ISOLATION AND CHARACTERIZATION OF A DEVELOPMENTALLY-REGULATED COTTON GENE

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Abstract

Identifying genes involved in the initiation of fiber development is one approach that will lead ultimately to improving cotton fiber yield. By comparing the RNA transcripts produced by a wild type line, Texas Marker-1 (TM1), and a near isogenic mutant, Naked Seed (N1), we hoped to identify genes that are important for the initiation of fiber development. TM1 is a normal upland cotton that is a long term inbred line (Kohel, et al., 1970). N1 mutants have only a small tuft of lint fiber at the chalazal end of the seed and fuzz fibers are completely absent (Carver, 1929).

Using differential display analysis (Liang and Pardee, 1992) to compare early fiber development between TM1 and N1, we have identified a number of differentially expressed gene fragments that are different in the two cotton lines. One of these gene fragments (GhGLP1) is expressed specifically in the immature locules of TM1, but not expressed in N1. The open reading frame for the full length cDNA corresponding to this fragment was obtained by 5´ RACE (rapid amplification of cDNA ends) PCR.

A comparison with public DNA sequence databases (GenBank) by BLAST searching showed that the primary sequence of GhGLP1 had high homology with the polypeptide sequence of *Prunus persica* auxin-binding proteins (ABPs) and another family of proteins known as germin and germin-like proteins.

Germins were first identified as proteins that are expressed very early during seed germination in wheat and barley (Lane, 1994). The native protein is a pentamer of identical 26 kDa subunits. In monocots, germin is glycosylated with highly substituted glucuronogalactoarabinoxylans, is highly resistant to protease activity, and is localized in the cell wall. A comparison of the germin deduced amino acid sequence with protein sequences in GenBank led to the discovery that germin functions as an oxalate oxidase. Generation of H_2O_2 by oxalate oxidase may function in plant cell walls as a fungicidal compound or as a catalyst of cell wall polymers by oxidative crosslinking.

In several plant species, cDNAs with sequence homology to germin have been found. Such germin-like proteins (GLPs) found in barley (HvGLP1) and in *Arabidopsis* (AtGLP) show no oxalate oxidase activity and their expression patterns are clearly distinct from germin gene expression. Despite the considerable sequence similarity, it is possible that the function of GLPs is different from germin (Vallelian-Bindschedler et al., 1998).

The auxin binding protein from *Prunus persica* contains regions of homology with germin-like proteins (Ohmiya et al., 1998). A 18 polypeptide region in GhGLP1 is partially homologous with the *Prunus persica* auxin binding domain (78 %) and the auxin binding domain of the highly characterized maize ABP1 gene (40 %). The physiological significance of a putative auxin binding domain in the germin-like proteins is unknown at present.

The primary and secondary structures of GhGLP1 are more similar to those of GLPs than germin protein. Northern blot analysis confirmed that GhGLP1 was expressed specifically in the immature locules of TM1, but not in N1. Expression of GhGLP1 was tissue-specific and developmentally regulated. Transcripts of approximately 1.3 kilobases were detected as early as 2 days postanthesis (DPA) in TM1 locules. Expression levels were high throughout the period of fiber cell elongation (4 to 14 DPA), but dropped dramatically when secondary cell wall synthesis began (16 DPA). The transcript was more abundant in fiber than in ovules, and was not detected in 10 day old cotton cotyledons, hypocotyl, root, or ungerminated pollen.

The GhGLP1 gene has been cloned in the expression vector, pET29a(+) for over-expression of this protein in *E. coli*. This recombinant protein will be used to generate polyclonal antibodies to the cotton germin-like protein for analysis of the sub-cellular localization and function of this gene in cotton fiber.

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