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INVITED REVIEW

Recent advances in understanding cotton fibre and seed development

Yong-Ling Ruan
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT, 2601, Australia

Abstract

The unique feature of the seed of tetraploid cotton (*Gossypium hirsutum* and *Gossypium barbadense*) is that about 30% of the seed coat epidermal cells develop into cellulose-enriched fibres, while the embryos synthesize oils and proteins. Hence, both the maternal and filial tissues of the cotton seed are of significant economic value. After initiation from the ovule epidermis at or just before anthesis, the single-celled fibres elongate to 2.5–6.0 cm long in the tetraploid species before they switch to intensive secondary cell wall cellulose synthesis. Thus, apart from its agronomic importance, the cotton fibre represents a model single-cell system to study the control of cell differentiation and elongation, carbon partitioning to cellulose synthesis and also the interaction between maternal (fibre) and embryonic tissues in seeds. Over the past decade or so, significant effort has been made to understand the cellular and molecular basis of cotton fibre development and oil biosynthesis in the embryo. Metabolic engineering of the oil biosynthetic pathway in cotton seed has successfully produced healthier and stable oils. A number of candidate genes and cellular processes that potentially regulate various aspects of fibre development have been identified. Further elucidation of the *in vivo* functions of those candidate genes could significantly deepen our understanding of fibre development and offer potential for improvement of fibre quality through genetic engineering or marker-assisted breeding approaches.

Keywords: cotton fibre, *Gossypium*, seed development

Correspondence
Fax: +61 2 62465000
Email: yong-ling.ruan@csiro.au

Introduction

Cotton (*Gossypium* spp.) is the most important fibre crop, as well as a major source of edible oil. Cotton seeds produce cellulose-enriched fibres (lint) from the seed coat epidermis and synthesize oils and proteins in the embryo. Hence, the cotton seed is perhaps the only seed type where both the maternal and filial tissues are of significant importance for the use by human society. In other crops, the major economically valuable tissue of the seed is filial, rather than maternal in origin (Ruan and Chourey, 2006). For example, the seeds of monocot crops, such as wheat, rice and maize, produce mainly starch and protein in the endosperm, while the dicot seeds (e.g. pea and bean) synthesize these products in another filial tissue, the embryo.

The genus *Gossypium* L. comprises about 45 diploid and 5 tetraploid species (Percival *et al.*, 1999). Among them, only four are cultivated: the tetraploid species of *G. hirsutum* L. [$n = 2x = 26$ $(AD)_1$] and *G. barbadense* L. [$n = 2x = 26$ $(AD)_2$], and two diploids, *G. herbaceum* L. ($n = x = 13$, A_1) and *G. arboretum* L. ($n = x = 13$, A_2) (Brubaker *et al.*, 1999). *G. hirsutum* L. is the dominant species for cultivation and accounts for about 90% of worldwide cotton fibre production (Han *et al.*, 2004). Not surprisingly, most of the research about cotton fibre and seed development has been conducted on *G. hirsutum* L. (see Arpat *et al.*, 2004; Ruan *et al.*, 2005 and references therein). Hence, the information discussed in this review derives mostly from studies of *G. hirsutum* L., unless otherwise stated.

Given the high economic value of cotton seeds as a major source of natural fibre and edible oil, extensive research has been carried out to understand cotton fibre and seed development over the past several decades. In this regard, excellent reviews have been published previously to address the cytology and structure of cotton fibre (e.g. Basra and Malik, 1984; Ryser, 1999) and the physiology of cotton seed

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development (Schubert *et al.*, 1986; Trelease *et al.*, 1986). However, despite these efforts, our understanding of cotton fibre and seed development is far from complete. Indeed, until the mid 1990s, little work had been published on the molecular events responsible for various aspects of fibre and seed development in cotton. This review aims to summarize and discuss some recent advances in this area. The focus is on the progress of understanding and manipulating cotton fibre and seed development at the cellular and molecular levels over the past decade.

The unique features of cotton seed anatomy

The developing cotton seed is unique because of the specialized hair-like fibres derived from the seed-coat epidermis (Fig. 1). The anatomy of the rest of the cotton seed is similar to that of other dicotyledonous seeds. The vascular bundle is located in the outer seed coat for delivery of assimilates, water and other nutrients for the development of the seed. The innermost layer of the seed coat develops into transfer cells at about 10 d after anthesis (DAA), with irregular wall ingrowth facing the seed apoplast, which encircles the filial tissues (Ryser *et al.*, 1988; Ruan *et al.*, 1997). Through their amplified plasma membrane surface area, the transfer cells facilitate the efflux of nutrients from the maternal tissue to the developing endosperm and embryo (Offler *et al.*, 2003). The endosperm is crushed and absorbed by the expanding embryo at about 25 DAA. By maturity, the dry weight of fibre accounts for about 40–50% of the whole seed (fibre plus the remaining seed), indicating that a substantial amount of assimilate has been channelled to the maternal fibre tissue, in addition to that delivered to the embryo.

The development of fibre cells from the maternal seed tissue

Each cotton fibre is a single cell, which initiates at, or just before, anthesis from the ovule epidermis. After initiation the single-celled lint fibres elongate rapidly to 2.5–3.0 cm for about 16 d before they switch to intensive secondary cell wall cellulose synthesis in the tetraploid species, *G. hirsutum* L. (Ruan *et al.*, 2001). By maturity, at about 60 DAA, more than 94% of fibre dry weight is pure cellulose, a β-1,4 polymer of glucose (Basra and Malik, 1984). Thus, apart from its economical importance, the cotton fibre represents a model single-cell system for studying the control of cell differentiation, elongation and carbon partitioning to cellulose synthesis (Haigler *et al.*, 2001; Kim and Triplett, 2001; Ruan *et al.*, 2001).

It should be noted here that a second wave of fibre initiation takes place 5–10 DAA (Stewart, 1975; Beasley, 1977). These fibres, termed fuzz, elongate to less than 10% of the length of the lint (Fryxell, 1963) and have little commercial value. So far, little is known about the basis of fuzz fibre development (Ruan *et al.*, 2005). However, it is possible that the fuzz may just be short lint, probably due to insufficient nutrients (e.g. sugars) being available by the time of lint initiation at anthesis (see Ruan *et al.*, 2005). Importantly, among all the natural mutants affecting fibre development, none of them show a lintless but fuzz normal phenotype. In addition, for fuzzless mutants, the growth of lint fibre is also reduced (T.-Z. Zhang and W.-Z. Guo, Cotton Research Institute, Nanjing Agricultural University, China, personal communication). These observations indicate a close genetic linkage between the fuzz and lint fibre development, and it is unlikely that fuzz initiation is controlled by a different molecular mechanism from that for lint. Given the sparse

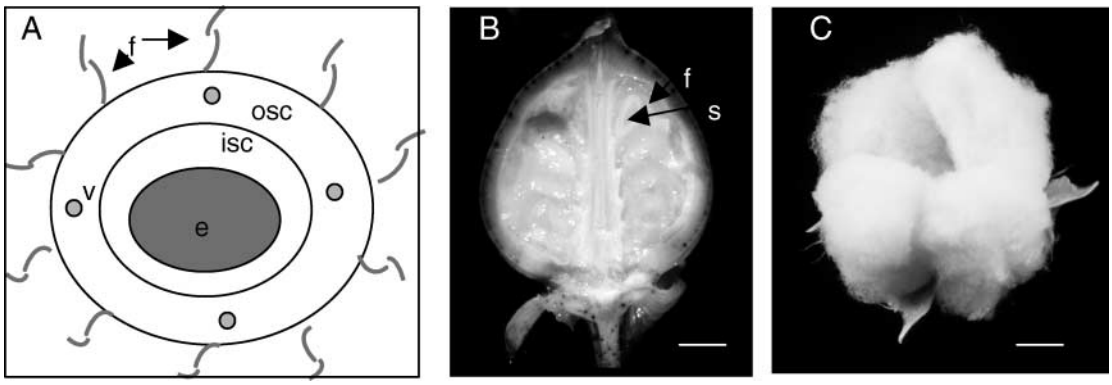


Figure 1. The anatomy of the developing cotton seed and fruit. (A) A schematic representation of a developing cotton seed. (B) A longitudinal section of a cotton fruit at 6 d after anthesis (DAA). Note the young fibres from the seed epidermis inside the fruit. Bar = 0.5 cm. (C) A fully mature cotton fruit at about 60 DAA, showing massive amounts of long fibres. Seeds are totally covered by the fibres. Bar = 1.3 cm. Abbreviations: e, endosperm and embryo; f, fibre; isc, inner seed coat; osc, outer seed coat; s, seed; v, vascular bundle.

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information on the molecular basis of fuzz fibre development, this paper concentrates only on lint fibre.

Genes controlling cotton fibre initiation

One intriguing phenomenon of fibre development is that only one in every 3–4 ovule epidermal cells becomes a fibre cell. The molecular event that triggers or represses fibre initiation from the ovule epidermis is a central issue in understanding fibre biology, and has been the subject of intensive investigations by several groups.

Due to the large genome size, the low density of the genetic map available and difficulties in transformation of cotton, researchers have turned to *Arabidopsis thaliana*, a species with a clear advantage in map-based cloning and transformation, for potential clues on the control of cotton fibre initiation (Wang *et al.*, 2004). In *Arabidopsis*, the development of the unicellular trichomes from leaves or stems is regulated by a set of transcription factors, belonging to the *R2-R3 MYB* gene family (Kirik *et al.*, 2004). Among them, transcription factors *GL1*, *GL3*, *TTG1* and *WD40* act as positive regulators for trichome development (Walker *et al.*, 1999), while *TRY* and *CPC* and their enhancers are negative regulators through lateral inhibition of trichome and root hair patterning (Schellmann *et al.*, 2002; Wada *et al.*, 2002; Kirik *et al.*, 2004; for a recent review, see Pesch and Hulskamp, 2004). To determine if *R2-R3 MYBs* are similarly involved in controlling cotton fibre development, Loguercio *et al.* (1999) screened a cotton ovule cDNA library using heterologous probes derived from the conserved region of *MYB* DNA-binding domains. Six *MYB* genes were found. However, none of them showed fibre-specific expression (Loguercio *et al.*, 1999). Using a similar approach, 55 more *MYB* cDNAs were isolated from cotton ovules by Suo *et al.* (2003). Thus, a total of 61 *MYB* genes has been found to be expressed in cotton ovules and fibre.

Despite the large number of *MYB* genes isolated from cotton, their role in cotton fibre development remains elusive. Since the cDNA libraries used (Loguercio *et al.*, 1999; Suo *et al.*, 2003) were prepared from RNA isolated from whole ovules −3 to +3 DAA, many of the *MYB* genes expressed may well be involved in regulation of female gamete formation and early embryogenesis, rather than in fibre initiation from the ovule epidermis. Nevertheless, one of them, *GhMYB109*, appears to be specifically expressed in fibre initials, pointing to its potential role in fibre development (Suo *et al.*, 2003). Notably, transformation of the trichomeless *GL1* mutant of *Arabidopsis* with *GaMYB2*, another cotton *MYB* gene isolated from the diploid species of *G. arboretum*, rescued trichome formation in leaves of the mutant, demonstrating the role of a fibre *MYB* gene in trichome development (Wang *et al.*, 2004). These authors also showed that by expression of this gene in wild-type *Arabidopsis* under the control of a 35S promoter, about 4% of the seeds from the T1 transgenic plants produced one hair per seed. Although interesting, this frequency of hair formation is too low to be used as evidence that *GaMYB2* can play a role in trichome development from seeds. Silencing the expression of *GaMYB2* or related genes in cotton may provide more direct evidence of their role in cotton fibre initiation.

While cotton fibres and trichomes share some degree of morphological similarities, it is important to keep in mind that there has been no compelling evidence thus far that cotton fibre initiation is indeed controlled in the same, or similar, way as that of trichomes. In contrast, some observations indicate that the two cell types are under the control of different molecular mechanisms. For example, there are several spontaneous mutants of cotton showing fibreless or fibre-reduced phenotypes (e.g. Ruan *et al.*, 2000; Du *et al.*, 2001). However, none of those mutants appear to be defective in trichome development. Indeed, cotton genes involved in regulating the development of seed fibres and leaf trichomes have been mapped to different loci (see Loguercio *et al.*, 1999). Clearly, further effort is needed to unravel the mechanisms controlling fibre initiation in the seed and trichome formation from other organs of the plant.

Cotton fibres are metabolically active sink cells that utilize sucrose and hexoses as metabolites or potential signals for their initiation and protrusion above the surface of the ovule epidermis. Several lines of evidence are consistent with or supportive of this assertion. First, developing cotton fibres contain about 150 mM glucose and fructose at the transition from elongation to secondary cell wall thickening (Ruan *et al.*, 1997). This hexose concentration is twice that in the seed coat, measured at the same stage (Ruan *et al.*, 1997), and 50 times higher than that of trichome-containing leaf epidermis (Koroleva *et al.*, 1998). The high hexose to sucrose ratio is indicative of active sucrose metabolism and strong sink activity (Ruan and Chourey, 2006). Secondly, fibre initiation starts 16–24 h preanthesis from the chalazal end of the ovule, where the vascular bundle terminates and phloem unloading occurs, while in the micropyle region, farthest away from the unloading area, there is little or no fibre cell initiation on the day of anthesis (Basra and Malik, 1984; Graves and Stewart, 1988; Nolte *et al.*, 1995; Tiwari and Wilkins, 1995). This asymmetric spatial pattern of fibre cell initiation suggests that the differentiation process may be triggered, or positively regulated, by phloem-unloaded sucrose or its derived hexoses. Consistent with this view is the fibre initial-specific localization of

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both the transcript (Ruan and Chourey, 1998) and protein (Nolte *et al.*, 1995; Ruan and Chourey, 1998) of sucrose synthase (Sus, EC 2.4.1.13), an enzyme that catalyses the first step in carbon use in sucrose-importing plant cells, by cleaving sucrose into UDP-glucose and fructose. In an *fls* mutant of cotton, lint is absent and Sus protein expression is delayed in the ovule epidermis (Ruan and Chourey, 1998). The delayed expression of Sus protein correlates temporally and spatially with the initiation of the short fibre cells seen in the mutant, indicating that the occurrence of short fibres may be related to delayed or insufficient expression of *Sus* (Ruan *et al.*, 2005). Consistently, microarray experiments also identified *Sus* to be one of the genes that was significantly reduced in the ovule epidermis of several fibreless mutants (Y.-R. Wu and D.J. Llewellyn, unpublished data). Importantly, transgenic suppression of the expression of *Sus* in cotton leads to decreased hexose content in ovules and inhibition of fibre initiation (Ruan *et al.*, 2003). This demonstrates the critical role of Sus in cotton fibre initiation.

The mechanisms of cotton fibre elongation

After initiation from the ovule epidermis, the fibres enter a period of very rapid elongation. The final length attained within a period of 3 weeks can reach 3.0 and 6.0 cm in *G. hirsutum* and *G. barbadense*, respectively (Ruan *et al.*, 2004). This probably makes the fibre the fastest growing and longest single cell known in higher plants (Ruan *et al.*, 2001). Elucidation of the cellular and molecular basis of fibre elongation would not only deepen our understanding of the mechanisms controlling rapid plant cell expansion, but also identify potential targets for genetic manipulation of fibre length, a key determinant of fibre yield and quality.

In contrast to root hairs and pollen tubes, which are typical cell types with tip growth characteristics, cotton fibres expand via a diffuse growth mechanism (Tiwari and Wilkins, 1995), as do leaf trichomes (e.g. Mathur *et al.*, 1999; Szymanski *et al.*, 1999). In diffuse growth, the cytoskeleton components (the cortical microtubules and microfilaments) are arranged transversely to the axis of growth, which provides a greater resistance to radial expansion than to longitudinal expansion (Martin *et al.*, 2001). Hence, in response to turgor pressure, the fibre cell elongates perpendicularly to the orientation of the cellulose microfibrils. This results in unidirectional growth from the surface of the seed epidermis (Seagull, 1990, 1992; Wilkins and Jernstedt, 1999; Martin *et al.*, 2001). Recently, silencing the expression of a fibre-specific actin gene, *ACTIN1*, has been shown to reduce fibre growth, demonstrating the essential role of the cytoskeleton in cotton fibre elongation (X.B. Li *et al.*, 2005).

Cotton fibre elongation is a specialized single-cell expansion, which has to be accomplished through a combination of 'pushing' against the cell wall exerted by turgor and 'loosening' of the wall mediated mainly by expansins (Ruan *et al.*, 2001). The relaxation of the cell wall appears to be achieved through the expression of endo-1,4,-β-glucanase and expansin genes (Shimizu *et al.*, 1997; Ruan *et al.*, 2001; Harmer *et al.*, 2002). The turgor in plants is achieved largely through the influx of water driven by a relatively high concentration of osmoticum within a cell (Cosgrove, 1997). To this end, a large number of aquaporins, including plasma membrane and tonoplast intrinsic proteins (PIP and TIP), are expressed in elongating cotton fibres (http://www.ncbi.nlm.nih.gov/dbEST/; Ferguson *et al.*, 1997). A recent study revealed that several PIP aquaporins are among the most highly expressed genes in 7–10 DAA elongating fibres of *G. arboretum* (Arpat *et al.*, 2004). These water-channel proteins are believed to play important roles in fibre elongation through exchange of water across the plasma membrane and tonoplast of the fibre cells. The major osmotically active solutes in elongating fibres are soluble sugars, K^+ and malate, which together account for about 80% of the fibre sap osmolality (Dhindsa *et al.*, 1975; Ruan *et al.*, 1997). The influx of sucrose and K^+ are mediated by their respective transporters in fibres (Ruan *et al.*, 2001), energized by the plasma membrane and tonoplast H^+-ATPases (Smart *et al.*, 1998). Within the fibres, sucrose is degraded into hexoses by Sus in the cytoplasm and acid invertase in the vacuole (Ruan *et al.*, 1997; Arpat *et al.*, 2004). The malate is synthesized locally by refixing CO_2 through the expression of phosphoenol-pyruvate carboxylase (PEPCase) (Basra and Malik, 1984; Smart *et al.*, 1998).

The fibre cells elongate 1600- to 3000-fold, accompanied by only 5- to 10-fold expansion of interconnecting seed coat cells, indicating a high degree of cell autonomy in fibres. It is intriguing how the turgor in the single-cell fibre is maintained and regulated. Recent studies revealed a transient closure of the plasmodesmata (PD) at the fibre base interconnecting the underlying seed coat epidermis. This PD closure coincides with the elevated expression of the sucrose and K^+ transporter genes (Ruan *et al.*, 2001). This symplastic isolation could provide a mechanism for fibres to maintain the high turgor for rapid elongation (Pfluger and Zambryski, 2001). Further comparison among different genotypes showed a positive correlation between the duration of PD closure and the extent of fibre elongation (Ruan *et al.*, 2004, 2005). The analyses also identified callose deposition and degradation as a likely basis for the closure and opening of PD, respectively, mediated

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through the expression of a fibre-specific β-1,3-glucanase gene (*GhGluc1*) (Ruan *et al.*, 2004). These observations establish a basis for engineering cotton fibre elongation through manipulation of PD gating and, in broad terms, provide an opportunity to study the *in vivo* role of PD gating in plant development at the single-cell level.

The switch to secondary cell wall cellulose synthesis

A remarkable feature of fibre development is the transition from primary to secondary cell wall synthesis at about 16–20 DAA. At this stage, the fibre is characterized by abrupt changes in the rate of cellulose synthesis, which rises over 100-fold as compared to the early period of elongation and primary cell wall synthesis (Delmer, 1999). Furthermore, the microtubule network, hence the direction of cellulose deposition, undergoes a shift from transverse to helical arrays (Seagull, 1990, 1992). These changes are also believed to limit the capacity of elongation (Delmer, 1999; Martin *et al.*, 2001).

Although a fascinating topic, the molecular basis of the switch from elongation and primary cell wall synthesis to intensive secondary cell wall cellulose production remains largely unknown. Two hypotheses have been suggested to regulate this transition process. One is a hormone theory, while the other involves an oxidative burst via the activity of NADPH oxidase and a small guanosine triphosphatase (GTPase).

Changes of hormonal balance in cotton fibres have been implicated in the transition to secondary cell wall thickening, although a majority of the hormone work focuses on the initiation and elongation period of fibres (reviewed by Basra and Saha, 1999; Davidonis, 1999). Yang *et al.* (2001) observed a sharp increase in the ratio of abscisic acid (ABA) to indole acetic acid (IAA) content in fibres from 30 to 44 DAA, a period of intensive cellulose synthesis. These authors speculated that the higher ratio of ABA to IAA might be beneficial for secondary wall thickening of the cotton fibre. However, it is possible that the rapid decrease of IAA and the rise of ABA may merely relate to senescence of the fibres at this time. Interestingly, alteration of the expression of several genes involved in either auxin or cytokinin biosynthesis produced no discernible phenotype of cotton fibre development in the resulting transgenic plants (John, 1999). There has been no compelling evidence thus far as to whether and how hormones regulate the transition of the cotton fibre to secondary wall cellulose synthesis.

Delmer *et al.* (1995) reported the highly enhanced expression of genes encoding two small GTPases of the Rho family, named *Rac9* and *Rac13*, just at the time of transition. Further evidence suggests that these proteins may be involved in signal transduction pathways leading to actin reorganization (see Delmer, 1999). Studies of cotton fibres and tracheary elements indicate that realignment of the actin network regulates the microtubule reorientation that sets the patterning of cellulose deposition (Seagull, 1990; Fukuda, 1992). The Rac proteins may also trigger the transition of the cotton fibre to cellulose production by activation of the NADPH oxidase at the plasma membrane to produce H_2O_2, followed by a rise of intracellular Ca^{2+} and protein phosphorylation/dephosphorylation (Diekmann *et al.*, 1994; Delmer, 1999; Potikha *et al.*, 1999). Evidence for the involvement of an oxidative burst in the signalling of this transition comes from the observations that hydrogen peroxide (H_2O_2) production in cotton fibres is initiated at the onset of secondary cell wall biosynthesis (Potikha *et al.*, 1999), and addition of an NADPH oxidase inhibitor to cultured ovules represses the production of H_2O_2 and the development of secondary cell wall formation (Delmer, 1999). Interestingly, a recent study identified a fibre-specific receptor-like kinase gene (*GhRLK*) that is a member of the leucine-rich repeat subfamily, which has dual specificity as a serine/threonine kinase and tyrosine kinase (Y.L. Li *et al.*, 2005). The expression of *GhRLK* is induced at the time of transition and remains high during secondary cell wall synthesis (Y.L. Li *et al.*, 2005). Microarray experiments also revealed elevated expression of a putative serine/threonine protein kinase at the beginning of the secondary cell wall stage, as compared to the early cell elongation phase (Arpat *et al.*, 2004). Analyses in *Arabidopsis* show that the target proteins of an RLK (CLV1, a protein regulating meristem activity) may include a Rho-GTPase or related proteins (Trotochaud *et al.*, 1999). Whether GhRLK interacts with GhGTPase, encoded by *Rac13*, in the cotton fibre is unknown. However, their similar temporal expression in the single-cell cotton fibre (Delmer *et al.*, 1995; Y.L. Li *et al.*, 2005) at least provides the possibility that the two proteins might interact with each other, an open question for further studies.

The cotton fibre is virtually a bio-factory for cellulose production after the switch to secondary cell wall formation. It has been estimated that up to 80% of total carbon entering the fibre at this stage is partitioned to cellulose (Mutsaers, 1976). How so much imported carbon is channelled into cellulose is not well understood. However, some recent progress has shed light on this process. Readers are referred to several excellent reviews on cellulose synthesis in general (Delmer, 1999; Haigler *et al.*, 2001; Doblin *et al.*, 2002). As mentioned previously, sucrose synthase (Sus) is the major enzyme in the cotton fibre that degrades incoming sucrose into fructose and UDP-glucose, an immediate substrate for cellulose

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synthesis (Ruan *et al.*, 1997). Sus has traditionally been considered as a soluble protein located in the cytoplasm. However, Amor *et al.* (1995) found that a substantial amount of the Sus protein is tightly bound to the plasma membrane of the cotton fibre. It has been estimated that sucrose entering the fibre cells is partitioned in a ratio of 3:1 between membrane (PM-) and soluble (S-) Sus (Delmer, 1999). Soon after the discovery of PM-Sus, Pear *et al.* (1996) reported the cloning of two cDNAs (*GhCelA-1* and *GhCelA-2*, later renamed as *GhCesA-1* and *GhCesA-2*; see Doblin *et al.*, 2002) from cotton fibres that encode the catalytic subunits of cellulose synthase genes. It is hypothesized that the PM-Sus forms a complex with the cellulose synthase to channel carbon directly from sucrose into cellulose on the plasma membrane of the cotton fibre (see Haigler *et al.*, 2001 for a recent review). However, protein–protein interaction screens with plant-specific regions of GhCesA1 have not yielded evidence for a direct interaction with Sus (Doblin *et al.*, 2002). The role of the plasma membrane association of Sus in cellulose synthesis remains hypothetical at this stage.

Apart from the *Sus* and *Ces* genes, many other genes are likely to be directly or indirectly involved in the cellulose synthesis process. For example, dissociation of cellulose-synthesizing 10-nm granules, which were isolated from plasma membranes of azuki bean, released eight polypeptides, including tubulin, CesA, Sus and Kor subunits (Doblin *et al.*, 2002). A putative lipid transfer protein (LPT), which is differentially expressed in developing cotton fibres (Ma *et al.*, 1995), interacts with the GhCesA1 zinc-binding domain (Doblin *et al.*, 2002). Subtractive polymerase chain reaction (PCR) and microarray techniques are being used to identify more genes specifically, or preferentially, expressed in cotton fibres during the secondary cell wall cellulose synthesis stage (e.g. Ji *et al.*, 2003; Haigler *et al.*, 2005). The challenge ahead is to validate the function of those candidate genes in cellulose synthesis.

Control of the diverse pattern of carbon partitioning in cotton seeds

The crucial role of Sus in the development of maternal and filial tissues in cotton seeds

During the stage of secondary cell wall thickening in the fibre, the cotton seed is actively synthesizing two carbon storage products in spatially separated tissues, namely cellulose in the maternal tissue fibres and oil in the embryos. The synthesis of both of these biopolymers utilizes sucrose, imported into seeds from photosynthetic leaves, as the initial and primary carbon source. After unloading from the phloem in the outer seed coat, sucrose is transported in two opposite directions: outwards to the fibre cells on the seed epidermis and inwards to the inner seed coat and embryo (see Fig. 2A). Sucrose moving towards the innermost seed coat is unloaded from the transfer cells to the embryos for synthesis of lipids and proteins. A small portion of sucrose is retained in the seed coat for temporary starch production (Ruan *et al.*, 1997). The starch is degraded at a late stage into hexoses for remobilization into the embryos, similar to the process found in canola seeds (King *et al.*, 1997) and grain legumes (Weber *et al.*, 1997).

After import into the seed or other sink tissues, sucrose must be degraded into hexoses, i.e. glucose and fructose, or their derivatives prior to its use in metabolism and biosynthesis. Two enzymes are responsible for the cleavage of sucrose: invertase and sucrose synthase (Sus). Although the role of invertase in cotton seed development has not been examined experimentally, a series of biochemical and molecular studies has demonstrated the critical role of Sus in both cotton fibre and embryo development. Sus protein is specifically expressed in the young fibres of the ovule or seed epidermis at 0–3 DAA, but also in the filial tissues at ~10 DAA onwards (Ruan *et al.*, 1997; Ruan and Chourey, 1998). Transgenic suppression of Sus activity by 70% or more in the seed coat represses fibre initiation and elongation, while additional silencing of Sus expression in the filial tissues blocks the development of the seed entirely (Ruan *et al.*, 2003; Fig. 2B). The linear relationship observed between the level of Sus suppression and the degree of the inhibition of fibre elongation demonstrates that fibre growth is highly sensitive to a small change of Sus activity. Thus, it would be logical and important to examine the impact of enhancing Sus expression on cotton fibre and seed development.

It is noteworthy that although Sus activity is generally required for starch synthesis in the seeds of many species, such as maize and *Vicia faba* (see Ruan and Chourey, 2006 for a recent review), the expression of Sus in cotton seeds does not spatially correlate with starch production at all (Ruan *et al.*, 1997; Fig. 2A). Rather, it appears to be tightly involved in cell wall biosynthesis of the cotton fibre (Ruan *et al.*, 1997; Haigler *et al.*, 2001), seed coat transfer cells and early embryogenesis (Ruan *et al.*, 2003). The role of Sus in early embryo development is uncertain. To clarify this issue, it would be necessary to determine the location of Sus protein in the endosperm and embryo during early development.

Carbon partitioning within the cotton seed may involve competition between the cellulose biosynthesis in the maternal tissue fibres and oil biosynthesis in the embryonic tissues (Fig. 2A). Thus, one might envisage that specific inhibition of fibre growth may enhance the development of the rest of the seed. In contrast to this

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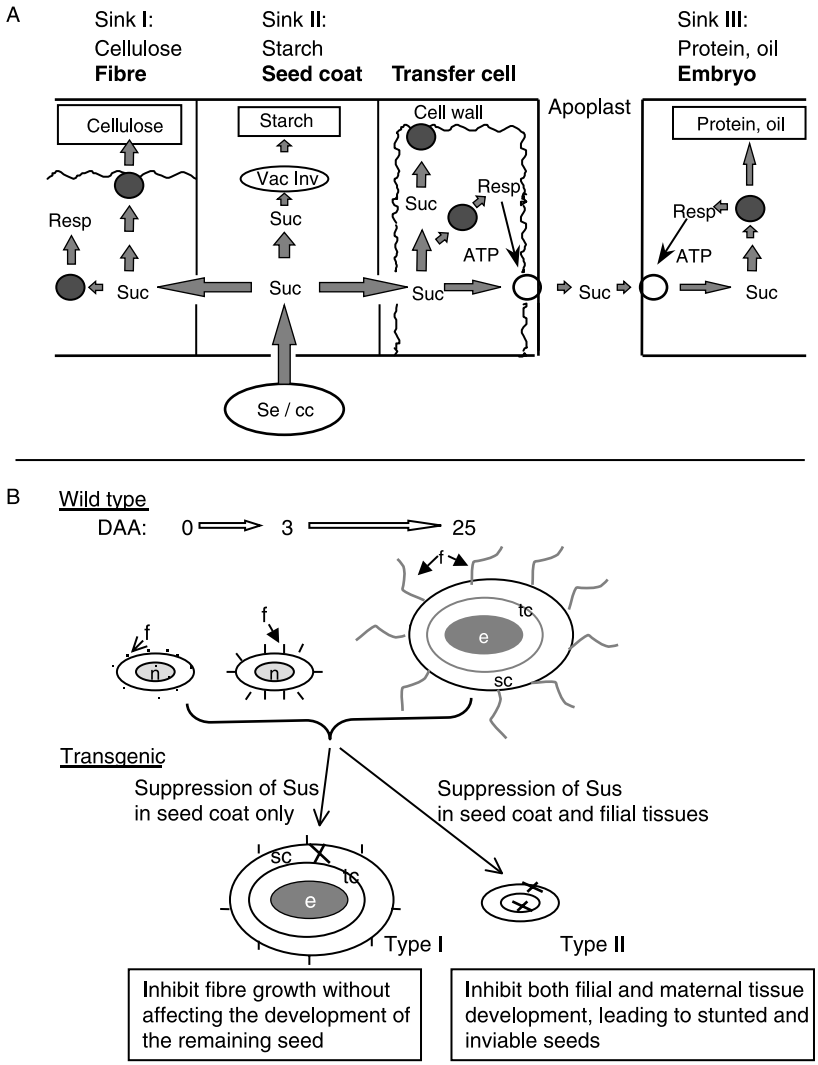


Figure 2. The control of diverse patterns of carbon partitioning in developing cotton seeds, mediated by sucrose synthase (Sus). (A) The pathway of sucrose flow into the developing cotton seed. The expression of Sus protein in fibre cells and transfer cells of the seed coat mobilizes sucrose into fibres for cellulose biosynthesis and into transfer cells for possible energy-coupled sucrose efflux into the apoplast. Sucrose is then taken up from the apoplast by the embryo and degraded by Sus for protein and oil biosynthesis. The remainder of unloaded sucrose moves into seed coat cells and is degraded by vacuolar invertase for temporary starch biosynthesis in this tissue. Abbreviations: Inv, invertase; Resp, respiration; Se/cc, sieve element/companion cell complex; Suc, sucrose; Vac, vacuole. ● = Sucrose synthase (Sus); ○ = putative sucrose transporter. Modified from Ruan *et al.* (1997). (B) The crucial role of sucrose synthase (Sus) in cotton fibre cell and seed development. In the wild-type cotton seed, Sus is expressed specifically in the fibres from 0 to 3 d after anthesis (DAA). From 10 DAA onwards, Sus is detectable both in the fibres and transfer cells of the seed coat, as well as in the filial tissues. In the Sus-suppressed transgenic lines, reduction of Sus activity by 70% or more results in a fibreless seed phenotype at 0–3 DAA. As the seed develops to 25 DAA, a portion of the transgenic seeds had Sus suppression only in maternal tissue due to transgene segregation or inactivation in the filial tissues (Type I). These seeds are identical to the wild seed except for much reduced fibre growth. The remaining seeds, however, showed Sus suppression in both maternal and filial tissues (Type II). These seeds are shrunken and non-viable. Abbreviations: e, endosperm and embryo; f, fibre; n, nucellus; sc, seed coat; tc, transfer cell. See Ruan *et al.* (2003) for more details.

expectation, suppression of fibre development, through either natural mutation (e.g. Ruan and Chourey, 1998) or gene silencing (Ruan *et al.*, 2003), did not lead to increases in either sucrose concentration in, or the weight of, the embryo. These observations suggest that the rate of phloem unloading of assimilates to the seed may be reduced proportionally in response to the decreased sink capacity in fibreless or fibre-reduced seed. Regulation of phloem unloading and post-phloem transport of sucrose through

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a feedback mechanism has been described in the seeds of *Vicia faba* (Patrick and Offler, 1995).

Oil biosynthesis and modification in the embryos of cotton seeds

The bulk of the sucrose taken up by the embryos at about 25 DAA onwards is utilized for oil biosynthesis in this tissue. From the crushing of the delinted seed, about 4 million tons of cotton seed oil are produced annually, making it the sixth most important plant oil in commerce (Liu *et al.*, 2002a). However, the oil composition from the cotton seed is not ideal for human consumption and industrial processing. It contains high amounts of palmitic and linoleic acids, but low levels of stearic and oleic acids. Palmitic acid (C16:0) is the main saturated fatty acid in cottonseed oil and has the undesirable property of raising the low-density lipoprotein form (LDL) of cholesterol. On the other hand, oleic acid (C18:1) has the beneficial property of reducing LDL-cholesterol, while stearic acid (C18:0) is LDL neutral (Dougherty *et al.*, 1995; Liu *et al.*, 2002a, and references therein). The polyunsaturated linoleic acid (C18:2), although nutritious for human health, is highly unstable during cooking and other processing. To develop stable and healthy cottonseed oil, Liu *et al.* (2002b) transformed cotton with gene silencing constructs against genes encoding either Δ^9- or Δ^{12}-desaturase. They found that down-regulation of the Δ^9-desaturase substantially increased stearic acid from the normal level of 2–3% up to as high as 40%, and silencing of the Δ^{12}-desaturase resulted in elevated oleic acid content, up to 77%, compared with about 15% in seeds from untransformed plants. In addition, palmitic acid was significantly reduced to about 15% in both high-stearic and high-oleic lines, as compared to 26% in the wild-type seeds. The large-scale introduction of these improved cotton oils has the potential to deliver substantial public health benefits through lowering the serum LDL-cholesterol levels and consequent reduction of cardiovascular disease (Liu *et al.*, 2002a). The healthy oil from the embryo, in addition to the cellulose-enriched mature fibres developed from the maternal tissue, would certainly increase the overall value of the cotton seed.

Interaction between maternal and filial tissues of the cotton seed

In all seeds, the development of the maternal and filial tissues must highly coordinate with each other to achieve full maturity. The maternally derived cotton fibres are homogeneous single cells, yet of a significant size for manipulation and observation. This feature renders the cotton seed an attractive system for studying the interaction between the maternal and filial tissues. Early *in vitro* studies revealed that *unfertilized* ovules are unable to produce fibres unless auxin (IAA) or gibberellic acid (GA_3) is added to the medium, and IAA promotes more fibre production than GA_3 alone (Beasley and Ting, 1974). On the other hand, *fertilized* ovules can grow fibres from the ovule epidermis without addition of hormones, and application of exogenous IAA does not promote additional fibre growth, while GA_3 does (Beasley and Ting, 1973; Beasley, 1977; for reviews on hormone regulation of cotton fibre development see Basra and Saha, 1999; Davidonis, 1999). These observations indicate that, although fibre precursors have the ability to initiate from the ovule epidermis at 1–2 dA (Basra and Malik, 1984; Nolte *et al.*, 1995), their protrusion from the ovule epidermis requires IAA or IAA-like signals, most likely from the newly formed embryo or endosperm through double fertilization. It is intriguing, then, that the fibres from the micropyle region, where the embryo forms, generally develop later and are smaller than fibres at the chalazal end (Nolte *et al.*, 1995; Tiwari and Wilkins, 1995). One possibility is that filial tissue-derived signals, such as IAA, may be essential, but not sufficient, for fibre growth from the ovule epidermis, and the fibre cells in the chalazal region may receive more 'fibre elongation' signals, possibly phloem-derived GA or sugars, than those at the micropyle end.

Regulation of maternal parts of a seed and fruit development by the embryo or endosperm has been proposed previously (Cowan *et al.*, 2001). Recent cytological studies on *haiku* mutants of *Arabidopsis* indicate control of seed coat development by endosperm-derived signals (Garcia *et al.*, 2003). The observation that suppression of *Sus* in cotton endosperm disrupts the formation of adjacent maternal seed coat transfer cells (Ruan *et al.*, 2003) suggests that these cells at the innermost layer of the seed coat could be the cellular sites that first perceive the vital signals from the filial tissues for coordinating the development of the maternal and filial tissues of seed. The identities and the transduction pathways of these signals are unknown, but probably involve sugar and hormone signalling and proteins secreted from the embryo (Farley *et al.*, 2000; Thompson *et al.*, 2001).

Conclusions and future perspectives

In recent years, strong efforts have been made to understand cotton fibre and seed development at the molecular level. Many candidate genes, potentially responsible for various aspects of fibre development, have been identified through either hypothesis-driven research (e.g. Pear *et al.*, 1996; Smart *et al.*, 1998; Ruan *et al.*, 2004) or analyses of gene expression profiles

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using microarrays (Arpat *et al.*, 2004). Quantitative trait loci (QTL) for fibre properties, such as length and strength, have been mapped to various chromosomes of the tetraploid cotton genome, and molecular markers linked with these QTL or important genes are being identified (see Jiang *et al.*, 1998; Paterson *et al.*, 2003; Zhang *et al.*, 2003; Han *et al.*, 2004). This progress provides a starting point for molecular-assisted breeding towards high-quality cotton fibre. The production of healthier oils from the embryos of transgenic cotton seeds (Liu *et al.*, 2002b) sets a promising example for the concept and value of genetic engineering of cotton, in addition to the successful commercialization of insect- or herbicide-resistant transgenic cotton (reviewed by John, 1997).

Despite the significant accomplishments made over the past decade or so, many fundamental questions remain to be answered regarding the molecular events underlying cotton fibre and seed development. What is the molecular basis that determines whether a given ovule epidermal cell will develop into a fibre or remain undifferentiated? To what degree, if any, do fibre development and trichome formation share the similar molecular mechanisms? What happens to fibre elongation if the gating properties of the fibre plasmodesmata are genetically altered? How do the aquaporin proteins regulate the influx of huge amounts of water into fibre cells during elongation? What are the signals that trigger the transition from primary to secondary cell wall synthesis? What are the mechanisms of plasma membrane association of Sus protein? Is the membrane-bound Sus encoded by a different *Sus* gene or derived from post-translational modification of the soluble Sus protein? How does the endosperm or embryo regulate the fibre development from the maternal tissue at different developmental stages of the seed?

Answers to these questions will only come from studies using multidisciplinary approaches. Gain- or loss-of-function studies, coupled with insightful analysis of the resultant phenotypes, are now emerging to provide novel information on the control of cotton fibre and seed development (e.g. Ruan *et al.*, 2003; Wang *et al.*, 2004). The physiological functions of a large number of candidate genes expressed in fibres and seeds (Arpat *et al.*, 2004; Haigler *et al.*, 2005) need to be evaluated carefully in cotton through transgenic analysis. This is particularly important since suppression of several 'fibre-specific' genes, such as *E6*, *H6* and *FbL2A*, surprisingly, shows no phenotypes in fibre development (John, 1999). Transient assays of gene expression in cultured ovules or seeds (Kim and Triplett, 2001), followed by stable transformation (Murray *et al.*, 1999), offer potential for efficient and reliable screening and determination of the roles of candidate genes in fibre and seed development *in vivo*. There is no doubt that cotton fibres and seeds are highly valuable systems from an applied perspective, as well as from a basic research viewpoint.

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