Identification and analysis of genes involved in cotton fibre initiation

Abstract

Cotton fibres are single-celled hairs, arising from the epidermal surface of the cotton ovule. One factor in determining the length of the mature cotton fibre is the timing of fibre initiation, which is therefore a crucial step in obtaining commercial cotton fibres. To achieve a greater understanding of the regulation of cotton fibre differentiation, more fundamental information is needed on the signals and mechanisms associated with fibre initiation. The extensive genetic knowledge of *Arabidopsis* leaf trichomes could aid in the elucidation of the genetic mechanisms controlling cotton fibre differentiation. Trichomes are small hairs on the plant surface, originating from single epidermal cells in a developmental process that appears very similar to that of cotton fibres. *Arabidopsis* trichome development has been extensively investigated, and several genes that control the process have been characterised. One gene essential for trichome initiation is *TRANSPARENT TESTA GLABRA1* (*TTG1*), and loss-of-function mutations in *TTG1* result in an almost complete absence of leaf trichomes. *TTG1* plays additional roles in numerous pathways in *Arabidopsis*, including root hair initiation, anthocyanin production and seed coat mucilage production. In order to isolate genes required for fibre initiation in cotton, functional homologues of *Arabidopsis* TTG1 in cotton have been sought.

Four putative homologues of *Arabidopsis TTG1* have previously been isolated in this laboratory by RT-PCR of mRNA prepared from cotton fibres, and are termed GhTTG1-4. Sequence comparisons between the four cotton deduced proteins and *Arabidopsis* TTG1 showed that they form two groups, with GhTTG1 and GhTTG3 being closely related to each other (87% identical and 93% similar) and to TTG1 (79% and 80% amino acid identity respectively). GhTTG2 and GhTTG4 formed the second group, with 95% amino acid identity to each other and lower (approximately 62%) identity to TTG1. An analysis of the genomic originis of the *GhTTG* genes demonstrated that each is derived from the same ancestral diploid genome.

Cross-species complementation experiments were performed to test for functional homology of these cotton *TTG1*-like genes to *AtTTG1*, by introducing the cotton genes into *Arabidopis ttg1-1* mutants via *Agrobacterium*-mediated transformation. This experiment showed that two of the four genes, *GhTTG1* and *GhTTG3* were able to restore trichome initiation in the *Arabidopsis* mutant plants, and a further investigation of *GhTTG3* transgenic plants demonstrated complementation of the full range of *ttg1* mutant phenotypes.

An analysis of the temporal and spatial expression of the *GhTTG* genes in cotton is also described. It was shown that each of the genes is expressed ubiquitously throughout the cotton plant, in common with many plant WD-repeat genes. A closer examination of transcript abundance in the developing cotton ovule utilising *in situ* hybridisation

revealed predominant expression of *GhTTG1/GhTTG3* in the epidermal cells destined to become cotton fibres.

A yeast two-hybrid assay was utilised to identify transcription factors that may interact with GhTTG3 during fibre development. This experiment identified three cotton fibre cDNAs encoding putative interacting proteins, including one with a similar secondary structure to several TTG1-interacting proteins in *Arabidopsis*, raising the possibility of similar regulatory complexes controlling trichome initiation in *Arabidopsis* and cotton.