Characterisation of α-expansin genes in *Gossypium hirsutum*

by

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A thesis submitted for the degree of Doctor of Philosophy

in

The University of Adelaide

School of Molecular and Biomedical Science

Discipline of Genetics

(Faculty of Sciences)

March, 2003

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Abstract

Cotton fibres arise from single epidermal cells of the ovule and initiate as protrusions on the day of flowering (anthesis). Elongation continues for approximately 20 days, resulting in fibres which are 2-3.5 cm in length. As fibres are single cells of homogenous tissue and their development is synchronous, they are an excellent model system for the analysis of the molecular processes involved in cell growth.

One type of cell wall protein, the α-expansins, has been implicated in cell wall modification. α-expansins have been characterised in a number of plant species and are encoded by large gene families (31 members in *Arabidopsis*), with family members often showing heterogenous patterns of expression (Wu *et al.*, 2001). In some plant species, α-expansins have specifically been implicated in cell elongation, such that a cotton fibre-specific α-expansin is an attractive target for cotton biotechnology. Differential screening of a cotton fibre cDNA library allowed the identification of a fibre-specific α-expansin mRNA (pFS14) of which accumulated at high levels during fibre cell elongation (Orford and Timmis, 1998). The aims of this project focused on the isolation, characterisation and analysis of the fibre-specific α-expansin gene and other cotton α-expansin family members.

Using, a combination of genomic library screening and PCR-based approaches six different cotton α-expansin gene family members were isolated (Chapter Three). One of the genes, *GhExp14.2*, was 99% similar to the pFS14 cDNA, and therefore it was considered to be the genomic representative of pFS14 cDNA.

RT-PCR analysis (Chapter Three) showed that five of the six α-expansins have distinctive expression patterns in different cotton tissues, and that the transcripts of four genes were detected in elongating fibres. *GhExp14.2* transcripts were detected at high levels in elongating fibres, consistent with the results described by Orford and Timmis (1998), suggesting that *GhExp14.2* plays an important role in fibre development.

Evolutionary analysis of α-expansin genes from cotton and other plant species (Chapter Four) confirmed previous studies that demonstrate the α-expansin family is divided into four ancient, distinct subclades. Each subclade was further divided into groups on the basis of gene orthology. In some cases 'orthologous groups' within clades contain multiple genes that appeared to be expressed in a similar manner to each other and the evolutionary analysis provides a foundation by which specific orthologous α-expansin genes could be identified and characterised.

The development of a transient assay for use in cotton fibres was undertaken (Chapter Five). Various parameters and conditions were tested on tobacco and cotton tissues until an appropriate method was refined, with fibres aged approximately 4 DPA providing the best results. The validity of the transient assay technique was tested using the *GhExp14.2* promoter, with the full available sequence (848 bp up to and including the ATG initiation codon) directing GUS reporter gene expression only in developing fibre cells and not other cotton tissues tested. This work describes an efficient, simple method for the functional dissection of cotton fibre-specific promoters.

Known *cis*-acting consensus elements in the *GhExp14.2* fibre-specific promoter were accurately mapped using transcription factor binding site websites (Chapter Six). Detailed comparisons of multiple fibre-specific promoters allowed identification of four new putative fibre-specific promoter elements. A deletion analysis was undertaken to dissect the functional regions of, and putative consensus bindings sites within, the *GhExp14.2* promoter. All *GhExp14.2* promoter deletions maintained fibre-specific expression of the GUS reporter gene, including the smallest, which consisted of only 149 bp. Within the *GhExp14.2* 149 bp minimal promoter five previously characterised consensus-binding sites (ACGT box, Q-element, RY pyrimidine repeat, an E-box and a GCN4 motif) and one novel binding site (PFE4) were present, and each was suggested to be involved in directing fibre-specific transcription.

In Chapter Seven, preparation of four constructs using the fibre-specific cDNA pFS14, under the control of different fibre-specific promoters, is described. These constructs were designed with a view of modifying fibre morphology by altering the temporal expression pattern of the α-expansin gene in transgenic cotton. Results from the transgenic cotton, although not available for incorporation into this thesis, will be used to define the important role that *GhExp14.2* plays in fibre elongation, and may also facilitate the production of cotton varieties with improved fibre morphology.