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Effect of GbKTN1 from *Gossypium barbadense* on cell elongation of fission yeast (*Schizosaccharomyces pombe*)

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Abstract **The *GbKTN1* gene was isolated from 10 DPA fiber cells of *Gossypium barbadense* using 5′RACE/3′RACE. Full-length cDNA of this gene is 2006 bp, including a 113 bp of 5′untranslated region, a 1563 bp of an open reading frame (ORF), and a 327 bp of 3′untranslated region (excluding the stop codon TAA). The ORF of *GbKTN1* encodes a 521-amino acid protein with a predicted size of 55 kD. Near C-terminal of the deduced protein there is a putative ATP binding site between amino acid residues from 233 to 414. Southern blot analysis indicated that the *GbKTN1* was a single copy gene in *G. barbadense*. Combining semi-quantitative RT-PCR with Southern blot hybridization revealed that *GbKTN1* expressed in all the organs detected such as roots, stems, leaves and fibers. However, the mRNA of *GbKTN1* was the most abundant in fiber cells, while it was the lowest in leaves. The *GbKTN1* cDNA was transformed into *S. pombe* to verify its function on cell elongation. Results showed that most yeast cells over expressing *GbKTN1* gene were elongated dramatically with an average length increase of 2.18 times than that of the non-induced cells. Even the morphology of some yeast cells appeared irregularly. To the best of our knowledge this is the first evidence that KTN1 is correlated with cell elongation *in vivo*.**

Keywords: Sea Island cotton (*G. barbadense*), *GbKTN1*, gene expression, fission yeast, cell elongation.

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Cotton fibers are important materials for textile industry with a long history. Recently, along with the innovation of air-spinning technology and the improvement of peoples' living standards, a much higher fiber quality is demanded.

Cotton fiber is a single, elongated and thickened cell of the ovule coat epidermis. The development of cotton fiber can be divided into four phases: initiation, elongation, secondary wall formation and maturation, which determine the lint yield, length, strength, fineness, etc.[1] These four phases are overlapped without definite boundaries. In general, fiber initiation occurs from several days of pre-anthesis to 1—2 d of post-anthesis (DPA). The elongation of fiber initiates on the day of anthesis and continues to do so for a period of ca. 20—30 d. Primary wall formation is at initiation and elongation phases, while secondary wall deposition begins from cessation of primary wall synthesis enabling fiber cells to function as an excellent mechanical tissue.

Fiber length, strength and fineness are essential traits of cotton fiber quality, which are mainly determined during fiber elongation and secondary wall deposition. Understanding regulatory mechanisms of cell elongation and cell wall biosynthesis at the molecular level will allow us to discover more functional genes that can be used in genetic engineering for cotton fiber quality improvement.

It is well known that cell walls are mainly composed of cellulose and hemicellulose. Researches on the mutants defective in genes are involved in cell wall synthesis and modification has shown that cell walls play essential roles in regulating cell morphology[2—7]. Microtubules, the important component of plant cytoskeleton, are key players in plant cell division, polar growth and differentiation[8,9]. Many lines of evidence have indicated that microtubules regulate cellulose-microfibril orientation, there by influencing cell wall biosynthesis and elongation. However, there is no direct evidence on how microtubules affect cell wall biosynthesis[3,13].

Katanin, a microtubule-severing protein firstly isolated from sea urchin, *Xenopus laevis*[14], is a heterodimer of 60- and 80-kD subunits and couples ATP hydrolysis to disassemble microtubules into tubulin subunits. The 60-kD subunit alone possesses both microtubule-stimulating ATPase activity and microtubule-severing activity[15—17]. Recently, the mechanisms of katanin-mediated microtubule severing in sea urchin have been proposed[18]. AtKTN1, the first and only known katanin-like microtubule-severing protein involved in cellular activity in higher plants, was identified from an *Arabidopsis* fragile fiber mutant *fra2*. The *fra2* mutant showed defection in the mechanical strength of interfascicular fibers in the inflorescence stems, due to significant reduction of both primary and secondary wall thickness, cellulose and hemicellulose content[3]. It has been shown that AtKTN1 possesses MT-severing activity *in vitro*[4,19] and is essential for normal cell wall biosynthesis and cell elongation in fiber cells of *Arabidopsis*[4].

The fibers of *Gossypium barbadense* are relatively fine but with compact structure and higher strength, most of them are longer than 33 mm. Its quality is superior among all the four cultivated species in the genus *Gossypium*[20]. Therefore, in this study we chose *G. barbadense* as a starting material, from which a full-length *GbKTN1* cDNA was isolated. The expression pattern of *GbKTN1* in different organs of cotton and the effect on cell elongation in *Schizosaccharomyces pombe* were analyzed.

1 Materials and methods

(ⅰ) Materials. For extraction of total RNA and genomic DNA, roots, leaves and stems of *G. barbadense* var. 7124 were harvested from three-week-old seedlings, fibers without ovules were collected from 10 DPA bolls and quickly frozen in liquid nitrogen and stored at −70℃.

(ⅱ) Primers. Primers were designed and synthesized as indicated in Table 1.

Table 1 Synthetic oligonucleotide primers and their corresponding locations in *GbKTN1* cDNA sequence

Primers	Nucleotide sequences	Corresponding locations in Fig.1/nt
p-RT	5′-Ⓟ-ACATTTGTGAGATC-3′	1458—1471(−)
p-1F	5′-GTGATGGTTTTGGCTGCAACTAACT-3′	1257—1282
p-1R	5′-CACCTTTCCATGGTCTCCTAATTCCC-3′	905—930(−)
p-2F	5′-TCGTCGAACAGAAGGATACAG-3′	1430—1450
p-2R	5′-GCTTCAGTCAAACCAGCAAC-3′	828—847(−)
p-F	5′-ATGGTTGGAAATTCGCTAGCT-3′	114—134
p-R	5′-GTTTATGCAGATCCGAATTCTG-3′	1660—1681(−)
Actin F	5′-TGGTCAAGGCTGGATTTGC-3′	—
Actin R	5′-TGGATAGCAACATACATAGC-3′	—

(−) represents low strand of *GbKTN*.

(ⅲ) Cloning of full length *GbKTN1* cDNA. The 3′ end of *GbKTN1* gene was obtained from cotton fibers with primer p-2F according to the manufacturer's protocol of 3′ RACE kit (Takara, Japan). Likewise, according to the protocol of 5′ RACE kit (Takara, Japan), the first strand cDNA was generated using RT-PCR with specific primer p-RT phosphorylated in its 5′end, followed by ligation with T4 RNA Ligase. Then by using circular cDNA as a template, a two-round of nest PCR was carried out with primers p-1F/p-1R in the first round and primers p-2F/p-2R in the second round. The RACE products were cloned into pMD18T-Vector (Takara, Japan) and positive clones were sequenced. Finally, the 5′ and 3′sequences were assembled with DNAMAN to generate full-length cDNA of *GbKTN1* gene.

(ⅳ) Semi-quantitative RT-PCR. First strand cDNA was synthesized with five micrograms of total RNA, oligo d(T) primer and superscript reverse transcriptase according to the manufacturer's protocol (Gibco/BRL, USA). Semi-quantitative RT-PCR was performed with primers of p-F/p-R as described by Burton[21]. One micro liter of first strand cDNA was used as template and PCR-amplification was carried out in a 50 μL reaction system with primers p-F/p-R. Conditions for PCR reaction were: 3 min at 96℃ 30 s at 94℃, 1 min and 20 s at 52℃, 1 min at 72℃ for 26 cycles and finally 72℃ for 10 s. Internal control *ACTIN1* was amplified with primers of ActinF/ActinR under conditions at 96℃ for 3 min, 94℃ for 30 s, 52℃ for 30 s, 30 s at 72℃ for 26 cycles and 72℃ for 10 min.

(ⅴ) Southern blot analysis. Fifteen micrograms of *G. barbadense* genomic DNA was extracted and digested with restriction enzyme *Hin*dⅢ and *Xho* Ⅰ, respectively, and then transferred onto Hybond^{TM}-N^{+} (Am-Pharmacia, UK) membrane. Southern blotting was conducted by using a partial sequence of the seventh exon (1381—1679 nt in Fig. 1) of *GbKTN1* gene as probe labeled with α-^{32}P-dCTP by using Primer-a-gene Labeling System (Promega, USA). The hybridization was performed at 68℃ for 12 h, then the X-ray film was exposed at −70℃ for 24 h.

(ⅵ) Expression of *GbKTN1* gene in fission yeast. The yeast expression vector pEKTN1 constructed by inserting *GbKTN1* gene into pESP-2M was transformed into *S. pombe*. *GbKTN1* gene expression was induced as described by Maundrell[22]. The morphology of yeast cells was investigated with Olympus BX51 to determine whether it was influenced by over expression of *GbKTN1* gene compared with that of the non-induced yeast cells. Totally 10 views with each containing 15—30 cells were investigated.

2 Results

(ⅰ) Cloning and sequencing of *GbKTN1* gene. The DNA sequence of *AtKTN1* was used to search Genbank by Blast$_n$, as a result, an EST clone (GenBank accession number: AI729407) from upland cotton (*G. hirsutum*) has shown significant homology to the *AtKTN1* gene. Based on this information, we designed primers described above and performed 5′RACE/3′RACE to clone 5′ and 3′ end and the full- length cDNA of *KTN1* from fiber cells of *G. barbadense*. The full-length cDNA is 2006 bp, including a 113 bp of 5′ untranslated region, a 1563 bp of an open reading frame (ORF) and a 327 bp of 3′ untranslated region (excluding the stop codon TAA). The ORF encodes a 521-amino acid protein with a molecular weight about 55 kD (Fig. 1, AY324647). Amino acid sequence analysis of this protein using Blast search indicates 82% identity to AtKTN1 (AF358779). In addition, we have also cloned *GhKTN1* from *G. hirsutum*, which has shown 95% identity of amino acid sequence to GbKTN1 (AY324646). Although the entire amino acid sequence of GbKTN1 has only 52% and 35% sequence identity with sea urchin (*Xenopus laevis*) katanin 60 kD subunit (AF177942) and nematode (*Caenorhabditis elegans*) MEI-1 (NM059856) (Fig. 2), a higher (77% and 59%) sequence identity has exhibited between 233 and 414 amino acid residues of GbKTN1 compared with the putative ATP binding site in that of the above two species (Fig. 3). The "Walker A" motif[23] of ATPases is also found in *GbKTN1* gene (Fig. 3).

(ⅱ) Copy number of *GbKTN1*. Fifteen micrograms of genomic DNA of *G. barbadense* were digested with *Hin*d Ⅲ or *Xho* Ⅰ, respectively. Southern blot showed that one band of size 2 to 3 kb both appeared in lines #1 and #2 (Fig. 4). Meanwhile, we have isolated and

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ACATATCCATTCGACCACTCAAAGCCCGAAAAAAAATAGATTTTTCTTCTTCAAATTTAGAACCCTAGAATTTGGGAGGTTTGCTGTTTTTGGATTTTCATGGT  104
GAATTCGAAATGGTTGGAAATTCGCTAGCTGGACTGCAAGATCACTTGAAATTGGCTCGAGAATACGCTCTCGAAGGCCTCTACGACACTTCCATTATCTTCTTT  209
          M  V  G  N  S  L  A  G  L  Q  D  H  L  K  L  A  R  E  Y  A  L  E  G  L  Y  D  T  S  I  I  F  F    32
GATGGTGCAATTGCTCAGATCAACAAGCATCTAAACACACTTGATGACCCGTTAATTCGATCCAAATGGATGAATGTGAAGAAAGCACTGTCTGAGGAGACAGAA  314
 D  G  A  I  A  Q  I  N  K  H  L  N  T  L  D  D  P  L  I  R  S  K  W  M  N  V  K  K  A  L  S  E  E  T  E    67
GTTGTGAAGCAACTGGATGCTGAGAGAAAGGCATTTAAGGAAGCTCCAAATGGGGCGGCGGTCCTTCTTCCCACCCGATCATGCCAAATCATCTTTTTGTGTTTC  419
 V  V  K  Q  L  D  A  E  R  K  A  F  K  E  A  P  N  G  A  A  V  L  L  P  T  R  S  C  Q  I  I  F  L  C  F  102
CACCCTCTTGATGAGTACCCAACTTCATCGGGTGCTCCAATGGATGATCCTGATGTGTGGAGGCCTCCAAGTCGGGACACATCAACTAGAAGACCTGCTAGGGGT  524
 H  P  L  D  E  Y  P  T  S  S  G  A  P  M  D  D  P  D  V  W  R  P  P  S  R  D  T  S  T  R  R  P  A  R  G  137
GGTCAAGCGGGAATGAGAAAGTCTCCCCAAGATGGGATTTCGGGTCGTGGTAATACTAGAACAGCTGCAACTGGACGTGGTGCTAAGGCTGGTGCTTCAAGTAGA  629
 G  Q  A  G  M  R  K  S  P  Q  D  G  I  S  G  R  G  N  T  R  T  A  A  T  G  R  G  A  K  A  G  A  S  S  R  172
ACTAACACGGGGGTCAGAGGATCTACCACTGGAAAAAAGGGTACTGGTTCTGGGAAATCTAGCAAAGGCGATTCGGCAAATGGTGATGCTGAAGATGGAAAGTTG  734
 T  N  T  G  V  R  G  S  T  T  G  K  K  G  T  G  S  G  K  S  S  K  G  D  S  A  N  G  D  A  E  D  G  K  L  207
AAGAGGTCACAGTATGAGGGGCCTGATCCAGATTTAGCTGAAATGCTGGAAAGGGATGTCTTAGAAACCACTCCTGGAGTGCGGTGGGATGATGTTGCTGGTTTG  839
 K  R  S  Q  Y  E  G  P  D  P  D  L  A  E  M  L  E  R  D  V  L  E  T  T  P  G  V  R  W  D  D  V  A  G  L  242
ACTGAAGCAAAAAGGCTTTTAGAGGAAGCTGTTGTTCTTCCTCTATGGATGCCTGAGTATTTTCAGGGAATTAGGAGACCATGGAAAGGTGTTCTTATGTTTGGC  944
 T  E  A  K  R  L  L  E  E  A  V  V  L  P  L  W  M  P  E  Y  F  Q  G  I  R  R  P  W  K  G  V  L  M  F  G  277
CCTCCTGGAACTGGCAAAACGCTCCTGGCTAAAGCAGTTGCCACTGAGTGCGGAACAACTTTTTTCAATGTTTCTTCTGCTACTTTAGCCTCAAAGTGGCGTGGG 1049
 P  P  G  T  G  K  T  L  L  A  K  A  V  A  T  E  C  G  T  T  F  F  N  V  S  S  A  T  L  A  S  K  W  R  G  312
GAGAGTGAACGCATGGTTCGGTGCTTGTTTGATCTTGCAAGAGCTTATGCACCCAGTACAATTTTTATTGATGAGATTGACTCTCTTTGCAATGCCCGTGGGGCT 1154
 E  S  E  R  M  V  R  C  L  F  D  L  A  R  A  Y  A  P  S  T  I  F  I  D  E  I  D  S  L  C  N  A  R  G  A  347
TCTGGTGAGCATGAGTCATCTAGGAGGGTTAAATCCGAACTTCTTGTTCAGGTAGATGGTGTAAATAATACTGGCACAAACGAAGATGGTAGCCGTAAGATTGTG 1259
 S  G  E  H  E  S  S  R  R  V  K  S  E  L  L  V  Q  V  D  G  V  N  N  T  G  T  N  E  D  G  S  R  K  I  V  382
ATGGTTTTGGCTGCAACTAACTTCCCATGGGACATAAATGAGGCTCTCAGGAAGCGACTGGAAAAGCGTATTTACATTCCTCTGCCTAATTTTGAAAGTCGTAAG 1364
 M  V  L  A  A  T  N  F  P  W  D  I  N  E  A  L  R  K  R  L  E  K  R  I  Y  I  P  L  P  N  F  E  S  R  K  417
GAGCTTATTCGGATCAATTTAAAAACAGTCGAAGTGGCTGCTGATGTGGATATTGATGAAGTGGCTCGTCGAACAGAAGGATACAGTGGGGACGATCTCACAAAT 1469
 E  L  I  R  I  N  L  K  T  V  E  V  A  A  D  V  D  I  D  E  V  A  R  R  T  E  G  Y  S  G  D  D  L  T  N  452
GTTTGTCGCGATGCTTCCTTGAATGGCATGAGACGAAAAATAGCGGGAAAGACACGAGATGAGATCAAGAACATGTCAAAAGATGAGATTTCAAAAGACCCTGTT 1574
 V  C  R  D  A  S  L  N  G  M  R  R  K  I  A  G  K  T  R  D  E  I  K  N  M  S  K  D  E  I  S  K  D  P  V  487
ACAATGTGTGACTTCGAAGAAGCCTTGGCGAAAGTCCAGCGAAGTGTTTCACAAGCCGATATCGAGAAACATGAAAAATGGTTTTCAGAATTCGGATCTGCATAA 1679
 T  M  C  D  F  E  E  A  L  A  K  V  Q  R  S  V  S  Q  A  D  I  E  K  H  E  K  W  F  S  E  F  G  S  A  *  521
ACAAATTCCTAAACCCTTTGATTTTTCTTTCTCGAGGACGATATATTGTTTTTGTTTGGTTTTGTGATATACATGCATCGGAAGAAAGGGTGTTCCAATTCATTG 1784
TTATTTCTTAGTTTTCTTGGCATTTGATTTGTTCCTTAAAAAAATGGTTGATTGATTGTAATTTTAGTGTTCAAGAAAGTTATTACATTGTTGTGCTATTTGTTT 1889
GTTTAATCGTTGTCGCTGAAACCTTATCATTGTTATCAAATATTATCAGTAAACTGTTTCCTTGAGTGAAATCCCATATATTTATTATAAAAAAAAAAAAAAAAA 1994
AAAAAAAAAAA                                                                                                  2006
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Fig. 1. Nucleotide and deduced amino acid sequence of *GbKTN1* cDNA from *G. barbadense*.

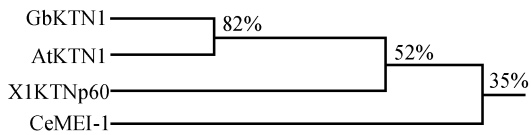

Fig. 2. Homology of the amino acid sequence of GbKTN1 with AtKTN1 (*A. thaliana*), XlKTNp60 (*X. laevis*) and CeMI-1 (*C. elegans*).

partially sequenced the *GbKTN1* genomic DNA, which contains six introns just like the *AtKTN1* (data not shown). Based on the size of signal band in Southern blot and the restriction sites in partial sequence of *GbKTN1* genomic DNA, it can be determined that *GbKTN1* is a single copy gene in *G. barbadense*.

(iii) *GbKTN1* expression in different organs of *G. barbadense*. To confirm the transcriptional expression of *GbKTN1* gene, semi-quantitative RT-PCR was performed. *GbKTN1* expression in *G. barbadense* could be detected in roots, stems and fibers, whereas it was absent in leaves (Fig. 5). However, a weak signal could be found by PCR-Southern blot analysis (data not shown). The result demonstrated that *GbKTN1* was expressed in all of the organs detected with a variable expression level. The most abundant expression was found in 10 DPA cotton fibers. If it was calculated as 100%, then the quantity of RNA in stems and roots was 65% and 44%, respectively. In RT-PCR amplification, the level of *GbACTIN1* RNA, which severed as an internal control, was similar in all the cases.

(iv) Over expression of *GbKTN1* gene promoted yeast cell elongation. *S. pombe* is a useful model system for analysis of microtubule function[24]. Since *GbKTN1* encodes a katanin-like protein, which suggests that it has a microtubule-severing function, probably related to the fiber cell elongation and cell wall biosynthesis. Therefore, it was transformed into *S. pombe* to verify its function. Totally 130 induced and non-induced yeast cells, respectively, were randomly chosen and the length of the cells was measured. Results showed that the mean value of the length of the non-induced yeast cells is 5.99±1.24 μm, while the length of the induced yeast cells is 13.07±2.88 μm. Statistic analysis indicated that the cell length of the two groups was significantly different ($P < 0.01$) (Table 2). The length of most yeast cells over expressing *GbKTN1* gene was increased by 2.18 times on average than that of

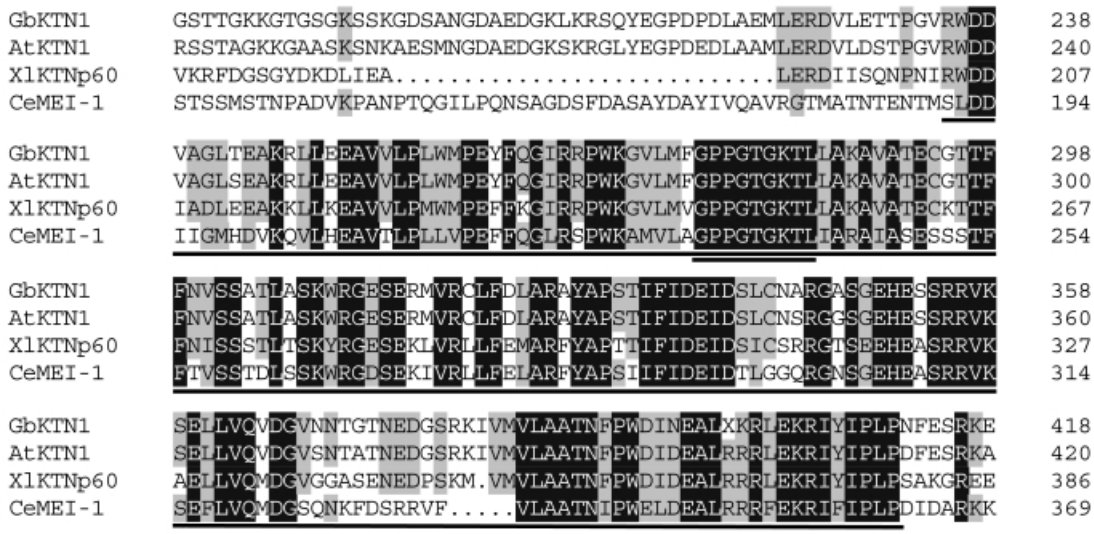

Fig. 3. Alignment of the ATP binding modules of GbKTN1(*G. barbadense*) with AtKTN1 (*A. thaliana*, AF358779), XlKTN1p60 (*X. laevis*, AF177942), and CeMEI-1 (*C. elegans*, NM059856). Amino acid residues in black are identical in all of the sequences. The conserved amino acid residues are shown in gray. The amino acid sequences underlined are the deduced ATP binding modules. "Walker A" motif is marked with double strand.

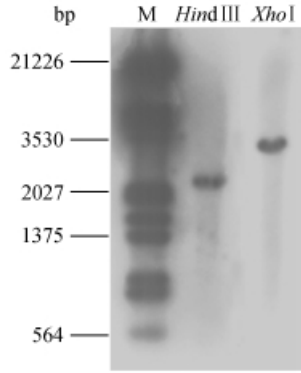

Fig. 4. Southern blot analysis of *G. barbadense GbKTN1* gene.

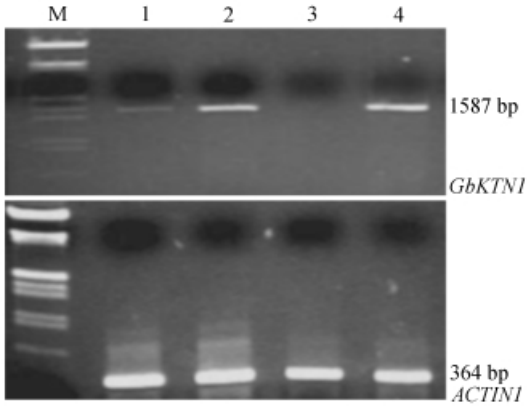

Fig. 5. Semi-quantitative RT-PCR analysis of *GbKTN1* expression in different organs of sea island cotton. Ethidium bromide-stained agarose gels show RT-PCR products. 1—4, Typical PCR products derived from roots, stems, leaves and fibers, respectively. *ACTIN1* transcripts are amplified as an internal control.

the non-expressing cells. The morphology of some induced-yeast cells even appeared irregularly (Figs. 6 and 7).

Table 2 Over-expression of *GbKTN1* gene enhances *S. pombe* cell elongation

	Non-induced	Induced
Average cell length/μm	5.99 ± 1.24	13.07 ± 2.88

* $F = 41.659 > F_{0.01} = 1.395$, indicating that the significant difference existed at 0.01 level.

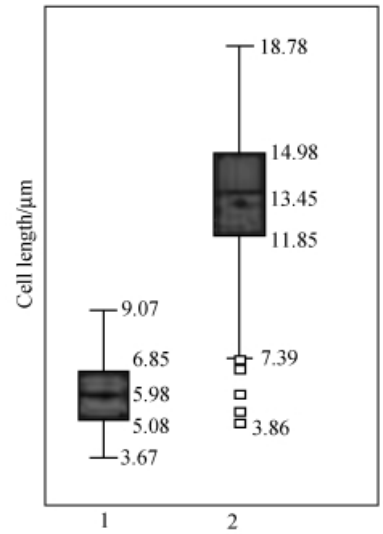

Fig. 6. Effect of *GbKTN1* over expression on cell length of *S. pombe* 1 and 2 represent the length of non-induced and induced yeast cells. Totally 130 cells of each group were measured. In group 1, cell lengths ranged from 3.67 to 9.07 μm, among which 50% cells are between 5.08 to 6.85 μm with a middle value of 5.98 μm. Cell lengths of group 2 ranged from 3.86 to 18.78 μm, with 50% cells from 11.85 to 14.98 μm and a middle value of 13.45 μm.

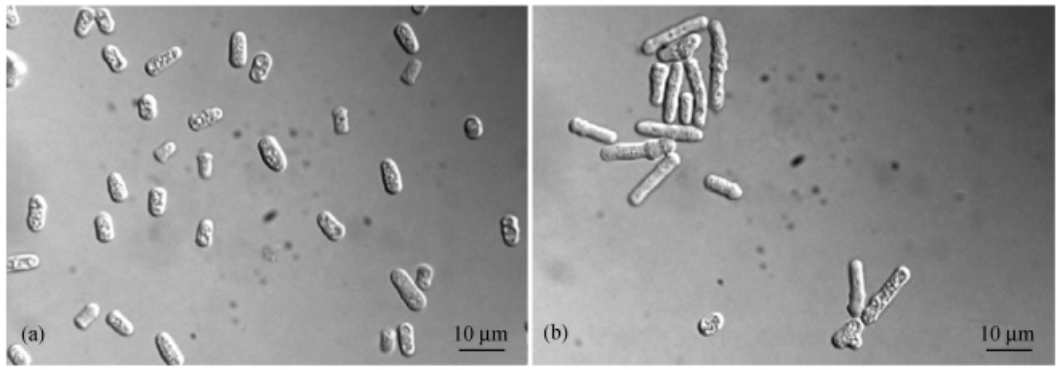

Fig. 7. Effect of *GbKTN1* over expression on cell morphology of *S. pombe* (a) Non-induced cells. (b) Induced cells with *GbKTN1* over expression (bar = 10 μm).

3 Discussion

The full-length cDNA of *GbKTN1* was isolated using 5′/3′RACE techniques. Analysis of amino acid sequence revealed that it shares certain homology with the AtKTN1[3], sea urchin katanin and *C. elegans* MEI-1[25, 26]. The highest sequence identity among these four homologous proteins is located in the ATP binding site of the C-terminal region, which is a distinct motif of katanin hydrolyzing ATP for its microtubule-severing activity. The module is highly conserved in the AAA (ATPase associated with diverse cellular activity) ATPase super-family, in which a "Walker A" motif is contained and it sometimes refers to "P loop". The "Walker A" motif, a glycine-rich region, forms a flexible loop, which interacts with one of the phosphate groups of ATP or GTP, and is a typical motif of AAA ATPase superfamily[23]. The "Walker A" motif of ATPases is also found in *GbKTN1* gene (Fig. 3). Above evidence strongly demonstrated that the *GbKTN1* we cloned is an ortholog of the *AtKTN1*, so it was named as *GbKTN1*. The GbKTN1 in *G. barbadense* is encoded by a single copy gene. At present, little is known about copy number of *KTN* gene in other higher plants including *Arabidopsis*.

It has been reported[3] that *AtKTN1* is expressed in all the organs tested such as stems, roots, leaves, flowers etc., however, no further analysis has been made to illuminate whether there is any difference among different organs. Our results showed that *GbKTN1* expression varied to some extent among different organs, with cotton fibers being the highest followed by stems. It is possible that different organs in different development stages consisted of different cell types and the *GbKTN1* is preferentially expressed in a certain type of cells. Plant cells can be grouped into three major types: parenchyma, sclerenchyma and collenchyma cells based on cell wall thickening. The former two are known to exist in the entire plant body, while collenchyma cells mainly exist in stems, venations and the cortex of petioles. Parenchyma and collenchyma cells only consist of primary wall, which provide main structure support for growing parts of the plant body, whereas sclerenchyma consists of primary walls and thickened secondary walls, which provides a major mechanical support[27]. Cotton fiber is a thickened seed hair and its plant stem serves as a mechanical supporting organ, which are abundant with sclerenchyma cells and phloem fibers. In this study we have demonstrated that GbKTN1 is highly expressed in cotton fibers and stems, therefore, we suspect that *GbKTN1* is preferentially expressed in sclerenchyma cells than in other types of cells, though defined proofs still await further investigation.

The *fra2* mutant displayed aberrant cortical microtubule orientation resulted in distorted deposition of cellulose microfibrils, which in turn led to a defect in cell elongation and cell wall biosynthesis. Further study revealed that the 3′ end deletion of *AtKTN1* was responsible for the phenotypic change, suggesting that AtKTN1 played an important role in the initiation, elongation and cell wall biosynthesis of fiber cells[3, 4]. It was known that animal katanin was responsible for the spindle microtubule severing at metaphase during mitosis, which allowed normal chromatid movement to guarantee cell division entering into anaphase[15–17]. Nevertheless, AtKTN1 acted as a player in the interphase and elongating cells, which quickly dissembled perinuclear microtubules and provided tubulin subunits for transition of perinuclear microtubule array into cortical microtubule array. The study on *Arabidopsis bot1* mutant also showed that the aberrant microtubule array had direct adverse effect on normal cell elongation[13]. As over expression of a full-length *GbKTN1* cDNA encoding a microtubule-severing protein has stimulated yeast cell elongation in this study, it is assumed that GbKTN1 may affect microtubules of yeast cells through influencing microtubule array and cell polar growth, which in turn lead to the increased cell length and irregular morphology in some cells. To the best of our knowledge, it is the first evidence that KTN1 is correlated with cell elongation *in vivo*. Based on the result of *GbKTN1* expression in 10 DPA cotton fibers and the related study on AtKTN1, we assumed that GbKTN1 might

play an important role in regulating cell elongation of cotton fibers.

So far, the AtKTN1 was the first and only known katanin-like microtubule-severing protein in higher plants[3,4]. Among all the known functions of KTN1, we are more interested in the function being required for normal cell wall biosynthesis and cell elongation in fiber cells. The correlation of GbKTN1 with cotton fiber development will be further studied in the future, which is expected to be useful for cotton fiber quality improvement through genetic engineering.

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