

The tissue-specific and developmentally regulated expression patterns of the *SAUR41* subfamily of *SMALL AUXIN UP RNA* genes: Potential implications

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The plant hormone auxin modulates cell proliferation and cell expansion in part by changing gene expression. Among the three primary auxin response gene families, *Aux/IAA*, *GH3* and *SAUR*, the function of *SAUR* genes remains unclear. *SAUR* transcripts were initially identified in epidermal and cortical cells of elongating tissues and thus were supposed to regulate cell expansion downstream of auxin transport and auxin signaling. Recent studies have proposed that SAUR proteins are able to modulate auxin transport and cell expansion by an unknown mechanism. We present our work on the *SAUR41* subfamily genes of *Arabidopsis* (*SAUR41*, *SAUR40*, *SAUR71* and *SAUR72*). Similar to the fusion protein between SAUR41 and EGFP, both SAUR40-EGFP and SAUR71-EGFP were identified in the cytoplasm of all types of root tip cells. This result indicated that the subcellular location pattern of SAUR proteins among the members of the same subfamily could be similar to each other, although the overall location pattern of SAUR proteins appeared to be highly diverse. *SAUR41* was distinctively expressed in the quiescent center and cortex/endodermis initials of root stem cell niches and in the endodermis of hypocotyls, whereas *SAUR71* and *SAUR72* were expressed in the steles of young roots and hypocotyls. In addition, *SAUR71* was differentially expressed during stomatal formation. The tissue-specific and developmentally regulated expression patterns of the *SAUR41* subfamily genes imply that SAUR transcripts or SAUR proteins might serve as signal molecules to ensure the coordination of cell proliferation and cell expansion. Finally, *Arabidopsis* seedlings expressing TAP (tandem affinity peptide) tagged SAUR41 displayed phenotypes, indicating that it was rational to use the TAP approach for identification of potential binding partners of SAUR41 proteins.

The plant hormone auxin modulates many aspects of plant growth and development and it regulates cell proliferation and cell expansion in part by changing gene expression. Primary auxin response genes consist of members of three gene families: *Aux/IAA* (*auxin/indole-3-acetic acid*), *GH3* (*gretchen hagen3*) and *SAUR* (*small auxin up RNA*).¹ Among these genes, the function of *SAUR* genes remains unclear, most likely due to genetic redundancy within the large *SAUR* gene family.^{1,2}

Recent studies have begun to address the function of *SAUR* genes in *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*). *Arabidopsis SAUR32* has been proposed to be involved in apical hook development.³ Rice *SAUR39* has been identified as a negative regulator of auxin synthesis and transport, since rice plants overexpressing this gene exhibit reduced lateral root development and shoot and root length.^{4,5} The *SAUR* gene family of *Arabidopsis* could be phylogenetically classified into three clades.⁶

More recent work has suggested that *SAUR19* (clade II) and *SAUR63* (clade I) promote cell expansion through the modulation of auxin transport.^{7,8} Most recently, we presented our work on *SAUR41*, a clade III *SAUR* gene with a distinctive expression pattern in root meristems.⁹ Overexpression of *SAUR41* from the CaMV 35S promoter led to pleiotropic auxin-related phenotypes, including long hypocotyls, increased vegetative biomass and lateral root development in young seedlings and expanded petals and twisted inflorescence stems in adult plants.⁹ Tissue-specific expression of *SAUR41* from promoters of auxin transporter genes and root meristem patterning genes (*PINI1*, *PIN2*, *WOX5*, *PLT2* and *ACR4*) differentially modulated root meristem development, root cell expansion and root gravitropic growth.⁹ In the meantime, *SAUR36*, another clade III *SAUR* gene of *Arabidopsis* has been identified as a positive regulator of leaf senescence and a negative regulator of leaf size.¹⁰ In an independent study,

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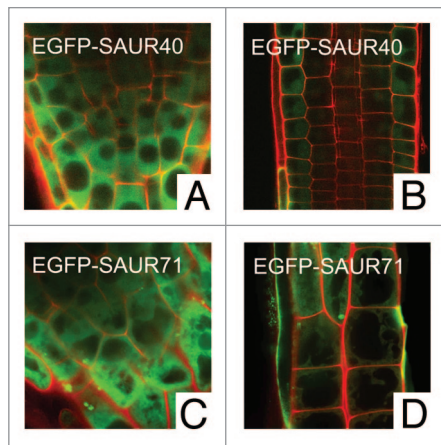


Figure 1. Subcellular location of SAUR40 and SAUR71 fusion proteins. Green EGFP (enhanced green fluorescent protein) signals and red PI (propidium iodide, staining cell wall here) signals were viewed by confocal microscopy.

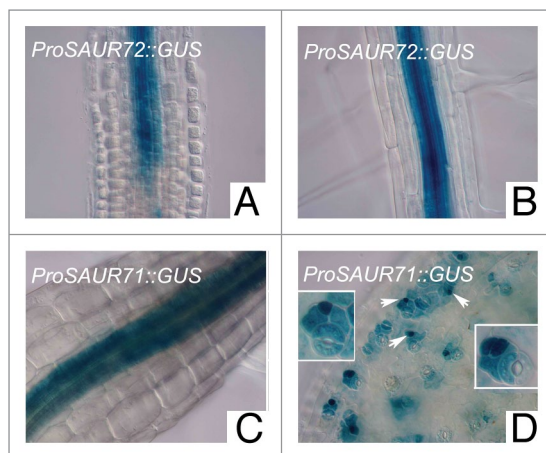


Figure 2. Expression patterns of SAUR71 and SAUR72. GUS (β-glucuronidase) activity stained in blue was viewed by DIC (differential-interference-contrast) microscopy. Arrows indicate guard mother cells.

SAUR36 has been characterized as a regulator of seed germination in response to gibberellins and abscisic acid.¹¹ Ectopic expression of *SAUR36* led to the absence of apical hooks in etiolated seedlings and significantly longer hypocotyls in light-grown seedlings. However, mature plants ectopically expressing *SAUR36* displayed dramatically wavy growth of inflorescence axes which could be linked to defects in auxin transport.¹¹ The mechanisms of how SAUR proteins modulate auxin transport and why they sometimes promote auxin transport but in other cases inhibit auxin transport remains unclear.

The subcellular location pattern of SAUR proteins appeared to be highly diverse. By examining GFP-tagged SAUR proteins, rice *SAUR39* was found exclusively in the cytoplasm.⁴ In *Arabidopsis*, *SAUR41* localized to the cytoplasm,⁹ *SAUR19* and

SAUR63 localized primarily to the plasma membrane,^{7,8} whereas *SAUR36* targeted the nucleus.¹²

The *SAUR41* subfamily contained four members: *SAUR41*, At1g16510; *SAUR40*, At1g79130; *SAUR71*, At1g56150; *SAUR72*, At3g12830. Their amino acid sequences differed from other SAUR families in the N-terminus. Herein, to investigate the subcellular localization of SAUR40 and SAUR71 proteins, we generated transgenic constructs in which the CaMV (cauliflower mosaic virus) 35S promoter drove C-terminal translational fusions between the full-length SAUR40 or SAUR71 and the EGFP proteins. Location of fusion proteins in stably-transformed *Arabidopsis* plants was examined by confocal microscopy. Similar to the SAUR41 fusion protein, SAUR40-EGFP and SAUR71-EGFP were identified in the cytoplasm of all types of root tip cells (Fig. 1A–D). Although we have not generated transgenic lines for the SAUR72-EGFP construction, we suggest that the subcellular location pattern of SAUR proteins among the members of the same gene subfamily could be similar to each other. As a support to this view, the SAUR19 subfamily members (*SAUR19*, 21, 23 and 24) have been reported to exhibit identical subcellular location patterns.⁷

According to microarray data, the *SAUR* genes of *Arabidopsis* in clades I and II had a tendency to display higher expression levels in leaves and lower expressions in roots, while the genes in clade III demonstrated opposite expression patterns.⁶ The *SAUR19* subfamily genes were expressed in growing hypocotyls in response to shade avoidance and in root elongation zones in response to auxin treatment.⁷ *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.⁸ We found that *SAUR41* displayed distinctive expression patterns in *Arabidopsis* roots; it was normally restricted to the quiescent center and cortex/endodermis initials of primary root meristems.⁹ During lateral root development, *SAUR41* was also expressed in prospective stem cell niches of lateral root primordial and in expanding endodermal cells surrounding the lateral root primordia.⁹

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.¹³ Using this system, together with the GATEWAY recombination approach, we generated promoter reporter lines for other members of the *SAUR41* subfamily genes. We found that the expression pattern of *SAUR40* was similar to that of *SAUR41*, while the expression pattern of *SAUR71* was similar to that of *SAUR72*. Results showed that *SAUR72* and *SAUR71* were highly expressed in the steles of *Arabidopsis* roots and hypocotyls (Fig. 2A–C). Interestingly, *SAUR71* was extensively expressed in guard mother cells and young guard cells of *Arabidopsis* cotyledons and leaves during stomatal formation (Fig. 2D).

SAUR transcripts were originally identified in tissues undergoing differential cell expansion during tropic growth and in the epidermal and cortical cells of elongating hypocotyls.^{14–16} Thus, they were supposed to regulate cell expansion downstream of auxin transport and auxin signaling. Recent work has suggested that they are able to modulate auxin transport and cell

expansion by an unknown mechanism.^{4,7-9} Our work revealed the tissue-specific and developmentally regulated expression patterns of the *SAUR41* subfamily genes. *SAUR41* was distinctively expressed in the quiescent center and cortex/endodermis initials of root stem cell niches and in the endodermis of hypocotyls. *SAUR71* and *SAUR72* were expressed in the steles of young roots and hypocotyls (Fig. 2A–C). In addition, *SAUR71* was differentially expressed during stomatal formation (Fig. 2D). It has also been reported that *SAUR36* was expressed in the regions of shoot apical meristems and in the distinct regions related to the formation of lateral roots.¹¹ Taken together, *SAUR* transcripts could be identified in stem cells and stele cells of roots and shoots. These results imply that *SAUR* transcripts or SAUR proteins may serve as signal molecules to ensure the coordination of cell proliferation and cell expansion by unknown pathway(s), because auxin plays a critical role in the integration of cell division, differentiation and expansion. Further investigations to better understand the molecular mechanism of SAUR proteins and to characterize more loss-of-function *SAUR* mutants are of great interest for expanding our knowledge of auxin biology.

Co-immunoprecipitation (CoIP) is a popular technique to identify physiologically relevant protein-protein interactions. SAUR19 and SAUR63 proteins are highly unstable in *Arabidopsis* and the addition of epitope tags to these proteins increases the stability of SAUR proteins.^{7,8} *Arabidopsis* plants displayed phenotypes only when they expressed stabilized SAUR19 or SAUR63 proteins.^{7,8} By contrast, *Arabidopsis* overexpressing untagged SAUR41 had strong phenotypes, similar to that of MYC-tagged SAUR41.⁹ Here, we found that *Arabidopsis* seedlings expressing TAP (tandem affinity peptide) tagged SAUR41 displayed phenotypes (Fig. 3), indicating that it was rational to use the

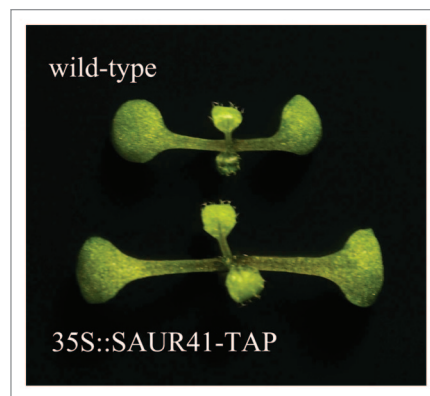


Figure 3. *Arabidopsis* seedlings expressing TAP (tandem affinity peptide) tagged SAUR41 had longer cotyledon petioles than the wild-type. Seedlings were 7-d-old.

TAP approach for identification of potential binding partners of SAUR41 proteins. Taken together, it could be helpful using different SAUR proteins to explore the mechanisms of SAUR proteins and these studies could complement and reinforce each other.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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