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Short communication

Co-expression and preferential interaction between two calcineurin B-like proteins and a CBL-interacting protein kinase from cotton

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Abstract

The CBL/CIPK signaling system mediates a variety of responses to environmental stimuli in plants. In this work, we identified four *CBL* genes from *Gossypium hirsutum*, two of which (designated *GhCBL2* and *GhCBL3*) showed preferential expression in the elongating fiber cells. Moreover, the expression patterns of these two *CBL* genes coincided with that of a putative CBL-interacting protein kinase gene (*GhCIPK1*) that we isolated in a previous study. Yeast two-hybrid assay indicated that among the four CBLs, GhCIPK1 interacted selectively with GhCBL2 and GhCBL3. The co-expression and interactions of these proteins suggest that they are components of the same signaling pathway. These findings strengthen our previous prediction that CBL/CIPK signaling plays a critical role in the regulation of cotton fiber elongation.

Keywords: Cotton fiber; Calcineurin B-Like protein; CBL-interacting protein kinase

1. Introduction

Calcium signaling plays important roles in a wide range of physiological processes and stress responses. Three major classes of Ca^{2+} sensors have been identified in plants, including calmodulin (CaM), Ca^{2+}-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs) [1]. The CBL proteins are specific to higher plants and function by interacting with a unique family of plant protein kinases called CIPKs [2]. *CBL* expression is regulated by a number of factors including abiotic stresses, plant hormones and nutrient deprivation. In addition, some CBL genes exhibit developmental and tissue-specific expression patterns [1,3]. Ten *AtCBLs* and 25 *CIPKs* were identified in *Arabidopsis* genome and 10 *CBLs* and 30 *CIPKs* in the rice genome [4]. It is believed that multiple combinations of CBL/CIPK proteins contribute to a complex network that functions in diverse signaling processes in plants [5].

Physiological functions of several plant CBL/CIPK proteins have been characterized. In *Arabidopsis*, for example, CBL4 (SOS3) and CIPK24 (SOS2) play a critical role in salt tolerance [6,7]; CBL1, CBL9 and CIPK1 regulate responses to diverse abiotic stresses and phytohormone abscisic acid (ABA) [8–12]; and CBL1/CBL9 and CIPK23 are crucial for K^+ uptake under low-K^+ conditions [13,14]. Compared to the notable progress made in discerning the roles of CBL/CIPK signaling in response to environmental cues, functions of these proteins in intrinsic plant growth and development processes under normal physiological conditions were rarely reported. It has been shown that OsCBL2 was involved in a GA-signaling pathway that led to vacuolation of the aleurone cell in rice [15].

Cotton fiber is a single and highly elongated ovule epidermal cell. The development of cotton fiber can be divided into

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four overlapping stages: initiation, elongation, secondary wall synthesis and maturation [16]. Fiber elongation starts on the day of anthesis and progresses for 20–30 days. Due to the extreme length and long duration of elongation, cotton fiber is considered as a unique system for studying the mechanisms controlling plant cell elongation [17]. Several fiber expansion-related genes have been functionally characterized in cotton plants. Among them, a sucrose synthase gene (*Sus*), an actin gene (*GhACT1*) and a gene (*GhDET2*) encoding a rate-limiting enzyme in brassinosteroid (BR) biosynthesis were shown to be crucial for fiber elongation [18–20]. Here, we report the cloning of four *CBL* genes from upland cotton (*G. hirsutum*), of which two exhibited fiber preferential expression patterns. To probe their functions, we investigated the interaction of these proteins with a putative CIPK partner by yeast two-hybrid and in vitro assays.

2. Results and discussion

2.1. Cloning of four cotton CBL genes and sequence analysis

In a previous study, a fiber-specific *CIPK* gene (*GhCIPK1*) was identified from *G. hirsutum* [21]. The results of this former study encouraged us to identify its possible partner proteins (CBLs). Publicly available express sequence tag (EST) databases were first queried using the sequences of *Arabidopsis CBL* genes. The query revealed cotton ESTs homologous to all the *Arabidopsis CBL* genes (*AtCBL1–10*), indicating that CBLs are encoded by a multigene family in cotton as in other higher plants studied. From these candidate ESTs, we then selected those that appeared with relatively higher frequency in the cotton fiber-derived EST libraries, and amplified their open reading frames (ORFs) from a cotton fiber cDNA library using gene-specific primers. Four cotton *CBL* genes were isolated and sequence analysis indicated that their coding

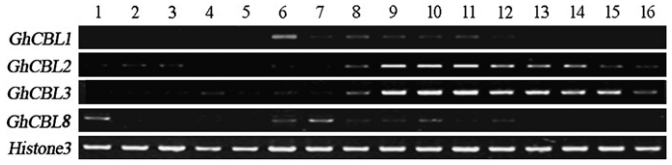

Fig. 2. RT-PCR analysis of expression profiles of GhCBL genes in cotton. Lane 1, roots; lane 2, hypocotyls; lane 3, leaves; lane 4, flowers; lane 5, ovules at −3 DPA; lane 6, ovules at 0 DPA; lane 7, ovules at 3 DPA; lane 8–16, 6, 9, 12, 15, 18, 21, 24, 27 and 30 DPA fibers, respectively. The histone gene was used as an internal control.

regions shared identities of 56–97% at the nucleotide level and 56–94% at the amino acid level (Fig. 1A). Based on the homology of the four cotton *CBLs* to *Arabidopsis CBL* genes, we designated them as following: *GhCBL1*, *GhCBL2*, *GhCBL3* and *GhCBL8* (EU085038–EU085041). A phylogenetic analysis was performed to estimate the relationships among the four cotton CBLs and 10 *Arabidopsis CBLs*. As shown in Fig. 1B, GhCBL1 is a sister subgroup of the AtCBL1/9, GhCBL8 is clearly allied with AtCBL8, while both GhCBL2 and GhCBL3 are most closely related to AtCBL2. This result suggests that the four cotton *CBL* genes may represent three distinct members of the *CBL* multigene family in *G. hirsutum* and the *GhCBL2*/*GhCBL3* gene pair may arise from a recent duplication event. The four GhCBL proteins all possessed four EF hand motifs that could act as calcium-binding sites [22], and the linker region size between the EF hands was conserved in all proteins. GhCBL2 and GhCBL3 contained identical amino acid residues in their EF hands. However, in the same EF hand regions, GhCBL1 and GhCBL8 displayed amino acid substitutions (Fig. 1A). This difference in the EF hand composition of these CBL proteins could lead to different affinities toward calcium ions. Another structural feature of the CBLs resided in their N-terminal sequences. Both GhCBL1 and GhCBL8 harbored conserved myristoylation motifs (MGXXXS/T), which often have an important functional role, either for protein–protein

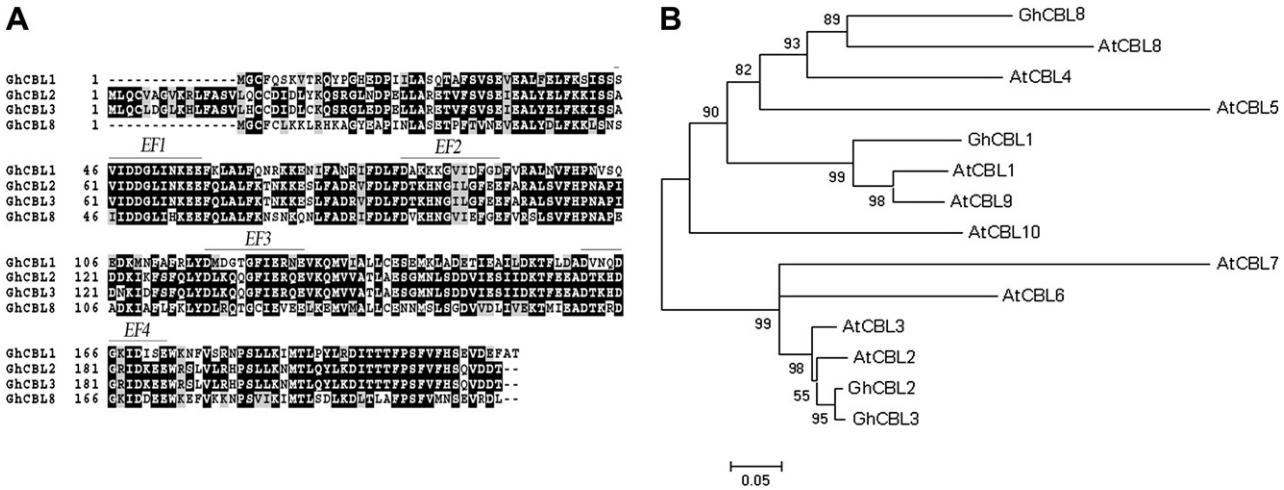

Fig. 1. Alignment of amino acid sequences of GhCBLs and phylogenetic analysis of cotton CBLs with Arabidopsis CBLs. A. The sequences were aligned with ClustalW and the boxshade was created by BOXSHADE 3.21 through EMBnet. (http://www.ch.embnet.org/software). B. The neighbor-joining tree was constructed in MEGA3.1 from 1000 bootstrap replicates (AtCBL1 At4g17615, AtCBL2 At5g55990, AtCBL3 At4g26570, AtCBL4 At5g24270, AtCBL5 At4g01420, AtCBL6 At4g16350, AtCBL7 At4g26560, AtCBL8 At1g64480, AtCBL9 At5g47100, AtCBL10 At4g33000).