Acta Botanica Sinica
植 物 学 报 2004, **46** (4): 472-479 http://www.chineseplantscience.com

Identification of an Endothelium-specific Gene *GhIAA16* in Cotton (*Gossypium hirsutum*)

SUO Jin-Feng [1], PU Li [2], LIANG Xiao-E [1*], XUE Yong-Biao [1**]

(1. *Institute of Genetics and Developmental Biology, The Chinese Academy of Sciences*, Beijing 100080, China;
2. *College of Life Sciences, Beijing Normal University*, Beijing 100875, China)

Abstract: Cotton (*Gossypium hirsutum* L.) fibers are derived from the outer integument of ovule, while little is known about cotton ovule development. In order to identify and analyze the expression of genes associated with cotton ovule development, a cDNA array approach was used to screen for genes with altered expression in cotton ovule before and after anthesis, and 25 differentially expressed genes were subsequently identified. Among them, *GhIAA16* encodes a predicted polypeptide of 208 amino acids highly homologous to *Arabidopsis IAA16*. Molecular analysis revealed that it is a single-copy gene in cotton genome and specifically expressed in the ovule endothelium. To our knowledge, *GhIAA16* is the first endothelium-specific gene isolated from cotton. Its possible function is discussed during cotton ovule formation.

Key words: cDNA array; GhIAA16; cotton ovule; endothelium

An angiosperm seed including embryo and endosperm is surrounded by seed coat, which develops from outer integument of ovule, the female reproductive organ and therefore is of maternal origin. Important functions of the seed coat include protecting embryo from biotic and abiotic stresses, providing nutrients for embryo during its development as well as water and oxygen during germination, and delaying germination by controlling the strength of dormancy (Boesewinkel and Bouman, 1995).

An ovule has a simple but nevertheless highly differentiated structure. The nucellus is the terminal region of the ovule and is the site of embryo sac formation, which consists of seven cells and four different cell types: three antipodal cells, two synergid cells, one egg cell, and one central cell. Surrounding the nucellus are one or two integuments, lateral structures that usually tightly encase the nucellus. The integuments are not fused at the apex of the nucellus but have an opening, the micropyle, through which a pollen tube can gain access to the embryo sac. The basal part of the ovule is the funiculus, a supporting stalk that attaches the ovule to the placental region within the carpel (Gasser *et al*., 1998). The innermost cell layer of the seed coat is also called endothelium. In *Arabidopsis*, the seed coat consists of five cell layers: two of them form the outer integument and three form the inner integument (Léon-Kloosterziel *et al*., 1994). Genetic analyses indicate that inner and outer integument development can occur independently and the late stage embryo sac development depends on the integuments. In every case in which the integuments do not enclose the nucellus, an embryo sac fails to form. So the diploid or sporophytic integument is indispensable for the formation of the haploid multicellular embryo sac (Gasser *et al*., 1998).

Aux/IAA genes are found throughout the plant kingdom and were first identified because many of them are rapidly induced as a primary response to auxin (Abel and Theologis, 1996). The *Arabidopsis thaliana* genome contains at least 29 *Aux/IAA* genes, many of which were identified because of their rapid induction after auxin treatment (Liscum and Reed, 2002). The *Aux/IAA* genes encode short-lived nuclear proteins, which have a relative molecular mass of 25–35 kD and share four conserved domains, designed **I-IV**. Domains **III** and **IV** mediate homo- and heterodimerization between Aux/IAA proteins and heterodimerization with members of a second large protein family called the auxin-response factors (ARFs), most of ARFs also contain domains **III** and **IV** as well. The ARF proteins are transcription factors that bind to auxin-response elements (AuxRE) located upstream of auxin-inducible genes (Leyser, 2001). Domain **II** has been demonstrated to act as a transferable protein degradation signal when fused to luciferase. Furthermore, mutations in domain **II** restored stability to

Received 5 Aug. 2003 Accepted 18 Nov. 2003
Supported by the National Natural Science Foundation of China (2001AA222051).
* Current address: Forest Biotechnology Group, Department of Forestry, North Carolina State University, 2500 Partner II Building, Campus Box 7247, Raleigh, NC 27695.
** Author for correspondence. Tel: +86 (0)10 62552880; Fax: +86 (0)10 62537814; E-mail:<ybxue@genetics.ac.cn>.

the luciferase fusion protein. Thus, a rapid turnover of Aux/IAA proteins is essential for normal auxin response (Tiwari *et al*., 2001).

Besides *Arabidopsis*, several members of this family have been found in other species. For example, *PS-IAA4/5*, *PS-IAA6* in pea and *Aux22* and *Aux28* in soybean are expressed in elongating, dividing, and differentiating cell types, indicating multiple functions during development (Ainley *et al*., 1988; Wong *et al*., 1996).

Cotton fibers are derived from the outer integument of ovule. But little is known about cotton ovule development. In this study, we isolated an *IAA* gene from cotton homologous to *Arabidopsis IAA16* and named it *GhIAA16*. Expressional analysis revealed that *GhIAA16*'s transcript is restricted to the most internal cell layer of the seed coat, that is, the endothelium. Its possible functions in cotton are discussed.

1 Materials and Methods

1.1 Plant materials

Vegetative and reproductive organs and tissues were harvested from the allotetraploid cotton species (*Gossypium hirsutum* L. cv. XZ142 and *G. hirsutum* L. cv. XZ142w) grown under a 30/21 ℃ day/night temperature regime in the greenhouse. The XZ142w is a fuzzless-lintless mutant identified by Zhang and Pan (1991) from the XZ142. Developing ovules were excised from flower buds or bolls on various days before/post-anthesis (dpa) relative to the day of anthesis (0 dpa).

1.2 cDNA array

cDNA library from cotton ovules was constructed using SMART cDNA Library Construction Kit (Clontech, K1051), and were randomly selected and printed from 384-well plates onto Hybond N^+ (Amersham Parmacia Biotech, UK) membranes. The cDNA array procedures were carried out basically as previously described (Bao *et al*., 2002). The high density filters were prepared using the Biomek 2000 HDRT system, and were probed with α-^{32}P-dCTP-labelled first strand cDNA. The filters were exposed to X-ray films (Kodak).

1.3 Semi-quantitative RT-PCR analysis

First-strand cDNA was synthesized from 5 μg total RNA using the SUPERSCRIPT Ⅱ RNase H^- Reverse Transcriptase (Gibco, USA). One tenth of the first strand cDNA was used as a template in 50 μL PCR reactions. Gene-specific RT-PCR primers were designed according to the cDNA sequences and synthesized commercially (Sangon, Shanghai, China). Parallel reactions using cotton ubiquitin primers served to normalize the amount of template added. The used primers are shown in Table 1.

1.4 Southern and Northern analyses

Genomic DNA was isolated from leaves of *G. hirsutum* L. cv. XZ142 and *G. hirsutum* L. cv. XZ142w using a cetyltrimethylammonium bromide (CTAB) extraction method (Paterson *et al*., 1993). The DNA (20 μg) was digested, separated on 0.8% agarose gel and transferred onto Hybond N^+ membrane. Prehybridization, hybridization, and washing of the blot were performed as recommended by the manufacturer. Total RNA was extracted from different tissues with the protocol according to RNeasy Plant Mini Kit (Qiagen, USA). RNA samples were separated on 1.2%

Table 1 Primers used for RT-PCR analysis of genes differentially expressed during cotton ovule development

Clone ID	3' primer	5' primer
ary1	CATTAAAATGAAATATGGTC	GGGCTTCGAGGGGGACCC
ary2	CTAATTAACAACAGTATG	CATTAAAGCTCAAATGCAACTCC
ary3	CACTTTGGAGCTATCAGT	GAGAAGGCCGGAGTGAAAG
ary4	GTTGTACAAGCCCTTGCC	AGAAGCGTAAGAAGCGCAAG
ary5	CATAGCAGAACAGGTAAATCCATC	TCACTAGATCTCTCATGGC
ary6	CGAGGCGACAAAGGGCTG	GAATCAGCGGGGAAAGAAGAC
ary10	CCATAGAACAACAACTGAGAACCG	CTCCGCTGCCCGAATTCCC
ary11	GTGAGAAAAGAGAGAGAGAGAG	CTGCTTTCTCGTATAGCAGCCTG
ary12	TACTCTTGTTTACATTACAGCTG	ATGGGGAGTTCTTGGGGCTG
ary13	CTCCTCTCACACATATGTCGCGC	CTTTTACTAGAGGCTAGAGCC
ary14	CTCTTAAAAACCTCTCTCAC	GCCTAAAAGTTAAAAGGTAAACAG
ary15	CCAAAGCCACTATACTGTGTGTG	GAAGATGGCCCGTACCAAGCAG
ary16	CGGAGAGTAAATTTCCAACAAGG	GCAACTGAGAAAAGGCCTC
ary27	CATTCTTATTACCGATACCCGAG	GGAGGCTTTGACTAAGGCATG
ary30	GCCGACTTCCCTTGCCTAC	GACGGTGGTCATGGAAGTCG
ary31	GCAAGAGCTACAAACGACG	ACTCGTGTACATCGCGTGGC
ary32	CACATTGCGTGAGCATCCGC	GCAGAACTGGCGATGCGGGATG
ary33	CTACTATATACTACTAGTTAACTTGG	GGAACTGAGTATTATCAAAAGAG
ary34	GCCGACTTCCCTTGCCTTAC	CGGTCCGACAGCGCGGTC

agarose/formaldehyde gels and transferred to Hybond N^+ membrane, and prehybridization, hybridization and washing of the blot were performed as recommended by the manufacturer. Probes were labeled with ^{32}P by random-priming using Prime-a-Gene Labeling system (Promega, USA). The blots were exposed to X-ray films (Kodak).

1.5 *In situ* RNA hybridization

Digoxygenin-labeled sense or antisense RNA probes were prepared following the manufacturer's recommendation (Boehringer, Mannheim). Tissue fixation and embedding, *in situ* hybridization and signal detection were essentially performed as described by Lai *et al*. (2002).

2 Results

2.1 Identification of *GhIAA16* expressed in cotton ovule

To identify genes involved in cotton ovule formation, we took a cDNA array approach to monitoring differential gene expression of cotton ovules. First strand cDNA probes reverse-transcribed from total RNAs of the ovules from –3 dpa, 0 dpa and +3 dpa were hybridized with 62 high-density filters containing 23 000 cDNA clones from the cDNA library constructed from developing cotton ovules and differentially expressed clones were selected. To reduce possible false positives caused by the difference of sample deposit among filters, hybridization was performed twice with changed filters and only those clones that showed similar expression patterns in the two hybridizations were chosen. An image of a portion of cDNA array is shown in Fig.1.

The hybridization results between different probes were similar, and most of the clones showed no significant changes among different probes. Subsequently, a total of only 25 differentially expressed cDNA clones were identified and subcloned into a pBluescript II SK (+) vector. Sequencing and homology analyses revealed that 10 of them are homologous to known genes in the GenBank (Table 2), whereas the rest has no hit.

To confirm the differential expression during cotton ovule development, they were subject to semi-quantitative RT-PCR and the results of 13 cDNA sequences are shown in Fig.2. Most of them displayed the same responsiveness as detected by the cDNA array and were highly expressed at the early stage of ovule formation. Among them, *ary11* encodes a 208 amino acid protein highly homologous to *Arabidopsis* IAA16 (AtIAA16) (Arabidopsis Genome Initiative, 2000) with 59.4% amino acids identity (Fig.3) and was therefore named *GhIAA16*. Sequence alignment between AtIAA16 and GhIAA16 revealed that they are identical in the conserved 4 domains of the IAA family with some differences in their amino termini (Fig.3A).

To investigate its gene structure, we obtained a genomic DNA sequence of *GhIAA16* of 934 bp corresponding to its

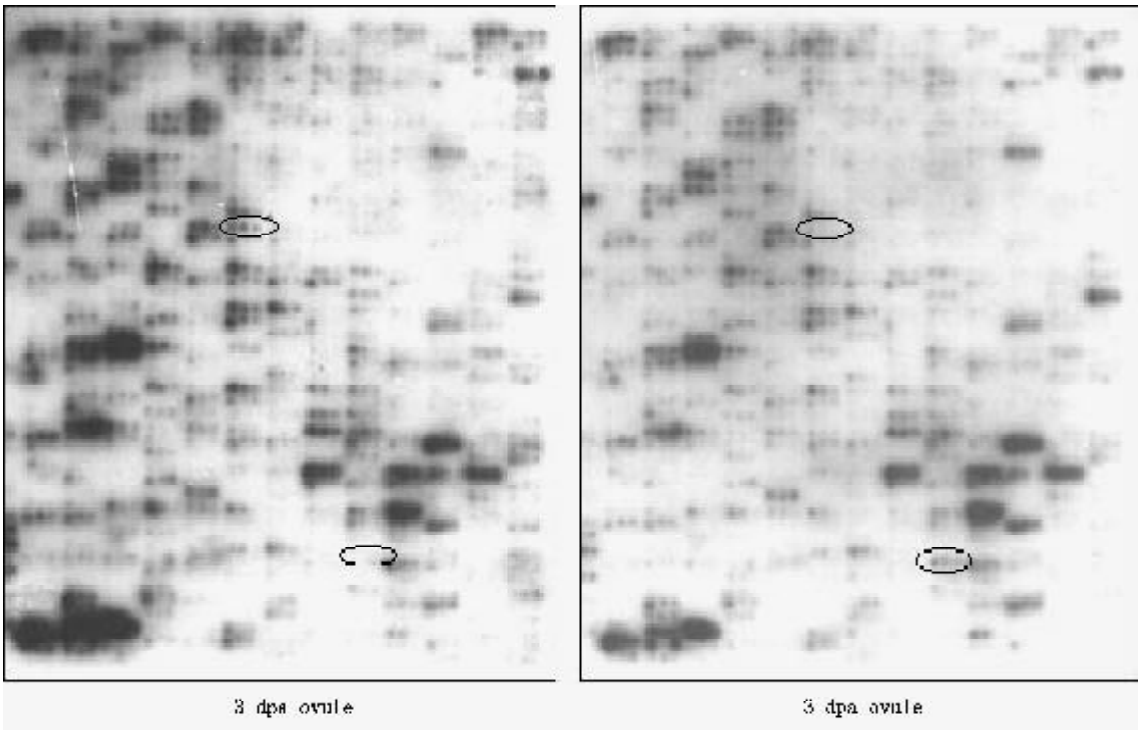

Fig.1. Identification of differential expressed genes during cotton ovule development by using cDNA array. A portion of a filter is shown here and was hybridized by probes from –3 dpa (upper) and +3 dpa (lower) ovules. Cycles indicate the differentially expressed clones.

Table 2 Genes that are differentially expressed during cotton ovule development

Clone ID	Accession number of the closest hit in database	Description	E-value
ary-3	Q42551	Ubiquitin-conjugating enzyme E2	3.1e-14
ary-4	Q42619	Ctp:phosphocholine cytidylyltransferase	3.3e-12
ary-6	O64410	Cytochrome P450 monooxygenase	4.0e-21
ary-11	O24407	Auxin-responsive protein Iaa16	2.7e-07
ary-12	Q9LWP7	Similar To *Arabidopsis thaliana* chromosome 2 bac clone F4l23	3.8e-05
ary-15	BAA31218	Histone H3	6.4e-56
ary-30	Q9NGQ4	Insecticide resistance-associated Cytochrome P450 (fragment)	0.010
ary-35	AAB97162	Histone H3	3.1e-65
ary-37	Q9AXD9	Mago nashi-like protein	1.2e-70
ary-38	O04263	Immunophilin	1.4e-51

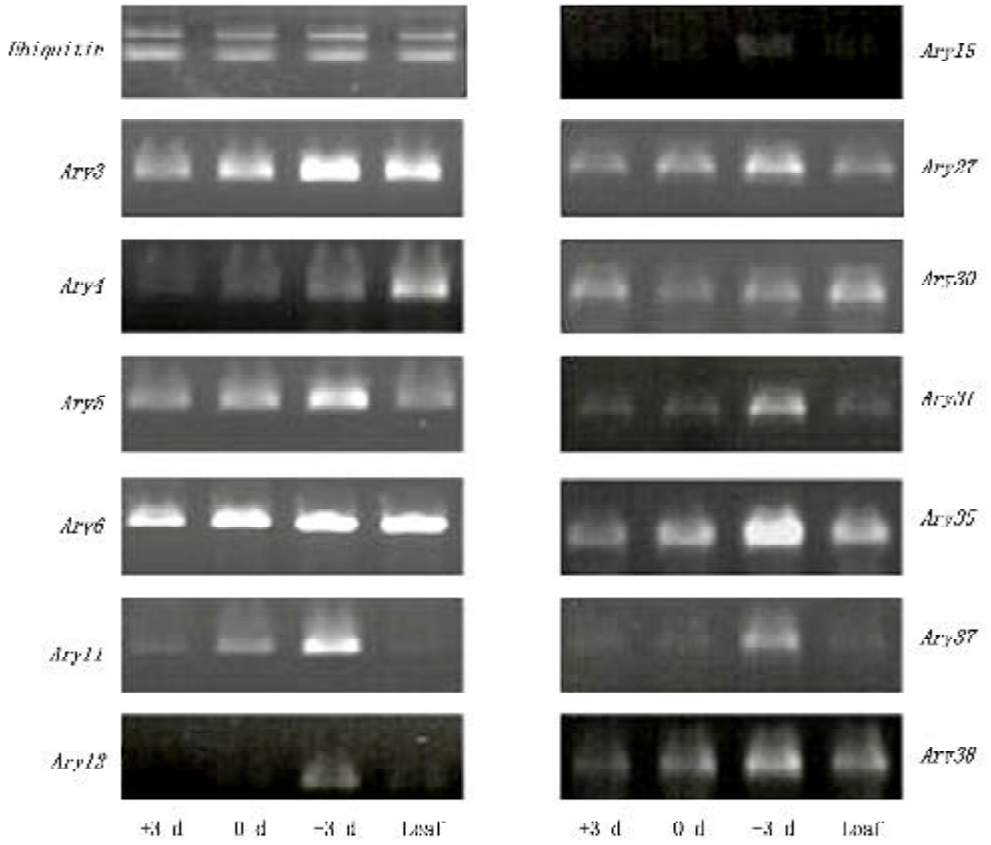

Fig.2. RT-PCR analysis of cotton ovule cDNAs. Total RNA (3 μg) isolated from – 3, 0 and +3 dpa ovules and leaves were used to synthesize cDNA for RT-PCR analysis using gene-specific primers. A cotton ubiquitin cDNA was used to normalize the amount of templates added in the PCR reactions. Days post anthesis (dpa) are shown at the bottom.

cDNA. Three introns with the lengths of 110, 103 and 94 bp and four exons with the lengths of 236, 182, 109 and 100 bp were identified, respectively, different from *AtIAA16* which contains four introns with the lengths of 450, 87, 94, and 81 bp and five exons with the lengths of 204, 271, 137, 61, 38 bp (Arabidopsis Genome Initiative, 2000), respectively. Sequence comparison showed that the former three introns are at the same positions between *AtIAA16* and *GhIAA16*, whereas the last one of *AtIAA16* is not present in *GhIAA16* (Fig.3B).

2.2 *GhIAA16* presents as a single-copy gene in cotton genome

To examine the organization of *GhIAA16* in cotton genome, DNA blotting analysis was performed using the coding region of *GhIAA16* as a probe (Fig.4). Single fragments of 6.0 kb and 8.0 kb were detected in *Bam*HⅠ and *Eco*RⅠ-restricted genomic DNA, respectively, and two fragments of 3.8 and 2.9 kb were found in the *Hin*dⅢ digest.

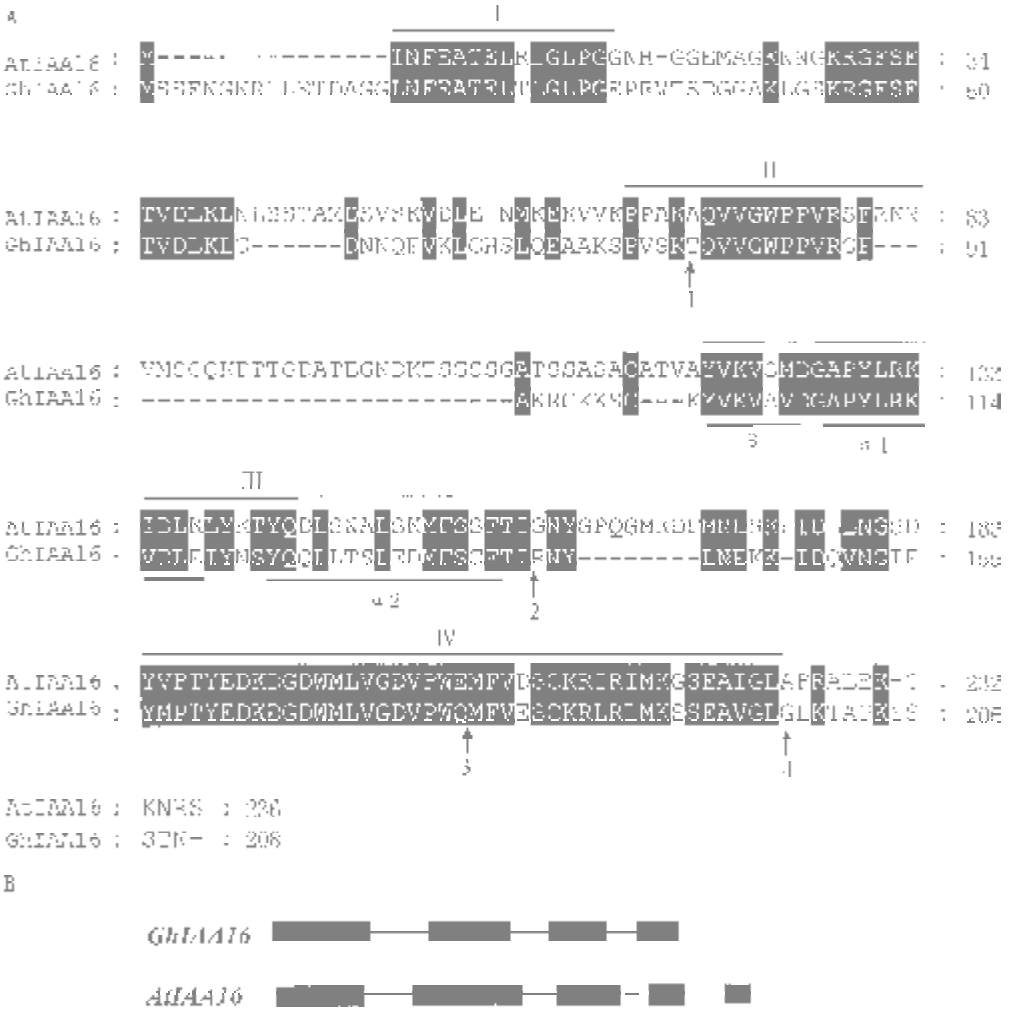

Fig.3. Comparison of amino acid sequences and gene structures of *GhIAA16* and *AtIAA16*. **A.** Alignment of predicted polypeptide sequences of GhIAA16 and AtIAA16. Identical amino acids are shaded. Dashes are introduced to maximize the alignment. Four conserved domains are indicated by I-IV, the amphipathic *b a a* structure are underlined. The arrows with number indicated the location of intron. **B.** The gene sequence structures of *GhIAA16* and *AtIAA16*. Boxes indicate exons and lines represent introns (intron not drawn to scale).

The two *Hin*d**III**-hybridizing bands were due to the presence of an internal *Hin*d**III** site of *GhIAA16* sequence. Thus, *GhIAA16* occurred as a unique gene in cotton genome. In addition, we also investigated the genomic organization of *GhIAA16* in a fuzzless-lintless (*fl*) mutant of cotton and detected no difference between the mutant and wild type (Fig.4).

2.3 *GhIAA16* is highly expressed in the endothelium of ovule

To examine the expression pattern of *GhIAA16*, Northern blotting analysis was performed using RNA from cotton ovules of –3 dpa, 0 dpa, +3 dpa and leaves respectively (Fig.5). The result showed that *GhIAA16* transcripts peaked at the –3 dpa ovule when cotton fibers are initiated, and no signal was detected in the leaves, suggesting that it is a cotton ovule-specific gene (Fig.5).

To further examine its tissue-specific pattern, *in situ* RNA hybridization was done with the ovule of 0 dpa. The 5' end of the gene with 450 bp was used as the probe, because the 3' ends of the IAA family are usually highly conserved. The result showed that it is specifically expressed in the endothelium of the cotton ovule (Fig.6).

3 Discussion

Here, we have isolated a cotton gene *GhIAA16* homologous to *Arabidopsis IAA16* and found that it is a single-copy gene in cotton genome and specifically expressed in the endothelium of the ovule.

Although genetic control of cotton ovule development remains unknown, a number of genes have been identified that are important for the growth and morphogenesis of the integuments in *Arabidopsis* (Schneitz *et al.*, 1998). Several

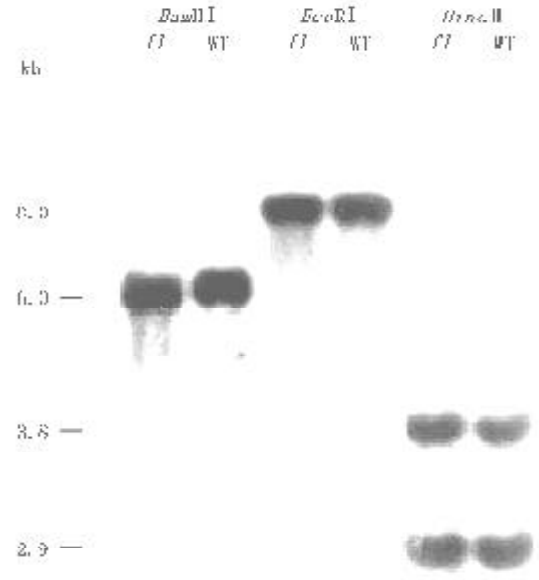

Fig.4. DNA blotting analysis of cotton genomic DNA of *GhIAA16*. Genomic DNA (20 μg/lane) of leaves of *Gossypium hirsutum* cv. XZ142 (WT) and *G. hirsutum* cv. XZ142w (*fl*) was completely digested with *Bam*HI, *Eco*RI and *Hin*dIII respectively and transferred on nylon membrane. The blot was hybridized with a gene-specific probe of *GhIAA16*. Molecular weight makers are indicated in kb.

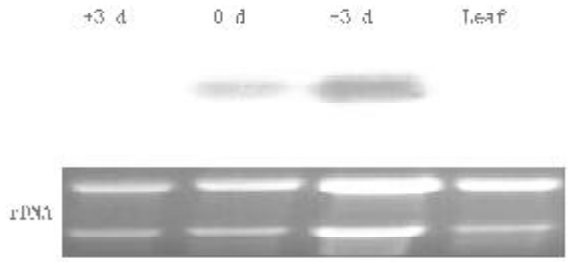

Fig.5. Northern blotting analysis of *GhIAA16*. Total RNA (20 μg/lane) from +3, 0, -3 dpa ovules and leaves was fractioned on a denaturing 1.2% (W/V) agarose gel and transferred to nylon membrane. The blot was hybridized with the full-length cDNA of *GhIAA16*. Loading control of total RNA was shown.

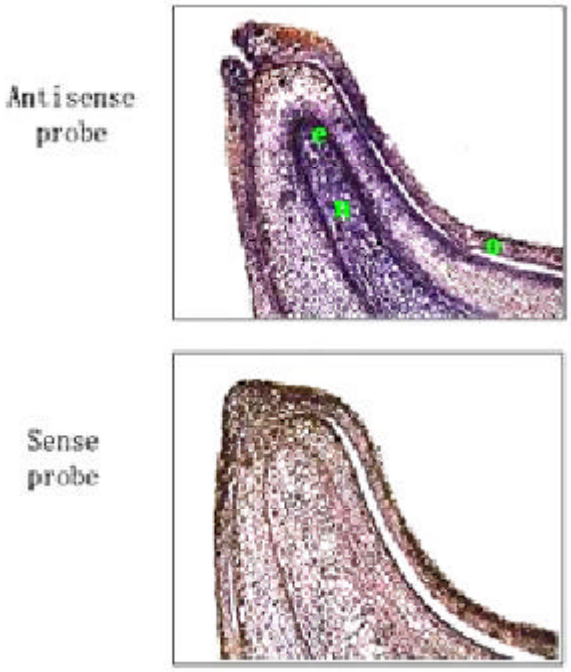

Fig.6. *In situ* RNA hybridization analysis of *GhIAA16*. Upper, a longitudinal section of 0 dpa ovule hybridized by an antisense probe of *GhIAA16*; lower, a longitudinal section of 0 dpa ovule hybridized by sense probe of *GhIAA16*. The blue color indicates the hybridizing signal. e, endothelium; i, inner seed coat; n, nucellus; o, outer seed coat.

genes for the initiation of the integuments and an increasing number of genes for their morphogenesis have been identified (Schneitz *et al.*, 1998). The first group comprises *HLL*, *ANT*, *BEL1*, *INO*, *ABERRANT TESTA SHAPE* (*ATS*) and *UNICORN* (*UCN*). They act as positive regulators of early integument development, with the possible exception of *UCN*. Genes of the second group include *STRUBBELIG* (*SUB*) and *BLASIG* (*BAG*), *LEUNIG* (*LUG*), *TOUSLED*, *SHORT INTEGUMENTS* (*SIN1*), *SUP*, *TSO1* and others. However, the genetic network of these genes is not clear although limited data indicate that the first group genes are likely to precede the activities of members of the second group (Schneitz, 1999).

Genes expressed in endothelium are not well documented. *Arabidopsis BANYULS* (*BAN*) gene, which most likely encodes a leucoanthocyanidin reductase and restricts its expression to the endothelium of immature seeds at the pre-globular to early globular stages of embryo development (Devic *et al.*, 1999). It represents a marker for early differentiation and development of the seed coat. BAN is probably involved in a metabolic channeling between the production of anthocyanins and pro-anthocyanidins in the seed coat (Devic *et al.*, 1999). Another *Arabidopsis* gene, *TT16*, encodes the ARABIDOPSIS BSISTER (ABS) MADS domain protein and is expressed mainly in the ovule (Nesi *et al.*, 2002). TT16/ABS is necessary for *BAN* expression and proanthocyanidin accumulation in the endothelium of the seed coat, with the exception of the chalazal-micropylar area. In addition, mutant phenotype and ectopic expression analyses suggested that TT16/ABS is also involved in the specification of endothelial cells. It appears that these two endothelium-expressed genes are all involved in flavonoids production of the seed coat as well (Nesi *et*

al., 2002).

Aux/IAA genes are early auxin response genes that encode short-lived nuclear proteins (Abel *et al*., 1994). Tiwari *et al*. (2001) found that most of the *Arabidopsis* Aux/IAA proteins repressed transcription on auxin-responsive reporter genes in protoplast transfection assays. Their work suggested that Aux/IAA proteins function as active repressors by dimerizing with auxin response factors bound to auxin response elements and that early auxin response genes are regulated by auxin-modulated stabilities of Aux/IAA proteins.

Whether the orthologs of these genes play roles in cotton integument development is not clear. The identification of *GhIAA16* as an endothelium-specific gene suggests that auxin plays a role in cotton ovule development. In fact, the initiation of cotton fiber development appears to be triggered by hormones, auxins and gibberellins. Gialvalis and Seagull (2001) have demonstrated that unfertilized ovules produced large numbers of fiber initials in the absence of any hormone treatment. But exogenous application of either indole-3-acetic acid or gibberellic acid induced significant increases in fiber production. But the molecular mechanism of this influence is unknown. GhIAA16 may provide a molecular bridge between hormone IAA and cotton fiber formation. The expression pattern of *AtIAA16* remains unclear. Direct transformation of Arabidopsis with GhIAA16 (sense or antisense) did not result any obvious phenotypic changes (data not shown), suggesting a diversified role of IAA16, also supported by their different genomic structures (Fig.3). Further genetic transformation of cotton likely reveals a role of GhIAA16, if any, in cotton ovule development.

Acknowledgements: We thank Dr. DU Xiong-Ming from Cotton Research Institute of The Chinese Academy of Agricultural Sciences for providing cotton *fl* mutants.

References:

Abel S, Theologis A. 1996. Early genes and auxin action. *Plant Physiol*, **111**:9-17.

Abel S, Oeller P W, Theologis A. 1994. Early auxin induced genes encode short-lived nuclear proteins. *Proc Natl Acad Sci USA*, **91**:326–330.

Ainley W M, Walker J C, Nagao R T, Key J L. 1988. Sequence and characterization of two auxin-regulated genes from soybean. *J Biol Chem*, **263**:10658-10666.

Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, **408**:796-815.

Bao F, Hu Y, Li J. 2002. Identification of auxin responsive genes in *Arabidopsis* by cDNA array. *Chin Sci Bull*, **47**:548-552.

Boesewinkel F D, Bouman F. 1995. The seed: structure and function. Kigel J, Galili G. Seed Development and Germination. Washington, D C: Library of Congress. 1–24.

Devic M, Guilleminot J, Debeaujon I, Bechtold N, Bensaude E, Koornneef M, Pelletier G, Delseny M. 1999. The *BANYULS* gene encodes a DFR-like protein and is a marker of early seed coat development. *Plant J*, **19**:387-398.

Du X M, Pan J J, Wang R H, Zhang T Z, Shi Y Z. 2001. Genetic analysis of present and absence of lint and fuzz in cotton. *Plant Breeding*, **120**:519-522.

Gasser C S, Broadhvest J, Hauser B A. 1998. Genetic analysis of ovule development. *Annu Rev Plant Physiol Plant Mol Biol*, **49**:1-24

Gialvalis S, Seagull R W. 2001. Plant hormones alter fiber initiation in unfertilized, cultured ovules of *Gossypium hirsutum*. *J Cotton Sci*, **5**:252-258.

Hagen G, Guilfoyle T. 2002. Auxin-responsive gene expression: genes, promoters and regulatory Factors. *Plant Mol Biol*, **49**: 373-385.

Lai Z, Ma W S, Han B, Liang L Z, Zhang Y S, Xue Y B. 2002. The self-incompatibility (*S*) locus encodes a novel F-box gene with a male gametophytic expression in *Antirrihium*. *Plant Mol Biol*, **50**:29-42.

Léon-Kloosterziel K M, Bunt G A, Zeevaart J A D, Koornneef M. 1994. Arabidopsis mutants with a reduced seed dormancy. *Plant Physiol*, **110**:233-240.

Leyser O. 2001. Auxin signalling: the beginning, the middle and the end. *Curr Opin Plant Biol*, **4**:382-386

Liscum E. Reed J W. 2002. Genetics of Aux/IAA and ARF action in plant growth and development. *Plant Mol Biol*, **49**:387-400.

Nesi N, Debeaujon I, Jond C, Stewart A J, Jenkins G I, Caboche M, Lepiniec L. 2002. The *TRANSPARENT TESTA16* locus encodes the ARABIDOPSIS BSISTER MADS domain protein and is required for proper development and pigmentation of the seed coat. *Plant Cell*, **14**:2463-2479.

Paterson A H, Brubaker C L, Wendel J F. 1993. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant Mol Biol Rep*, **11**:122-127.

Schneitz K, Balasubramanian S, Schiefthaler U. 1998. Organogenesis in plants: the molecular and genetic control of ovule development. *Trends Plant Sci*, **3**:468-472.

Schneitz K. 1999. The molecular and genetic control of ovule development. *Curr Opin Plant Biol*, **2**:13-17.

Tiwari S B, Wang X J, Hagen G, Guilfoyle T J. 2001. AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. *Plant Cell*, **13**:2809-2822.

Wong L M, Abel S, Shen N, Foata M, Mall Y, Theologis A. 1996. Differential activation of the primary auxin response genes, *PS-IAA4/5* and *PS-IAA6*, during early plant development. *Plant J*, **9**:587-599.

Zhang T-Z, Pan J-J. 1991. Genetic analysis of a fuzzless-lintless mutant in *Gossipium hirsutum* L. *Jiangsu Agric Sci*, **7**:13-16. (in Chinese with English abstract)

(Managing editor: ZHAO Li-Hui)