Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Isolation and identification of two new alleles of *STICHEL* in Arabidopsis

Ali Xi [1], Xuying Yang [1], Meng Deng, Yu Chen, Jingxia Shao, Jun Zhao, Lijun An*

State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi Province, 712100, China

ARTICLE INFO

Article history:
Received 24 March 2018
Accepted 26 March 2018
Available online xxx

Keywords:
Branching
Cell cycle
Endoreduplicaton
Protein interaction
Trichome

ABSTRACT

Plant trichome has long been selected as a model system to study the regulatory mechanism during cell development due to its unique growth characteristics. In an effort to explore new components that contribute to trichome development, we performed large-scale forward genetic screening in Arabidopsis, and found two recessive mutants with significant trichome branching defects, both of which display one branched trichomes. After map-based cloning, genetic complementation experiments, we confirmed that they are new alleles of *STICHEL* (*STI*) that has been reported to act as a key regulator of trichome branching. The predicted product of *STI* gene is comprised by three functional domains including a large domain with sequence similarity to eubacterial DNA polymerase III γ-subunit in the middle, two PEST domains in the N terminal, and two nuclear localization signals (NLS) at the very N terminal and C terminal, respectively. Our biochemistry and molecular analysis indicated that a PEST domain in the N-terminal could be important for *STI* functioning in regulating trichome branching. It can directly interact with BRACHLESS TRICHOME (BLT), an important linker of cell shape and endoreplication. Because the previously data showed that the function of *STI* in regulating trichome branching was not linked to DNA replication and actin or microtubule cytoskeleton configuration, our findings of the direct interaction domains between STI and BLT veiled the probable roles of *STI* in cell morphogenesis.

1. Introduction

Cell differentiation and proliferation are two crucial features for the acquisition of different cell types of plant forms. Trichomes are special structures differentiated from epidermal cells of plant aerial parts, the morphology of which vary considerably among the species [1–4]. In model plant Arabidopsis, trichomes are specialized single cells, covering the epidermis of leaves, petioles, sepals and stems [1,5,6]. They have unique branched cellular architectures: on leaves, they typically bear three or four branches, while on stems, petioles and sepals, they always display one or two branches [1,5,6]. The developmental processes of trichomes in Arabidopsis have been well investigated during the past years. A remarkable feature during the earlier trichome development is the switch from the mitosis to endoreplication, and the developing trichome typically undergoes three to four rounds of DNA replication and makes the nuclear DNA reach to 32C (C equals haploid DNA content per nucleus) [1,7–9]. Accompanying the continuous DNA endoreplication, the developing trichome cell expands from the plane of the epidermis and typically complete two to three branching events, resulting in a total of three to four branches in the mature trichome [1,10]. In general, mutations in genes that increase or decrease the number of rounds of endoreplication elevate or reduce the trichome cell size and the number of branches, respectively [1,7–9]. However, abnormal trichome branching and cell shape may also independent of endoreplication, and the underline mechanisms are still unclear. Recessive loss of function mutations in *STICHEL* (*STI*), which encodes a protein with the sequence similarity to ATP-binding eubacterial DNA polymerase III γ-subunits, result in trichomes that are completely one branched [11]. Interestingly, despite the presence of DNA polymerase III γ-subunits implicated that *STI* might be involved in regulation of DNA replication, molecular and genetic analysis indicated that *STI* is not facilitate to the regulation of endoreplication during trichome branching [11]. The *sti* mutants appeared to have the similar DNA content to that of wild type, and overexpression of *STI* resulted in hyperbranched

* Corresponding author. 22 Xinong Rd, State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi, 712100, China.
E-mail address: lijunan@nwsuaf.edu.cn (L. An).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.03.197
0006-291X/

trichomes but did not alter the nuclear DNA content [11]. *STI* was also indicated not affect the actin or microtubule organization during cell morphorgenesis [11], so the real molecular functions of *STI* keep to a mystery for a long time. Recently, Kasili and collaborators [12] showed that STI could interact both genetically and physically with BRANCHLESS TRICHOMES (BLT), which encodes a protein with no similarity to proteins of known function [12,13]. Similar to the impacts that *STI* imposed on trichome branching, loss of function of *BLT* also displays almost one branched trichomes without affect unclear DNA content [12], but there are also differences. Unlike that of *STI*, overexpression of *BLT* resulted in an additional round of endoreplication [12], indicated that *BLT* might function in regulating cell cycle process during trichome branching. As expected, the later studies demonstrated that recessive mutation of *BLT* could enhance the multicellular trichome phenotypes of mutants of the *SIAMESE* (*SIM*) gene, which was reported to encode a cyclin-dependent kinase inhibitor and acted as a regulator of endoreplication onset during trichome cell differentiation [14,15]. These data implicated that *BLT* might function in linking the cell shaping and nuclei DNA content. Moreover, both of BLT and STI were showed to expressed in the initiating trichome branch points [12], suggesting that STI and BLT might be key regulators for branch sites determination.

Here, we report the isolation of two new *STI* alleles, *sti-58* and *sti-59*. Either of these two alleles contains a G to A mutation in *STI* gene that could theoretically led to the pre-maturation of STI protein. Both of these two new alleles display completely one branched trichomes on leaves. Our further investigations showed N terminal 454 amino acids of STI protein could strongly interact with BLT, while the predicted DNA polymerase III γ-subunits domain in the middle only showed a weaker interaction with BLT. Moreover, one of the PEST-domain in the N terminal domain of STI might essential for its biological function.

2. Material and methods

2.1. Plant material and growth conditions

The wild type plants used were all in the *Columbia-0* (Col-0) background unless indicated otherwise. The *F04-01abs3-1D* and *F08-02abs3-1D* plants were isolated from an Ethyl methanesulfonate (EMS)-mutagenized population in the Arabidopsis *abs3-1D* mutant background [16], respectively. The *F04-01/abs3-1D* and *F08-02/abs3-1D* plants were crossed respectively with the Col-0 wild type plants to remove the *abs3-1D* background. The mutant phenotypes and homozygous status of *F04-01* and *F08-02* were examined and confirmed in the F2 to F3 generations. The *F04-01F08-02* double mutants were generated by crossing *F04-01* with *F08-02*, and the phenotype were examined in the F1 generation.

The *sti-57* mutant is a point mutation allele of *STI* [11], obtained from the Arabidopsis Biological Resources Center (ABRC).

All of the plants were grown at $22 \pm 1\,^{\circ}C$ under continuous illumination (~100 $\mu mol\ m^{-2}\ s^{-1}$).

2.2. Trichome branching phenotype characterization

The trichome branching phenotypes of the 3rd and 4th rosette leaves of three-week-old plants in different genotypes backgrounds were examined with a SZ61sterescope (Olympus). Numbers of branches of all the trichomes on each leaf were counted and recorded. For each genotype, at least 16 plants were used for phenotypic analysis. Data were presented as mean ± s.d. Student's *t*-test was used to assess the difference between wild type and mutants. The experiments were repeated as least twice.

For scanning electron microscopy (SEM) observation, the 3rd or 4th fresh rosette leaves were directly taped on the sample stage and the morphologies of the trichomes were viewed and photographed using a tabletop SEM TM3030 (Hitachi, Japan).

2.3. Cloning of F04-01 and F08-02

The *F08-02* locus was identified by using map-based cloning [17]. First-pass mapping located *F08-02* mutation in an interval covering the molecular markers T23K3#1 and T20K24#1 on chromosome II. By scanning the candidate genes in this chromosomal region, *AT2g02480*, which corresponds to *STI* gene, was found. Because of the phenotypic similarity, the *STI* gene in the *F08-02* mutant background was sequenced directly to confirm the mutation site.

Due to the phenocopy of trichome branching of *F04-01* to *F08-02* mutant, the allelic complementation between the *F04-01* and *F08-02* loci were tested by detecting the trichome branching phenotypes of *F04-01 F08-02* double mutants, and then the mutation site in *F04-01* mutant was identified by sequencing the *STI* gene.

2.4. Plasmid construction and plant transformation

To construct the *35S:STI* plasmid for complementation experiments, the full-length sequences spanning the open reading frame (ORF) of *STI* gene was amplified and cloned into the pBI111L binary vector with restriction enzyme *Bam*HI. The primers used were described in the Supplemental Table 3. For plant transformation, the *35S:STI* plasmids were transformed to the plants via *Agrobacterium tumefaciens* GV3101 by floral-dip method [18]. The presences of *35S* promoter sequence were detected by genomic PCR to confirm the transgenic plants. Then the trichome branching phenotypes and expression levels of *STI* gene were examined in T2 plants. At least 10 transgenic lines were observed for each transformation, and two of them were used for detailed analysis.

2.5. Yeast two-hybrid assays and subcellular localization

The split-GAL4 system was used to examine the interaction between STI and BLT. The different *STI* fragments as indicated in Fig. 3B were cloned into the bait vector pGBDK7, respectively, and the full length of *BLT* gene was inserted into the prey vector pGADT7. The confirmed bait and prey constructs were cotransforrmed into yeast strain GOLD [19,20]. The primers used were described in the Supplemental Table 3. Cotransfermation of the empty bait and prey vectors were used as negative control and the interaction between pGADT7/AtwT7 and pGBKT7/AtwT7 was used as positive control. Transformants were firstly grown on SD medium lacking the amino acid Trp and Leu. Positive colonies were then transferred to the QDO medium lacking Leu, Trp, His, and Ade to confirm the interactions.

For subcellular localization experiments, expression cassettes harbouring the 35S promoter and the coding sequences of GFP were first cloned into vectors of pBS backbone. The ORF of STI was subcloned into this vector to have the fluorescent proteins fused to the C-terminus of STI proteins. The confirmed plasmid was transiently expressed in WT leaf protoplasts. Leaf protoplast transient expression assays were performed as described [21]. An A1 confocal microscope (Nikon) was used to monitor the signals of fluorescent fusion proteins.

Please cite this article in press as: A. Xi, et al., Isolation and identification of two new alleles of *STICHEL* in Arabidopsis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.197