Analysis of Gene Expression During Cotton Fibre Development

by

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A thesis submitted for the degree of

Doctor of Philosophy

in

The University of Adelaide

Department of Genetics

(Faculty of Science)

May, 1996

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ABSTRACT

Cotton fibres represent a coordinated process of gene regulation within a single cell type and are especially suitable for studies of cellular and developmental events. In addition, the commercial desirability of long fibres has stimulated studies of the factors involved in controlling the extent of fibre growth. The aims of this project were to isolate and characterise cDNA clones of mRNAs which are specific to, or important in, cotton fibre development and to elucidate ribosomal gene expression and its role in fibre development.

Screening and sequencing of several low abundance cDNA clones from an initial cDNA library constructed from total RNA led to the isolation of a cDNA which is likely to encode a member of the superfamily of translation elongation factor 1α (EF-1α) proteins. EF-1α is universally conserved and is abundant in tissues which are active in protein synthesis. Southern analysis suggests that EF-1α is encoded in the cotton genome by a family of related genes, six of which were isolated within genomic clones.

Differential screening of a 13 DPA cotton fibre cDNA library constructed from poly(A)$^+$ RNA resulted in the identification of six putative fibre-specific messages of which five (pFS1, pFS3, pFS6, pFS17 and pFS18) showed preferential hybridisation to transcripts in fibre RNA. The mRNAs corresponding to these cDNAs exhibited differing patterns of temporal accumulation during fibre development.

The nucleotide sequences of three of the fibre-specific clones were similar to known sequences. The sequence of pFS1 showed similarity to a previously reported fibre-specific cDNA, encoding a protein of unknown function. pFS17 encodes a member of a class of well-characterised proline-rich structural proteins (PRPs) which are present in the walls of plant cells and a role for PRPs during elongation of the fibre cell is envisaged.

Messenger RNA corresponding to the remaining clone, pFS6, was the most abundant fibre-specific transcript. The pFS6 nucleotide sequence and its derived amino acid sequence

showed substantial similarity to phospholipid transfer proteins (LTPs), a class of plant proteins which has been implicated in the biogenesis of cellular membranes or in the deposition of cutin. A gene family of at least six members potentially encodes LTP-like mRNAs in *G. hirsutum*, of which two were isolated in a single 18 kb genomic clone. Sequence analysis of this clone suggested that neither is likely to encode the pFS6 group of fibre-specific transcripts. Screening of nucleotide databases was uninformative for the remaining two clones, pFS18 and pFS3.

Previous studies suggested that ribosomal RNA metabolism is related to final fibre length in cotton. The nucleolar size in fibres from three *G. hirsutum* varieties, differing in their final fibre lengths, was measured at early stages of growth. The nucleolar growth profiles differed between the three varieties, confirming previous work. One of several levels at which accumulation of rRNA may be controlled is by quantitative variation in the number of copies of the rRNA gene. The rDNA repeat unit from *G. hirsutum* var. Deltapine 90 was cloned and used to estimate the number of rRNA gene copies in six cotton varieties. Significant differences were observed between some of the varieties, but these did not correlate with final fibre length.

Results from this work clearly have commercial potential. Manipulation of the structure of fibre-specific genes provides exciting prospects for the modification of cotton fibre characteristics in transgenic plants. Alternatively, promoters isolated as a result of this study could be used to control the expression of heterologous genes specifically within the cotton fibre.