Analysis of Gene Expression During Cotton Fibre Development

Sharon J. Orford and Jeremy N. Timmis

Department of Genetics, The University of Adelaide, S.A. 5005

Introduction

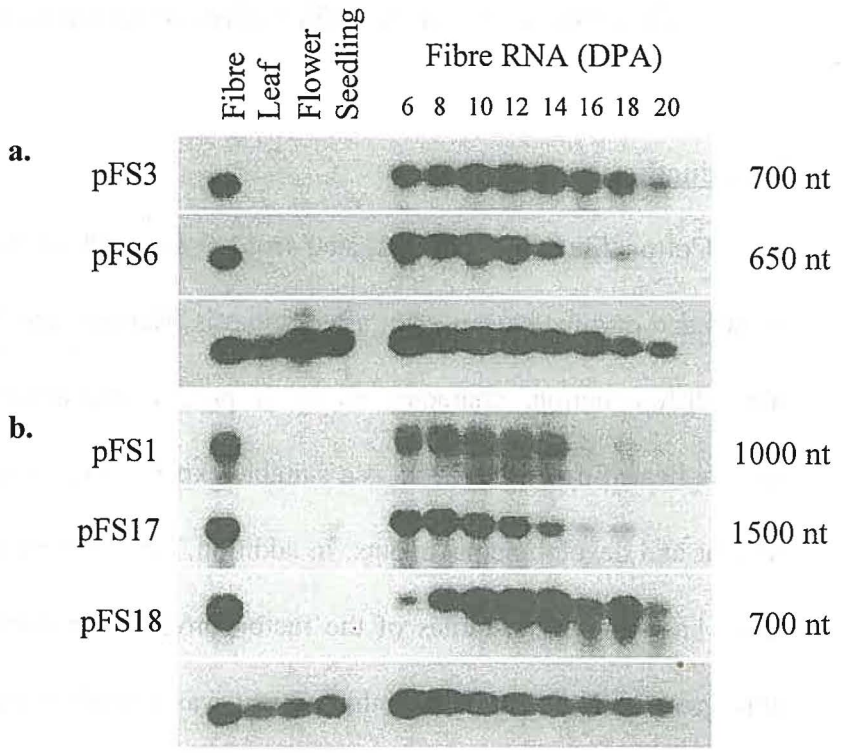
Cotton fibres are differentiated from single cells of the outer epidermis of ovules and originate at, or soon after, anthesis (Ramsey and Berlin, 1976). Cotton fibre differentiation, characterised by a precise and synchronous growth and uncomplicated by cell division, is a suitable experimental system in which to study 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 main aim of this project was to isolate and characterise cDNA clones of mRNAs which are specific to, or important in, cotton fibre development.

Results and Discussion

Differential screening of a 13 DPA cotton fibre cDNA library constructed from poly(A)$^+$ RNA of *G. hirsutum*, cv. Siokra 1-2 resulted in the identification of 24 putative fibre-specific messages. Cross-hybridisation eliminated duplicate clones, reducing the clone population to six different sequence types, of which five (pFS1, pFS3, pFS6, pFS17 and pFS18) showed tissue-specificity, hybridising strongly to transcripts in fibre RNA but not to RNAs from leaf, whole flower or seedling tissue (Figure 1). The temporal pattern of transcript accumulation in fibre development varied between clones (Figure 1).

Sequence information for cDNA clones corresponding to the five

Figure 1
Northern analysis of five putative fibre-specific cDNA clones.



a. and b. represent independent Northern blots with 10 µg of RNA in each lane, sequentially hybridised with the specific probes indicated. Fibre ages are indicated in days post-anthesis (DPA). Total RNA loadings in each track were monitored by hybridisation with a ribosomal probe and shown in the lower panel of each blot. Each clone detected a single transcript, the approximate sizes of which are indicated on the right in nucleotides.

differentially-expressed mRNAs was used to determine potential functions of the encoded proteins. The sequence of pFS1 showed similarity to a cDNA previously isolated from cotton fibres (John and Crow, 1992). The protein is thought to have a unique enzymatic or structural role in primary cell wall deposition, compatible with predominance of pFS1 transcripts early in fibre development (Figure 1).

pFS17 encodes a member of a class of well-characterised proline-rich proteins or PRPs. PRPs play a structural role in the walls of plant cells and are

thought to have a cross-linking or defense-related function (Showalter, 1993). A role for PRPs during elongation of the fibre cell is therefore envisaged. PRP genes are tightly controlled and often exhibit cell type specificity, such as the one isolated from cotton fibres. Southern analysis showed that PRPs in *Gossypium hirsutum* may form a small gene family, typical of PRP genes in other plants.

Messenger RNA corresponding to clone pFS6 was the most abundant fibre-specific transcript in 13 DPA fibres. 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 (Arondel and Kader, 1990; Pyee and Kolattukudy, 1995). The LTP isolated in this study differed significantly in sequence from that previously isolated from *G. hirsutum*, cv. DES119 (Ma *et al.*, 1995). A gene family of at least six members potentially encodes LTP-like mRNAs in *G. hirsutum*, cv. Siokra 1-2, 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.

As for pFS17 and pFS6, rapid amplification of cDNA ends (5'-RACE) was used to obtain the 5' end of pFS3. However, screening of nucleotide databases was uninformative for the remaining two clones, pFS3 and pFS18.

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

References

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