Tissue-Specific Expression of SMALL AUXIN UP RNA41 Differentially Regulates Cell Expansion and Root Meristem Patterning in Arabidopsis

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Among the three primary auxin-induced gene families, Auxin/Indole-3-Acetic Acid (Aux/IAA), Gretchen Hagen3 (GH3) and SMALL AUXIN UP RNA (SAUR), the function of SAUR genes remains unclear. Arabidopsis SAUR genes have been phylogenetically classified into three clades. Recent work has suggested that SAUR19 (clade II) and SAUR63 (clade I) promote cell expansion through the modulation of auxin transport. Herein, we present our work on SAUR41, a clade III SAUR gene with a distinctive expression pattern in root meristems. SAUR41 was normally expressed in the quiescent center and cortex/endodermis initials; upon auxin stimulation, the expression was provoked in the endodermal layer. During lateral root development, SAUR41 was expressed in prospective stem cell niches of lateral root primordia and in expanding endodermal cells surrounding the primordia. SAUR41–EGFP (enhanced green fluorescent protein) fusion proteins localized to the cytoplasm. Overexpression of SAUR41 from the Cauliflower mosaic virus 35S promoter led to pleiotropic auxin-related phenotypes, including long hypocotyls, increased vegetative biomass and lateral root development, expanded petals and twisted inflorescence stems. Ectopic SAUR41 proteins were able to promote auxin transport in hypocotyls. Tissue-specific expression of SAUR41 from the PIN1, WOX5, PLT2 and ACR4 promoters induced the formation of new auxin accumulation/signaling peaks above the quiescent centers, whereas tissue-specific expression of SAUR41 from the PIN2 and PLT2 promoters enhanced root gravitropic growth. Cells in the root stem cell niches of these transgenic seedlings were differentially enlarged. The distinctive expression pattern of the SAUR41 gene and the explicit function of SAUR41 proteins implied that further investigations on the loss-of-function phenotypes of this gene in root development and environmental responses are of great interest.

Keywords: Arabidopsis • Auxin transport • Cell expansion • Root meristem patterning • SAUR.

Abbreviations: ACR4, ARABIDOPSIS CRINKLY4; ARF, AUXIN RESPONSE FACTOR; Aux/IAA, AUXIN/INDOLE-3-ACETIC ACID; CaMV, Cauliflower mosaic virus; DIC, differential interference-contrast; EGFP, enhanced green fluorescent protein; GH3, Gretchen Hagen3; GUS, β-glucuronidase; LRC, lateral root cap; NAA, 1-naphthaleneacetic acid; PI, propidium iodide; PIN, PINFORM; PLT2, PLETHORA2; RT–PCR, reverse transcription–PCR; SAUR, SMALL AUXIN UP RNA; TIR1/AFB, TRANSPORT INHIBITOR RESPONSE1/AUXIN-BINDING F-BOX PROTEIN; WOX5, WUSCHEL-RELATED HOMEOBOX 5.

Introduction


The SAUR41 subfamily contained four members: SAUR41 (At1g16510), SAUR40 (At1g79130), SAUR71 (At1g56150) and SAUR72 (At3g12830). Their amino acid sequences differed from those of other SAUR families in the N-terminus. In previous microarray experiments, the expression of SAUR41 has been reported to be regulated by circadian rhythm (Mazzella et al. 2005, Darrah et al. 2006), biotic stress (Zhang et al. 2011, Pellet et al. 2011), and mitochondrial dysfunction and reactive oxygen species (Carrie et al. 2010, Gleason et al. 2011). The expression of SAUR40 and SAUR71 was responsive to ABA signaling (Leonardt et al. 2004, Zeng et al. 2012) and the functional status of chloroplasts (Bosco et al. 2004, Estavillo et al. 2011). SAUR71 and SAUR72 were expressed in vascular development (Nagawa et al. 2006, Shirakawa et al. 2009).

We found that SAUR41 was normally expressed in the quiescent center and cortex/endodermis initials, but it was provoked in the endodermal layer upon an auxin or gravitropic stimulation. During lateral root development, SAUR41 was distinctively expressed in prospective stem cell niches of lateral root primordia and in expanding endodermal cells surrounding the primordia. SAUR41–enhanced green fluorescent protein (EGFP) fusion proteins localized to the cytoplasm, unlike EGFP–SAUR19 and SAUR63–EGFP which localized predominantly to the plasma membrane. Interestingly, although the gene expression pattern and the protein localization pattern of SAUR41 were different from those of SAUR19 and SAUR63, the phenotypes resulting from overexpression of SAUR41 driven by the Cauliflower mosaic virus (CaMV) 3SS promoter shared many similarities with those of SAUR19 and SAUR63 subfamily genes. Tissue-specific expression of SAUR41 from promoters of auxin transporter genes and root meristem patterning genes differentially modulated root meristem development, root cell expansion and root gravitropic growth. The distinctive expression pattern of the SAUR41 gene and the explicit function of ectopic SAUR41 proteins implied that further investigations on the loss-of-function phenotypes of this gene family in root development are of great interest.

**Results**

**SAUR41 had a distinctive expression pattern in Arabidopsis root meristems**

Previously, to counteract possible position effects in plant promoter analysis, we used the gypsy-Su(Hw) system of Drosophila in a novel approach that facilitated high and precise expression of reporter genes (She et al. 2010). Using this system, together with the GATEWAY recombination approach, we generated sets of promoter reporter lines for the PIN (PINFORM) gene family encoding auxin carriers and the TIR1/AFB gene family encoding auxin receptors (She et al. 2010). We extended this system to promoter analysis of certain SAUR genes, and found that the SAUR41 (At1g16510) gene showed a distinctive expression pattern in root meristems.
In generating promoter reporter lines for SAUR41, an ~1,800 bp DNA fragment upstream of the ATG start codon of SAUR41, as predicted by AtcisDB (Davuluri et al. 2003), was fused with the EGFP-GUS reporter gene. Viewed by confocal microscopy, SAUR41 was found to be specifically expressed in the quiescent center and cortex/endodermis initials of root stem niches (Fig. 1A). Considering that SAUR genes had been proposed to function in auxin-mediated growth events, we examined the expression of SAUR41 under gravitropic stimulation and auxin treatment. Gravitropism allows plant roots to grow directionally, whereas auxin is an essential regulator in this process (Harrison and Masson 2008). During the gravitropic response, the expression of SAUR41 was provoked on both sides of the endodermis at the proximal meristem region and on the upper side of the endodermis at the distal elongation zone, indicating that this gene might act to coordinate root elongation rather than simply respond to auxin redistribution (Fig. 1B). SAUR41 was specifically induced in the endodermis with 1 h of auxin [10 μM 1-naphthalene acetic acid (NAA)] treatment (Fig. 1C). Since the endodermis was recently reported as the primary responsive tissue for gibberellins to coordinate root growth (Ubeda-Tomàs et al. 2008, Ubeda-Tomàs...
et al. 2009, Ubeda-Tomás et al. 2012), we then tested whether or not SAUR41 was a gibberellin-responsive gene. As shown in Fig. 1D, SAUR41 was unresponsive to gibberellin treatment. This result was in agreement with the results of a microarray experiment (Josse et al. 2011).

Extending the duration of auxin treatment to 12 h, and using detection by overnight histochemical staining, SAUR41 was found to be induced in multiple cell layers at the root meristem and transition zone (Fig. 1E). However, in the elongation zone, it was specifically induced in the endodermal cells (Fig. 1F).

During lateral root development, SAUR41 was expressed in the prospective quiescent center of lateral root primordia (Fig. 1G, H). Interestingly, SAUR41 was also specifically expressed in the endodermal cells surrounding the lateral root primordia during the process of the lateral root primordia breaking through the endodermis (Fig. 1G). In the newly formed lateral roots, SAUR41 was strongly expressed in the quiescent center and initial cells, and weakly expressed in the endodermis (Fig. 1I). In hypocotyls, petioles and cotyledons, SAUR41 was predominantly expressed in the vascular tissues (Fig. 1J–L).

**SAUR41–EGFP fusion protein accumulated in the cytoplasm**

To check the subcellular localization of the SAUR41 protein, we generated a transgenic construct in which the CaMV 35S promoter drove a C-terminal translational fusion between the full-length SAUR41 and the EGFP protein. Location of the fusion protein in hypocotyls and root tips of stably transformed Arabidopsis plants was examined by confocal microscopy. Results showed that the EGFP fluorescence was identified at the cytoplasm of epidermal and cortical cells in hypocotyls (Fig. 1M, N), and at the cytoplasm of all types of cells in root tips, including quiescent center cells, cortex/endodermis initial cells and endodermal cells (Fig. 1O, P).

**Overexpression of SAUR41 conferred pleiotropic auxin-related phenotypes**

To explore potential roles for SAUR41 in Arabidopsis growth and development, we first screened T-DNA and transposon insertion lines from the Arabidopsis Biological Resource Center (ABRC) and the Rikagaku Kenkyusho Bioresource Center (RIKEN-BRC), respectively. The line SALK_056968 from the ABRC stocks contained a T-DNA at the promoter region of SAUR41. However, reverse transcription–PCR (RT–PCR) analysis revealed that this insertion did not impair the expression of SAUR41 (data not shown). In three additional lines (SALK_121397, PST_11030 and PST_17947), we failed to identify DNA insertions inside the SAUR41 gene.

We then used a gain-of-function approach and generated transgenic Arabidopsis plants overexpressing the untagged (35S:SAUR41) or MYC-tagged (35S:SAUR41-MYC) SAUR41 gene, in addition to the 35S:SAUR41-EGFP plants described above. More than 20 independent lines were obtained for each transgenic construct. Plants overexpressing untagged SAUR41 had strong phenotypes, similar to that of MYC-tagged SAUR41. In contrast, plants overexpressing EGFP-tagged SAUR41 had the weakest phenotypes. We chose 35S:SAUR41 plants for detailed study.

The 35S:SAUR41 transgenic lines displayed pleiotropic auxin-related phenotypes. Light-grown seedlings exhibited 1.7- to 2.0-fold longer hypocotyls than wild-type controls (Fig. 2A, B, E; P < 0.01, t-test). Surprisingly, these seedlings also displayed 1.3- to 1.5-fold longer primary roots compared with wild-type controls (Fig. 2C, D, F; P < 0.05, t-test). In addition, overexpression of SAUR41 increased root waving on vertically oriented agar plates (Fig. 2D). After 10 d of growth, 35S:SAUR41 seedlings had a 1.5- to 1.9-fold increase in lateral root numbers (P < 0.05, t-test) and a 20–30% increase in vegetative biomass as measured by the fresh weight of shoots (Fig. 2G, H; P < 0.05, t-test).

Adult 35S:SAUR41 plants had twisted inflorescence stems (Fig. 2I). Furthermore, the petals of transgenic flower organs were overexpanded and defective in opening completely (Fig. 2J–L), resulting in reduced seed setting in many siliques (Fig. 2I).

To determine if the elongated hypocotyl phenotype induced by ectopic SAUR41 was due to increased cell expansion, we measured hypocotyl epidermal cell length in 6-day-old seedlings. The change in hypocotyl epidermal cell length was parallel to the change in hypocotyl length (Figs. 3A, 2E). We then directly measured [3H]IAA transport in hypocotyls and detected a 40–70% increase in basipetal IAA transport in hypocotyls of 35S:SAUR41-EGFP, 35S:SAUR41-MYC and 35S:SAUR41 seedlings (Fig. 3B).

**Tissue-specific expression of SAUR41 from the PIN1 promoter induced alterations in root meristem patterning**

As SAUR41 had a distinctive expression pattern in Arabidopsis root meristems, rice and Arabidopsis SAUR proteins have been proposed to modulate auxin transport (Kant et al. 2009, Chae et al. 2012, Spartz et al. 2012), we next implemented tissue-specific expression of SAUR41 from promoters of auxin transporter genes and root meristem patterning genes. To facilitate the examination of auxin signaling and distribution, all transgenic plants were generated in a DR5rev::GFP background (Friml et al. 2003, Fig. 4B).

We first expressed SAUR41 from the PIN1 and PIN2 promoters. In Arabidopsis roots, PIN1 promoter activity was strong in stele cells and weak in endodermis and the quiescent center (She et al. 2010). Ectopic expression of SAUR41 driven by the PIN1 promoter led to auxin retention in stele initials transporting auxin, resulting in a large auxin accumulation/signaling peak (Fig. 4D–G). In addition, PIN1:SAUR41 roots had additional tiers of distal stem cells (Fig. 4E, G), while wild-type roots typically had one tier of distal stem cells (Ding and Friml 2010, Fig. 4A, B). Non-differentiated distal stem cells below the quiescent center were characterized by
Fig. 2 Overexpression of SAUR41 from the CaMV 35S promoter conferred pleiotropic auxin- or cell expansion-related phenotypes. (A–E) Light-grown 6-day-old seedlings. (F–H) Light-grown 10-day-old seedlings. (I–L) Adult plants. Hypocotyls of wild-type controls (A) and 35S::SAUR41 seedlings (B). Roots of wild-type controls (C) and 35S::SAUR41 seedlings (D). (E–H) Statistical comparison of hypocotyl length (E), primary root length (F), lateral root number (G) and vegetative biomass (H) of wild-type controls with 35S::SAUR41 seedlings. (I–L) Adult 35S::SAUR41 plants had twisted inflorescence stems (I) and overexpanded petals (J–L). Roots or flowers marked by arrows were enlarged in the subsequent images. **P < 0.01; *P < 0.05, t-test.
the absence of starch grains (Ding and Friml 2010; Fig. 4H, L). Finally, PIN1::SAUR41 roots had supernumerary cell layers (Fig. 4E, G), while wild-type roots exhibited distinctive root radial patterns (Fig. 4A, B). Statistically, 80–90% of PIN1::SAUR41 roots displayed abnormalities in root meristem patterning (n = 40, P < 0.05, t-test).

PIN2 promoter activity was detected in the epidermis, cortex and lateral root cap (LRC; She et al. 2010; Fig. 4I). Ectopic expression of SAUR41 from the PIN2 promoter induced expansion of epidermal and cortical cells, but the auxin accumulation/signaling pattern appeared normal (Fig. 4I). PIN2::SAUR41 roots exhibited a root-waving phenotype on vertically oriented agar plates, and the epidermal and cortical cells in the two sides of the root transition zones were irregularly and asymmetrically elongated (Fig. 4K).

Comparison of tissue-specific expression of SAUR41 and IAA2P65S from promoters of root meristem patterning genes

We also expressed SAUR41 from the promoters of root meristem patterning genes. Three promoters were chosen: WOX5 (WUSCHEL-related homeobox 5), ACR4 (ARABIDOPSIS CRINKLY4) and PLT2 (PLETHORA2). WOX5 encoded a homeodomain transcription factor (Sarkar et al. 2007), PLT2 encoded an AP-2 type transcription factor (Aida et al. 2004, Galinha et al. 2007) and ACR4 encoded a receptor-like kinase (De Smet et al. 2008). They were three of the major regulators of root stem cell activity (Aichinger et al. 2012, Perilli et al. 2012, Petricka et al. 2012). To compare the effects between tissue-specific expression of a SAUR protein and a stabilized AUX/IAA protein, we introduced IAA2P65S that harbored a site-directed mutation in the proline residue of conserved domain II. As one of 29 Aux/IAA genes of Arabidopsis, IAA2 was highly auxin inducible and expressed in vascular tissues and auxin accumulation/signaling peaks (Swarup et al. 2001).

WOX5 was expressed in the quiescent center of root stem cell niches (Sarkar et al. 2007; Fig. 5A). Tissue-specific expression of SAUR41 from the WOX5 promoter induced the formation of additional auxin accumulation/signaling peaks in stele initials above the quiescent center (Fig. 5B–F). In addition, WOX5::SAUR41 roots had supernumerary cell layers (Fig. 5C, D, F), like those observed in PIN1::SAUR41 roots (Fig. 4E, G). As expected, tissue-specific expression of IAA2P65S driven by the WOX5 promoter inhibited auxin accumulation/signaling in the quiescent center (Fig. 5G, H). Interestingly, it also inhibited auxin accumulation/signaling in the stele initials (Fig. 5G, H), opposite to the effects observed in tissue-specific expression of SAUR41 from the WOX5 promoter (Fig. 5B–F). ACR4 was expressed in the root stem cell niche and its surrounding cells, including young epidermal cells, cortical cells and columella cells (De Smet et al. 2008; Fig. 5I). Ectopic expression of SAUR41 from the ACR4 promoter led to the expansion of cells expressing ACR4 (Fig. 5J, K). Formation of auxin accumulation/signaling peaks in stele initials was visible (Fig. 5J, K), but not as dramatically as that in the WOX5::SAUR41 roots (Fig. 5B–F), indicating that the balanced expression of SAUR41 in the entire root stem cell niche attenuated the effects of ectopic SAUR proteins on auxin transport, compared with the selected expression of SAUR41 in the quiescent center by the WOX5 promoter. On the other hand, tissue-specific expression of IAA2P65S from the ACR4 promoter had a more remarkable impact on root meristem development compared with that seen in WOX5::IAA2P65S roots. As shown in Fig. 5, ACR4::IAA2P65S roots had two separate auxin accumulation/signaling maxima: one in the quiescent center and the other in the young columella cells (Fig. 5L).

PLT2 was gradiently expressed in root meristems and lateral cap cells (Aida et al. 2004, Galinha et al. 2007; Fig. 5M). Tissue-specific expression of SAUR41 from the PLT2 promoter exhibited triple effects as addressed above: additional auxin accumulation/signaling peaks in stele initials and supernumerary cell layers in the proximal meristem zone (Fig. 5N, O). On the other hand, tissue-specific expression of IAA2P65S from the PLT2 promoter inhibited auxin accumulation/signaling in the quiescent center, promoted auxin accumulation/signaling in stele initials and led to establishment of new auxin accumulation/signaling peaks in the adjacent provascular cells (Fig. 5P), similar to that seen for relocalization of auxin accumulation/signaling peaks upon polar auxin transport inhibitor treatment (Sabatini et al. 1999).
Ectopic SAUR41 proteins differentially regulated root cell expansion and root gravitropic growth

We measured cell areas of stele initial cells, quiescent center cells and distal stem cells in root meristems of transgenic seedlings expressing SAUR41 from promoters of auxin transporter genes and root meristem patterning genes. The results showed that PIN1::SAUR41, WOX5::SAUR41 and PLT2::SAUR41 roots had a 30–60% increase in cell areas of stele initial cells and quiescent center cells, while ACR4::SAUR41 roots had enlarged quiescent center cells and distal stem cells (Fig. 6A; P < 0.05, t-test).

The root gravitropic responses in these transgenic seedlings were also analyzed. ACR4::SAUR41 roots showed delayed gravitropic growth (Fig. 6B), coinciding with their higher auxin accumulation/signaling in young columella cells but lower auxin accumulation/signaling in LRC cells (Fig. 5I). PLT2::SAUR41 roots had advanced gravitropic growth 2 h after the gravitropic stimulation, while PLT2::SAUR41 roots displayed enhanced gravitropic growth 3 h after the gravitropic stimulation (Fig. 6B). It has been suggested that auxin transport in the lower side of lateral cap cells and auxin accumulation in the lower side of epidermal and cortical cells were essential for the root gravitropic response (Ottenschläger et al. 2003). Our results were consistent with the expression pattern of PIN2 and PLT2 promoters. PIN2 was expressed in both lateral cap cells and epidermal and cortical cells (Fig. 4I), while PLT2 was expressed in lateral cap cells (Fig. 5M).

Discussion

The expression pattern of SAUR41 in root meristems was distinctive. SAUR19 subfamily genes were expressed in growing hypocotyls in response to shade avoidance, and in root
elongation zones in response to auxin treatment (Spartz et al. 2012). SAUR63 and members of its clade were expressed in growing regions of hypocotyls, cotyledons, petiole, young rosette leaves and inflorescence stems, but not in roots (Chae et al. 2012). Herein, we found that the expression of SAUR41 was normally restricted to the quiescent center and cortex/endodermis initials of root meristems; upon an auxin or gravitropic stimulation, it was provoked in the endodermis at the proximal meristem region and transition zone of Arabidopsis roots (Fig. 1A–F). Furthermore, SAUR41 was differentially expressed...
during lateral root development, as manifested by β-glucuronidase (GUS) activity in prospective stem cell niches of lateral root primordia, and in expanding endodermal cells surrounding the lateral root primordia (Fig. 1G–I). In previous microarray experiments, the expression of SAUR41 has been reported to be regulated by multiple environmental signals (Mazzella et al. 2005, Darrah et al. 2006, Zhang et al. 2007, Peltier et al. 2011). Taken together, it seemed that Arabidopsis SAUR proteins have some similarities but also specificity in terms of molecular functions. Alternatively, SAUR proteins may share a similar molecular function, but different SAUR proteins require different tissue-specific partners.

Tissue-specific expression of SAUR41 from PIN1, WOX5, ACR4 or PLT2 promoters caused new auxin accumulation/signaling peaks in stele initial cells transporting auxin (Figs. 4D–G, 5B–F, N, O). Roots of PIN1::SAUR41 seedlings had additional tiers of distal stem cells below the quiescent center and supernumerary cell layers in root meristems (Fig. 4D–H, L, Q). It has been reported that auxin regulates distal stem cell differentiation in Arabidopsis roots, and defects in auxin transport would lead to additional tiers of distal stem cells (Ding and Friml 2010). Thus, it seemed likely that SAUR41 induced perturbation of auxin transport in root meristems as it was expressed above the stem cell niches (from the PIN1 promoter). In contrast, tissue-specific expression of SAUR41 from the PIN2 promoter did not induce alterations in root meristem patterning, but caused alterations in cell expansion in the corresponding cell lineages (Fig. 4J, K). In addition, PIN2::SAUR41 and PLT2::SAUR41 roots had enhanced gravitropic growth (Fig. 6B), indicating that ectopic SAUR41 proteins promoted root basipetal auxin transport for root gravitropic responses. Taken together, it seems likely that ectopic SAUR41 proteins retard auxin transport in root stem cell niches, but promote auxin transport in LRC cells and epidermal and cortical cells.

Previously, it has been proposed that rice SAUR39 acted as a negative regulator of organ growth and auxin transport (Kant et al. 2009), while Arabidopsis SAUR19 and SAUR63 acted as positive regulators of cell expansion and auxin transport (Chae et al. 2012, Spartz et al. 2012). Herein, in terms of cell expansion, we found that SAUR41 promoted cell expansion, as it was constitutively expressed from the CaMV 35S promoter (Figs. 2, 3). In addition, stele initial cells, quiescent center cells and distal stem cells in root meristems of transgenic seedlings expressing SAUR41 from promoters of auxin transporter genes and root meristem patterning genes were differentially enlarged (Fig. 6A). Thus, similarly to SAUR19 with N-terminal tags and SAUR63 with C-terminal tags, untagged SAUR41 promoted cell expansion, as it was ectopically expressed. However, in terms of auxin transport, the functions of SAUR proteins appeared to be more complicated. The observed higher flow rate of labeled IAA in hypocotyls could be an indirect effect of SAUR protein overexpression. Two questions, why ectopic SAUR41 proteins retarded auxin transport in root stem cell niches but promoted basipetal auxin transport, and why rice OsSAUR39 (analogous to Arabidopsis SAUR63, clade I) inhibited auxin transport but Arabidopsis SAUR63 promoted auxin transport, remained
unanswered. It should be noted that the DRS::GFP marker basically indicated the status of auxin signaling but not the auxin transport. Currently, direct measurement of auxin transport in root stem cell niches is unavailable. It was tempting to speculate that SAUR41 proteins used different mechanism to regulate auxin transport for cell elongation and for root meristem patterning.

Tissue-specific expression of IAA2P65S from WOX4, ACR4 and PLT2 promoters displayed fundamentally different effects on root meristem patterning compared with that observed for SAUR41 (Fig. 5). The mechanism of stabilized Aux/IAA proteins is clear. They impaired the SFTIR1 pathway of auxin signaling to regulate cell division, differentiation and elongation. They also disturbed auxin transport by transcriptional modification of the auxin export machinery (Hayashi 2012, Scherer et al. 2012). In contrast, the precise mechanism by which SAUR proteins regulate cell expansion and auxin transport remains unclear. It will be interesting to learn whether there exist epistatic interactions between the IAA2 and the SAUR41 gain-of-function phenotypes. We are currently crossing the IAA2P65S lines with the corresponding SAUR41 lines to answer this question.

The SAUR41 function reported here was solely dependent on the ectopic expression data, while its endogenous role in stem cell maintenance remained unclear. The gene could be involved in the regulation of cell sizes of quiescent center and cortex/endodermis initials, and/or in the modulation of auxin transport in these cells. In addition, the SAUR41 subfamily contains four members: SAUR40, SAUR41, SAUR71 and SAUR72. Further investigations on promoter activity and protein localization patterns of other SAUR41 subfamily members, as well as on loss-of-function phenotypes of the SAUR41 gene family, are required and of great interest.

Materials and Methods

Plant materials and growth conditions
Arabidopsis thaliana ecotype Columbia-0 and the DRSrev::GFP background (Friml et al. 2003) were used as sources of wild-type plant materials. Reporter promoter lines for the PIN gene family and the TIR1/AFB gene family (She et al. 2010) have been donated to ABRC. Seeds were surface sterilized and cultured aseptically on 9 cm Petri dishes containing Gamborg’s BS medium with 1% (w/v) sucrose and 1% (w/v) agar. The plates were maintained at 4°C for 2 d, and then transferred to a culture room (23°C, 80 μmol m⁻² s⁻¹ irradiance with a 16 h photoperiod and 30–40% relative humidity).

Vector construction and plant transformation
We used the GATEWAY™ system for vector construction. Entry vectors were created using the pENTR™/D-TOPO kits (Invitrogen). The PCR primers for construction of entry vectors for the coding region of SAUR41 and for promoter regions of SAUR41, WOX5, ACR4 and PLT2 are listed in Supplementary Table S1. IAA2P65S was generated by overlapping PCR using the primers given in Supplementary Table S1. Each entry clone was confirmed by DNA sequencing. GATEWAY™-compatible destination vectors for protein subcellular localization, overexpression, MYC tagging and promoter analysis were used (Karimi et al. 2002, Earley et al. 2006, She et al. 2010). The LR reaction was conducted to generate different expression vectors.

To facilitate tissue-specific gene expression from various promoters, the CaMV 35S promoter in the overexpression constructs (35S::SAUR41 and 35S::IAA2P65S) was replaced with a ccdB fragment by a method described previously (Yang et al. 2012). Briefly, the ccdB fragment was PCR amplified using pH7FWG2.0 as a template, with the primers ccdB-Up and ccdB-Dn, containing an introduced HindIII and SpeI site, respectively. The ccdB fragment was then digested to replace the 35S promoter sequence, thus forming new destination vectors for tissue-specific expression.

All of the expression vectors were electroporated into Agrobacterium tumefaciens strain GV3101. Plants were transformed using the vacuum infiltration method (Bechtold et al. 1993). Transgenic plants were selected on BS plates with 12.5 μg ml⁻¹ hygromycin or 25 μg ml⁻¹ kanamycin depending on the selection markers. Single-locus and homozygous transgenic lines were characterized as we described previously (She et al. 2010).

Microscopic analysis and histochemical detection
For histochemical detection of GUS activities, young seedlings at different developmental stages and different parts from transgenic plants were collected. They were stained at 37°C overnight in 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc), 1 mM potassium ferri cyanide, 0.1% Triton X-100 and 0.1 M sodium phosphate buffer, pH 7.0 with 10 mM EDTA. Samples were washed in 70% ethanol to remove Chl. Differential interference contrast (DIC) images were visualized using a microscope (Nikon Eclipse 80i) with a DXM1200 CCD camera and EclipseNet software. For the localization of fluorescence fusion proteins, a confocal microscope system (Zeiss LSM510) was used. Without specification, 5-day-old seedlings were mounted in water. Starch grains in columella cells were stained with I₂–KI as described previously (Ding and Friml 2010).

Hypocotyl IAA transport assay
IAA transport in hypocotyls was measured as previously described (Chae 2012). [³H] IAA was a product of American Radiolabeled Chemicals, Inc. The radioisotope counts of [³H] IAA were detected using a low-noise scintillation counter (MicroBeta 2, Perkin Elmer).

Growth and cell measurement, statistical analysis and image processing
After incubations for the durations indicated in the text, the plates were digitally photographed. Root and hypocotyl length
was measured using magnified images. Lateral root (>1 mm) numbers were counted using each seedling as an individual sample. For hormone treatment, seedlings were transferred onto a medium containing 10 μM NAA or GA₃ for 1 or 12 h. For gravitropism assays, the protocol of Weijers et al. (2005) was adopted. The mean hypocotyl epidermal cell length and the cell area of root stem cell niches were measured as described by Spartz et al. (2012). Each treatment contained 30–50 seedlings and was replicated three times. Statistical analysis of the data was performed using Microsoft Excel and Student’s t-test. Images were processed using Adobe Photoshop CS2.

**Supplementary data**

**Supplementary data** are available at PCP online.

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**References**


