm; (F) = 300 nm.

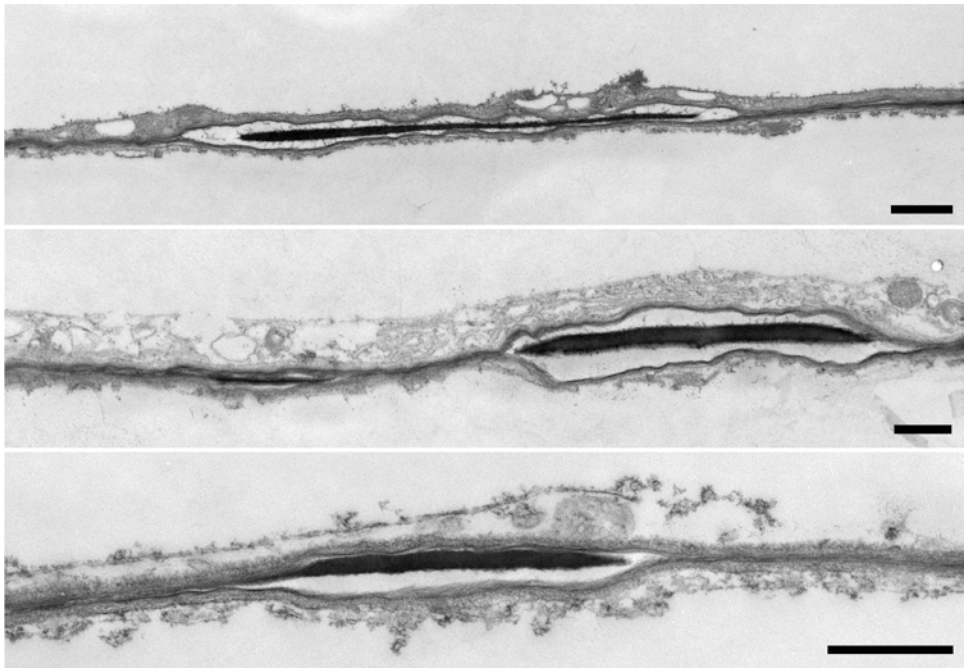

Figure S3. TEM views of CFML-filled bulges between 15 DPA fibers observed in longitudinal orientation. Each of the three micrographs shows parts of two fibers, with the coherent wall of each fiber pair in the middle of the micrograph. Sometimes, as shown here, the CFML material (stained darkly above) separated from the primary walls during specimen preparation, whereas in other cases it filled the space entirely. (See also Fig. 1F and Fig. 3.) All bars are 1 μm.

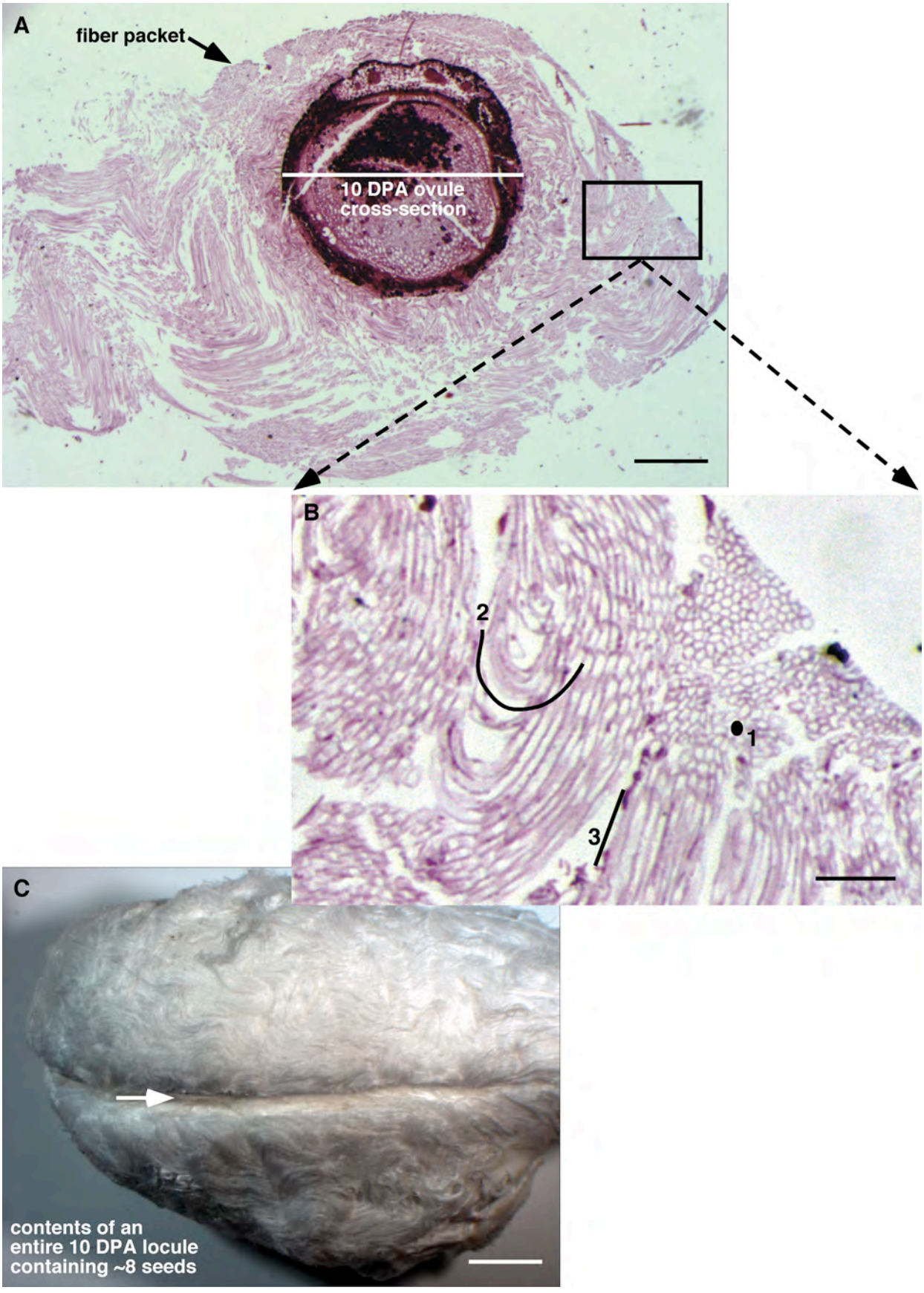
A
fiber packet
10 DPA ovule
cross-section
B
2
1
3
C
contents of an
entire 10 DPA locule
containing ~8 seeds

Figure S4. Images showing the packing of fiber around a developing ovule and within a locule at 10 DPA. **(A)** A higher magnification micrograph (taken using a compound microscope) of the cross-sectioned ovule in Figure 2F surrounded by a packet of its own fiber (stained with Safranin O). **(B)** The boxed area in A showed bundled fiber within the fiber packet with three major patterns (highlighted with black shapes and numbers) relative to the plane of the section: 1, perpendicular, so that fiber cross-sections were revealed; 2, in arcs that mimicked those in the upper fiber layer appressed to the boll wall shown in C and Figure 2G; and 3, parallel, so that fiber longitudinal sections were revealed. Although some fiber separation and loosening of the fiber packet occurred (see A, lower left side), the specialized protocol for paraffin histology (Supplemental Protocol S3) preserved many regions of fiber bundling and organized packing. This image was processed using the Filter/Sharpen/Unsharp Mask in Adobe Photoshop CS2 in order to increase the contrast and clarity of the fiber patterns. **(C)** A micrograph taken using a dissecting microscope of the seed/fiber contents of an entire locule that was removed from the boll, frozen in slushed nitrogen, then freeze dried. The ovule/fiber mass retained its original shape, as emphasized by the indentation created by the suture line (where the boll wall splits upon opening) running down the middle (arrow). The surface of the excised ovule/fiber tissue, which was appressed to the inner boll wall in situ, showed the tight organization of fiber in wave-like patterns as also observed within the boll by cryo-FE-SEM. The waves can be seen more clearly when the image is enlarged two fold on the computer screen. The bar in A = 500 μm, the bar in B = 100 μm, and the bar in C = 2 mm.

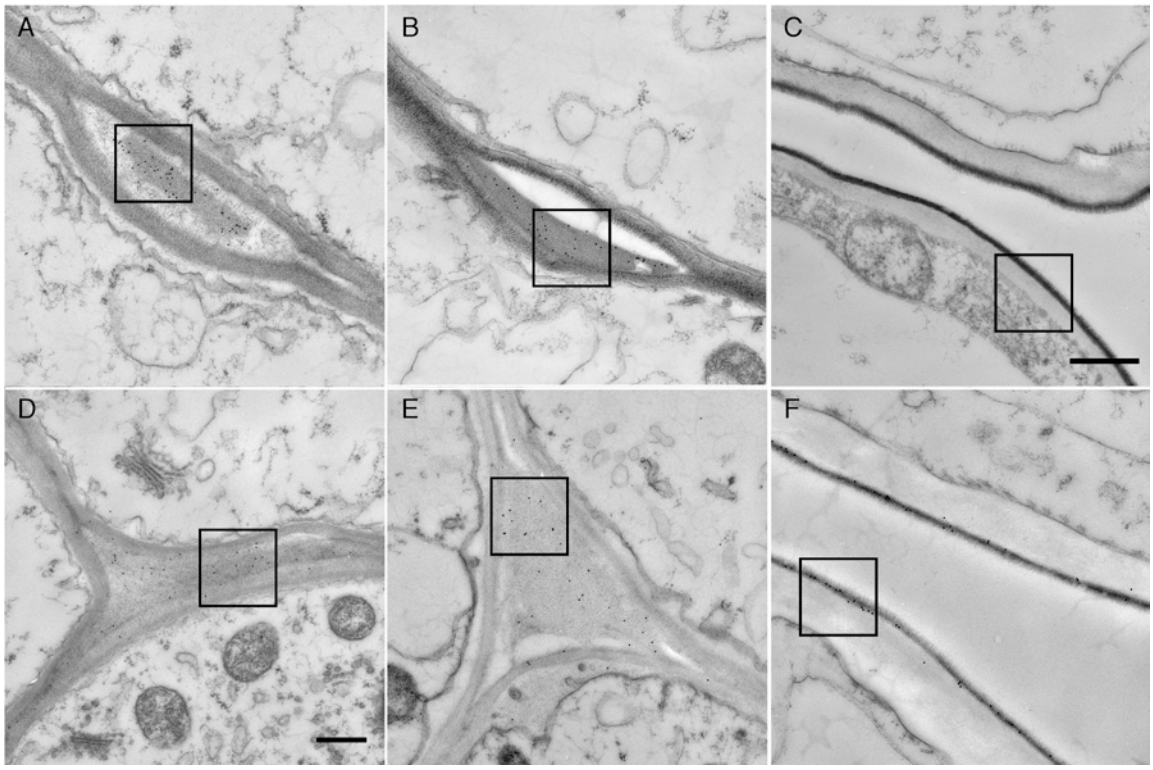

Figure S5. Broader TEM views of fibers immunolableled in Figure 3. The boxed areas were excerpted to form Figure 3, D to I, and the legend of Figure 3 contains information about age of fibers and probes used. The gold label becomes more visible when the figure is enlarged 2 to 3 fold on the computer screen. Stronger immunolabeling occurred in regions where the CFML material was more spread out in bulges or at the meeting point of three fibers, similarly to data from red clover *(Trifolium pratense* L.) (Moore and Stahelin 1988). Bars in: (C) = 600 nm for A, B, C, and F; and (D) = 600 nm for D and E.