Auxin regulation of the microRNA390-dependent transacting small interfering RNA pathway in Arabidopsis lateral root development

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ABSTRACT

MicroRNA (miR)390 cleaves the non-coding TAS3 precursor RNA for the production of tasiRNA-ARF, a group of endogenous trans-acting small-interfering RNAs which cleave the transcripts of auxin response factor (ARF) 3/4. miR390-cleaved TAS3 RNA is polymerized and diced into tasiRNA-ARF by RNA-dependent RNA polymerase6 (RDR6) and Dicer-like4 (DCL4), respectively. tasiRNA-ARF-dependent post-transcriptional gene silencing (PTGS) of ARF3/4 is involved in auxin-mediated polarity establishment in the development of aerial lateral organs, such as leaf and flower. To understand how auxin regulates ARF4 expression, we examined auxin responsiveness of miR390 expression, which comprises a regulatory step for the biogenesis pathway of tasiRNA-ARF (the tasiRNA-ARF pathway), in Arabidopsis thaliana lateral root (LR) development. The results of this study provide evidence that miR390 expression is sensitive to TIR1-dependent transcriptional regulation and auxin concentration, and also that mutual negative-regulation between the tasiRNA-ARF pathway and ARF4 modulates the spatiotemporal expression of ARF4. We propose that, together with auxin concentration sensing through miR390 transcription, the tasiRNA-ARF pathway mediates the auxin response and ARF4-mediated LR developmental processes.

INTRODUCTION

During the development of multicellular organisms, cells detect local concentrations of pattern-forming substances within a concentration gradient and respond by exhibiting the corresponding developmental fates. In plants, the phytohormone auxin is considered to be a pattern-forming substance which participates in many aspects of plant development, including morphogenic triggering of lateral organs. Various lateral organs appear to adopt a similar pattern of auxin distribution with high concentrations at the apexes of the respective primordia (1–3). However, our knowledge of how auxin concentrations are translated into pattern formation is rudimentary.

Once delivered into target cells, auxin binds to the F-box protein TIR to initiate its downstream signaling pathway (4,5), although, formally, alternative auxin signaling pathways may also exist. Auxin signaling employs two classes of antagonistic regulatory transcription factors: auxin/indole-3-acetic acid (AUX/IAA) and auxin response factor (ARF) (6,7). There are 23 known ARFs in Arabidopsis, and this diversity suggests that they have roles in different tissues and/or developmental stages (8). Similarly, AUX/IAA forms a large family with 29 members (9). When auxin levels are low, AUX/IAA represses ARF activity by directly binding to ARF. Conversely, when auxin levels are high, degradation of AUX/IAA is induced and the released ARF becomes active in transcriptional control (10,11). Direct auxin binding causes the AUX/IAA protein to interact with SCF:TIR, a member of an E3 ubiquitine ligase complex, inducing degradation (12,13).
In the cambium cells of the poplar tree, the distribution of AUX/IAA depends on auxin concentration, suggesting that the AUX/IAA genes have roles in interpreting auxin concentration (14). Among the 23 family members of ARFs in Arabidopsis, ARF3 (ETT) and its close homologue ARF4 (ARF3/4) are known to be transcriptional repressors (11), and are implicated in pattern formation in gynoecium (15) and leaf primordium development (1,16,17). As abaxial determinants, ARF3/4 have been suggested to be mediators for auxin signaling to partition adaxial and abaxial domains during leaf primordium formation (17). It has thus been hypothesized that ARF3/4 interprets auxin concentrations in the leaf.

A unique feature of ARF3/4 regulation is that its transcripts are post-translationally cleaved by tasiRNA-ARF, an endogenous transacting small-interfering RNA (18–21). Biogenesis of tasiRNA-ARF (the tasiRNA-ARF pathway) is initiated by cleavage of the protein-non-coding TAS3 RNA by miR390. The miR390 gene in Arabidopsis comprises two family members: miR390a and miR390b (22). The miR390-cleaved transcript bound to Argonaute7 (AGO7) (22) is used as a template for polymerization by RNA-dependent RNA polymerase6 (RDR6). The resulting double-stranded RNA is cleaved in phase by Dicer-like4 (DCL4) to generate 21-nucleotide-long tasiRNA-ARF. Defects in this pathway cause phenotypic abnormalities in flower gynoecium formation (15) and leaf heteroblasty (23), suggesting that regulation of ARF3/4 plays a role in the development of these lateral organs. Furthermore, sided adaxial localization of tasiRNA-ARF correlates with the restriction of ARF3/4 localization in the abaxial domain during leaf development in Arabidopsis (17,23–25). The degree of conservation across the Arabidopsis genome (higher level) and abaxial domains (lower level) during leaf primordium formation (26). It was recently demonstrated that tasiRNA-ARF forms a concentration gradient across the adaxial (higher level) and abaxial domains (lower level) during leaf development in Arabidopsis (27). tasiRNA-ARF was suggested to be mobile by demonstrating that the localization of tasiRNA-ARF is different from the place of its biogenesis. In contrast to the gradient distribution of tasiRNA-ARF and broad distribution of miR390, ARF3 expression occurs in the abaxial domain with a sharp boundary (28). Although the molecular mechanism underlying the boundary formation of ARF3 is not understood, a connection to tasiRNA-ARF has been suggested (27,28).

It was recently shown that miR390 is the restrictive factor for the tasiRNA-ARF accumulation in maize shoot (29,30). Based on the roles of tasiRNA-ARF in auxin-triggered morphogenesis in lateral organs, we hypothesized that the tasiRNA-ARF pathway interacts with auxin signaling. To determine how auxin signaling and the tasiRNA-ARF pathway might interact, we used lateral root (LR) development in Arabidopsis as a model system. Auxin is known to be the primary trigger of LR development, but the tasiRNA-ARF pathway has not been reported to act in LR development. Auxin accumulates in the founder cells adjacent to the xylem pole prior to initiation of anticlinal and periclinal cell divisions of these cells to form primordia. Following the formation of early primordia, autonomous meristems of three to five cell layers are established (31–34). Further cell divisions and changes of cellular architecture lead to about eight cell layers of primordium at which point the LR emerges out of the primary root epidermis. Spatio-temporal correlation of auxin maxima with the initiation of LR suggests that auxin concentration is an instructive signal for positioning the site of initiation (35,36). In order to gain insight into how auxin regulates the tasiRNA-ARF pathway and ARF3/4 expression, we examined how miR390 expression is regulated by auxin. We suggest that miR390 expression plays a role in sensing auxin concentration, potentially leading to tasiRNA-ARF-dependent post-transcriptional gene silencing (PTGS) of ARF4 and LR development.

**MATERIALS AND METHODS**

Plant materials and growth conditions

Following germination on Murashige and Skoog (MS) media, plants (Arabidopsis thaliana ecotype Columbia-0) were germinated on MS media and then the plants were grown under long-day conditions (16 h-light/8 h-dark) at 22°C for two weeks. The plants were transferred to media containing the designated hormones for the indicated length of time. To analyze the effect of the 26S proteasome inhibitor MG132, 10-day-old seedlings were dipped into MS media containing 10 μM IAA and/or 10 μM MG132 for 6 h. The numbers of emerged LR were counted by eye. The pMIR390a:GUS and pMIR390b:GUS constructs are described in Montgomery et al. (22) and the seeds carrying these constructs were kindly provided by Dr James Carrington.

**RT-PCR, qRT-PCR and northern analysis**

RNA was extracted with Trizol reagent (MRC) and poly d(T) cDNA was prepared from 2 μg of total RNA with MMLV reverse transcriptase (Fermentas) and quantified on the Chromo-4-apparatus (Bio-Rad) using the Power SYBR green PCR Master Mix (Applied Biosystems). Cycling conditions were as follows: 95°C for 15 s and 60°C for 1 min. The primers used in this study are listed in Supplementary Table 1.

For northern blots analyses, total RNA (15μg) extracted from the roots was transferred to Hybond-N+ membranes (Amersham Biosciences). Hybridization was performed at 65°C using Rapid-Hyb buffer (Amersham Biosciences) with probes labeled with 32P-dCTP using the Random Primers DNA Labeling System (Invitrogen). Blots were washed once for 20 min in 2× SSC and 0.1% SDS at room temperature; once for 15 min in 0.5× SSC and 0.1% SDS at 65°C; and once for 15 min in 0.1× SSC and 0.1% SDS at 65°C. Low molecular weight (LMW) RNAs were precipitated with 5% PEG8000 and 0.5 M NaCl, separated in a 15% denaturing polyacrylamide gel, and subjected to blot hybridization analysis. DNA oligonucleotides complementary to miR390 and tasiR-ARF were end-labeled with [γ-32P] ATP with T4

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polynucleotide kinase (TaKaRa) for hybridization. After hybridization at 45°C, the membrane was washed twice in a non-stringent solution [3× SSC, 25 mM NaHPO₄ (pH 7.5), 5% SDS and 10× Denhardt’s solution] and once in a stringent solution (1× SSC and 1% SDS) at 45°C. The membrane was dried and exposed to X-ray film at −70°C.

**Transient expression assay using protoplasts**

*Arabidopsis* protoplasts were prepared from three-week-old seedlings. Leaves were collected and soaked in an enzyme solution (1% cellulose R-10, 0.25% macerozyme R-10, 400 mM mannitol, 8 mM CeCl₂, 5 mM MES at pH 5.7) at 22–25°C for 3–4 h. Transient expression assays using *Arabidopsis* mesophyll protoplasts were performed according to the method of Yoo et al. (37). The transfected protoplasts were lysed in lysis reagent (Promega), and luciferase activity was assayed as described by the manufacturer (Promega).

**Whole mount in situ hybridization**

A DNA fragment of 0.45 kb containing the *tasiR-ARF* cleavage site of the *ARF4* gene was used to make digoxigenin-labeled antisense/sense RNA probes. The sense and antisense RNA probes were labeled with digoxigenin-11-UTP using SP6 and T7 polymerase (Ambion) and the hybridized probe was detected by using an anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche). Whole mount *in situ* hybridization was performed according to the method of Jan Hejátko et al. (38). *tasiR-ARF* and *miR390* were identified using the locked nucleic acids (LNA) oligonucleotide probe (IDT): 5’- T(+G)GGG(+T)CT T(+A)C(AA)+G(T)GCA(+A)GAA-3’ and 5’-GG(+)GC(+T)AT+(+)CC(+T)CC(+T)GA(+G)CT(+T)-3’, respectively (23,26).

**RESULTS**

**miR390 expression in response to auxin and auxin concentration**

To examine how *miR390* expression is regulated by auxin concentration, *miR390* levels were monitored in the wild-type Col-0 seedling roots exposed to the different IAA concentrations (Figure 1A). The results showed that the levels increased only at high concentrations such as 10 and 50 μMs (Figure 1A). Consistent with this, the levels increased as the duration of exposure was extended at the fixed concentration of 10 μM, whereas at 10 nM, the levels remained relatively unchanged, suggesting that *miR390* transcription is induced at high auxin concentrations. In contrast to *miR390*, IAA up-regulated *tasiRNA-ARF* levels remained relatively constant throughout the different IAA concentrations, when compared to the untreated control (Figure 1B, upper). The IAA (10 μM)-induced up-regulated level of *tasiRNA-ARF* remained constant during the exposure period of 24 h (Figure 1B, bottom). *tasiRNA-ARF* was not detected over the basal level in *rdr6-11* mutants, where the *tasiRNA-ARF* pathway is blocked (Figure 1B, bottom). *In situ* hybridization of *tasiRNA-ARF* using LNA antisense probe shows that when compared to Col-0, overall *tasiRNA-ARF* accumulation is significantly weak in *tir1-1*, further substantiating auxin control of *tasiRNA-ARF* level (Figure 1C). The response to high IAA concentrations seems to be restricted to *miR390*, and does not appear for *ARF4*, *RDR6*, *DCL4*, or *AGO7* (Supplementary Figure 1). These results collectively suggest that *miR390* expression is a potential regulatory step for auxin regulation of the *tasiRNA-ARF* pathway.

We monitored promoter activity of the *miR390* genes to investigate how the expression and localization of *miR390* are regulated by auxin. Promoter activity (GUS signal) in both *pMIR390a : GUS* and *pMIR390b : GUS* seedlings was observed in the primary root and LR primordia (Figure 2A and B). The auxin-responsive synthetic promoter DR5 was used an indirect measure of IAA concentration, and showed a weak concentration maximum at the LR apex (Figure 2C). Whereas the GUS signal in the LR primordia of *pMIR390a : GUS* appears to be inversely correlated with DR5:GUS expression, the GUS signal in *pMIR390b : GUS* primordia positively correlates with DR5:GUS expression (Figure 2A and B). These results imply that promoter activity of the *MIR390b* gene may correlate with a putative auxin gradient present in the LR primordium. Whole-mount *in situ* hybridization analyses with an LNA antisense probe revealed that mature *miR390* is broadly distributed within the LR primordia of wild-type Col-0 seedlings, also in an auxin-dependent manner, in both earlier (upper) and later (bottom) stages of LR development prior to emergence (Figure 2D). Unlike the promoter activities, the distribution of mature *miR390* appears to be restricted to the LR (Figure 2D). In addition, the distribution of mature *miR390* in response to auxin was significantly broader than that of GUS expression, implying that the site of *miR390* transcription is different from the site of *miR390* processing and/or final localization. Similar to the distribution of mature *miR390*, *tasiRNA-ARF* was also broadly distributed in LR primordia (Figure 2E), suggesting that *miR390* is spatially associated with *tasiRNA-ARF* accumulation. These results collectively show that *miR390* expression is induced by auxin in a concentration-dependent manner, leading to a speculation that auxin may influence the activity of the *tasiRNA-ARF* pathway through its concentration-dependent regulation of *miR390* expression. *miR390* expression was shown to be sensitive only to auxin, and not to the other hormones such as abscisic acid, gibberellins and cytokinin (Figure 2F), suggesting that *miR390* expression and possibly the *tasiRNA-ARF* pathway are specifically involved in the auxin response. In contrast, RT-PCR analyses showed that expression of *HYLI*, which is involved in biogenesis of microRNA, and *TAS3* was induced by the various hormones applied including auxin (Supplementary Figure 2).

To further analyze auxin regulation of *miR390b* expression, we performed a transient transcription assay in *Arabidopsis* protoplasts transformed with a *pMIR390b : LUC* construct. The results show that
luciferase activity increased with exogenous IAA (Figure 3A). Consistent with this, qRT-PCR analyses indicate that the levels of the precursor-MIR390b are up-regulated by IAA in Col-0 seedling roots exposed to IAA (10 μM) for 24 h (Figure 3B). In addition, up-regulation by IAA was inhibited in the mutant tir1-1 in which TIR1-mediated protein degradation is compromised (Figure 3B). Consistent with these results, MG132, a protein degradation inhibitor, was shown to decrease miR390 levels in both the absence and presence of exogenous IAA (Figure 3C). In situ hybridization also showed that miR390 expression appeared to be restricted to the LR base in tir1-1, while it was more broadly dispersed in Col-0 (Figure 3D). It was also shown that miR390 was accumulated mainly in the matured LR region toward the LR apex in Col-0 seedling roots treated with the auxin transport inhibitor N-1-naphthyphthalamic acid (NPA), while was broadly dispersed in LR in the untreated control (Figure 3E). These results collectively indicate that miR390 expression is regulated by both TIR1-dependent auxin signaling and auxin transport.

**Mutual negative-regulation between the tasiRNA-ARF pathway and ARF4**

To test the possibility of tasiRNA-ARF involvement in ARF4 regulation, we compared the levels of ARF4 transcripts in Col-0 and rdr6-11 seedling roots exposed to exogenous IAA (10 μM) for 12 or 24 h (Figure 4A). In both Col-0 and rdr6-11, ARF4 transcript levels increased at 12 h. However, after 24 h exposure to IAA, they decreased in Col-0, whereas an increase was observed in rdr6-11, indicating that tasiRNA-ARF-mediated PTGS is affecting ARF4 expression in Col-0. A transient transcription assay, which was performed by transfecting the pARF4:LUC construct into Arabidopsis protoplasts, showed that ARF4 promoter activity was up-regulated by IAA (Figure 4B), suggesting that transcriptional control is involved in the auxin-mediated up-regulation of ARF4 expression. The higher auxin sensitivity observed for ARF4 compared to RDR6 may explain the initial ARF4 up-regulation followed by PTGS in Col-0 (Figure 4C). It required 10 μM IAA to attain a 6-fold increase of RDR6 transcripts compared to the untreated control, whereas only 10 nM was required to attain a 7-fold increase of ARF4 transcripts. These results show that auxin-dependent ARF4 expression is modulated through transcriptional up-regulation and tasiRNA-ARF-dependent down-regulation.

To understand the auxin-dependent regulatory network of ARF4 expression, temporal changes in ARF4 transcript levels were evaluated in Col-0 and rdr6-11 seedlings over 72 h-periods, following exposure to 2,4-D (10 μM), a chemically stable form of auxin (Figure 5A). In Col-0 seedlings, an initial increase in ARF4 transcript level was observed at 6 h, followed by a slight decrease and maintenance of levels during 24–72 h. In contrast, in rdr6-11, ARF4 transcript levels increased more dramatically at 6 h than Col-0 and peaked at 24 h, followed by a continuous decrease without maintenance of a constant level. This implies that the tasiRNA-ARF pathway provides the regulation to modulate ARF4 expression at a constant level. This finding is consistent with the in situ hybridization signal of the enlarged ARF4 domain in rdr6-11, when compared to Col-0 (Figure 5B). To examine whether the tasiRNA-ARF pathway is regulated by ARF4, the mRNA levels of RDR6, AGO7 and DCL4 were compared between Col-0, arf4-2 and ARF4-OX, in which ARF4 is overexpressed under the control of 35S promoter (Figures 5C and 6A). qRT-PCR analyses showed that while the levels of AGO7 and DCL4 mRNAs were not significantly different between Col-0 and rdr6-11.
Figure 2. miR390 expression in response to auxin concentration. The promoter activity of pMIR390a:GUS (A) and pMIR390b:GUS (B) seedlings. GUS activity was observed in the LR primordia of seedlings treated with the designated concentrations of IAA for 12h, at comparable developmental stages. (C) GUS activity driven by the DR5 promoter in LR primordia of seedlings at comparable developmental stages, treated with the designated concentrations of IAA for 12h. (D) In situ hybridization analyses of miR390 distribution in LR primordia at early (upper) and later pre-emergence stages (bottom) in Col-0 seedlings treated with the designated concentrations of IAA for 12h. (E) In situ hybridization of tasiRNA-ARF using a LNA antisense probe at different developmental stages of Col-0 LR. (F) Northern analyses of the levels of miR390 in Col-0 seedling roots exposed to various hormones (each 10μM) for 12h.
and arf4-2, the level of RDR6 mRNA was high in arf4-2 and was at a basal level in ARF4-OX (Figure 5C). In addition, miR390 levels, measured by northern hybridization, were repressed in ARF4-OX, and were higher in arf4-2, albeit less significantly (Figure 5D). These results indicate that ARF4 inhibits the activity of the tasiRNA-ARF pathway by repression of RDR6 and/or miR390, forming a feedback loop. To examine ARF4 repression of miR390 expression during LR development, the distribution of miR390 was compared between arf4-2 and Col-0 by in situ hybridization (Figure 5E). In arf4-2, the miR390 signal was observed to appear at the LR initiation site, and a tail of expression stemming from the LR apex of the fully mature LR was also observed (Figure 5E, bottom). In contrast, in Col-0, miR390 was not detected in the initiation site, and there was no tailing expression of miR390 in the apex region in the fully matured LR (Figure 5E, upper). The intensity of miR390 level in the fully matured LR was weaker in ARF4-OX than in Col-0, further supporting ARF4 repression of miR390 (Figure 5F). These results suggest that the distribution of miR390 is associated with ARF4 repression of miR390. Together with the tasiRNA-ARF-dependent regulation of ARF4 expression described above (Figure 5B), these results suggest that mutual negative regulation between the tasiRNA-ARF pathway and ARF4 determines the distributions of ARF4 and miR390. We speculate that the ability to maintain the levels of ARF4 transcripts in Col-0 may represent a dynamic and balanced up- and down-regulation of ARF4 expression throughout the developmental stages.

**ARF4 mediates LR development**

To investigate ARF4 participation in LR development, expression of genes which are involved in the pattern-formation of lateral organs, such as HD-ZIPIII and YABBY, was compared between arf4-2 and ARF4-OX (Figure 6A). RT-PCR analyses showed that the YABBY genes FILAMENTOUS FLOWER (FIL) and YAB3 were up-regulated in ARF4-OX, while they were repressed in arf4-2 (Figure 6A). In contrast, the HD-ZIPIII genes PHABULOSA (PHB), PHAVOLUTA (PHV) and REVOLUTA (REV) were shown to be insensitive to ARF4 regulation (Figure 6A). Consistent with the
RT-PCR analyses, in situ hybridization of a YABBY FIL gene indicates that its expression is up-regulated in ARF4-OX, but repressed in arf4-2 (Figure 6B). These results suggest that ARF4-up-regulation of FIL is involved in auxin regulation of LR development. The results of an LR assay indicate that the tasiRNA-ARF pathway and ARF4 are involved in LR development. The numbers of emerged LR in rdr6-11 and arf4-2 were...
less than in Col-0 by ~50%, while the lengths of primary roots were relatively constant in these plants (Figure 6C and D). It was also noted that growth of the emerged LRs in rdr6-11 was significantly retarded, compared to Col-0 and arf4-2. The number of LRs in the ARF4-OX line was higher than in Col-0 (Supplementary Figure 3). These results collectively suggest that tasiRNA-ARF regulation of ARF4 expression plays a role in LR development.

In conclusion, this study shows that the tasiRNA-ARF pathway and ARF4 mediate auxin regulation of LR development, and proposes that the auxin signal interacts with miR390 expression, the tasiRNA-ARF pathway and ARF4 expression during LR development.

DISCUSSION

In this study, we have analyzed how the auxin signal interacts with the tasiRNA-ARF-dependent PTGS of ARF4 during LR development. We suggest that miR390 expression is a potential regulatory component for the sensing of auxin concentration and for the modulation of ARF4 expression and tasiRNA-ARF level. Additionally, auxin signal is also known to regulate miR164 expression, which down-regulates the NAC1 transcription factor during LR initiation (39). Accumulation of data identifying other hormone-responsive microRNAs such as miR393 (ABA), miR160 (auxin) or miR159 (GA) suggest that microRNAs may be a key component in hormonal regulation of development in various organs (40–42). We previously reported that auxin-sensitive miR167 down-regulates ARF8 and OsGH3-2, a free auxin conjugating enzyme, to regulate the free auxin level in cultured rice cells (43). Taking into consideration that microRNA target genes are also subject to transcriptional control, what could be the developmental role of microRNA-mediated down-regulation of target genes? In triple mutants of miR164, where miR164 expression is completely abolished, the expression domain of CUC, a target of miR164, becomes less precise, leading to the varying degrees of abnormal phenotypes in inflorescence meristem and flower primordia (44). Thus, it has been suggested that miR164 reduces fluctuations of its target transcripts to increase developmental precision and stability. Stabilization of developmental processes by microRNA has also been proposed in animal development (45). Therefore, we speculate that miR390-mediated modulation of the levels of tasiRNA-ARF and ARF4 transcripts may play a role in stabilizing developmental processes during LR development.

Our findings in this study suggest that the expression of ARF4 and tasiRNA-ARF are mutually dependent (Figure 5B and E), leading to a speculation that distribution of ARF4 is defined by tasiRNA-ARF. This study also suggests that the capacity to maintain constant levels of ARF4 transcripts is derived from a complex regulatory network involving ARF4 transcription, PTGS of ARF4 and ARF4 repression of RDR6/miR390 (Figure 5A). The tasiRNA-ARF pathway may provide the ability to fine-tune the spatiotemporal expression of ARF4 in response to auxin, and thus we propose that the tasiRNA-ARF pathway may play a role in buffering and increasing precision of ARF4-mediated developmental processes. We propose that transcriptional control alone is not sufficient for the accurate positioning and expression.

Figure 6. ARF4 control of the YABBY genes. (A) RT-PCR analyses of the YABBY and HD-ZIPIII genes in arf4-2, ARF4-OX and Col-0. (B) In situ hybridization of FIL transcripts using an antisense probe in arf4-2, ARF4-OX and Col-0. (C, D) Numbers and picture of emerged LRs following 10-d growth of germinated seedlings in auxin-free media. The numbers are an average of 10 seedlings for each line (Col-0, arf4-2, rdr6-11). The asterisks indicate a significant difference from the wild-type control (P < 0.01, Student’s t-test). Standard errors are indicated.
of ARF4. To make the regulatory network even more complicated, miR390 maturation and tasiRNA-ARF biogenesis may also participate in the spatiotemporal regulation of ARF4 expression (27). It is interesting to note that ARF4 repression of RDR6, but not AGO7 and DCL4, may represent an additional regulatory step in regulating the tasiRNA-ARF pathway (Figure 5C).

Our results suggest that the level of miR390 expression appears to reflect auxin concentration, and thus may act as a sensor of auxin concentration. miR390 transcription is regulated by TIR1-dependent auxin signaling, implying that degradation of an AUX/IAA may play a role in sensing auxin concentration. Identification of this putative AUX/IAA and its auxin-dependent distribution should help us understand the mechanism underlying miR390 sensing of auxin concentration. It is possible that different AUX/IAA genes are involved in miR390 transcription at different developmental stages. We speculate that the tasiR-ARF and ARF4 transcripts are auxin-directed positional signals that specify subregions within LR. Conservation of the tasiR-ARF pathway in land plants suggests that PTGS-mediated modulation of ARF4 expression may be a common and fundamental event in auxin-mediated LR development in plants. Although deviations are likely to occur depending on plant lineages and developmental contexts, conveying of the auxin signal to development through tasiRNA-ARF-dependent PTGS of ARF4 may represent a conserved pathway underlying the development of lateral organs in plants.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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