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Molecular regulation of fibre-specific gene expression in cotton (*Gossypium hirsutum*)

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Abstract

Cotton fibre growth and development are regulated by the expression of several thousand genes in the fibre cell. These genes are often expressed in both cotton fibres and other plant tissues, although a proportion are 'fibre-specific' (expressed predominantly or exclusively in the fibre). Many fibre-specific genes have important functions in fibre development, and their expression is generally regulated at the level of transcription. However, the mechanisms that restrict gene expression to the cotton fibre have not been well characterised. An understanding of these mechanisms is central to a molecular model of fibre development, and may be important in the generation of improved cotton varieties with fibre-specific transgene expression.

The aim of this project was to identify the promoter sequences and transcription factors involved in regulating the fibre-specific expression of *FSltp6*, a gene encoding a cotton lipid transfer protein (LTP). The *FSltp6* gene is abundantly and specifically transcribed in elongating cotton fibres. In this project, the fibre-specificity of the *FSltp6* promoter was analysed using constructs containing the *FSltp6* promoter sequence driving expression of the reporter gene β-glucuronidase (GUS). Cotton fibres and other cotton tissues were transiently transformed with an *FSltp6*::GUS construct and analysed for GUS expression. The *FSltp6* promoter restricted GUS expression primarily to the cotton fibre. Successive 5' deletions of the *FSltp6* promoter were then used to isolate the regions necessary for fibre-specific expression. An 84 bp fibre-specificity region (FSR) was found to be essential for GUS expression exclusively in the cotton fibre, while a 49 bp region was necessary for expression in any of the tissues tested. The fibre-specificity of the *FSltp6* promoter was also analysed by the stable transformation of tobacco with *FSltp6*::GUS. The transgenic tobacco plants demonstrated strong GUS expression in the leaf trichomes. This result provided further support for the fibre-specificity of the *FSltp6* promoter and (in line with previous studies) suggested the utility of tobacco trichomes as a model for cotton fibre development.

A yeast one-hybrid assay was used to identify transcription factors that may regulate fibre-specific expression by interacting with the FSR. This experiment identified three novel classes of cotton protein with potential roles in fibre specificity: HMGA-like proteins, *Mutator* transposase-like proteins and an AT-hook protein. The full-length cDNA of the AT-hook protein was isolated and analysed for its potential function as a transcription factor and regulator of fibre-specificity.

This project has identified a promoter region and several novel transcription factors with a potential role in the regulation of fibre-specific gene expression. These results provide further insight into the molecular regulation of gene transcription in cotton fibres. Application of these results in the generation of transgenic cotton may produce varieties with enhanced gene expression in the cotton fibre.