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Letter to the Editor

An Effective and Inducible System of TAL Effector-Mediated Transcriptional Repression in *Arabidopsis*

Dear Editor,

Gene regulation in model organisms is critical for gene function analysis and is thus essential for human health and agricultural production. Therefore, several molecular tools have been developed to regulate gene expression at the transcriptional and post-transcriptional levels. In recent years, the transcription activator-like effector (TALE), which is predominantly found in *Xanthomonas*, has been proved powerful for not only genome editing but also gene regulation in different organisms (Huang et al., 2011; Cong et al., 2012; Li et al., 2012; Wang et al., 2014). TALEs are composed of an N-terminal translocation signal, a central DNA-binding domain, a C terminus containing a nuclear localization signal, and an acidic activation domain (Morbitzer et al., 2010). The specificity of TALE DNA-binding proteins depends on a variable number of tandemly arranged, imperfect 34/35-amino-acid repeat units (Moscou and Bogdanove, 2009). The repeat variable di-residues (RVD) at positions 12 and 13 of each repeat dictate the specificity of the repeat domain to one nucleotide in the targeted DNA (Boch et al., 2009).

TALEs can be fused to different domains and developed into a variety of molecular tools. For example, the activator domain VP64 was fused to TALEs and successfully activated the expression of the endogenous genes *Sry-box 2* (*SOX2*) and *Krüppel-like transcription factor 4* (*KLF4*) in human cells (Zhang et al., 2011). In addition, by binding the promoter region, TALEs can be utilized to repress gene expression via fusion to the EAR repression domain in plant cells (Mahfouz et al., 2012). Notably, the TALE DNA-binding domain is responsible for precise binding between TALE proteins and the targeted DNA, both in TALE activators and TALE repressors. Therefore, it is meaningful to further reveal the function and broaden the application of TALEs, which play a role in precisely regulating gene expression in mammalian and plant cells.

Unit assembly employs a simple strategy and is easy to handle for construction of TALEs. This method requires only a few starting materials, and all operations are based on a standard molecular cloning procedure (Huang et al., 2011). In this study, we generated a novel repression system to demonstrate the repressor function of TALEs in *Arabidopsis thaliana*, based on the unit assembly method. We first designed and constructed a TALEN vector that targeted a specific sequence of *deetiolated 2* (*DET2*), which encodes a pivotal biosynthesis enzyme of brassinosteroid in *Arabidopsis* (Supplemental Figure 1). The *det2* seedlings grown in the dark displayed short hypocotyls and retarded growth. When screening for the stably inherited mutants, we found that the lines TALEN-1-DET2-2 (TD1-2), TD1-3, TD1-13, TD1-14, and TD1-21 exhibited different hypocotyl lengths, which was related to the variable expression of *DET2* (Figure 1A). Interestingly, we did not detect any mutation in the *DET2* genomic sequence in TD1-2, TD1-3, TD1-13, TD1-14, or TD1-21, although the transcriptional levels of *DET2* in these lines were affected to different degrees (Supplemental Figure 2A). Real-time quantitative PCR (qPCR) analysis showed that *DET2* was highly repressed in TD1-2 while its expression was hardly affected in TD1-21, which was correlated with the hypocotyl lengths of the five lines (Supplemental Figure 2B). In addition, after examining the abundance of TALEN in TD1-2, TD1-3, TD1-13, TD1-14, and TD1-21, we found that the expression of the left arm of TALEN-DET2 was much higher in TD1-2 than that in the other four lines. Only a small portion of the left arm was detected in TD1-14 and TD1-21 (Figure 1B). These findings suggested that the shorter hypocotyls in TD1-2, TD1-3, TD1-13, TD1-14, and TD1-21 seedlings resulted from reduced *DET2* expression caused by the expression of TALEN-DET2.

As the transcription of the target gene *DET2* was suppressed in different TD1 lines, we presumed that this repression was induced mainly by the binding of the TALEN left arm on the sense strand of the *DET2* coding sequence. To confirm this hypothesis, we designed and generated a vector, TALE-repressor (TALER), which expressed the TALE under the control of the cauliflower mosaic virus 35S promoter in the backbone vector pCAMBIA1300, without a functional domain fused to the C terminus. As a *Nhe*I (GCTAGC) digestion site was detected at the pVS1 RepA locus of pCAMBIA1300, we performed a same-sense mutation of this restriction site into GCTCGC to make TALER compatible with the unit assembly system (Supplemental Figure 3A). Therefore, the assembled tandem RVDs in pMD18-T could be digested by *Nhe*I and *Spe*I, then directly ligated into the TALER vector. By PCR-amplifying the TALE scaffold sequence including the hemagglutinin (HA) tag, nuclear localization sequence SV40, N terminus, and C terminus from the pCS2-FokI vector, followed by subcloning into the mutated pCAMBIA1300, we obtained the TALE-based repression vector TALER (Supplemental Figure 3B).

To verify the repression effect of TALEs further, we ligated the TALE sequence of the TD1 sense strand to the TALER system. Initially we transiently expressed TALER-DET2 in 4-week-old *Arabidopsis* leaves to evaluate the effect of TALE on repression of the target gene *DET2* (Figure 1C). Using real-time qPCR analysis, we found that the expression of target gene *DET2* in

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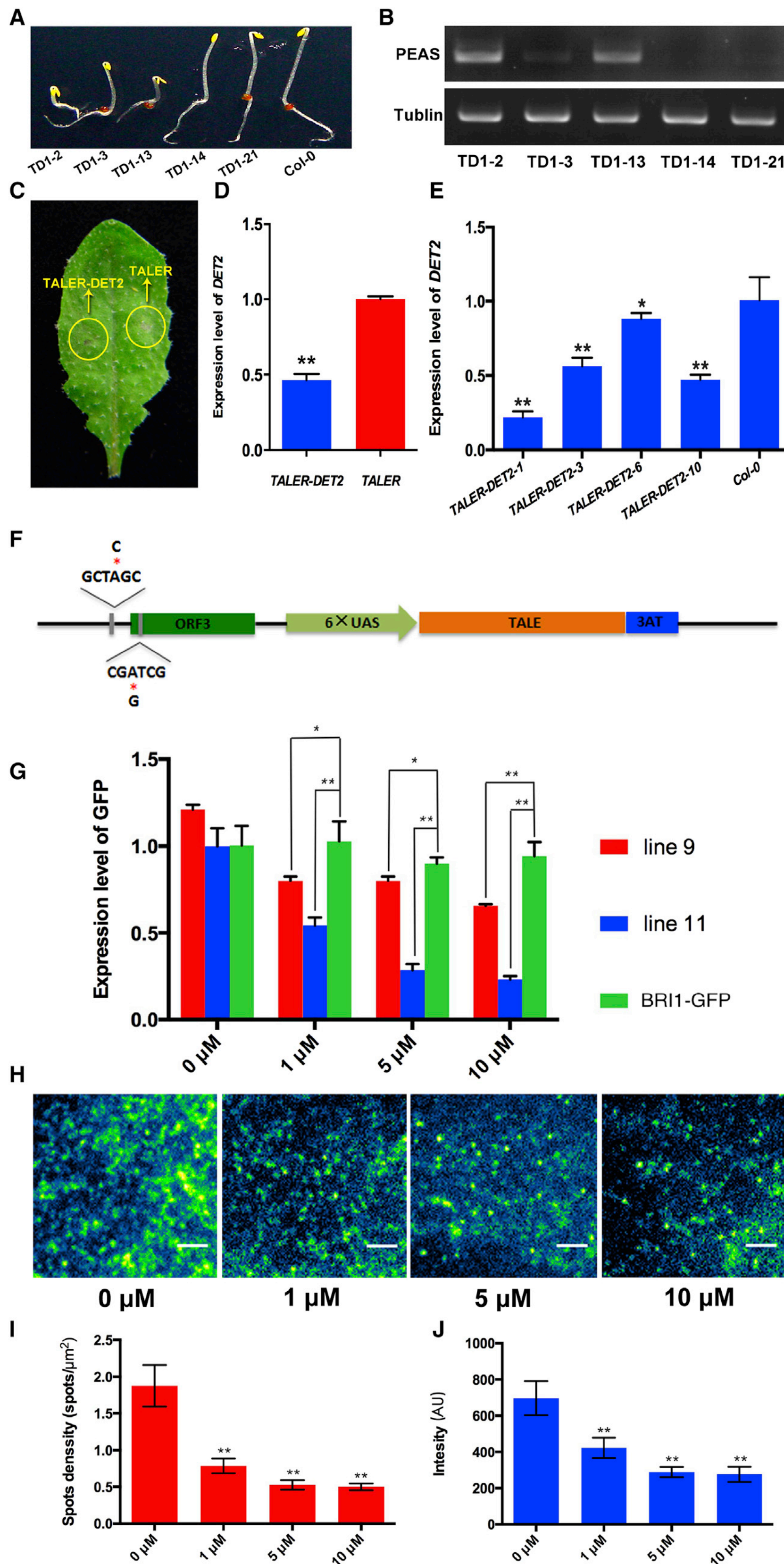

Figure 1. The Repression of the Target Gene by TALE-Only in *Arabidopsis*.

(A) The phenotype of TD1-2, TD1-3, TD1-13, TD1-14, and TD1-21 (from left to right) grown on 1/2 MS medium in the dark for 5 days.

(B) The expression level of the TALEN-DET2, left arm. Semi-quantitative RT–PCR expression levels were normalized to *β-tubulin*.

(C) Transient expression of TALER-DET2 in *Arabidopsis* leaves. TALER-DET2 was infiltrated into the left side of leaves, and the TALER vector was infiltrated into the right side of leaves as a control, as indicated in the yellow circles.

(D) The expression level of *DET2* in *Arabidopsis* leaves after infiltration of TALER-DET2 and TALER for 48 h.

(E) *DET2* expression level in four T3 lines of TALER-DET2.

(F) The structure of the iTALER vector. The expression of TALE was driven by the UAS promoter, the adenine nucleotides at two NheI sites in the pTA7002 backbone vector were mutated to cytosine and guanine as indicated by the red star.

(G) GFP expression levels in lines 9 and 11 of iTALER-BRI1-GFP seedlings. The seedlings were treated with 0, 1, 5, and 10 μM dexamethasone. All seedlings were 5 days old and treated with dexamethasone for 2 days. $^{**}P < 0.01$, $^{*}P < 0.05$, t-test. Error bars represent the mean ± SD, n = 3.

(H) Live images of hypocotyl epidermal cells from iTALER-BRI1-GFP-11 seedlings treated with 0, 1, 5, and 10 μM dexamethasone. Scale bar represents 2 μm.

(I and J) BRI1-GFP density **(I)** and fluorescence intensity **(J)** on the plasma membrane of iTALER-BRI1-GFP-11 seedlings treated with 0, 1, 5, and 10 μM dexamethasone, five live cells from 10 representative *Arabidopsis* hypocotyls (120 × 120 pixels). The seedlings in **(I and J)** were 5 days old and treated by dexamethasone for 2 days. $^{**}P < 0.01$, $^{*}P < 0.05$, t-test. Error bars represent the mean ± SD, n ≥ 3.

TALER-DET2-infiltrated leaves was repressed by 54% compared with the control infiltrated with the TALER vector only (Figure 1D). In addition, to examine the TALER effect further, we performed a parallel experiment to generate another transiently expressed plasmid, TALER-BKI1, which binds to the 20-bp target sequence CCACTTGTTCTTCTCCTTCT localized in the exon-only region of *BRI1 kinase inhibitor 1* (*BKI1*), whose product is a substrate of brassinosteroid-insensitive 1 (BRI1) and negatively regulates BR signaling in *Arabidopsis* (Supplemental Figure 4A). After transiently expressing TALER-BKI1 in *Arabidopsis* leaves for 48 h (Supplemental Figure 4B), we found that the expression of *BKI1* was reduced by 46% in comparison with the control, which was infiltrated with the TALER vector (Supplemental Figure 4C). Our results indicated that the designed TAL effectors repressed the transcription of target genes in the transient assays.

We further analyzed the expression level of *DET2* in the stably inherited TALER-DET2 seedlings by real-time qPCR. The result showed that the expression of *DET2* was differentially inhibited in the four TALER-DET2 lines evaluated, and TALER-DET2-1 showed the highest repression (Figure 1E). Also, we obtained stable lines that constitutively expressed the TALE protein, targeting the *BKI1* sequence specifically. It is evident that the expression of *BKI1*was repressed by 48% in all of the four lines evaluated (Supplemental Figure 4D). Moreover, we engineered two more TALEs, which bind to another site at *DET2* and *BZR1*, respectively (Supplemental Figure 5A). By performing the transient expression assays in the leaves of *Arabidopsis*, we found that the transcription levels of target genes *DET2* and *BZR1* were reduced by 31.1% and 40.7% (Supplemental Figure 5B). In addition, we obtained the TALEN-DET2-R (TDR) lines, which overexpress the right arm of TALEN-DET2, but the mRNA level was decreased in none of them (Supplemental Figure 6). Our results confirmed that the designed TALEs binding at the sense strand effectively repressed the expression of the target gene.

In parallel, we generated an inducible TALER system (iTALER), based on the pTA7002 plasmid, to further evaluate the effect of TALEs on repressing gene expression. We selected pTA7002 as the backbone vector and mutated two *Nhe*I digestion sites that were separated by 547 base pairs. One *Nhe*I site was mutated from GCTAGC to GCCAGC. The other *Nhe*I site, localized in the ORF3 region, was mutated to GCGAGC. We then amplified the N- and C-termini of TALE in the pCS2-PEAS plasmid by PCR and subcloned the TALE sequence into the mutated pTA7002 vector, following the same procedure as described previously (Figure 1F). We then designed and selected the 18-bp TALE-binding site TTACCCTTAAATTTATTT, localized within the *GFP* gene (Supplemental Figure 7). After transforming the iTALER-GFP plasmid into $\mathrm{Pro}_{\mathrm{BRI1}}$-BRI1-GFP seedlings, we assessed the efficiency of this inducible TALE repression vector in iTALER-BRI1-GFP lines. Using real-time qPCR to measure mRNA levels of *GFP* in iTALER-BRI1-GFP seedlings, which were cultured in 1/2 Murashige–Skoog (MS) medium and treated with different concentrations of dexamethasone, we found that the expression of *GFP* was effectively repressed by 1 μM dexamethasone, and was repressed the most under treatment with 10 μM dexamethasone (Figure 1G).

Given that the brassinosteroid receptor BRI1 is localized in the plasma membrane, we assessed BRI1-GFP in iTALER-BRI1-GFP-11 seedlings using variable-angle total internal reflection fluorescence microscopy (VA-TIRFM) (Figure 1H). VA-TIRFM has a high sensitivity and signal-to-noise ratio and can minimize photodamage in plant cells (Li et al., 2013). Single-particle fluorescence imaging showed that the fluorescence intensity of BRI1-GFP in the plasma membrane was generally reduced by 58%, 72%, and 74% after treatment with 1, 5, and 10 μM dexamethasone, respectively (Figure 1I). Furthermore, by single-particle analysis of the BRI1-GFP fluorescence intensity in the membranes of iTALER-BRI1-GFP-11 seedlings, we found that it was reduced to 39% upon treatment with 10 μM dexamethasone (Figure 1J). These results were consistent with the *GFP* mRNA levels observed by real-time qPCR.

A previous study showed that fusing the repressor domain with TALE can repress the target gene by its binding the specific region in the promoter (Mahfouz et al., 2012). Here, our study provided several lines of evidence for the repressor effects of TALEs. We found that TALEs repressed the transcription of target genes by binding to the exon region of the sense strand. Further analyses revealed that TALEs effectively repressed the expression of both endogenous and exogenous genes. More importantly, this repression effect was independent of a functional domain fused to TALEs. Based on our results, we proposed that the mechanism that TALEs inhibited the transcription of the target genes was probably due to the binding blocking, or at least partially blocking, the transcription process. Taken together, we set up an effective and inducible system of TALE-based transcriptional repression, which could effectively extend the application of TALEs and provide a new approach to gene regulation in plants.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

J.L. and S.L. conceived the study. S.L. performed the experiments. Y. Zhao ran the data analysis. S.L. drafted the manuscript. J.L. reviewed and edited the manuscript. Y. Zhao, Y. Zhu, M.G., X.D., and X.W. participated in the correction of the manuscript. J.L. acquired the funding support. All authors read and approved the final manuscript.

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