

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

Yingying Kong^{1,2,3}, Yubin Zhu^{1,2,3}, Chen Gao¹, Wenjing She¹, Weiqiang Lin¹, Yong Chen¹, Ning Han¹, Hongwu Bian¹, Muyuan Zhu¹ and Junhui Wang^{1,*}

¹Institute of Genetics, College of Life Sciences, Zhejiang University, Zijingang Campus, Hangzhou 310058, China

²Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China

³These authors contributed equally to this work.

*Corresponding author: E-mail, junhuiwang@zju.edu.cn; Fax: +86-571-88206535.

(Received August 20, 2012; Accepted February 3, 2013)

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 HOMEBOX 5*.

Introduction

The plant hormone auxin modulates many aspects of plant growth and development, and recent decades have seen rapid progress in our understanding of the molecular mechanism of auxin biology (Woodward and Bartel 2005, Santner and Estelle 2009, Vanneste and Friml 2009). For instance, in the field of auxin control of root development, numerous fundamental insights have been achieved on the contribution of shoot-derived auxin and root-generated auxin in root development (Overvoorde et al. 2010), establishment and maintenance of root stem cell niches (Aichinger et al. 2012, Perilli et al. 2012, Petricka et al. 2012), hormone interactions in coordinated root growth (Petricka et al. 2012, Ubeda-Tomás et al. 2012) and root growth in response to the environment (Jones and Ljung, 2012, Petricaka et al. 2012).

Auxin regulates cell division, differentiation and elongation in part by changing gene expression (Woodward and Bartel 2005, Paponov et al. 2008, Santner and Estelle 2009, Vanneste and Friml 2009, Hayashi 2012). Primary auxin response genes consist of members of three gene families (Hagen and Guilfoyle 2002): *Aux/IAA* (*Auxin/Indole-3-Acetic Acid*), *GH3* (*Gretchen Hagen3*) and *SAUR* (*SMALL AUXIN UP RNA*). *Aux/IAA* proteins are transcription repressors (Ulmasov et al. 1997, Worley et al. 2000, Gray et al. 2001, Ouellet, et al. 2001, Ramos et al. 2001,

Plant Cell Physiol. 54(4): 609–621 (2013) doi:10.1093/pcp/pct028, available online at www.pcp.oxfordjournals.org

© The Author 2013. Published by Oxford University Press on behalf of Japanese Society of Plant Physiologists.

All rights reserved. For permissions, please email: journals.permissions@oup.com

Reed 2001, Rogg et al. 2001, Tiwari et al. 2001). Their domain II interacts with the TRANSPORT INHIBITOR RESPONSE1/AUXIN-BINDING F-BOX PROTEIN (TIR1/AFB) family of auxin receptors, while their domains III and IV dimerize with the AUXIN RESPONSE FACTOR (ARF) family transcription factors (Kim et al. 1997, Gray et al. 2001). Upon an auxin stimulus, Aux/IAA proteins are ubiquitinated by the TIR1/AFB proteins and then degraded, which releases the activity of ARF proteins (Dharmasiri et al. 2005, Kepinski and Leyser 2005, Tan et al. 2007). The GH3 family of acyl acid amido synthetases contributes to the amino acid conjugation of IAA, jasmonic acid or salicylic acid (Ljung et al. 2002, Staswick et al. 2005, Park et al. 2007). The crystal structure and putative mechanisms of two GH3 proteins had been reported recently (Westfall et al. 2012). In contrast to Aux/IAA and GH3 proteins, the function of SAUR proteins remains unclear, most probably due to genetic redundancy within the large SAUR gene family (Hagen and Guilfoyle 2002, Jain et al. 2006).

Recent studies have begun to address the function of SAUR proteins in rice (*Oryza sativa*) and Arabidopsis. *OsSAUR39* has been identified as a negative regulator of auxin synthesis and transport, since rice plants overexpressing *OsSAUR39* exhibit reduced lateral root development and shoot and root length (Kant et al. 2009). The SAUR19 subfamily proteins (SAUR19–SAUR24) of Arabidopsis have been reported to be highly unstable; however, the addition of an N-terminal tag increases the stability of these proteins (Spartz et al. 2012). Arabidopsis plants overexpressing these stabilized SAUR fusion proteins exhibited increased hypocotyl elongation and larger leaf size, while seedlings expressing an artificial microRNA targeting the SAUR19 subfamily genes exhibit opposite phenotypes (Franklin et al. 2011, Spartz et al. 2012). In another report on Arabidopsis, transgenic plant lines overexpressing *SAUR63:GFP* or *SAUR63:GUS* displayed long hypocotyls, petals and stamen filaments; in contrast, transgenic plants expressing artificial microRNA constructs targeting the SAUR63 subfamily genes (*SAUR61–SAUR68* and *SAUR75*) had slightly reduced hypocotyl and stamen filament elongation (Chae et al. 2012). The SAUR19 subfamily genes were expressed in elongating tissues, including the root elongation zone (Spartz et al. 2012), while the SAUR63 subfamily genes were not expressed in roots (Chae et al. 2012). As far as the mechanism is concerned, Arabidopsis SAUR proteins have been proposed to promote cell expansion through the modulation of auxin transport (Chae et al. 2012, Spartz et al. 2012).

The SAUR gene family could be phylogenetically classified into three clades (Kodaira et al. 2011). The genes in clades I and II had a tendency to display higher expression levels in leaves and lower expression in roots, while the genes in clade III demonstrated opposite expression patterns (Kodaira et al. 2011). The SAUR63 subfamily and the SAUR19 subfamily are clade I and clade II SAUR genes, respectively (Kodaira et al. 2011). Herein, we present our work on SAUR41, a clade III SAUR gene displaying a distinctive expression pattern in Arabidopsis root meristems.

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. 2007, Peltier 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) 35S 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 μ M 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-Tomás et al. 2008, Ubeda-Tomás

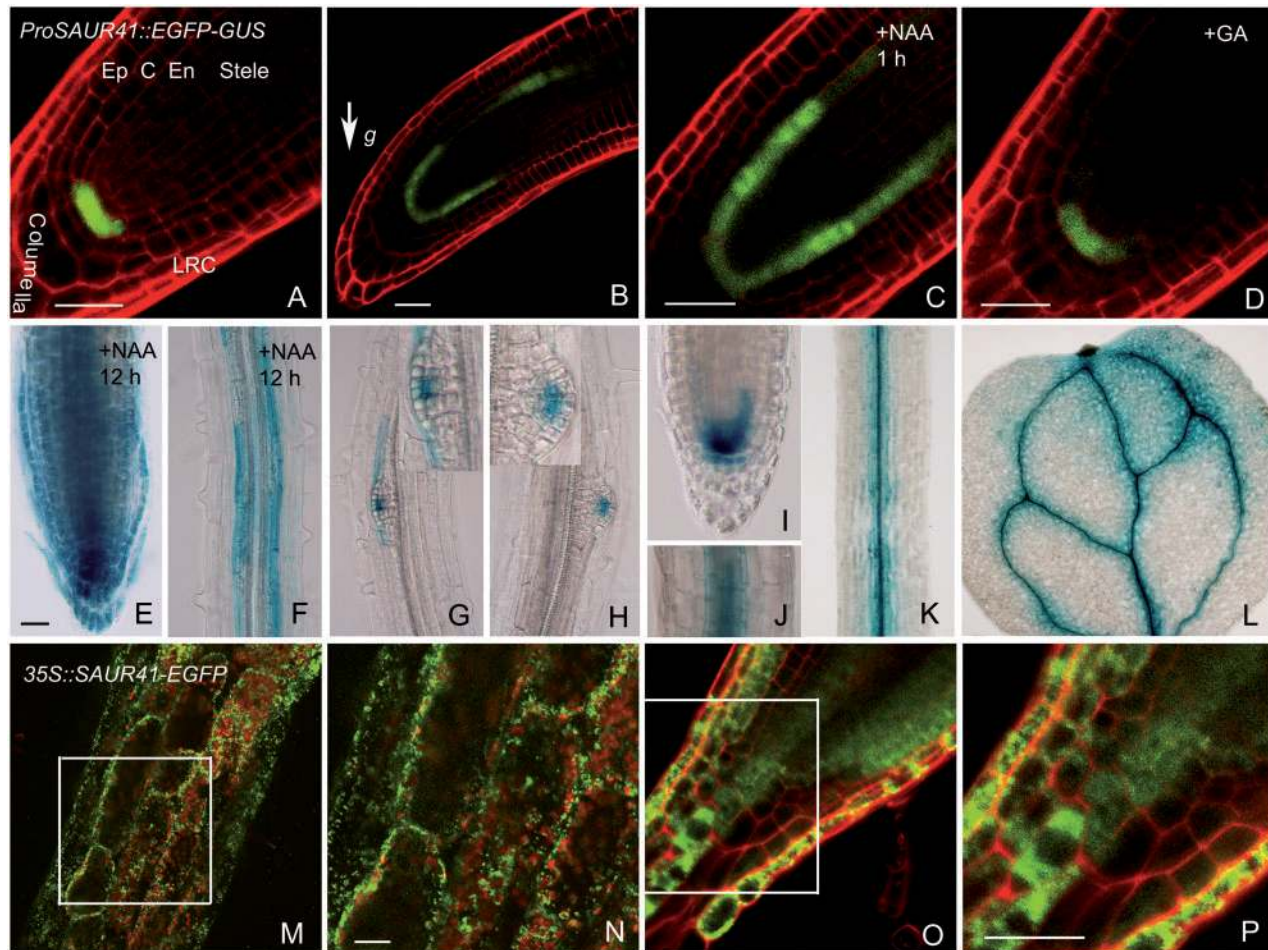


Fig. 1 The expression pattern of the *SAUR41* gene, and the subcellular localization of SAUR41 fusion protein. An ~1,800 bp promoter region of *SAUR41* was fused with the *EGFP-GUS* reporter gene. Green EGFP (enhanced green fluorescent protein) signals and red PI (propidium iodide, staining the cell wall here) signals were viewed by confocal microscopy. GUS (β -glucuronidase) activity stained in blue was viewed by DIC (differential interference contrast) microscopy. *SAUR41* was normally restricted to the quiescent center and cortex/endodermis initials at the root meristems (A). Upon a gravitropic (B) or auxin (C) stimulation, *SAUR41* was provoked in the endodermis layer. (D) *SAUR41* was unresponsive to gibberellin treatment. After extended auxin treatment, *SAUR41* was induced in multiple cell layers at the root meristem (E); however, in the elongation zone, it was specifically induced in the endodermal cells (F). During lateral root development, *SAUR41* was expressed in the prospective quiescent center and initial cells of lateral root primordia (G, H) and in the endodermal cells surrounding the lateral root primordia (G). Insets are enlargements of lateral root primordia. In a new lateral root, *SAUR41* was strongly expressed in the quiescent center and initial cells (I). *SAUR41* was predominantly expressed in the vascular tissues of hypocotyls (J), petioles (K) and cotyledons (L). SAUR41-EGFP fusion proteins localized to the cytoplasm of epidermal cells and cortical cells in hypocotyls (M, N) and to the cytoplasm of quiescent center cells, cortex/endodermis initial cells and epidermal cells in root tips (O, P). LRC, lateral root cap; Ep, epidermis; C, cortex; En, endodermis. Bars: 25 μ m.

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 epidermal 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, and 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; **Fig. 4C**). 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

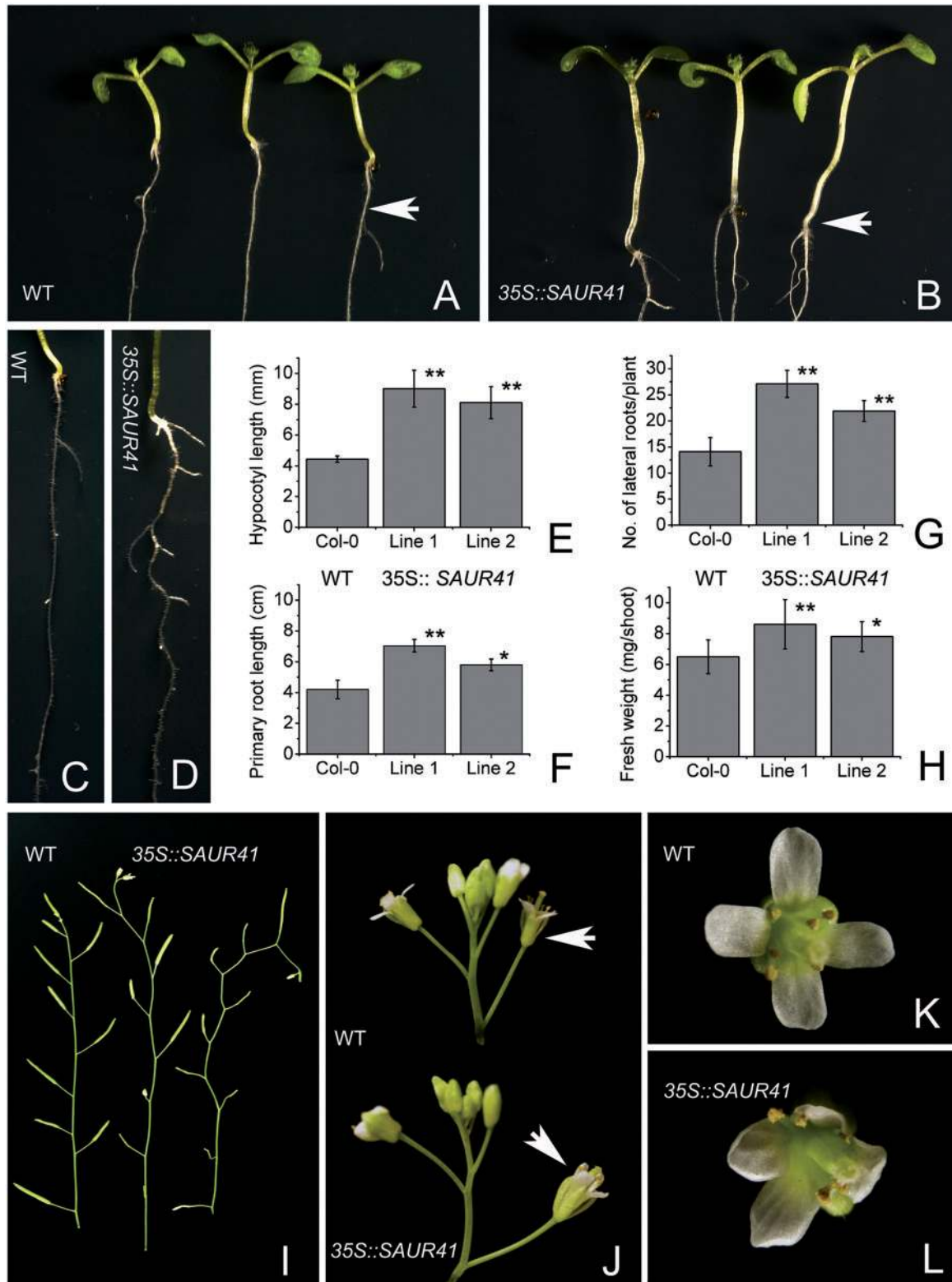


Fig. 2 Overexpression of *SAUR41* from the CaMV 35S promoter conferred pleiotropic auxin- or cell expansion-related phenotypes. (A–E) Light-grown 6-day-old seedlings. (F–H) Light-grown 10-day-old seedlings. (I–L) Adult plants. Hypocotyls of wild-type controls (A) and 35S::*SAUR41* seedlings (B). Roots 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.

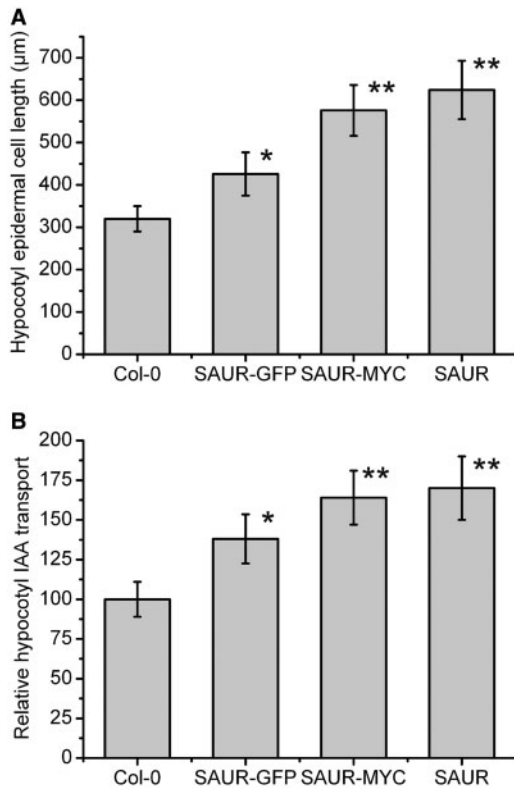


Fig. 3 Ectopic *SAUR41* promoted hypocotyl epidermal cell expansion and IAA transport. (A) Mean hypocotyl epidermal cell length in 6-day-old seedlings. (B) Relative [^3H]IAA basipetal transport in hypocotyls of 7-day-old seedlings. ** $P < 0.01$; * $P < 0.05$, *t*-test.

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. 4J). *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 *IAA2^{P65S}* 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 *IAA2^{P65S}* 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 *IAA2^{P65S}* 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 *IAA2^{P65S}* from the *ACR4* promoter had a more remarkable impact on root meristem development compared with that seen in *WOX5::IAA2^{P65S}* roots. As shown in Fig. 5, *ACR4::IAA2^{P65S}* 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 gradually 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 *IAA2^{P65S}* 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).

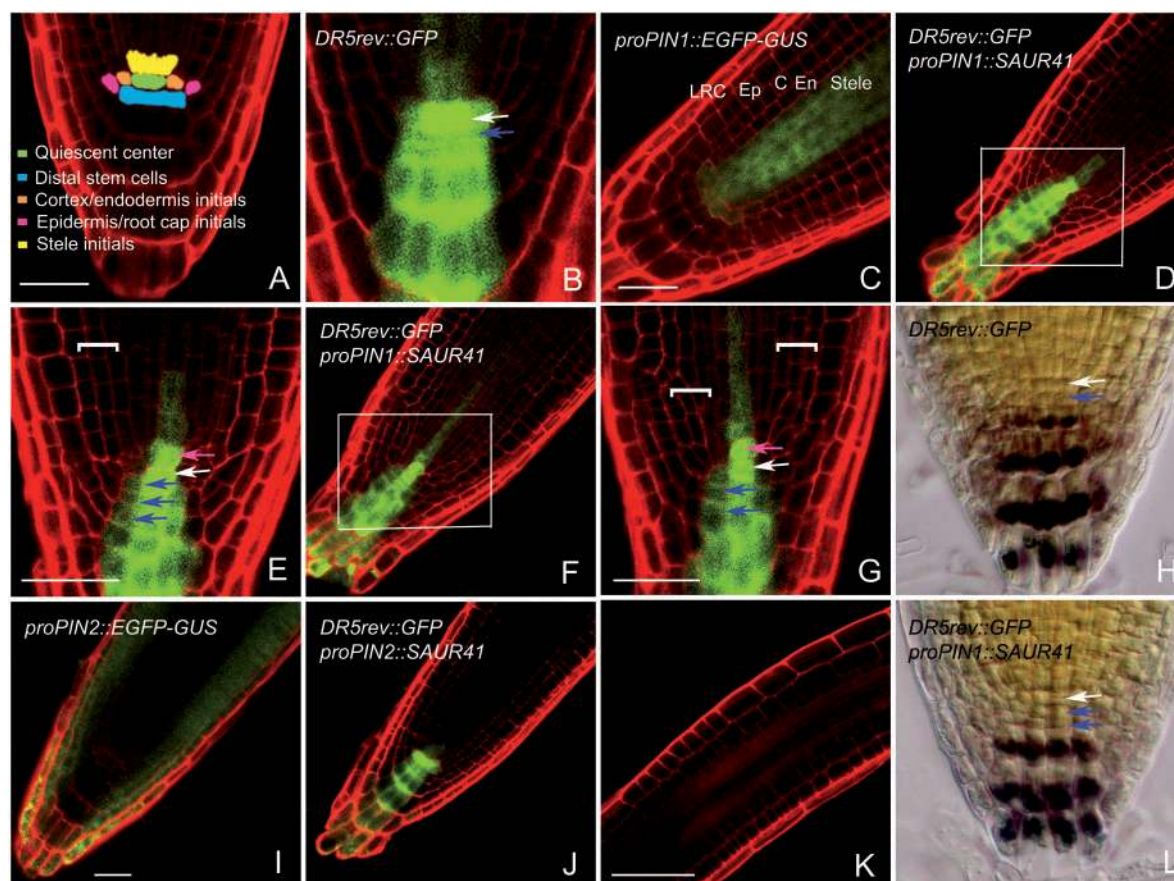


Fig. 4 Tissue-specific expression of SAUR41 from the PIN1 and PIN2 promoters. Green EGFP (enhanced green fluorescent protein) signals and red PI (propidium iodide, staining the cell wall here) signals were viewed by confocal microscopy. Purple starch grains stained by Lugol's solution were viewed by DIC (differential interference contrast) microscopy. (A) The root stem cell niches. (B) Auxin accumulation/signaling pattern in root meristems of the *DR5rev::GFP* background line. A white arrow indicates the quiescent center, while a blue arrow indicates the tier of distal stem cells. Boxed areas were magnified in the subsequent images. (C) Promoter activity of PIN1. (D–F) Tissue-specific expression of SAUR41 from the PIN1 promoter led to auxin retention in stele initials transporting auxin (pink arrow), additional tiers of distal stem cells (blue arrow) and supernumerary cell layers (white bracket). (H, L) Differentiation status of columella cells in control roots (H) and PIN1::SAUR41 roots (L) determined by Lugol's solution staining. (I) Promoter activity of PIN2. (J, K) Tissue-specific expression of SAUR41 from the PIN2 promoter did not induce auxin retention but caused irregular cell expansion in the corresponding cells. LRC, lateral root cap; Ep, epidermis; C, cortex; En, endodermis. Bars: 25 μ m.

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). PIN2::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

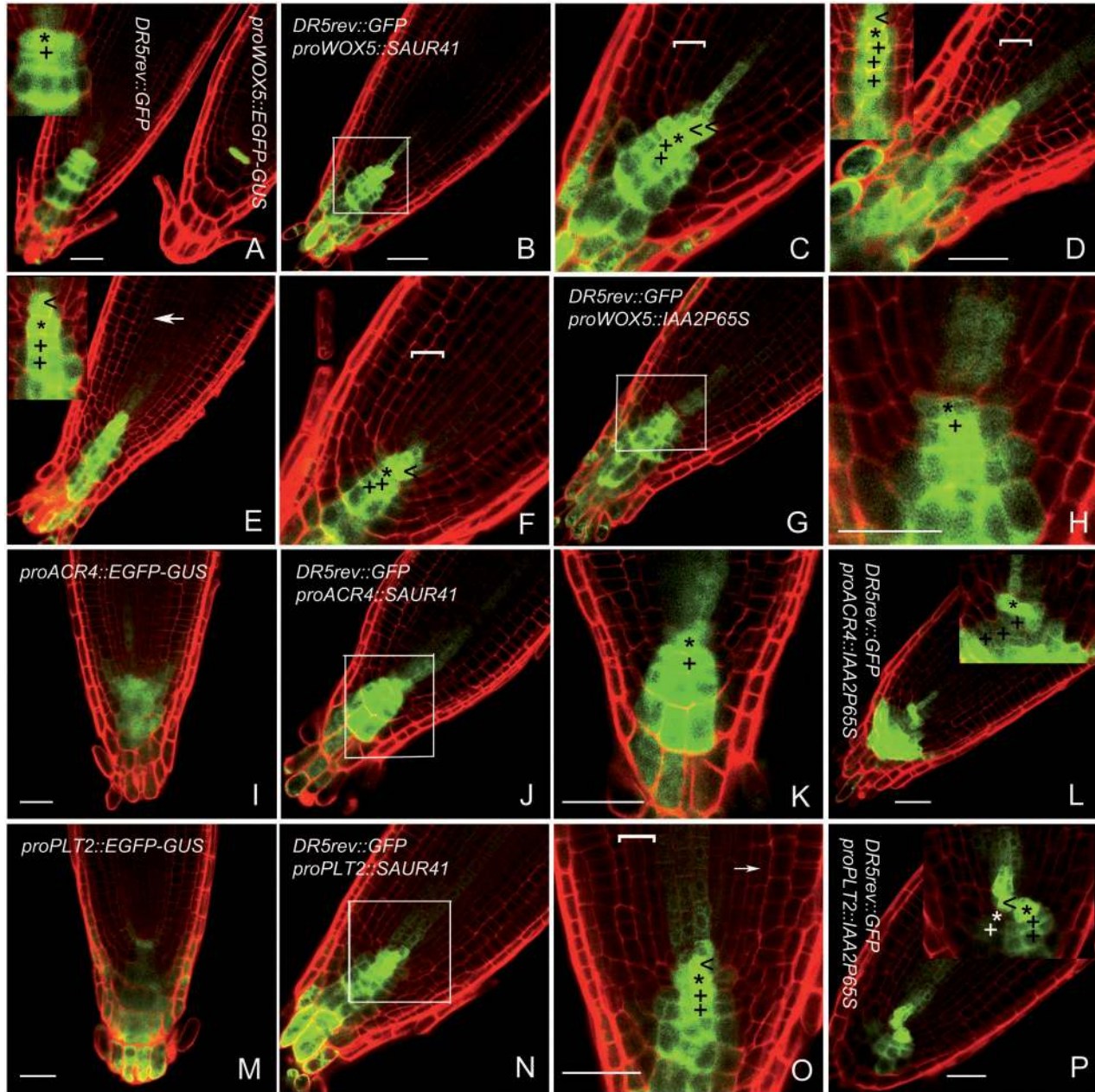


Fig. 5 Tissue-specific expression of *SAUR41* and *IAA2^{P65S}* from promoters of root meristem patterning genes. Green EGFP (enhanced green fluorescent protein) signals and red PI (propidium iodide, staining the cell wall here) signals were viewed by confocal microscopy. Boxed areas were magnified in the subsequent images. Insets were enlargements of root stem cell niches. (A) Auxin accumulation/signaling pattern in root meristems of the *DR5rev::GFP* background line (left) and the promoter activity of *WOX5* (right). (B–F) Tissue-specific expression of *SAUR41* from the *WOX5* promoter. (G, H) Tissue-specific expression of *IAA2^{P65S}* from the *WOX5* promoter. (I) Promoter activity of *ACR4*. (J, K) Tissue-specific expression of *SAUR41* from the *ACR4* promoter. (L) Tissue-specific expression of *IAA2^{P65S}* from the *ACR4* promoter. (M) Promoter activity of *PLT2*. (N, O) Tissue-specific expression of *SAUR41* from the *PLT2* promoter. (P) Tissue-specific expression of *IAA2^{P65S}* from the *PLT2* promoter. The arrow indicates the division of endodermal cells. ∇ indicates additional auxin accumulation/signaling peaks; * indicates the quiescent center; + indicates tiers of distal stem cells; and [indicates the supernumerary cell layers. Bars: 25 μ m.

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

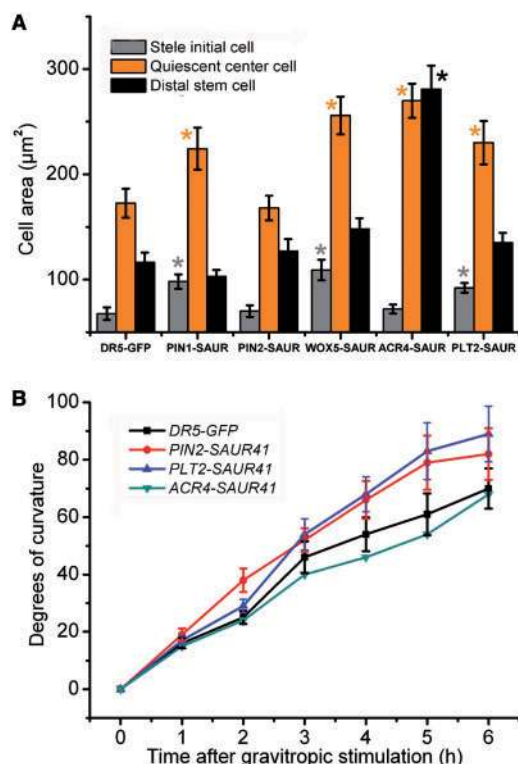


Fig. 6 Ectopic SAUR41 proteins differentially regulated root cell expansion and root gravitropic growth. (A) Cell areas of stele initial cells, quiescent center cells and distal stem cells in roots of 5-day-old seedlings. (B) Root gravitropic responses in 6-day-old seedlings. * $P < 0.05$, t -test.

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 SAUR41 was actively expressed in response to both developmental and environmental cues.

SAUR41–EGFP fusion proteins localize to the cytoplasm (Fig. 1M–P), while EGFP–SAUR19 and SAUR63–EGFP localize predominantly to the plasma membrane (Chae et al. 2012, Spartz et al. 2012). Although the protein localization pattern and the gene expression pattern of SAUR41 were different from those of SAUR19 and SAUR63, the phenotypes resulting from overexpression of SAUR41 driven by the CaMV 35S promoter shared many similarities with those of SAUR19 and SAUR63 subfamily genes. In all three cases, transgenic seedlings had elongated hypocotyls, expanded hypocotyl epidermal cells and enhanced hypocotyl basipetal IAA transport compared with the wild type (Figs. 2A, B, E, 3; Chae et al. 2012, Spartz et al. 2012). In the cases of SAUR41 and SAUR19, transgenic seedlings displayed waving roots and increased vegetative

biomass (Fig. 2C, D, H; Spartz et al. 2012); while in the cases of SAUR41 and SAUR63, transgenic plants had expanded petals and twisted inflorescence stems (Fig. 2I–L; Chae et al. 2012). Interestingly, only in the case of SAUR41 did the transgenic seedlings have increased primary root length and lateral root development compared with wild-type controls (Fig. 2F, G). Thus, it seems likely 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 *DR5::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 *IAA2^{P65S}* from *WOX5*, *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 SCF^{TIR1} 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 *IAA2^{P65S}* 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 *DR5rev::GFP* background (Friml et al. 2003) were used as sources of wild-type plant materials. Promoter reporter 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 B5 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 GATEWAYTM system for vector construction. Entry vectors were created using the pENTRTM/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**. *IAA2^{P65S}* was generated by overlapping PCR using the

primers given in **Supplementary Table S1**. Each entry clone was confirmed by DNA sequencing. GATEWAYTM-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::IAA2^{P65S}*) 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 B5 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 ferricyanide, 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 μ M 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.

Funding

This work was supported by the National Natural Science Foundation of China [grant No. 31170211 to J.W.].

Acknowledgments

We are grateful to ABRC and RIKEN-BRC for the distribution of *Arabidopsis* materials. No conflict of interest is declared.

References

- Aichinger, E., Kornet, N., Friedrich, T. and Laux, T. (2012) Plant stem cell niches. *Annu. Rev. Plant Biol.* 63: 625–636.
- Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C. et al. (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. *Cell* 119: 109–120.
- Bechtold, N., Ellis, J. and Pelletier, G. (1993) *In planta* Agrobacterium-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *CR Acad. Sci. Ser. III Sci. Vie* 316: 1194–1199.
- Bosco, C.D., Lezhneva, L., Biehl, A., Leister, D., Strotmann, H., Wanner, G. et al. (2004) Inactivation of the chloroplast ATP synthase gamma subunit results in high non-photochemical fluorescence quenching and altered nuclear gene expression in *Arabidopsis thaliana*. *J. Biol. Chem.* 279: 1060–1069.
- Carrie, C., Giraud, E., Duncan, O., Xu, L., Wang, Y., Huang, S. et al. (2010) Conserved and novel functions for *Arabidopsis thaliana* MIA40 in assembly of proteins in mitochondria and peroxisomes. *J. Biol. Chem.* 285: 36138–36148.
- Chae, K., Isaacs, C.G., Reeves, P.H., Maloney, G.S., Muday, G.K., Nagpal, P. et al. (2012) *Arabidopsis* *SMALL AUXIN UP RNA63* promotes hypocotyl and stamen filament elongation. *Plant J.* 71: 684–697.
- Darrah, C., Taylor, B.L., Edwards, K.D., Brown, P.E., Hall, A. and McWatters, H.G. (2006) Analysis of phase of *LUCIFERASE* expression reveals novel circadian quantitative trait loci in *Arabidopsis*. *Plant Physiol.* 140: 1464–1474.
- Davuluri, R.V., Sun, H., Palaniswamy, S.K., Matthews, N., Molina, C., Kurtz, M. et al. (2003) AGRIS, Arabidopsis Gene Regulatory Information Server, an information resource of Arabidopsis cis-regulatory elements and transcription factors. *BMC Bioinform.* 4: 25.
- De Smet, I., Vassileva, V., De Rybel, B., Levesque, M.P., Grunewald, W., Van Damme, D. et al. (2008) Receptor-like kinase ACR4 restricts formative cell divisions in the *Arabidopsis* root. *Science* 322: 594–597.
- Dharmasiri, N., Dharmasiri, S. and Estelle, M. (2005) The F-box protein TIR1 is an auxin receptor. *Nature* 435: 441–445.
- Ding, Z. and Friml, J. (2010) Auxin regulates distal stem cell differentiation in *Arabidopsis* roots. *Proc. Natl Acad. Sci. USA* 107: 12046–12051.
- Earley, K.W., Haag, J.R., Pontes, O., Opper, K., Juehne, T., Song, K. et al. (2006) Gateway-compatible vectors for plant functional genomics and proteomics. *Plant J.* 45: 616–629.
- Estavillo, G.M., Crisp, P.A., Pornsiriwong, W., Wirtz, M., Collinge, D., Carrie, C. et al. (2011) Evidence for a SAL1–PAP chloroplast retrograde pathway that functions in drought and high light signaling. *Plant Cell* 23: 3992–4012.
- Franklin, K.A., Lee, S.H., Patel, D., Kumar, S.V., Spartz, A.K., Gu, C. et al. (2011) PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proc. Natl Acad. Sci. USA* 108: 20231–20235.
- Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T. et al. (2003) Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* 426: 147–153.
- Galinha, C., Hofhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R. et al. (2007) PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. *Nature* 449: 1053–1057.
- Gleason, C., Huang, S., Thatcher, L.F., Foley, R.C., Anderson, C.R., Carroll, A.J. et al. (2011) Mitochondrial complex II has a key role in mitochondrial-derived reactive oxygen species influence on plant stress gene regulation and defense. *Proc. Natl Acad. Sci. USA* 108: 10768–10773.
- Gray, W.M., Kepinski, S., Rouse, D., Leyser, O. and Estelle, M. (2001) Auxin regulates SCF^{TIR1}-dependent degradation of AUX/IAA proteins. *Nature* 414: 271–276.
- Hagen, G. and Guilfoyle, T. (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant Mol. Biol.* 49: 373–385.
- Harrison, B.R. and Masson, P.H. (2008) ARL2, ARG1 and PIN3 define a gravity signal transduction pathway in root statocytes. *Plant J.* 53: 380–392.
- Hayashi, K. (2012) The interaction and integration of auxin signaling components. *Plant Cell Physiol.* 53: 965–975.
- Jain, M., Tyagi, A.K. and Khurana, J.P. (2006) Genome-wide analysis, evolutionary expansion, and expression of early auxin-responsive SAUR gene family in rice (*Oryza sativa*). *Genomics* 88: 360–371.
- Jones, B. and Ljung, K. (2012) Subterranean space exploration: the development of root system architecture. *Curr. Opin. Plant Biol.* 15: 97–102.
- Josse, E.-M., Gan, Y., Bou-Torrent, J., Stewart, K.L., Gilday, A.D., Jeffree, C.E. et al. (2011) A DELLA in disguise: SPATULA restrains the growth of the developing *Arabidopsis* seedling. *Plant Cell* 23: 1337–1351.
- Kant, S., Bi, Y.M., Zhu, T. and Rothstein, S.J. (2009) SAUR39, a small auxin-up RNA gene, acts as a negative regulator of auxin synthesis and transport in rice. *Plant Physiol.* 151: 691–701.

- Karimi, M., Inzé, D. and Depicker, A. (2002) GATEWAYTM vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci.* 7: 193–195.
- Kepinski, S. and Leyser, O. (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature* 435: 446–451.
- Kim, J., Harter, K. and Theologis, A. (1997) Protein–protein interactions among the Aux/IAA proteins. *Proc. Natl Acad. Sci. USA* 94: 11786–11791.
- Kodaira, K.-S., Qin, F., Tran, L.-S.P., Maruyama, K., Kidokoro, S., Fujita, Y. et al. (2011) Arabidopsis Cys2/His2 zinc-finger proteins AZF1 and AZF2 negatively regulate abscisic acid-repressive and auxin-inducible genes under abiotic stress conditions. *Plant Physiol.* 157: 742–756.
- Leonhardt, N., Kwak, J.M., Robert, N., Waner, D., Leonhardt, G. and Schroeder, J.I. (2004) Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. *Plant Cell* 16: 596–615.
- Ljung, K., Hull, A.K., Kowalczyk, M., Marchant, A., Celenza, J., Cohen, J.D. et al. (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. *Plant Mol. Biol.* 50: 309–332.
- Mazzella, M.A., Arana, M.V., Staneloni, R.J., Perelman, S., Batiller, M.J.R., Muschietti, J. et al. (2005) Phytochrome control of the Arabidopsis transcriptome anticipates seedling exposure to light. *Plant Cell* 17: 2507–2516.
- Nagawa, S., Sawa, S., Sato, S., Kato, T., Tabata, S. and Fukuda, H. (2006) Gene trapping in Arabidopsis reveals genes involved in vascular development. *Plant Cell Physiol.* 47: 1394–1405.
- Ottenschläger, I., Wolff, P., Wolverton, C., Bhalerao, R.P., Sandberg, G., Ishikawa, H. et al. (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. *Proc. Natl Acad. Sci. USA* 100: 2987–2991.
- Ouellet, F., Overvoorde, P.J. and Theologis, A. (2001) IAA17/AXR3, biochemical insight into an auxin mutant phenotype. *Plant Cell* 13: 829–841.
- Overvoorde, P., Fukaki, H. and Beeckman, T. (2010) Auxin control of root development. *Cold Spring Harb. Perspect. Biol.* 2: a001537.
- Paponov, I.A., Paponov, M., Teala, W., Menges, M., Chakrabortee, S., Murray, J.A.H. et al. (2008) Comprehensive transcriptome analysis of auxin responses in Arabidopsis. *Mol. Plant* 12: 321–337.
- Park, J.-E., Park, J.-Y., Kim, Y.-S., Sraswick, P.E., Jeon, J., Yun, J. et al. (2007) GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in Arabidopsis. *J. Biol. Chem.* 282: 10036–10046.
- Peltier, C., Schmidlin, L., Klein, E., Tsconnat, L., Prinsen, E., Erhardt, M. et al. (2011) Expression of Beet necrotic yellow vein virus p25 protein induces hormonal changes and a root branching phenotype in Arabidopsis thaliana. *Transgenic Res.* 20: 443–466.
- Perilli, S., Mambro, R.D. and Sabatini, S. (2012) Growth and development of the root apical meristem. *Curr. Opin. Plant Biol.* 15: 17–23.
- Petricka, J.J., Winter, C.M. and Benfey, P.N. (2012) Control of Arabidopsis root development. *Annu. Rev. Plant Biol.* 63: 563–564.
- Ramos, J., Zenser, N., Leyser, O. and Callis, J. (2001) Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent. *Plant Cell* 13: 2349–2360.
- Reed, J.W. (2001) Roles and activities of Aux/IAA proteins in Arabidopsis. *Trends Plant Sci.* 6: 420–425.
- Rogg, L.E., Lasswell, J. and Bartel, B. (2001) A gain-of-function mutation in IAA28 suppresses lateral root development. *Plant Cell* 13: 465–480.
- Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J. et al. (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell* 99: 463–472.
- Santner, A. and Estelle, M. (2009) Recent advances and emerging trends in plant hormone signaling. *Nature* 459: 1071–1078.
- Sarkar, A.K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K. et al. (2007) Conserved factors regulate signaling in Arabidopsis thaliana shoot and root stem cell organizers. *Nature* 446: 811–814.
- Scherer, G.F.E., Labusch, C. and Effendi, Y. (2012) Phospholipases and the network of auxin signal transduction with ABP1 and TIR1 as two receptors: a comprehensive and provocative model. *Front. Plant Sci.* 3: 56.
- She, W., Lin, W., Zhu, Y., Chen, Y., Jin, W., Yang, Y. et al. (2010) The gypsy insulator of *Drosophila melanogaster* together with its binding protein Su(Hw) (Suppressor of Hairy-wing) facilitate high and precise expression of transgenes in Arabidopsis thaliana. *Genetics* 185: 1141–1150.
- Shirakawa, M., Ueda, H., Shimada, T., Nishiyama, C. and Hara-Nishimura, I. (2009) Vacuolar SNAREs function in the formation of the leaf vascular network by regulating auxin distribution. *Plant Cell Physiol.* 50: 1319–1328.
- Spartz, A.K., Lee, S.H., Wenger, J.P., Gonzalez, N., Itoh, H., Inzé, D. et al. (2012) The SAUR19 subfamily of SMALL AUXIN UP RNA genes promote cell expansion. *Plant J.* 70: 978–990.
- Staswick, P.E., Serban, B., Rowe, M., Tiryaki, I., Maldonado, M.T., Maldonado, M.C. et al. (2005) Characterization of an Arabidopsis enzyme family that conjugates amino acids to indole-3-acetic acid. *Plant Cell* 17: 616–627.
- Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. et al. (2001) Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes Dev.* 15: 2648–2653.
- Tan, X., Calderon-Villalobos, L.I.A., Sharon, M., Zheng, C., Robinson, C.V., Estelle, M. et al. (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* 446: 640–645.
- Tiwari, S.B., Wang, X.J., Hagen, G. and Guilfoyle, T.J. (2001) AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. *Plant Cell* 13: 2809–2822.
- Ubeda-Tomás, S., Beemster, G.T.S. and Bennett, M.J. (2012) Hormonal regulation of root growth: integrating local activities into global behavior. *Trends Plant Sci.* 17: 326–331.
- Ubeda-Tomás, S., Federici, F., Casimiro, I., Beemster, G.T., Bhalerao, R., Swarup, R. et al. (2009) Gibberellin signaling in the endodermis controls Arabidopsis root meristem size. *Curr. Biol.* 19: 1194–1199.
- Ubeda-Tomás, S., Swarup, R., Coates, J., Swarup, K., Laplaze, L., Beemster, G.T. et al. (2008) Root growth in Arabidopsis requires gibberellin/DELLA signaling in the endodermis. *Nat. Cell Biol.* 10: 625–628.
- Ulmasov, T., Murfett, J., Hagen, G. and Guilfoyle, T. (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* 9: 1963–1971.
- Vanneste, S. and Friml, J. (2009) Auxin: a trigger for change in plant development. *Cell* 136: 1005–1016.
- Weijers, D., Benkova, E., Jäger, K.E., Schlereth, A., Hamann, T., Kientz, M. et al. (2005) Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators. *EMBO J.* 24: 1874–1885.

- Westfall, C.S., Zubieta, C., Herrmann, J., Kapp, U., Nanao, M.H. and Jez, J.M. (2012) Structural basis for prereceptor modulation of plant hormones by GH3 proteins. *Science* 336: 1708–1711.
- Woodward, A.W. and Bartel, B. (2005) Auxin: regulation, action, and interaction. *Ann. Bot.* 95: 707–735.
- Worley, C.K., Zenser, N., Ramos, J., Rouse, D., Leyser, O., Theologis, A. et al. (2000) Degradation of Aux/IAA proteins is essential for normal auxin signaling. *Plant J.* 21: 553–562.
- Yang, Y., Jin, H., Chen, Y., Lin, W., Wang, C., Chen, Z. et al. (2012) A chloroplast envelope membrane protein containing a putative LrgB domain related to the control of bacterial death and lysis is required for chloroplast development in *Arabidopsis thaliana*. *New Phytol.* 193: 81–95.
- Zeng, Y., Zhao, T. and Kermode, A.R. (2012) A conifer ABI3-interacting protein plays important roles during key transitions of the plant life cycle. *Plant Physiol.* 161: 179–195.
- Zhang, H., Kim, M., Krishnamachari, V., Payton, P., Sun, Y., Grimson, M. et al. (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. *Planta* 226: 839–851.