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Direct activation of *EXPANSIN14* by LBD18 in the gene regulatory network of lateral root formation in Arabidopsis

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Abbreviations: ARF, auxin response factor; ASL, asymmetric leaves 2-like; AUX1, auxin permease 1; Aux/IAA, auxin/indole-3-acetic acid; EXP, expansin; LAX, like-AUX1; LBD, lateral organ boundaries domain; LRP, lateral root primordia; TIR1, transport inhibitor response1

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Root system architecture is important for plants to adapt to a changing environment. The major determinant of the root system is lateral roots originating from the primary root. The developmental process of lateral root formation can be divided into priming, initiation, primordium development and the emergence of lateral roots, and is well characterized in Arabidopsis. The hormone auxin plays a critical role in lateral root development, and several auxin response modules involving AUXIN RESPONSE FACTORS (ARFs), transcriptional regulators of auxin-regulated genes and Aux/IAA, negative regulators of ARFs, regulate lateral root formation. The *LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE* (*LBD/ASL*) gene family encodes a unique class of transcription factors harbouring a conserved plant-specific lateral organ boundary domain and plays a role in lateral organ development of plants including lateral root formation. In our previous study, we showed that *LBD18* stimulates lateral root formation in combination with *LBD16* downstream of *ARF7* and *ARF19* during the auxin response. We have recently demonstrated that LBD18 activates expression of *EXP14*, a gene encoding the cell-wall loosening factor, by directly binding to the *EXP14* promoter to promote lateral root emergence. Here we present the molecular function of LBD18 and its gene regulatory network during lateral root formation.

Arabidopsis lateral roots initiate from pericyle founder cells after the priming of the xylem pole pericycle cells to divide by auxin signaling in the basal meristem and undergo a series of anticlinal divisions, producing a few initial cells.[1] Inner and outer cell layer are then formed by periclinal cell divisions. Further anticlinal and periclinal divisions create a lateral root promordia (LRP) that continue to grow and emerge through the cortex and epidermal layers of the parental primary root. The hormone auxin plays a major role in lateral root development. The auxin transporter AUX1 regulates the initiation of lateral roots by basipetal auxin transport,[2-4] whereas LAX3, the AUX1-like auxin influx carrier, promotes lateral root emergence by affecting auxin influx in the outer endodermis and cortex cells.[5] LAX3 promotes lateral root emergence by auxin-dependent induction of a selection of cell-wall-remodeling enzymes that likely promote cell separation in advance of the developing LRP.[5] Two auxin response modules, IAA14-ARF7-ARF19 and IAA12-ARF5, control lateral root initiation and the patterning process.[6-8] ARF7 and ARF19 regulate lateral root formation by activating *LBD16* and *LBD29*.[9] *LBD18* regulates lateral root formation in conjunction with *LBD16* downstream of *ARF7* and *ARF19*.[10,11] *LBD18* was shown to regulate lateral root initiation by transcriptionally activating the E2Fa transcription factor that activates the cell cycle.[12]

We have previously shown that the number of emerged lateral roots of *lbd16* or *lbd18* single mutants decreased significantly, and that *lbd16 lbd18* double mutants exhibited an additively reduced number of emerged lateral roots,[11]

indicating that LBD18 might also play an important role in lateral root emergence. We isolated putative target genes of LBD18 using microarray analysis to investigate transcriptional response downstream of LBD18, which is responsible for lateral root development. Dexamethasone (DEX)-induced nuclear localization of LBD18 fused to the glucocorticoid steroid hormone binding domain (GR) was utilized to isolate the genes differentially regulated by LBD18 using the Affymetrix Arabidopsis full genome array. Although an early time point at a 2.5 h was used to identify the genes regulated downstream of LBD18, a substantial number of downstream genes might be secondary response genes and some of them could be primary response genes. *EXP14* which exhibited robust expression in Pro_{35S}*:LBD18:GR* Arabidopsis plants by DEX treatment was found to be a direct target of LBD18.[13] Reduced GUS expression in the promordium and overlaying tissues of Pro_{EXP14}*:GUS* by loss-of-function mutation in *LBD18* suggested that *EXP14* is an endogenous LBD18 target. Transient gene expression assays with Arabidopsis protoplasts, yeast one-hybrid system, chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assays demonstrated that LBD18 directly binds to a specific region of the *EXP14* promoter in vivo and in vitro. *EXP14* overexpression in Arabidopsis resulted in enhanced lateral root formation at the emergence step, whereas loss-of-function in *EXP14* reduced auxin-stimulated lateral root formation. These results together suggested that LBD18 activates expression of *EXP14* by directly binding to the *EXP14* promoter as a part of the transcriptional response promoting lateral root emergence. We noted that the *EXP14* promoter region that LBD18 bound in vitro and in vivo did not contain the LBD motif, in which LBD4 and AS2 bind in vitro,[14] suggesting that there are variable *cis*-acting elements that LBD proteins *trans*-act for functional diversity.

Although LBD18 possesses a molecular function as a transcriptional activator, it directly downregulates *ANTEGUMENTA* encoding a gene regulating plant organ size.[15] Thus LBD18 may act as an activator or a repressor by interacting with a coactivator or a corepressor depending on the sequence variation in the promoter to which LBD18 binds. A variety of studies on mammalian transcription factors have demonstrated that although some factors are pure activators or repressors, many others can both activate and repress transcription.[16] For example, Pit-1 activates growth hormone gene expression in one cell type, the somatotrope, whereas the allosteric effect on Pit-1 generated by other DNA binding factors results in the recruitment of a corepressor for active repression of the growth hormone gene in another cell type, the lactotrope.[17] In another example, the glucocorticoid receptor binds as a dimer to the glucocorticoid response element (GRE) following hormone treatment and activates transcription, but the receptor binds as a trimer to the distinct negative GRE sequence and represses transcription.[18] Further study is necessary to determine the dual molecular function of LBD18 as an activator or a repressor.

Genes with fold-change > 1.5 and a low-stringency/high-sensitivity *FDR* value < 0.15 following DEX treatment were regarded as the genes differentially regulated by LBD18 in our microarray analysis.[13] Under these conditions, 381 genes were upregulated and 585 genes were downregulated by LBD18, indicating that a large number of genes are subject to regulation by LBD18. The genes classified into metabolism, signal transduction and transcription factor categories constitute 48 and 58% among the genes up and downregulated, respectively. Expression of numerous transcription factor genes and protein kinase genes was up or downregulated by LBD18. Such an early transcriptional cascade may impact later developmental and physiological changes. We have previously identified 27 candidate genes that might be involved in lateral root emergence among the LBD18-upregulated genes.[13] *LAX3*, *EXP17* and *AIR3*, which are dependent upon *LAX3*, belong to the emergence group. We also found 70 candidate genes among the LBD18-upregulated genes, that might be involved in lateral root initiation, (data not shown), from the genes that display transcriptional changes in the xylem pole pericycle cells during lateral root initiation.[5] This analysis supports the previous notion that *LBD18* plays a role in lateral root initiation, as it activates *E2Fa* expression promoting lateral root initiation.[12] *ARF19* belongs to the initiation group, indicating that *LBD18* might upregulate *ARF19* through a positive feedback loop. *LAX3* was shown previously to be involved in regulating the expression of a selection of cell-wall-remodeling enzymes including EXP17 in promoting lateral root emergence.[5] Our microarray data showed that *LBD18* upregulated *EXP17* and *LAX3*. *LBD18* is positively regulated downstream of *ARF7* and *ARF19*.[10,11] *ARF19* overexpression in Arabidopsis results in stimulation of lateral root formation.[19] *LAX3* is auxin-inducible.[5] Taken together, these data led us to hypothesize that once this pathway is activated by auxin, lateral root formation might be reinforced in part by a *LAX3*-ARF7/*ARF19-LBD18* positive feedback regulatory network to ensure continued lateral root growth. Such a positive feedback regulatory loop might override the negative feedback regulatory loop by Aux/IAA-ARF system during the auxin response for developmental determination of lateral root formation. Genetic, biochemical and developmental approaches are underway to confirm this hypothesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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