Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Plant Biology

How cotton fibers elongate: a tale of linear cell-growth mode

Yong-Mei Qin and Yu-Xian Zhu

Cotton fibers (cotton lint) are single-celled trichomes that differentiate from the ovule epidermis. Unidirectional and fast-growing cells generally expand at the dome-shaped apical zone (tip-growth mode); however, previous studies suggest that elongating fiber cells expand via a diffuse-growth mode. Tip-localized Ca^{2+} gradient and active secretary vesicle trafficking are two important phenomena of tip-growth. Recently, a high Ca^{2+} gradient is found in the cytoplasm of fast-elongating cotton fiber cells near the growing tip. Several protein coding genes participating in vesicle coating and transport are highly expressed in elongating fiber cells. Taken together with the observation that ethylene acts as a positive regulator for cotton fiber and several Arabidopsis tissues that are known to elongate via tip growth prompted us to propose a linear-growth mode for similar cell types.

Address
The National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, 100871, China

Corresponding author: Zhu, Yu-Xian (zhuyx2@pku.edu.cn)

Current Opinion in Plant Biology 2011, **14**:106–111

This review comes from a themed issue on
Cell signalling and gene regulation
Edited by Jiayang Li and Nam-Hai Chua

Available online 11th October 2010

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2010.09.010

Introduction

Cotton fiber is the most prevalent natural raw material used in the textile industry and serves as one of the mainstays for the global economy. Cotton belongs to the genus *Gossypium* in the family Malvaceae. It is one of the angiosperm species in which the formation of polyploidy through genome-wide duplications occurred ~1.5 million years ago [1,2,3•]. There are four cultivated species: *G. arboreum*, *G. herbaceum*, *G. hirsutum*, and *G. barbadense* (with representative *G. arboretum* and *G. hirsutum* plants at the time of flowering shown in Figure 1). The first two are diploids ($2n = 26$) and the last two are allotetraploids ($2n = 52$). Upland cotton (*G. hirsutum*) fibers generally grow to about 30–40 mm in length and 15 μm in thickness at full maturity and account for 90% of fiber production in the world [4,5]; an additional 5–8% is produced from *G. barbadense*.

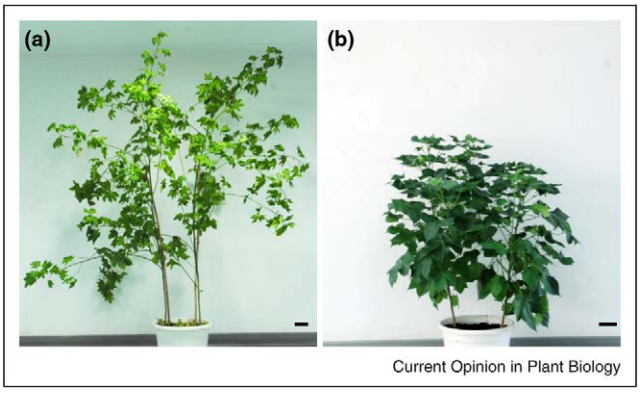
Fiber development consists of four overlapping stages (initiation, elongation, secondary cell wall biosynthesis, and maturation), which are defined on the basis of the number of days post-anthesis (dpa) [6,7•]. Fiber initiation is characterized by trichome protrusion and enlargement on the epidermal surface that occurs from 3 days before anthesis to 3 dpa. Only 25–30% of epidermal cells differentiate into the mature long-fiber cells commonly known as cotton lint, whereas others may develop into short fibers called fuzz (5–6 mm in length). In the developing fiber, initiation and elongation proceed nearly synchronously on the same ovule. During the most active elongation period (5–25 dpa), vigorous cell expansion with peak growth rates of >2 mm/day is observed in upland cotton, coupled with cell expansion and a specific set of metabolite syntheses. Cellulose synthesis dominates the period of secondary cell wall biosynthesis (20–45 dpa), which is followed by a dehydration period (45–50 dpa) to produce mature lint fibers. Here we focus on recent advances pertinent to the second growth stage and try to clarify the mode of fiber cell elongation.

Special metabolic processes during cotton fiber elongation

Cotton fiber serves as an excellent model system for understanding mechanisms of cell elongation and differentiation [6,8••,9•]. Rapid elongation of fiber cells is associated with cell turgor pressure, plasmodesmatal regulation, and transporter activities [10,11]. Large-scale transcriptome analysis revealed that during fiber cell initiation and elongation, several metabolic pathways are specifically and significantly up-regulated [8••,12•,13–15]. On the basis of the systematic microarray hybridization of 12,233 Uni-ESTs obtained by sequencing 102,000 expressed sequence tags (ESTs) from a cotton ovule cDNA library, ethylene biosynthesis and signaling are the most highly up-regulated biochemical pathway [8••]. Conversion of *S*-adenosyl-L-Met (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) and the oxidative cleavage of ACC to produce ethylene, which are catalyzed by ACC synthase (ACS) and ACC oxidase (ACO), respectively, comprise the two key steps in ethylene biosynthesis. Transcripts of three *GhACOs* are specifically accumulated in 10-dpa fibers as compared with 10-dpa ovules. Exogenously applied ethylene stimulates fiber growth, whereas L-[2-aminoethoxyvinyl]-glycine (AVG), an ethylene biosynthesis inhibitor, inhibits fiber growth [8••].

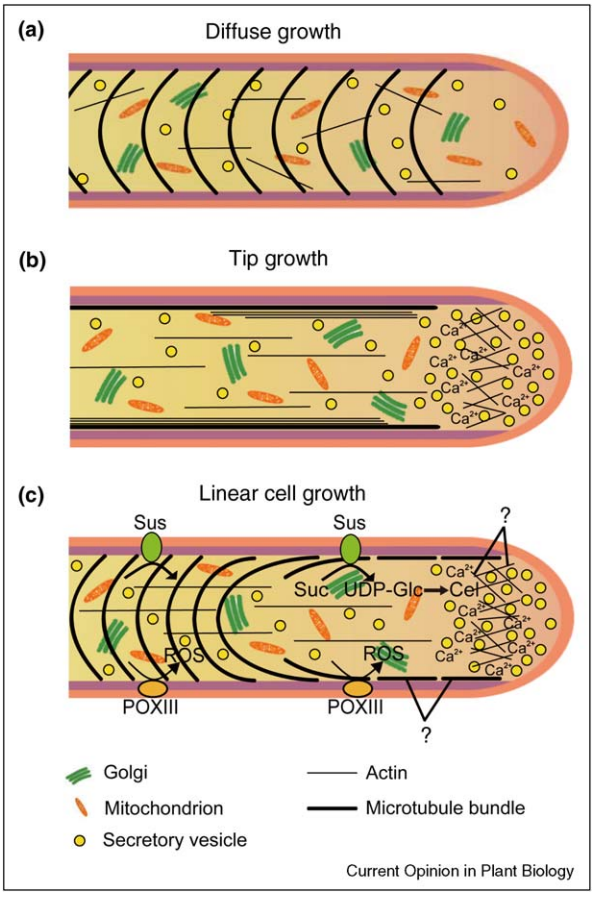
The biosynthesis of saturated or monounsaturated very-long-chain fatty acids (VLCFAs, fatty acids > C18) — which are precursors of sphingolipids, seed triacylglycerols, suberins, and cuticular waxes [16] — has important

Figure 1



Phenotypes of the two cultivated *Gossypium* species. (**a**) *G. arboreum*; A diploid cotton that can grow more than 2 m so that it is nick-named 'tree cotton'. (**b**) *G. hirsutum*; An allotetraploid cotton that produces more than 90% of the world's commercial cotton lint. Bars = 10 cm.

Figure 2



A schematic drawing of (**a**) diffuse-growth, (**b**) tip-growth, and (**c**) linear cell-growth modes. (**a**) and (**b**) are modified from [26•,28••]. Most obviously, in tip growth, there is a zonation of mitochondria, Golgi, and ER in the subapical region of the cells with an obvious accumulation of secretory vesicles. Also, the microtubules are arranged in bundles along the longitudinal axis of the cell. In diffuse growth, no clear-cut zonation is evident, and the microtubules are oriented transversely to the growth axis. In the linear cell-growth mode, the microtubules are oriented transversely to the growth axis only in those parts of the cell with secondary cell wall deposition. High levels of Ca^{2+}, ROS, and even secretory vesicles (as shown by up-regulated gene expression) are observed in the tip of the apical zone, which is normally associated with tip growth. The existence of vertical microtubule bundles and actin meshwork, as indicated by two question marks, in the apical zone of cotton fiber cells and other related cell-types with a common linear cell-growth mode has not been experimentally verified. Suc, sucrose; Cel, cellulose.

roles in plant growth and development [17•,18•,19••]. It has also been speculated that free fatty acids or their derivates may serve directly as signaling molecules in plants. Fast-elongating fiber cells contain three to five times the amount of VLCFAs (from C20 to C26) and a higher amount of unsaturated fatty acid (α-linolenic acid: C18:3) than do ovules [19••], consistent with previous analyses of cotton lipids [20•]. VLCFAs may promote fiber cell elongation by activating ethylene synthesis [19••]. The inhibitory effect of exogenous acetochlor, an inhibitor of VLCFA biosynthesis, on fiber cell growth is reversed by adding ethylene into the ovule culture medium, indicating that VLCFAs or VLCFA derivates may exert their functions by modulating ethylene biosynthesis. Twenty-one different KCS genes encoding the first and rate-limiting step in VLCFA biosynthesis are found in the Arabidopsis genome, with discrete tissue-specific, temporal-specific or spatial-specific expression patterns that reflect its multiple roles in plant growth and development [21]. A study of *GhFAD2*, which encodes a desaturase that introduces a double bond between carbons 12 and 13 of monounsaturated oleic acid to form linoleic acid, suggests that particular unsaturated fatty acids may be required to support the specific membrane structure required at the time of fast fiber cell elongation [22]. VLCFA biosynthesis also affects cell growth in Arabidopsis and embryo development in maize [17•,23].

Experimental evidence supporting the diffuse-growth mode for fiber cells

Plant cells expand via either diffuse growth or tip growth (Figure 2a and b); the mode of cotton fiber cell elongation has not been definitively established and is still a topic of argument for scientists working in this field [6]. Typically, unidirectional and fast-growing cells such as pollen tubes, root hairs, leaf trichomes, and fungal hyphae follow a tip-growth pattern that confines expansion to the dome-shaped apical zone. Rapidly elongating fiber cells, however, seem to expand via a diffuse-growth mode (Figure 2a) according to the following observations. First, organelle zonation does not occur in the tips of elongating cotton fiber cells; scanning electron microscopy and transmission electron microscopy performed on 2-dpa cotton ovules using a rapid freeze-fixation and freeze-substitution protocol revealed that the apical part of a fast-growing fiber cell entirely lacks organelle zonation. No

secretory vesicles accumulate in this region, indicating that the addition of new wall material for surface expansion may not be restricted to the apex of the cell [4]. Second, the cortical microtubules and the newly deposited cellulose microfibrils are transversely oriented with respect to the growth axis in fiber cells, which provides greater resistance to radial expansion than to longitudinal expansion [24]. Thus, in response to increased turgor pressure, fibers elongate perpendicular to the orientation of cellulose microfibrils, leading to unidirectional outgrowth from the epidermis of the developing ovule. In a tip-growing cell such as the pollen tube, microtubules are arranged in bundles parallel to the growth axis and are absent from the apical dome (Figure 2b).

Actin filaments, which are the main components of microfilaments, play an important role in cytoskeleton maintenance by supporting essential processes such as cytoplasmic streaming, organelle orientation and intracellular trafficking, and vesicle secretion [25]. They are arranged as long bundles along the length of cells during tip growth and are arranged randomly during diffuse growth [4,26•]. In addition, they form a meshwork in the subapical region of tip-growth cells that is not found in fiber cells [24]. This actin meshwork is suggested to facilitate vesicle transport and docking within the apex to prevent large organelles from entering the apical region, and it is required for producing the so-called 'clear zone' in the tip. Disruption of the actin cytoskeleton by Rac/Rop GTPase overexpression in Arabidopsis pollen tubes converts the typical polar growth into isotropic growth [25].

Data that support the tip-growth mode

Polar growth that is due to localized vesicle targeting and exocytosis to the growth site is termed tip growth (Figure 2b), which is a common phenomenon in all eukaryotic kingdoms. The establishment of tip growth requires a tip-high Ca^{2+} gradient, a polarized actin cytoskeleton, and tip-directed vesicle trafficking [27]. In the subapical region of a tip-growth cell, the endoplasmic reticulum (ER), Golgi body, and mitochondrion content is characteristically high. Studies of tip-growth mechanisms in pollen tubes have focused on three connected aspects: cytosolic calcium gradient, the production of reactive oxygen species (ROS), and tip-localized dynamic Rho GTPase signaling [28••]. A large number of genes implicated in vesicle coating and trafficking, such as syntaxin, clathrin, and vesicle transport SNARE, are overexpressed throughout various stages of fiber cell development, indicating their importance in maintaining the rapid growth of this unique cell type [29••,30].

Calcium-mediated signal transduction plays crucial roles in a wide array of growth and developmental processes and is especially important in tip growth. In plants, the temporal and special changes in cellular Ca^{2+} concentrations are transmitted through several calcium sensors including calmodulins (CaMs), calcium-dependent protein kinases, and calcineurin B-like proteins. A highly elevated Ca^{2+} concentration gradient is observed in the tips of rapidly elongating root hairs but not in non-growing root hairs [31]. Likewise, when *in vitro*-cultured elongating fiber cells are incubated with the calcium indicator Fluo-3/AM, significant Fluo-3-fluorescence is observed in the cytoplasm of cotton fiber cells near the growing tip. Obvious inhibition of fiber growth occurs when cotton ovules are cultured in the presence of the CaM antagonist TFP or in the absence of exogenous Ca^{2+} ions [32], indicating that Ca^{2+} influx to the fiber tips is required for sustaining fast cell elongation.

ROS include the superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH). As visualized by the 2′,7′-dichlorodihydrofluorescein diacetate staining method, a significant ROS burst occurs at the time of fiber initiation and early elongation [33]. Addition of the NADPH oxidase inhibitor DPI or the peroxidase inhibitor SHAM to the ovule culture medium inhibits ROS production with simultaneous suppression of fiber elongation [33]. Transcripts of *GhPOX1*, which encodes a class III plant peroxidase involved in oxidoreductions of H_2O_2, predominantly accumulate in fast-elongating fibers, suggesting that class III peroxidase activities are involved in fiber growth, probably by mediating ROS content [33]. Another H_2O_2 scavenging enzyme, ascorbate peroxidase (APX), important for maintaining the cellular ROS concentration, is also reported to play a role during cotton fiber growth. Exogenous H_2O_2 or ethylene stimulates *GhAPX1* expression and increases total APX activity, which leads to extended fiber cell elongation [34]. A study of the root hair mutant *rhd2*, which is defective in root hair elongation, provides convincing evidence that ROS generated by NADPH oxidase regulate Ca^{2+} channels localized on root hair tips [35••]. The production of high levels of ROS in transgenic Arabidopsis plants by epidermis-specific expression of *FAE1*, an ortholog of the KCS family, leads to the death of trichome cells by seriously damaging cell membranes [36•].

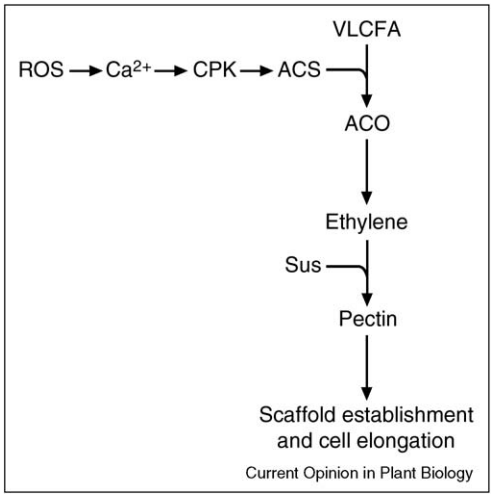
Silencing of fiber-preferential *GhACTIN1* inhibits fiber cell elongation by reducing the amount of F-actin in the cell, suggesting that F-actin arrays are crucial for fiber elongation in a fashion similar to what has been reported for Arabidopsis root hair growth [37,38•]. *GhRac1* — which encodes Rac/Rop GTPase — is highly expressed during the fast fiber elongation stage [39•], and *GhRac9* and *GhRac13* are highly expressed during the transition stage between primary and secondary cell wall synthesis, suggesting that Rho family GTPases may regulate cell polarity through cytoskeleton organization and vesicle transport during fiber growth, similar to the mechanism proposed in Arabidopsis [28••].

Experiments that support a common linear cell-growth mode mediated by the ethylene pathway

Recently, nucleotide sugar metabolism was reported to be the most significantly up-regulated biochemical process during fiber elongation on the basis of comparative proteomic and bioinformatic analyses [40••]. Ethylene may act as a positive regulator for cotton fiber cell elongation as well as for Arabidopsis root hair, apical hook, and hypocotyl development [8••,41]. Several enzymes, including UDP-4-keto-6-deoxy-d-glucose 3,5-epimerase 4-reductase (UER), UDP-D-glucose pyrophosphorylase (UGP), and UDP-D-glucose dehydrogenase (UGD), which are potentially involved in pectic cell wall polysaccharide biosynthesis, are specifically accumulated in wild-type samples in an ethylene-dependent or lignoceric acid-dependent way, suggesting that these two compounds may promote fiber elongation by modulating the production of cell wall polymers. When added exogenously to ovule culture medium, UDP-L-rhamnose (UDP-Rha), UDP-D-glucuronic acid (UDP-GlcA), or UDP-D-galacturonic acid (UDP-GalA) promotes significant fiber growth. The short root hairs of Arabidopsis *uer1-1* and *gae6-1* mutants, which lack the ability to synthesize UDP-Rha and UDP-GalA, respectively, are complemented by adding the specific pectin precursor to the growth medium. Wild-type root hair lengths are observed in both *cut1* and *ein2-5* Arabidopsis mutants when both types of the pectin precursors, UDP-Rha and UDP-GalA, are used in a chemical complementation assay [40••]. A mutation in the Arabidopsis Rab GTPase *RABA4D* disrupts normal pollen tube growth by altering the pattern of pectin deposition so that it is no longer present exclusively in its growing tip [42••]. These results indicate that ethylene and C24:0 may promote Arabidopsis root hair growth and, in a similar manner, cotton fiber growth by activating the pectin biosynthesis network, and the pectin layer may serve as a scaffold to support secondary cell wall biosynthesis and cell elongation (Figure 2c).

Exogenously applied C24:0 significantly increases the lengths of the main root, lateral roots, and root hairs of Arabidopsis seedlings [19••]. In cotton, VLCFAs act upstream of ethylene biosynthesis because the acetochlor inhibition of fiber elongation is reversed by exogenous ethylene, whereas VLCFAs do not revert the inhibitory effect of AVG in the culture medium [19••]. QRT-PCR analysis reveals also that genes in ethylene biosynthesis are up-regulated very rapidly upon addition of C24:0 in the medium, whereas ethylene application has little effect on the expression of VLCFA biosynthesis genes [19••]. A schematic model that depicts the biochemical pathway that leads to fiber cell elongation is proposed in Figure 3. The connection between ethylene signaling, plant cell wall biosynthesis, and elongation is also supported by the finding that the Arabidopsis root cell expansion defect in the *fei1 fei2* mutant is suppressed by inhibition of ACS expression [43•].

Figure 3



Signaling pathway for the linear cell-growth mode. Ethylene plays a key role in fiber growth [8••]. VLCFAs promote fiber growth by activating ethylene biosynthesis [19••], whereas ethylene stimulates pectin biosynthesis and scaffold establishment [40••]. Ca^{2+}, CPK, and ROS are involved in fiber cell, Arabidopsis root, and root hair growth [32–34,35••]. The importance of Sus was elaborated in [44••]. CPK, Ca^{2+}-dependent protein kinase; Sus, sucrose synthase.

The involvement of Sus in fiber elongation

Sucrose synthase (Sus; Ec 2.4.1.13) is encoded by one of the cotton genes that is the earliest up-regulated gene during fiber initiation and elongation [44••]. *Sus* is preferentially expressed in elongating fiber cells but not in adjacent normal epidermal cells, and it is induced significantly upon exogenous ethylene treatment [8••]. Antisense suppression of *Sus* expression reduces hexose levels and the osmotic potential in ovules of transgenic plants, leading to a fiberless phenotype [44••]. These authors proposed that suppression of *Sus* expression impairs the integrity of the fiber cell wall by reducing the supply of UDP-D-glucose (UDP-Glc) that is essential for the synthesis of cellulose and many non-cellulose cell wall components [44••]. Cellulose biosynthesis, which uses UDP-Glc as the primary substrate, is, however, very slow in the early phases of fiber development, and the amount of cellulose increases only after the onset of secondary cell wall synthesis around 15–20 dpa [7•,45]. Therefore, biosynthesis of pectin precursors, which is activated early in development, may be responsible for utilizing the large amounts of UDP-Glc initially produced by Sus throughout the primary cell wall synthesis and fiber elongation stages. Cellulose biosynthesis may start to function at the end of the primary cell wall extension period to utilize the UDP-Glc that is continuously produced by Sus and UGP for secondary cell wall biosynthesis and deposition (see also Figure 2c).

Conclusions

On the basis of the currently available results, we suggest that fiber cells may elongate via a combination of both tip-growth and diffuse-growth modes, which can be termed the linear cell-growth mode (Figure 2c). Many types of cells, such as cotton fibers, pollen tubes, root hairs, and trichomes, may grow via this mode. These cells may all possess a unique feature in that they respond positively, in some way, to the plant hormone ethylene for elongation [8••,19••,40••]. By contrast, leaves, stems and petals may undergo two-dimensional enlargement using a different mechanism given that they respond very differently to exogenous ethylene.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bowers JE, Chapman BA, Rong J, Paterson AH: **Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events**. *Nature* 2003, **422**:433-438.

2. Paterson AH, Bowers JE, Chapman BA: **Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics**. *Proc Natl Acad Sci USA* 2004, **101**:9903-9908.

3. • Wendel JF, Cronn RC: **Polyploidy and the evolutionary history of cotton**. *Adv Agron* 2003, **78**:139-186.
Gossypium species exhibit extraordinary morphological variation, ranging from herbaceous perennials to small trees with a diverse array of reproductive and vegetative characteristics. This review article deals with cytogenetic and genomic diversity during global radiation of the genus, in evolutionary terms.

4. Tiwari SC, Wilkins TA: **Cotton (*Gossypium hirsutum*) seed trichomes expand via diffuse growing mechanism**. *Can J Bot* 1995, **73**:746-757.

5. Ji S, Lu Y, Li J, Wei G, Liang X, Zhu Y: **A β-tubulin-like cDNA expressed specifically in elongating cotton fibers induces longitudinal growth of fission yeast**. *Biochem Biophys Res Commun* 2002, **296**:1245-1250.

6. Kim HJ, Triplett BA: **Cotton fiber growth in planta and in vitro. Models for plant cell elongation and cell wall biogenesis**. *Plant Physiol* 2001, **127**:1361-1366.

7. • Ji SJ, Lu YC, Feng JX, Wei G, Li J, Shi YH, Fu Q, Liu D, Luo JC, Zhu YX: **Isolation and analyses of gene preferentially expressed during early cotton fiber development by subtractive PCR and cDNA array**. *Nucleic Acids Res* 2003, **31**:2534-2543.
Probably this is the first systematic analysis of genes involved in cotton fiber development at the transcriptome level that reported more than one hundred fiber-preferentially expressed cDNAs. The data suggest that a concerted mechanism involving multiple cellular pathways is responsible for cotton fiber cell elongation.

8. •• Shi YH, Zhu SW, Mao XZ, Feng JX, Qin YM, Zhang L, Cheng J, Wei LP, Wang ZY, Zhu YX: **Transcriptase profiling, molecular biological, and physiological studies reveal a major role for ethylene in cotton fiber cell elongation**. *Plant Cell* 2006, **18**:651-664.
Microarray hybridization of UniESTs obtained from *Gossypium hirsutum* reveals the key importance of ethylene in fiber cell elongation. The authors propose that ethylene may promote fiber cell elongation by activating genes that mediate cell wall synthesis, wall loosening, or cytoskeleton arrangement.

9. • Singh B, Avci U, Inwood SEE, Grimson MJ, Landgraf J, Mohnen D, Sorensen I, Wilkerson CG, Willats WGT, Haigler CH: **A specialized outer layer of the primary cell wall joins elongating cotton fibers into tissue-like bundles**. *Plant Physiol* 2009, **150**:684-699.
This paper shows that adhesion modulated by an outer layer of the primary wall of the fiber cell coordinates the extensive growth. It also reports dynamic changes in primary wall structure and composition during differentiation and fiber cell growth.

10. Ruan YL, Xu SM, White R, Furbank RT: **Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover**. *Plant Physiol* 2004, **136**:4104-4113.

11. Zhu YP, Xu KX, Luo B, Wang JW, Chen XY: **An ATP-binding cassette transporter GhWBC1 from elongating cotton fibers**. *Plant Physiol* 2003, **133**:580-588.

12. • Arpat AB, Waugh M, Sullivan JP, Gonzales M, Frisch D, Main D, Wood T, Leslie A, Wing RA, Wilkins TA: **Functional genomics of cell elongation in developing cotton fibers**. *Plant Mol Biol* 2004, **54**:911-929.
This paper reports the transcriptome of expanding fiber cells in *Gossypium arboretum*, a cultivated diploid cotton species. The authors analyzed 14,000 unique genes out of 46,603 ESTs using fiber mRNAs from different developmental stages.

13. Yang SS, Cheung F, Lee JJ, Ha M, Wei NE, Sze SH, Stelly DM, Thaxton P, Triplett B, Town CD *et al.*: **Accumulation of genome-specific transcripts, transcription factors and phytohormonal regulators during early stages of fiber cell development in allotetraploid cotton**. *Plant J* 2006, **47**:761-775.

14. Gou JY, Wang LJ, Chen SP, Hu WL, Chen XY: **Gene expression and metabolite profiles of cotton fiber during cell elongation and secondary cell wall synthesis**. *Cell Res* 2007, **17**:422-434.

15. Wu Y, Llewellyn DJ, White R, Ruggiero K, Al-Ghazi Y, Dennis ES: **Laser capture microdissection and cDNA microarrays used to generate gene expression profiles of the rapidly expanding fibre initial cells on the surface of cotton ovules**. *Planta* 2007, **226**:1475-1490.

16. Blacklock BJ, Jaworski JG: **Substrate specificity of Arabidopsis 3-ketoacyl-CoA synthases**. *Biochem Biophys Res Commun* 2006, **346**:583-590.

17. • Zheng H, Rowland O, Kunst L: **Disruptions of the Arabidopsis enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis**. *Plant Cell* 2005, **17**:1467-1481.
This report shows that VLCFA synthesis plays an essential role in cell expansion. Disruption of the At3g55360 gene, which encodes the enoyl-CoA reductase required for VLCFA synthesis, results in a reduction of cuticular wax load and affects VLCFA composition.

18. • Chen M, Han G, Dietrich CR, Dunn TM, Cahoon EB: **The essential nature of sphingolipids in plants as revealed by the functional identification and characterization of the Arabidopsis LCB1 subunit of serine palmitoyltransferase**. *Plant Cell* 2006, **18**:3576-3593.
This paper demonstrates that sphingolipids are essential components of plant cells and contribute to growth and development.

19. •• Qin YM, Hu CY, Pang Y, Zhu SW, Kastaniotis AJ, Hiltunen JK, Zhu YX: **Saturated very-long-chain fatty acids promote cotton fiber and Arabidopsis cell elongation by activating ethylene biosynthesis**. *Plant Cell* 2007, **19**:3692-3704.
VLCFA biosynthesis is specifically up-regulated during early cotton fiber development, and in both cotton and Arabidopsis plants, exogenously applied C24:0 stimulates significant elongation. VLCFAs may exert their growth-promoting effect through enhanced ethylene biosynthesis and signaling. The scenario outlined in this paper suggests that the current paradigm for the mechanism of cell elongation should be reassessed.

20. • Wanjie SW, Welti R, Moreau RA, Chapman KD: **Identification and quantification of glycerolipids in cotton fibers: reconciliation with metabolic pathway predictions from DNA databases**. *Lipids* 2005, **40**:773-785.
This paper reports the first lipid profiles from elongating and maturing cotton fiber cells and forms a foundation for future studies of the functional contribution of lipid metabolism to fiber development.

21. Napier JA, Graham IA: **Tailoring plant lipid composition: designer oilseeds come of age**. *Curr Opin Plant Biol* 2010, **13**:330-337.

22. Kargiotidou A, Deli D, Galanopoulou D, Tsaftaris A, Farmaki T: **Low temperature and light regulate delta 12 fatty acid desaturases (FAD2) at a transcriptional level in cotton (*Gossypium hirsutum*)**. *J Exp Bot* 2008, **59**:2043-2056.

23. Dietrich CR, Perera MA, Yandeau-Nelson M, Meeley RB, Nikolau BJ, Schnable PS: **Characterization of two GL8 paralogs reveals that the 3-ketoacyl reductase component of fatty acid elongase is essential for maize (*Zea mays* L.) development**. *Plant J* 2005, **42**:844-861.

24. Seagull R: **The effect of microtubule and microfilament disrupting agents on cytoskeletal arrays and wall deposition in developing cotton fibres**. *Protoplasma* 1990, **159**:44-59.

25. Chen CY, Cheung AY, Wu HM: **Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth**. *Plant Cell* 2003, **15**:237-249.

26. • Ruan YL: **Rapid cell expansion and cellulose synthesis regulated by plasmodesmata and sugar: insights from the single-celled cotton fiber**. *Funct Plant Biol* 2007, **34**:1-10.
This review concludes that the branching of plasmodesmata that occurs toward the secondary cell wall stage functions as a molecular sieve for tightly controlling the trafficking of macromolecules into fibers to sustain intensive cellulose synthesis.

27. Cole RA, Fowler JE: **Polarized growth: maintaining focus on the tip**. *Curr Opin Plant Biol* 2006, **9**:579-588.

28. •• Yang ZB: **Cell polarity signaling in Arabidopsis**. *Annu Rev Cell Dev Biol* 2008, **24**:551-575.
This review focuses on signaling mechanisms for the formation of cell polarity in Arabidopsis, with an emphasis on Rho GTPase signaling in polarized cell growth, and on how these mechanisms compare with those for cell polarity signaling in yeast and animal systems.

29. •• Hovav R, Udall JA, Chaudhary B, Hovav E, Flagel L, Hu G, Wendel JF: **The evolution of spinnable cotton fiber entailed prolonged development and a novel metabolism**. *PLoS Genet* 2008, **4**:e25.
The authors conducted comparative gene expression profiling across a developmental time-course of fibers using microarrays with 22,000 genes. Gene expression and Gene Ontology analyses showed that genes involved in the regulation of ROS production and homeostasis may have had an important role during the evolution of long spinnable fibers.

30. Hovav R, Udall JA, Hovav E, Rapp R, Flagel L, Wendel JF: **A majority of cotton genes are expressed in single-celled fiber**. *Planta* 2008, **227**:319-329.

31. Wymer CL, Bibikova TN, Gilroy S: **Cytoplasmic free calcium distributions during the development of root hairs of *Arabidopsis thaliana***. *Plant J* 1997, **12**:427-439.

32. Huang QS, Wang HY, Gao P, Wang GY, Xia GX: **Cloning and characterization of a calcium dependent protein kinase gene associated with cotton fiber development**. *Plant Cell Rep* 2008, **27**:1869-1875.

33. Mei WQ, Qin YM, Song WQ, Li J, Zhu YX: **Cotton GhPOX1 encoding plant class III peroxidase may be responsible for the high level of reactive oxygen species production that is related to cotton fiber elongation**. *J Genet Genomics* 2009, **36**:141-150.

34. Li HB, Qin YM, Pang Y, Song WQ, Mei WQ, Zhu YX: **A cotton ascorbate peroxidase is involved in hydrogen peroxide homeostasis during fiber cell development**. *New Phytolog* 2007, **175**:462-471.

35. •• Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD *et al.*: **Reactive oxygen species produced by NADPH oxidase regulate plant cell growth**. *Nature* 2003, **422**:442-446.
The authors report that mutants for Arabidopsis *rhd2*, which encodes an NADPH oxidase involved in the formation of ROS, have short root hairs and stunted roots that are mainly due to a defect in Ca^{2+} uptake. This is the first demonstration of a direct linkage between ROS production, activation of Ca^{2+} ion channels, and plant cell expansion.

36. • Reina-Pinto JJ, Voisin D, Kurdyukov S, Faust A, Haslam RP, Michaelson LV, Efremova N, Franke B, Schreiber L, Napier JA *et al.*: **Misexpression of fatty acid elongation1 in the Arabidopsis epidermis induces cell death and suggests a critical role for phospholipase A2 in this process**. *Plant Cell* 2009, **21**:1252-1272.
This paper reports that production of high-level ROS in transgenic Arabidopsis plants by epidermis-specific expression of FAE1 is accompanied by the death of trichome cells, resulting in seriously damaged cell membranes.

37. Gilliland LU, Pawloski LC, Kandasamy MK, Meagher RB: **Arabidopsis actin gene ACT7 plays an essential role in germination and root growth**. *Plant J* 2003, **33**:319-328.

38. • Li XB, Fan XP, Wang XL, Cai L, Yang WC: **The cotton ACTIN1 gene is functionally expressed in fibers and participates in fiber elongation**. *Plant Cell* 2005, **17**:859-875.
GhACT1 is expressed predominantly in fiber cells, and RNA interference of GhACT1, which significantly reduces its mRNA and protein levels, disrupts the actin cytoskeleton network in fibers with abolished fiber growth.

39. • Kim HJ, Triplett BA: **Characterization of GhRac1 GTPase expressed in developing cotton (*Gossypium hirsutum* L.) fibers**. *Biochim Biophys Acta* 2004, **1679**:214-221.
These authors suggest that the association of high levels of GhRac1 expression with stages of maximal cotton fiber elongation indicates that GhRac1 GTPase may be a potential regulator of fiber elongation by controlling cytoskeletal assembly.

40. •• Pang CY, Wang H, Pang Y, Xu C, Jiao Y, Qin YM, Western TL, Yu SX, Zhu YX: **Comparative proteomics indicate that biosynthesis of pectic precursors is important for cotton fiber and Arabidopsis root hair elongation**. *Mol Cell Proteomics* 2010, **9**:2019-2033.
The authors report that ethylene and VLCFA promote fiber cell and Arabidopsis root hair elongation by activating the expression of several enzymes, including UER, UGP, and UGD, which are involved in pectic cell wall polysaccharide biosynthesis. These authors suggest that the pectin layer may serve as a scaffold to support secondary cell wall biosynthesis.

41. Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM: **A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis**. *Plant Cell* 2005, **17**:2230-2242.

42. •• Szumlanski AL, Nielsen E: **The Rab GTPase RabA4d regulates pollen tube tip growth in *Arabidopsis thaliana***. *Plant Cell* 2009, **21**:526-544.
RABA4D, a member of the Arabidopsis RabA4 subfamily of Rab GTPases, is localized to the tips of growing pollen tubes, and pollen from RABA4D mutants displays bulged pollen tubes with a reduced growth rate.

43. • Xu SL, Rahman A, Baskin TI, Kieber JJ: **Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in Arabidopsis**. *Plant Cell* 2008, **20**:3065-3079.
Mutations in FEI1 and FEI2 (named after the Chinese word for fat) disrupt anisotropic expansion and cell wall polymer synthesis. The defect is suppressed by inhibition of ACC synthase, an enzyme that converts S-adenosine methionine to ACC.

44. •• Ruan YL, Llewellyn DJ, Furbank RT: **Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development**. *Plant Cell* 2003, **15**:952-964.
The authors report that the suppression of the sucrose synthase gene (Sus) in cotton correlates with the inhibition of fiber initiation and elongation. Suppression of Sus activity by 70% or more in the ovule epidermis leads to a fiberless phenotype.

45. Haigler CH, Zhang DS, Wilkerson CG: **Biotechnological improvement of cotton fiber maturity**. *Physiol Plant* 2005, **124**:285-294.