Expression of a cotton profilin gene *GhPFN1* is associated with fiber cell elongation*

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Abstract Profilin is an important actin-binding protein involved in regulating the organization of actin filaments. A cotton profilin gene (*GhPFN1*) that shares 71% identity to *profilin1* of *Arabidopsis* in its amino acid sequence was isolated. Semi-quantitative RT-PCR showed that *GhPFN1* was expressed preferentially in the developing cotton fibers and reached the highest level at the fast elongation stage. The function of *GhPFN1 in vivo* was analyzed using the *S. pombe* system, and results suggested that *GhPFN1* plays a role in fiber cell elongation.

Keywords: actin cytoskeleton, profilin, cotton fiber, *S. pombe*.

In plant cells, several cellular processes like cell division, differentiation, polar growth, and response to pathogen attack depend on rapid reorganization of the actin cytoskeleton[1~3]. The reconstruction of actin filaments is controlled by a wide variety of actin-binding proteins, of which, profilin is one of the key modulators.

Profilins, small proteins that bind to monomer actin (G-actin) in 1∶1 molar ratio, are ubiquitously present in organisms ranging from fungi to higher plants and mammals. In addition to actin, profilins also interact with poly-L-proline (PLP), proline-rich proteins, and several multi-protein complexes. It has been shown that profilins have two opposite effects on the assembly of actin filaments. On one hand, profilins can promote actin polymerization at the barb ends by lowering the critical concentration[4]. On the other hand, these proteins can cause the depolymerization of actin filaments by binding and sequestering G-actin[5]. In plants, profilins were found to be encoded by a multigene family in many species such as maize, tomato, tobacco and *Arabidopsis*[6~9]. Although a number of profilin genes have been isolated and characterized from several plants, the cellular roles of profilins are poorly understood. At present, there are only a few reports on this aspect[10,11].

Cotton fiber is a single epidermal cell of the outer integument of the ovule. In the early developmental stage, fiber cells elongate in an extremely high speed and can reach to a length of 3~5 cm. Several striking features are exhibited in the elongation of cotton fiber including: (1) cotton fiber is unicellular, therefore cell elongation is independent from cell division; (2) cell elongation lasts for a fairly long period (about 3 weeks); (3) cells elongate synchronously. Because of these characters, cotton fiber is considered as an ideal model for studying the mechanism of plant cell elongation.

It has been shown that the organization of microtubules and actin cytoskeleton changes actively in fiber cells during the stages of cell elongation and the second cell wall synthesis. Therefore, cotton fiber is also thought to be uniquely suited to study the function of the cytoskeleton in plant cell elongation and cell wall synthesis[12].

By searching the cotton EST database, we found several cDNA sequences which encode different putative profilins. In this study, we isolated a cDNA clone representing a member of this gene family from 6DPA (days post anthesis) fibers and designated the gene as *GhPFN1*. We conducted a semi-quantitative RT-PCR to analyze the expression of *GhPFN1* in various organs and fiber cells of *Gossypium hirsutum* and found that this gene was preferentially expressed in the cotton fibers with a highest expression level at the stage of cell elongation. As a first step, we inves-

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tigated the functions of *GhPFN1 in vivo* using the *Schizosaccharomyces pombe* system. The results suggest that *GhPFN1* plays a role in cell elongation and cell shape maintenance.

1 Materials and methods

Gossypium hirsutum TM-1 was provided by Prof. He Jianxing in the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. *S. pombe* strain Q-01 was purchased from Stratagene.

The cDNA of *GhPFN1* was isolated from the total RNA of 6DPA cotton fibers. The primers were designed based on the sequence information in the cotton EST database. The total RNAs of root, leave, hypocotyl, flower and fibers of *Gossypium hirsutum* were extracted by the method of ultracentrifugation[13]. Reverse transcriptions for the first-strand cDNA synthesis were performed using 5 μg of total RNA as the templates. The same amounts of the first-strand cDNAs were used for semi-quantitative PCR. The upstream primer for *GhPFN1* mRNA was 5′-GCTCTAGAATGTCGTGGCAAACAT-3′, and the downstream primer was 5′-CCTCCCACCTAAACAATC-3′. PCR reactions were conducted under the following conditions: 94 ℃ 3 min, 1 cycle; 94 ℃ 1 min, 52 ℃ 1 min, 72 ℃ 1 min, 45 cycles; 72 ℃ 10 min. The upstream primer for *histon* mRNA was 5′-CCCGTAAGTCTACTGGTG-3′, and the downstream primer was 5′-TCTAAGCGACTGATCCAC-3′.

DNA sequence was determined by an automated DNA sequencer (GeneCore, Shanghai). Protein sequences were aligned with NCBI Blast and Genedoc programs at website http://www.ncbi.nlm.nih.gov/.

The cDNA of *GhPFN1* was subcloned into the yeast vector pREP1 that contained a thiamine repressible promoter *nmt-1*, the recombinant plasmid was transformed into *S. pombe* cells by electroporation. Cells from the selected clone were grown to the mid-exponential phase in minimal medium containing 2 μmol/L thiamine, washed three times with the minimal medium without thiamine to derepress the *nmt-1* promoter, and then incubated at 28 ℃ for 22 h to express the protein. The cells were fixed and stained for nuclear (DAPI), septum material (Calcofluor) and then examined by fluorescence microscopy according to standard protocols[14].

2 Results and discussion

2.1 Cloning of *GhPFN1* cDNA and protein sequence alignment

The cDNA of *GhPFN1* was cloned from the total RNA of 6DPA cotton fibers as described in the materials and methods. Sequence analysis revealed that the cDNA encoded an open reading frame of 393 bp. Protein sequence alignment showed that GhPFN1 was highly homologous to the profilins from other plants and animals (Fig. 1), with an identity of 71% to *profilin1* of *Arabidopsis*. GhPFN1 contained actin-binding residues similar to those found in profilins of *Arabidopsis*. Apart from this, GhPFN1 also comprised the highly conserved residues implicated in PLP binding. It is known that PLP-binding is one of the most important biochemical activities of profilin proteins.

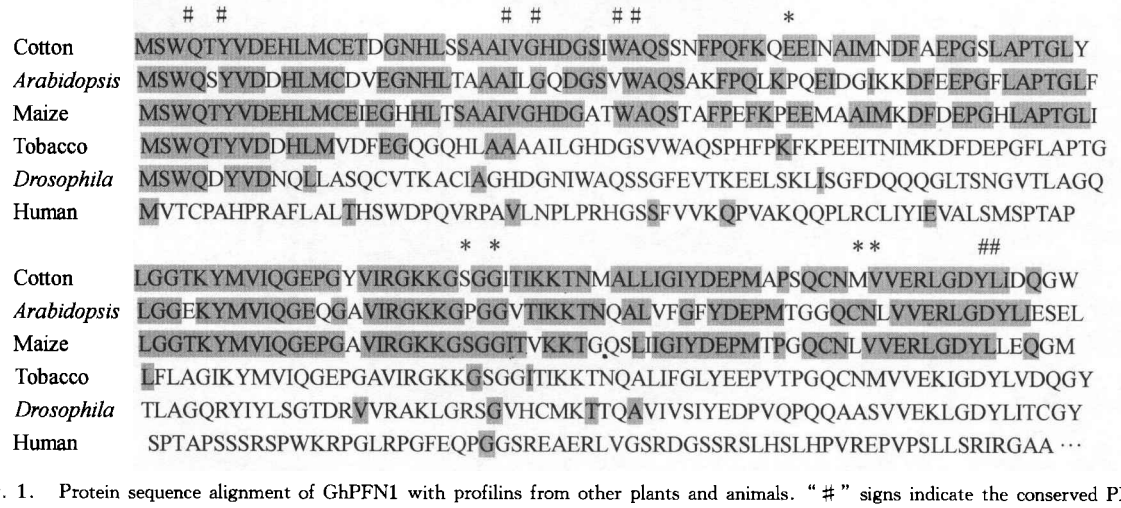

Fig. 1. Protein sequence alignment of GhPFN1 with profilins from other plants and animals. "#" signs indicate the conserved PLP binding residues. Actin-binding residues are marked with asterisks.

2.2 Expression analysis of *GhPFN1* in different organs and fiber cells

Because of the low expression level of *GhPFN1*, we performed a semi-quantitative RT-PCR to analyze the *GhPFN1* expression in different organs and fibers. As shown in Fig. 2, *GhPFN1* gene was expressed mainly in the fiber cells (lanes 1~8), the expression level was rather low in roots and hypocotyls, and almost no signals were found in other organs. During fiber development, *GhPFN1* transcripts were most abundant at the fast elongation stage (lanes 1~4). These results were confirmed by several independent experiments. As an internal control, we used the *histon* gene which has been shown to express constitutively in all organs of *Gossypium hirsutum*.

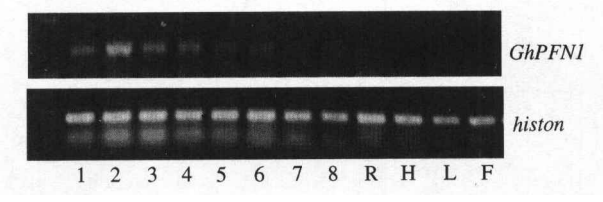

Fig. 2. Semi-quantitative RT-PCR analysis of *GhPFN1* expression in different organs and fibers. 1~8, 6, 9, 12, 15, 18, 21, 24, 27DPA fibers respectively; R, root; H, hypocotyl; L, leave; F, flower.

2.3 Effects of *GhPFN1* overexpression on the cellular morphogenesis of *S. pombe*

The fission yeast (*S. pombe*) has been shown to be a feasible system to study the function of the evolutionarily conserved plant genes[15]. In order to understand the cellular function of *GhPFN1*, *GhPFN1* cDNA was cloned into the yeast plasmid pREP1 under the control of the inducible promoter *nmt1* and the recombinant plasmids were transformed into the yeast cells. As shown in Plate I, the yeast cells were dramatically elongated when the expression of *GhPFN1* was induced. Some of them were more than 3-fold longer than the cells before induction. Furthermore, the cell shapes were largely deformed and many cells became dumbbell-shaped. These results indicated that GhPFN1 was functional in yeast cells and suggested a role of this protein in the cell elongation and cell shape maintenance.

3 Discussion

As one of the key modulators of the actin organization, profilin has been shown to be essential in the polar growth of plant cells such as pollen tube and root hair cells[16]. Quader et al.[17] and Seagull[18] observed that the organization of the cortical microtubules and cellulose microfibrils of the cell wall correlated precisely in the elongating cotton fibers, and disruption of microtubule arrays always resulted in defects in the deposition of cell wall microfibrils. Furthermore, they also found that microfilaments might be involved in regulating microtubule organization in fiber cells. Based on these observations, microtubules and actin cytoskeleton are thought to play pivotal roles in cotton fiber elongation.

In this paper, we report that the *GhPFN1* gene of cotton expressed preferentially in the fiber cells, and the transcripts appeared most abundant at the stage of fast elongation. Since profilin is an important modulator of actin cytoskeleton, we speculate that *GhPFN1* is involved in the elongation of fiber cells by regulating microfilament organization which affects the orientation of microtubule cytoskeleton.

It has been shown that many evolutionarily conserved plant genes have similar functions in yeast, thus yeast can be used as a fast and efficient system to study the cellular functions of certain plant genes. In our study, overexpression of GhPFN1 protein in yeast cells caused a dramatic change in the cell length and shape, this supported our speculation on the function of *GhPFN1* in the process of cotton fiber elongation. Xia et al.[15] found that the expression level of transformed plant genes in the yeast cells increased at least 10-fold after induction. Although we did not measure the quantity of profilin proteins directly, our induction experiment and the re-transformation experiment (data not shown) indicated that the changes in the cell length and cell shape of the yeast cells were results of *GhPFN1* overexpression.

Our result provides a piece of evidence about the function of profilin gene in the elongation of cotton fiber cells. Since *GhPFN1* is mainly expressed at the stage of fiber elongation, its promoter may be used as a regulatory element of the gene expression in the genetic engineering of cotton fiber.

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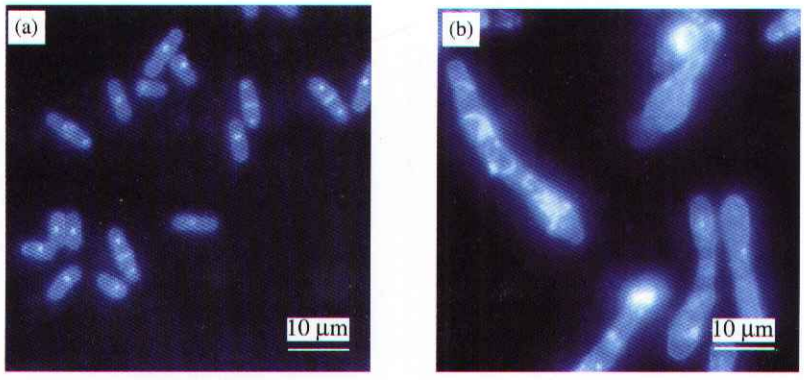

Plate Ⅰ. Effect of *GhPFN1* overexpression on the cell morphogenesis of yeast. (a) *S.pombe* cells, before induction of *GhPFN1* cDNA; (b) *S.pombe* cells, after induction of *GhPFN1* cDNA.