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TCP1 positively regulates the expression of *DWF4* in *Arabidopsis thaliana*

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Abbreviations: TCP, TEOSINTE BRANCHED 1, CYCLOIDEA, and PCF; DWF4, DWARF4; BRs, brassinosteroids; CPD, CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM; BR6ox1, BR-6-oxidase 1; DET2, DE-ETIOLATED 2; ROT3, ROUNDFOLIA 3

Brassinosteroids (BRs) are a group of major phytohormones playing critical roles in plant growth and development. Within the last two decades, key events of BR biosynthesis and signal transduction have been gradually elucidated. The detailed molecular mechanisms controlling bioactive levels of BRs, however, are not fully understood. TCP1 is a member of class II TCP proteins in *Arabidopsis thaliana*. The role of TCP1 in BR biosynthesis was discovered by an activation tagging analysis aiming to screen for genetic suppressors of an intermediate allele named *bri1-5* of the BR receptor gene *BRI1*. Overexpression of *TCP1* partially suppresses the defective phenotypes of *bri1-5* via direct upregulation of *DWF4*, one of the target genes of TCP1.

Previous studies using pea plants indicated that BRs cannot be transported in a long distance way as other phytohormones do.[1] BR homeostasis, therefore, must be precisely regulated within a plant cell. Both excessive and deficient amount of BRs are inadequate to optimal plant growth and development. Generally speaking, bioactive levels of BRs are mainly controlled by three different mechanisms, positive regulation, negative feedback regulation and catabolism. Positive/negative feedback regulation controls the speed of biosynthesis, whereas catabolism mediates the rate of degrading excessive amount of BRs. It was found that the expression levels of a number of crucial BR biosynthetic genes are controlled by negative feedback mechanisms.[2-8] For example, when endogenous BRs are depleted by a specific BR biosynthetic inhibitor, brassinazole (BRZ),[9] at least five known BR biosynthetic genes including *DET2*, *DWF4*, *CPD*, *BR6ox1* and *ROT3* are upregulated.[6] Whereas, four of these five genes (*DWF4*, *CPD*, *BR6ox1* and *ROT3*) were found to be downregulated upon the supplementation of exogenous BL, the final product of BR biosynthetic pathway and the most active BR.[6] It is now clear that the negative feedback response of BRs is through two dual-role transcription factors, BZR1 and BES1.[3,7] Each of the aforementioned five BR biosynthesis gene promoters contains at least one specific binding site for either BZR1 or BES1 or both.[10,11] BZR1 and BES1 act either as repressors or activators for thousands of downstream BR response genes.[8] Within the past decade, a number of proteins involved in BR catabolism have also been identified. For example, BNST3 from *Brassica napus* was found to inactivate BRs by sulfonation.[12] Arabidopsis BAS1, SHK1/SOB7/CHI2, UGT73C5, BEN1 are thought to be involved in inactivating BRs with distinctive mechanisms.[13-18] Positive regulation of BR biosynthesis had been less understood until TCP1 and CESTA were identified as transcription factors directly stimulating the transcription of *DWF4* and *CPD*, respectively.[19,20] In this addendum, we discuss the information of TCP1 in regulating BR biosynthesis by upregulating the expression of a key BR biosynthesis gene, *DWF4*.

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Both *dwf4-1D* and *tcp1-1D* were Identified as Dominant Genetic Suppressors of *bri1-5*

In higher plants, most of the genes contain at least one additional copy, which makes reverse genetics a less powerful strategy to reveal the biological roles of these genes. To overcome this problem, we employed a gain-of-function approach called activation tagging to identify novel genes regulating BR homeostasis and signal transduction via screening for suppressers of *bri1-5*, an intermediate BR receptor mutant. From a large scale activation tagging transgenic pool, we identified *dwf4-1D* and *tcp1-1D* as two authentic genetic suppressors of *bri1-5*.[19] Overexpression of *DWF4* and *TCP1* can partially suppress the defective phenotypes of *bri1-5* including the flowering time, petiole length, rosette width and plant height (**Fig. 1**). Overexpression of *TCP1* also suppresses biosynthetic mutant *det2* but cannot suppress null alleles of *BRI1*, indicating that *TCP1* is indeed involved in BR signal transduction or biosynthesis. Because there lacks the T-DNA insertion lines for *TCP1* in the Arabidopsis Biological Resource Center (ABRC), we constructed a chimeric repressor mutant *tcp1-SRDX*. Overexperession of *tcp1-SRDX* resulted in a typical dominant negative phenotype which is similar to the BR deficient or signaling mutants such as *det2* or *bri1-5*. These results further suggested that TCP1 has a role in a BR related pathway.

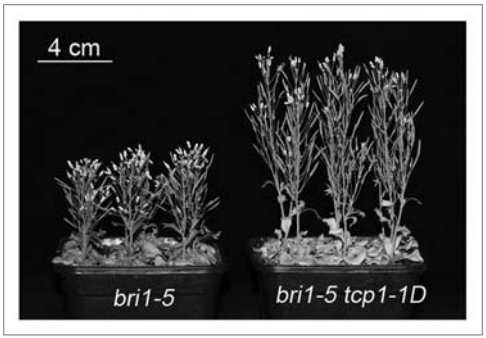

Figure 1. Phenotypes of *bri1-5* and *bri1-5 tcp1-1D*. The plants were photographed five weeks after germination.

TCP1 Positively Regulate BR Biosynthesis via its Direct Regulation of the Transcription of *DWF4*

Interestingly, the dwarfed *tcp1-SRDX* transgenic plants can be rescued by exogenously supplied BL, but not by any other tested major plant hormones such as auxins, gibberellins or cytokinins, hinting that dwarfed *tcp1-SRDX* plants may have resulted from the disruption of BR biosynthesis rather than BR signal transduction. Because TCP proteins usually act as transcription factors, we proposed that TCP1 may directly regulate the expression of a gene/genes involved in BR biosynthesis. To discover the true TCP1 target gene, we compared the amount of BR biosynthetic intermediates in WS2, *tcp1-1D* and *tcp1-SRDX* plants to examine which specific BR biosynthetic step has been altered. Our results showed that the catalytic capability of *DWF4* is obviously upregulated in *tcp1-1D* and downregulated in *tcp1-SRDX* plants. ChIP analysis indicated that TCP1 can interact with the promoter region of *DWF4*. Electrophoretic mobility shift assays (EMSA) solidified the hypothesis that TCP1 directly regulate the transcription of *DWF4* via its association with its binding site within the *DWF4* promoter (unpublished data). The facts that *tcp1-1D* cannot suppress *dwf4* and the identification of *dwf4-1D* as one of the genetic suppressors of *bri1-5* are consistent with the hypothesis that TCP1 is a positive regulator mediating the transcription of the key BR biosynthetic gene *DWF4*.

Perspectives

Regarding the BR homeostasis, positive regulation of BR biosynthesis is the least understood research area in the field. Identification of TCP1 in regulating *DWF4* transcription and CESTA in mediating *CPD* expression opens up a novel avenue in BR homeostasis research.[19,20] In the future, the true TCP1 binding sequence in *DWF4* promoter has to be confirmed by a series of EMSA analyses. In addition, how *TCP1* expression can respond various endogenous and exogenous cues will be another attractive focus to understand how BR biosynthesis is stimulated by internal and environmental signals. We are also testing all 23 TCP1 homolgs in Arabidopsis genome to determine all possible functional redundant genes of *TCP1*. Once determined, we will further use reverse genetic tools to test their significance in regulating plant growth and development.

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References

1. Symons GM, Reid JB. Brassinosteroids do not undergo long-distance transport in pea. Implications for the regulation of endogenous brassinosteroid levels. Plant Physiol 2004; 135:2196-206.
2. Goda H, Shimada Y, Asami T, Fujioka S, Yoshida S. Microarray analysis of brassinosteroid-regulated genes in Arabidopsis. Plant Physiol 2002; 130:1319-34.
3. Wang ZY, Nakano T, Gendron J, He J, Chen M, Vafeados D, et al. Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell 2002; 2:505-13.
4. Mathur J, Molnár G, Fujioka S, Takatsuto S, Sakurai A, Yokota T, et al. Transcription of the Arabidopsis *CPD* gene, encoding a steroidogenic cytochrome P450, is negatively controlled by brassinosteroids. Plant J 1998; 14:593-602.
5. Müssig C, Fischer S, Altmann T. Brassinosteroid-regulated gene expression. Plant Physiol 2002; 129:1241-51.
6. Tanaka K, Asami T, Yoshida S, Nakamura Y, Matsuo T, Okamoto S. Brassinosteroid homeostasis in Arabidopsis is ensured by feedback expressions of multiple genes involved in its metabolism. Plant Physiol 2005; 138:1117-25.
7. Yin Y, Wang ZY, Mora-Garcia S, Li J, Yoshida S, Asami T, et al. BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell 2002; 109:181-91.
8. He JX, Gendron JM, Sun Y, Gampala SSL, Gendron N, Sun CQ, et al. BZR1 Is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science 2005; 307:1634-8.
9. Asami T, Yoshida S. Brassinosteroid biosynthesis inhibitors. Trends Plant Sci 1999; 4:348-53.
10. Sun Y, Fan XY, Cao DM, Tang W, He K, Zhu JY, et al. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev Cell 2010; 19:765-77.
11. Yu X, Li L, Zola J, Aluru M, Ye H, Foudree A, et al. A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in *Arabidopsis thaliana*. Plant J 2011; 65:634-46.
12. Rouleau M, Marsolais F, Richard M, Nicolle L, Voigt B, Adam G, et al. Inactivation of brassinosteroid biological activity by a salicylate-inducible steroid sulfotransferase from *Brassica napus*. J Biol Chem 1999; 274:20925-30.
13. Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, et al. *BAS1*: A gene regulating brassinosteroid levels and light responsiveness in Arabidopsis. Proc Natl Acad Sci USA 1999; 96:15316-23.
14. Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, et al. CYP72B1 inactivates brassinosteroid hormones: an intersection between photomorphogenesis and plant steroid signal transduction. Plant Physiol 2003; 133:1643-53.
15. Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, et al. BAS1 and SOB7 act redundantly to modulate Arabidopsis photomorphogenesis via unique brassinosteroid inactivation mechanisms. Plant J 2005; 42:23-34.
16. Nakamura M, Satoh T, Tanaka SI, Mochizuki N, Yokota T, Nagatani A. Activation of the cytochrome P450 gene, *CYP72C1*, reduces the levels of active brassinosteroids in vivo. J Exp Bot 2005; 56:833-40.
17. Takahashi N, Nakazawa M, Shibata K, Yokota T, Ishikawa A, Suzuki K, et al. *shk1-D*, a dwarf Arabidopsis mutant caused by activation of the *CYP72C1* gene, has altered brassinosteroid levels. Plant J 2005; 42:13-22.
18. Yuan T, Fujioka S, Takatsuto S, Matsumoto S, Gou X, He K, et al. *BEN1*, a gene encoding a dihydroflavonol 4-reductase (DFR)-like protein, regulates the levels of brassinosteroids in *Arabidopsis thaliana*. Plant J 2007; 51:220-33.
19. Guo Z, Fujioka S, Blancaflor EB, Miao S, Gou X, Li J. TCP1 Modulates Brassinosteroid Biosynthesis by Regulating the Expression of the Key Biosynthetic Gene DWARF4 in *Arabidopsis thaliana*. Plant Cell 2010; 22:1161-73.
20. Poppenberger B, Rozhon W, Khan M, Husar S, Adam G, Luschnig C, et al. CESTA, a positive regulator of brassinosteroid biosynthesis. EMBO J 2011; 30:1149-61.