Brassinosteroid Selectively Regulates PIN Gene Expression in Arabidopsis

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When brassinosteroid (BR)-deficient mutant (det2) or wild-type (WT) seedlings were treated with brassinolide (BL), the most active BR, for 3 h, the abundance of PIN4 and PIN7 transcripts decreased, and there were fewer PIN4 and PIN7 transcripts in det2 than in the WT. This suggests that BL selectively regulates the PIN gene in a complex manner.

Key words: PIN; brassinosteroid

The members of the PIN protein family (PIN1, PIN2/AGR/EIR1, PIN3, and PIN4) participate in polar auxin transport and regulate plant development, including differential growth, embryo and root patterning, and vascular tissue differentiation.1,2) It was recently suggested that PIN6 and PIN7/AEH1 also participate in polar auxin transport.3,4) Physiological studies have demonstrated that the actions of brassinosteroids (BRs) are related to those of auxin.5,6) Recently, studies have begun to reveal the molecular mechanism of auxin-BR interactions. Using DNA microarray analysis, we reported that the most bioactive BR, brassinolide (BL), regulated a number of genes in auxin-related gene families in Arabidopsis; BL down-regulated PIN7.7) In this experiment, we studied the expression of PIN genes in response to BL.

To study the regulation of PIN genes by BL, we treated 7-day-old light-grown Arabidopsis BR-deficient mutants (det2),8) grown in 1/2 Murashige and Skoog (MS) medium,9) with 10 nM BL for 3 h. Total RNA was extracted from whole seedlings and then the levels of PIN1, PIN2, PIN3, PIN4, PIN6, and PIN7 transcripts were quantified, using real-time quantitative (RTQ) TaqMan RT-PCR. RTQ RT-PCR analysis was done as described previously7,10) using gene-specific primers and probes (Table 1). We found that no probe-primer set recognized the cDNA template of the other genes (data not shown). Within 3 h, BL decreased the levels of PIN2, PIN4, and PIN7 transcripts to 74, 48, and 46%, respectively (Fig. 1A). To analyze whether BL decreases transcripts of PIN2, PIN4, and PIN7 in wild-type plants (WT, Col-0), 7-day-old WT plants were treated with 10 nM, 100 nM, or 1 μM BL for 3 h. The levels of PIN4 and PIN7 transcripts decreased in a dose-dependent manner in the WT (Fig. 1B). Only a modest decrease was observed in the 10-nM BL treatment. By contrast, at 100 nM and 1 μM BL, the levels of PIN4 and PIN7 transcripts were approximately 30% of the initial levels. Conversely, there was no obvious decrease in PIN2 transcripts with BL in WT plants. We also compared the levels of PIN2, PIN4, and PIN7 transcripts in WT and det2 plants. Since the level of endogenous BR is lower in det2 than in the WT,11) we expected that PIN2, PIN4, and PIN7 expression would be higher in det2. Contrary to our expectations, the levels of PIN4 and PIN7 transcripts in the det2 mutant were decreased to 69% and 54% of the WT respectively (Fig. 1C). To solve this discrepancy, we studied expression of the PIN7 gene in response to BL in a time course. Transcript abundance of PIN7 was decreased in 3 h, then recovered to the initial level in 12 h, and slightly induced (about 1.2-fold) in a 24-h BL treatment (data not shown). Therefore, we

Table 1. Primers and TaqMan Probes Used for RT-PCR

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward Primer Sequence</th>
<th>Reverse Primer Sequence</th>
<th>TaqMan Probe Sequence</th>
</tr>
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<tbody>
<tr>
<td>PIN1</td>
<td>AAACCACCAAGCCGATTACT</td>
<td>TTTCCCTGAGGTACAGGATTTAAAC</td>
<td>CACCGTACGAAACGATCATCATAAAAGGA</td>
</tr>
<tr>
<td>PIN2</td>
<td>GGGCGGCTCTAATCCCAAG</td>
<td>CACCTGGAACCGCTCCA</td>
<td>AACCGTGAGAGGCGTGGAGCTT</td>
</tr>
<tr>
<td>PIN3</td>
<td>GGCGGCGCTCTAATCCCAAG</td>
<td>GGCGGCGGCGCGATTTTTTT</td>
<td>TGACTGTCGCTGCTCCTCCTGAGGCTG</td>
</tr>
<tr>
<td>PIN4</td>
<td>CACCCCAAATTGGTCTGGTG</td>
<td>CCGACCGGGTATATAATGCGTACC</td>
<td>ACTCTGGTGCACAGTTGGCATG</td>
</tr>
<tr>
<td>PIN6</td>
<td>CCGGGAAAGACGACAACTC</td>
<td>CATCATTAAATGCACTGGCCGCA</td>
<td>TGCCGGCAGATGCCGACGCCAC</td>
</tr>
<tr>
<td>PIN7</td>
<td>CCGTGGAATATTTTACGGACCA</td>
<td>CACGGCTAGTTGCTCCAGTAAT</td>
<td>TAATGGCGGTGGCCAGATGGCTA</td>
</tr>
</tbody>
</table>

*The complementary sequence was used as the TaqMan probe.

Abbreviations: BR, brassinosteroid; BL, brassinolide; RTQ RT-PCR, real-time quantitative reverse transcriptase polymerase chain reaction; WT, wild-type
speculate that a transient increase in the BL level down-regulates expression of the PIN4 and PIN7 genes, while a continuous decrease in the BL up-regulates expression of these genes. The level of PIN2 transcripts was similar in det2 and the WT. It is unclear whether BL regulates PIN2 expression. The results suggest that BL selectively regulates PIN4 and PIN7, although the direction of BL regulation is not one-way and the regulatory mechanism is complex.

The PIN4 and PIN7/AEH1 genes have been observed in every tissue tested. PIN4 protein is expressed in the quiescent center and surrounding cells of developing and mature root meristems, and the protein is localized toward the auxin maximum. The localization of PIN4 and the analysis of the pin4 mutant suggest that PIN4 is involved in maintaining and establishing an auxin gradient. Very recently, it was demonstrated that PIN7 is expressed in the basal cell lineage in the embryo. PIN7 is also involved in axis formation during early embryogenesis concomitant with PIN1 and PIN4. During lateral root primordium development, at least six PIN genes are expressed in specific, partially overlapping patterns. Although the organ-specific regulation of PIN4 and PIN7 expression by BL remains unclear, we speculate that BL participates in plant morphogenesis via the regulation of auxin flow by selectively regulating the expression of PIN genes. Previously, we suggested that BR and auxin regulate the same early auxin-inducible genes, IAA5, IAA19, and SAUR-AC1, which suggests that auxin and BR share several signaling components. BR regulation of auxin flow via the selective regulation of PIN expression might be another mechanism that accounts for the interaction of BR and auxin.

References


