TOWARDS GENETIC ENGINEERING OF SUCROSE SYNTHASE TO ENHANCE FIBRE CELL INITIATION AND CELLULOSE BIOSYNTHESIS IN COTTON

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Introduction

Despite the great potential for increasing cotton productivity through the genetic engineering of fibre development, little progress has so far been made in this area. This is in sharp contrast to the success of pest and herbicide resistant transgenic cotton that have already made a large impact on agriculture in both the U.S. and Australia (2). The major impediment to fibre engineering is our poor understanding of the biology of the cotton fibre, particularly, the identities and functions of genes controlling various fibre developmental processes (1,2,3,4). Consequently, a recent major thrust has been on elucidating the molecular and cellular basis for fibre development and the cloning of a number of genes highly expressed in developing cotton fibres (1,3,4).

The cotton fibre is almost pure cellulose so the biosynthetic event, the polymerisation of glucose residues from UDP-glucose to form the homopolymer ß-1,4-D-glucan (1), is one of the key features of and perhaps limitations to fibre growth. UDP-glucose is derived by cleavage of the sucrose that is produced in photosynthetic carbon fixation and transported to the seed for use in the production of starch, oil and cellulose. As a number of biosynthetic reactions must compete for this supply of UDP-glucose, an understanding of the control of carbon partitioning into cotton fibres would be essential for designing genetic engineering approaches to improve fibre development. To this end, a cDNA encoding sucrose synthase (SuSy) has recently been cloned from developing cotton fibres (3). Detailed expression analysis, together with relevant cellular and biochemical studies, have indicated the important role played by SuSy in controlling not only cellulose biosynthesis (3) but perhaps also fibre cell initiation (4). These studies shed light on how to engineer SuSy to increase fibre yield and quality. Here, we firstly reviewed the diverse role played by SuSy in developing cotton seeds and then propose strategies to genetically engineer the SuSy gene to potentially increase fibre cell initiation and cellulose biosynthesis.

The Role of SuSy in Fibre Development

(1) Fibre cell initiation requires high level of SuSy expression in the ovule epidermis

Each fibre cell initiates from the outermost layer of the ovule epidermis during the flowering stage of cotton and then undergoes rapid cell elongation over the next 14 days before it switches to massive secondary cell wall cellulose biosynthesis which lasts for about three weeks (2,3,4). Thus, on a per seed basis, the frequency of fibre cell initiation from the ovule epidermal cells and the length and wall thickness (cellulose content) of each fibre are the key determinants of cotton fibre yield and quality.

It has been long recognised that although all epidermal cells are potential fibres, only about 30% of these cells actually initiate into fibres (2,4). The molecular basis determining which epidermal cells differentiate into fibres is unknown. In this regard, the cell-specific *In Situ* analyses of SuSy gene expression in ovule sections of both wild type (*FLS*) and a fibre-less seed (*fls*) mutant have yielded some important clues (4). By using the cDNA encoding cotton SuSy and a polyclonal antibody raised against this protein, Ruan and Chourey (3) found no detectable SuSy mRNA or protein in the ovule epidermis of the *fls* mutant, which correlates with the lack of fibre cell initiation from these ovules (4). In the wild type ovules (*FLS*), there was much heterogeneity in the SuSy expression between the ovule epidermal cells: those showing high and low levels of SuSy expression protrude as big and small fibres respectively, while those with no SuSy expression do not initiate into fibres (4). Together, these observations provide strong correlative evidence that SuSy could play a critical role for fibre cell initiation.

There are several possibilities as to why high level expression of SuSy might be required for ovule epidermal cells to initiate into fibres. This enzyme catalyses a reversible reaction but is thought to preferentially convert sucrose into fructose and UDP-glucose, which are the precursors for energy metabolism and the immediate substrate (UDP-glucose) for cellulose biosynthesis in cotton fibres (1,3,4). As invertase, the other sucrose-cleavage enzyme that occurs in plants, is undetectable in both its cell wall and cytoplasmic forms in developing cotton fibres, SuSy must be the key enzyme in degrading symplastically imported sucrose in initiating fibres (3,4). Thus, the SuSy-deficienct epidermal cells of the mutant or those FLS cells which are not initiated into fibres may lack both energy and precursors required for the metabolic and biosynthetic processes necessary for fibre cell development (4).

It is also possible that the enzyme SuSy may contribute to turgor related functions required for initiation and associated protrusion of the ovular epidermal cells (4). Sucrose cleavage into fructose and UDP-glucose by SuSy would double the osmotic contribution of sucrose raising the turgor pressure within the initial cells and driving their elongation into a fibres (4). The high ratio of hexose to sucrose in developing fibres (3) supports this viewpoint. In the *fls* mutant, the lack of SuSy in the ovule epidermal cells may lead to low turgor and consequently no fibre protrusion (4).

(2) SuSy controls Carbon partitioning to fibre cellulose

The massive cellulose biosynthesis in cotton fibres requires an efficient and continuous supply of photoassimilate (3). Several lines of evidence have shown the critical role of SuSy in mobilising sucrose to fibre cellulose during secondary cell wall biosynthesis. First, sucrose import into fibre follows a symplastic route (through cells) at this stage and the cytoplasmic SuSy is the key enzyme to degrade and thus to mobilise phloem-unloaded sucrose into fibres (3). Secondary, fibre cellulose biosynthesis is highly correlated with the level and activity of the SuSy protein (3). Finally, about 50% of SuSy protein is tightly associated with the plasma membrane of fibres and is thought to form a complex with cellulose synthase to channel carbon directly from imported sucrose to cellulose via UDP-glucose (1).

In developing cotton seed, however, the phloem unloaded sucrose is not only partitioned to fibres for cellulose biosynthesis but is also mobilised to the seed coat and cotyledons for starch and lipid biosynthesis, respectively (Fig.1). Hence, a three-way competition for unloaded sucrose is evident among the three tissues with fibre being the weakest sink in this competition, particularly under sub-optimum conditions when plants are stressed. Potential therefore exists for enhancing carbon flow to fibres for cellulose biosynthesis through genetic manipulation by partitioning more of the available carbon away from the developing embryo. In this context, the expression of SuSy protein at the innermost cell layer of the seed coat (transfer cells) and in cotyledons is believed to play a key role in mobilising sucrose to cotyledons for protein biosynthesis (Fig.1). Therefore SuSy could be a target for manipulation of carbon partitioning within the seed (see below).

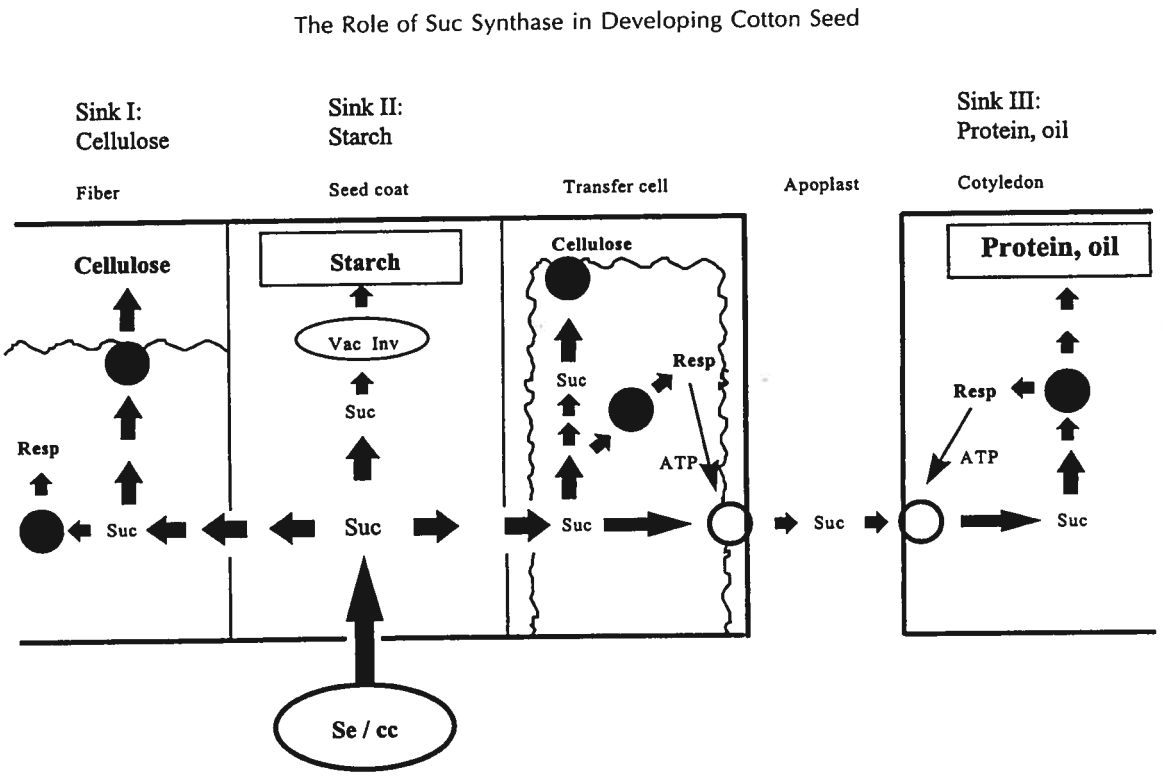

Figure 1. An intergrated model for the role of SuSy in controlling diverse patterns of carbon partitioning in developing cotton seed. ● = SuSy; ○ = Putative sucrose transporter. The arrow indicates the main direction of carbon flow.

The differential expression of SuSy protein in fibre cells and transfer cells of the seed coat plays a key role in mobilizing sucrose symplastically into fibres for massive cellulose biosynthesis and into transfer cells for possible energy coupled sucrose efflux into the apoplast where it is then taken up by cotyledonary cells and degraded by SuSy for protein and oil biosynthesis. The remainder of unloaded sucrose moves into the seed coat cells and is degraded by vacuolar invertase for starch biosynthesis in this tissue. Inv, invertase; Resp, respiration; Se/cc, sieve element / companion cell complex; Suc, sucrose; Vac, vacuole.

This figure was extracted from Ruan et al (1997) Plant Physiology115: 375-385.

Engineering Strategies of SuSy to Enhance Fibre Cell Initiation and Cellulose Synthesis

Based on the above analysis, we proposed two strategies to improve fibre development through genetic engineering of SuSy. The first is to over-express SuSy gene specifically in the ovule and seed epidermis from the period of fibre cell initiation to secondary cell wall cellulose biosynthesis. The application of this approach may significantly increase the

frequency of fibre cell initiation from ovule epidermis and enhance fibre sink strength in mobilising sucrose into this tissue for cellulose biosynthesis. A strong fibre-specific promoter is required to drive SuSy expression in the ovule/seed epidermis from a period of fibre initiation to secondary cell wall biosynthesis. As such a promoter is currently not available, other constitutive promoters, such as the 35S promoter from cauliflower mosaic virus, may be used as an alternative. The second strategy is to inhibit or reduce SuSy expression in cotyledons to reduce sucrose flow to this tissue to increase the availability of sucrose for fibre cellulose biosynthesis (see Figure 1). To achieve this, cotyledon specific promoters such as those of the soybean Lectin or pea Vicilin genes have been selected to drive an antisense of the SuSy cDNA. In both approaches, however, the cell-specificity and developmental profile of the promoter expression must be examined in cotton ovules and seeds to ensure that they are appropriate. Transgenic cotton expressing a reporter gene driven by either of these seed-specific promoters have been generated and are being analysed and a seed-specific antisense SuSy construct should shortly be introduced into transenic cotton.

References

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