

Article Addendum

Roles of DLT in fine modulation on brassinosteroid response in rice

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Accumulated evidences suggest that brassinosteroid (BR) response is fine-tuned by multiple strategies. Recently, a GRAS protein, DLT, was identified to be involved in rice BR signaling. Transcription analysis of most known BR-related genes, including biosynthetic genes, signaling genes and also several downstream BR responsive genes, reveals DLT has extensive effects on BR-related genes expression, indicating its important role in modulating BR response. By promoter and protein sequence analysis, a complicated and subtle network was proposed that DLT may be involved in fine-modulating BR response at both transcriptional and protein levels. This provides new insights into BR signaling pathway and also gives a clear direction for the future work.

Although a very low concentration of BR can promote plant growth, a relatively higher concentration of BR may inhibit it, including both shoot and root.¹ The exact threshold value of this concentration is obscure, which likely depends on the plant cultivar or other unknown factors. In addition, unlike to GA or auxin, rice height has no remarkable or relatively slower response to exogenous BR treatment. Consistently, most of BR up or down-regulated genes have delayed and also mild changes of expression to BR treatment in Arabidopsis.^{2,3} Similar expression pattern was also implied in rice BR regulated genes.^{4,5} On the other hand, unlike some seriously dwarf phenotype caused by BR deficiency, ectopic expression of a BR biosynthetic gene in rice led to only slightly taller than wild-type while with remarkably enlarged leaf angles.⁶ Our unpublished data also show that overexpression of a positive signaling gene, *DLT*, leads to confused phenotype, with either slightly higher or even shorter than wild-type, implying the

insensitivity to a certain extent of rice cell elongation/division to alteration of endogenous BR level. It was believed that rice adopts a subtle and complicated mechanism to fine-tune the plant response to BR, either exogenous or endogenous. Recently, our identification of a new GRAS family protein, DLT, provides new evidence of this modulation strategy.⁷

Function of DLT seems very similar to OsBZR1. Both of them play dual roles in mediating upstream signaling to activate downstream gene expression, and in addition inhibiting the expression of BR biosynthetic genes.^{8,9} This gives out the possibility that DLT may interact with OsBZR1 directly to form protein complex to execute their regulatory functions. However, our preliminary yeast two-hybrid tests disaffirmed this presumption. It is interesting that three BRREs (BR-Response Elements) and two E-boxes were found in *DLT* promoter, implying the role of *DLT* as the potential target of OsBZR1 which could possibly both repress and activate its transcription.^{10,11} Our data also suggest that DLT and OsBZR1 regulate each other in opposite ways: while OsBZR1 binds to *DLT* promoter to inhibit its expression, DLT seems to promote *OsBZR1* expression, indicating that the complex regulatory network may be necessary for the fine-tuning of BR response.

What's more, DLT is a GRAS protein, which provides possibilities of regulation at protein level by phosphorylation and dimerization.¹² Results by mini-motif search (<http://sms.engr.uconn.edu/servlet/SMSSearchServlet>) of OsBZR1 and DLT protein show that there are 11 'GSK3-ALPHA consensus phosphorylation sites in DLT protein comparable to OsBZR1's 15 sites; and a 14-3-3 motif at 171 amino acid of DLT similar to OsBZR1's at 152 (Table 1). These hints give out important information that DLT could be the target of possibly existed rice GSK3-like kinase, potentially homolog of BIN2,¹³ as evidences show BR signaling pathway should be conserved between monocotyledon and dicotyledon,^{8,14} and in addition that DLT may shuttle between cytoplasm and nucleus with the help of 14-3-3 proteins.

This assumption brings highly complicated possibilities into the relations between DLT and other BR signaling factors and also BR biosynthetic genes, suggesting an intricate network involving the regulations on both transcriptional and protein levels. At transcriptional level, mediating through DLT and OsBZR1, BR response can feedback-regulate both the BR biosynthetic gene

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Table 1 Minimotif search of OsBZR1 and DLT by minimotif miner

	Known motif	Annotation	Positions
OsBZR1	S/T-?-?-?	GSK3-ALPHA consensus phosphorylation site (C-term Ser must first be phosphorylated)	89, 93, 98, 102, 106, 110, 114, 118, 122, 160, 165, 192, 218, 233, 248
	R?-S/Y/F/W/T/Q/A/D-?-S/T-?-P/L/M	14-3-3 motifs, Ser must be phosphorylated	152
DLT	S/T-?-?-?	GSK3-ALPHA consensus phosphorylation site (C-term Ser must first be phosphorylated)	6, 28, 63, 170, 172, 174, 176, 182, 604, 608, 610
	R/K/H-S/T/A/V/L-?-S/T-?-P/E/S/R/D/I/F	14-3-3 motif	171

expression and BR signaling gene expression. While at the same time, *DLT* and *OsBZR1* themselves are regulated each other. This compensation mechanism used to balance the BR response may partially explain that the change folds of the whole genome gene expression caused by BR deficiency or BR treatment are comparably very modest, as indicated by several microarray tests in *Arabidopsis*,^{2,3,15,16} which is quite different from observed in other phytohormone treatments. With respect to protein level, *DLT* could be phosphorylated, subcellular transported, interacted with other proteins or degraded,¹¹ representing multiple strategies for hormone signaling. The subtle network may provide some cues for the tricky response of rice to increased exogenous or endogenous BR level.

Whatever is true or not, this deduction provides new insights into BR signaling pathway in rice, and also direct our further efforts to testify this hypothesis to finally uncover the underlying mechanism of rice fine modulation on BR response.

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