5′-non-transcribed flanking region and 5′-untranslated region play distinctive roles in sulfur deficiency induced expression of SULFATE TRANSPORTER 1;2 in Arabidopsis roots

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Abstract  Plants increase sulfate uptake activity under sulfur deficiency (−S). In Arabidopsis, SULTR1;2 is the major high-affinity sulfate transporter induced in epidermis and cortex of roots for mediating sulfate uptake under −S. Though it is known that transcript levels of SULTR1;2 increase under −S largely due to the function of 5′-upstream region, contributions of 5′-non-transcribed flanking region and 5′-untranslated region (UTR) to transcriptional and post-transcriptional regulations have not yet been individually verified. To investigate the roles of 5′UTR of SULTR1;2 in −S responses, transcript levels and activities of firefly luciferase (Luc) were analyzed in transgenic plants expressing Luc under the control of the 2,160-bp long 5′-upstream region of SULTR1;2 with (PL2160) or without (PL2160ΔUTR) the 154-bp 5′UTR. Both transgenic plants expressed similar levels of Luc mRNAs that showed significant accumulations under −S relative to +S regardless of presence of the 5′UTR. In contrast, Luc activities were detected only in PL2160 plants, suggesting presence of 5′UTR of SULTR1;2 being necessary for translational initiation while its absence impairing translation of functional Luc protein in PL2160ΔUTR. These results indicate an essential role of the 5′-non-transcribed flanking region of SULTR1;2 at positions −2160 to −155 in −S-responsive transcriptional regulation.

Key words: sulfate transporter, SULTR1;2, −S-inducible expression, translation, 5′UTR.

Sulfur is an essential macronutrient for all organisms. It is taken up by plants as sulfate, which is activated, reduced, and assimilated into an amino acid cysteine. Following cysteine biosynthesis, a wide variety of sulfur-containing compounds, such as glutathione, methionine, proteins, lipids, coenzymes, vitamins, and various secondary metabolites are synthesized in plants (Leustek et al. 2000; Saito 2004; Takahashi et al. 2011). Thus, sulfur in these essential compounds derives from sulfate which is taken up from the soil environment through the function of plasma membrane-localizing sulfate transporters.

The initial uptake of sulfate is facilitated by two high-affinity sulfate transporters, SULTR1;1 and SULTR1;2, expressed in epidermis and cortex of roots in Arabidopsis (Maruyama-Nakashita et al. 2003; Shibagaki et al. 2002; Takahashi et al. 2000; Vidmar et al. 2000; Yoshimoto et al. 2002, 2007). In both sulfur sufficient (+S) and deficient (−S) conditions, the transcript levels of SULTR1;2 are higher than those of SULTR1;1 (Maruyama-Nakashita et al. 2003; Rouached et al. 2008; Shibagaki et al. 2002; Yoshimoto et al. 2002, 2007). In addition, the growth phenotypes and sulfate uptake activity as well as the sulfate, cysteine, and GSH levels of knockout lines deficient in SULTR1;1 and SULTR1;2 indicate that SULTR1;2 is the main contributor determining sulfate uptake capacity of Arabidopsis roots under both +S and −S conditions (Maruyama-Nakashita et al. 2003; Shibagaki et al. 2002; Yoshimoto et al. 2002, 2007). The −S-induced expression of SULTR1;1 and SULTR1;2 depends on the promoter activities of their 5′-upstream regions (Maruyama-Nakashita et al. 2004a, 2004b). Further studies have indicated that both SULTR1;1 and SULTR1;2 are controlled by the activity of a transcription factor, SLIM1, which coordinates
the expression of a wide range of −S-responsive genes in Arabidopsis (Maruyama-Nakashita et al. 2006). However, the cis-acting elements in 5′-upstream regions responding to sulfate availabilities appear to be different between SULTR1;1 and SULTR1;2. A putative auxin response factor binding sequence, SURE1, is present in the SULTR1;1 promoter region to control its sulfur response, while the identical sequences have not been identified in the SULTR1;2 promoter region (Maruyama-Nakashita et al. 2005). These previous findings implicate the importance of transcriptional regulation of SULTR1;1 and SULTR1;2, although the regulatory pathways may involve slightly different mechanisms. In addition to regulation at mRNA levels, yet unknown post-transcriptional mechanisms can be essential for the maintenance of SULTR1;1 and SULTR1;2 protein abundance under −S (Yoshimoto et al. 2007). Thus, multiple mechanisms are involved in regulation of sulfate uptake systems in roots in order to obtain adequate amount of sulfate under −S conditions.

We previously reported that transgenic plants expressing GFP under the control of a 2160-bp 5′-upstream region of SULTR1;2 accumulated GFP concomitantly with an increase in endogenous SULTR1;2 mRNA under −S (Maruyama-Nakashita et al. 2004b). The 2160-bp 5′-upstream region used in our previous study, however, includes the 5′ UTR of SULTR1;2 flanking 154-bp upstream of the transcriptional start codon according to the sequences deposited in the TAIR database (http://www.arabidopsis.org; Shibagaki et al. 2002; Yoshimoto et al. 2002). Several studies provide evidence that presence of 5′ UTR can substantially contribute to regulation of mRNA stability and translational efficiency in plants (Bailey-Serres and Dawe 1996; Gutierrez et al. 1999; Hulzink et al. 2002; Kawaguchi and Bailey-Serres 2002; Mardonava et al. 2008). In addition, the existence of two splicing variants in SULTR1;2 cDNA, which comprised of 60 bp 5′ UTR containing one splicing site between −107 and −12 bp (At1g78000.1) and with 55 bp 5′ UTR (At1g78000.2) without splicing site (http://www.arabidopsis.org; Figure 1), seems to be suggestive for the regulatory role of 5′ UTR. To verify the function of 5′ UTR in transcriptional and post-transcriptional regulations of SULTR1;2 in response to sulfate availabilities, we analyzed the transcript levels and the activities of firefly luciferase (Luc) reporter in transgenic plants expressing the Luc gene under the control of the 5′ upstream region of SULTR1;2 with or without the 5′ UTR.

The chimeric gene constructs named PL2160 and PL2160ΔUTR were designed to contain the 2,160 bp 5′-upstream region of SULTR1;2 or the same region with a deletion of the 154-bp 5′ UTR respectively fused to the coding sequence cassette of Luc and nopaline synthase terminator (Figures 1, 2A). For these constructs, the 5′-regions of SULTR1;2, starting from the positions 2160 and terminating before the translational start codon or the 5′-end of the 5′ UTR of SULTR1;2, were amplified from genomic DNA of Arabidopsis thaliana (Col-0 accession) by polymerase chain reaction (PCR) using KOD-Plus DNA polymerase (TOYOBO, Osaka, Japan) and primer combinations comprised of the forward primer 1;2ProFSal: 5′-GTC GAC TTG ATT TGG AGC CAG TGG CAT TGT CGT-3′ and the reverse primer 1;2ProRBam: 5′-GGTAACC ACATGTGAG GC TGG CAT TGT CGT-3′ paired with either 1;2ProRBam: 5′-GGATAACC ACATGTGAG GC TGG CAT TGT CGT-3′ or 1;2ProRBam(− ΔUTR): 5′-GGATAACC ACATGTGAG GC TGG CAT TGT CGT-3′ as the reverse primer. Following cloning of PCR fragments into pCR-BluntII-TOPO (Invitrogen, Carlsbad, CA, USA) and sequencing, the Sali-BamHI fragments of SULTR1;2 promoter region were cloned between the Sali-BamHI sites of pBI101-Luc (Maruyama-Nakashita et al. 2005; Figures 1, 2A). The resultant binary plasmids were transferred to Agrobacterium tumefaciens GV3101 (pMP90) (Koncz and Schell 1986), and used for the transformation of Arabidopsis plants (Clough and Bent 1998). The transgenic plants were selected on GM media (Valvekens et al. 1988) containing 50 mg l−1 kanamycin sulfate.

The T2 progenies of PL2160 and PL2160ΔUTR transgenic lines were grown for 10 days on the agar medium (Hirai et al. 1995) supplied with 1500 µM (+S) or 50 µM (−S) sulfate. Agar plates were set vertically in a growth chamber controlled at 22°C and 16/8 h light and dark cycles, and the root tissues from the 10-day-old seedlings were used for the analysis. The transcript levels of Luc were determined by real-time PCR using SYBR Green Perfect Real Time kit (Takara) and Thermal Cycler.
and (C), 1976; Maruyama-Nakashita et al. 2005, 2015). In (B) luminescence units per mg protein. The Luc activities and protein (SEM, determining the average values of mRNA levels and their ratios between S and −S conditions in each plant line. *p < 0.01) between S and +S (−S/+S ratio) are presented. The Luc activities are shown as relative luminescence units per mg protein. The Luc activities and protein concentration were determined as described previously (Bradford 1976; Maruyama-Nakashita et al. 2005, 2015). nd, not detected. In (B) and (C), T2 progenies of five independent PL2160 and PL2160ΔUTR transgenic lines were grown for 10 days on agar medium containing 1500µM (+S, white bar) or 50µM of sulfate (−S, black bar) as described previously (Maruyama-Nakashita et al. 2005, 2015). Root tissues from 20 plantlets were pooled as one sample and used for determining the Luc mRNA levels by real-time PCR or for assaying the Luc activities. Error bars denote the standard error of the mean (SEM, n = 5). Asterisks indicate significant differences (Student’s t-test; *p < 0.01) between +S and −S conditions in each plant line.

Figure 2. The 5′-upstream flanking sequence of SULTR1;2 affects mRNA and protein expression in response to sulfate availabilities. (A) Schematic presentation of the constructs used in this study. The diagram shown on the top indicates the structure of 5′-upstream sequence of SULTR1;2. The lower two diagrams show the fusion gene constructs, PL2160 and PL2160ΔUTR, used for plant transformation. (B) Effect of deletion of 5′UTR on transcript levels of Luc. Average values of mRNA levels and their ratios between −S and +S (−S/+S ratio) are presented. (C) Effect of deletion of 5′UTR on Luc activities. Average values of Luc activities and the ratios between −S and +S (−S/+S ratio) are presented. The Luc activities are shown as relative luminescence units per mg protein. The Luc activities and protein concentration were determined as described previously (Bradford 1976; Maruyama-Nakashita et al. 2005, 2015). nd, not detected. In (B) and (C), T2 progenies of five independent PL2160 and PL2160ΔUTR transgenic lines were grown for 10 days on agar medium containing 1500µM (+S, white bar) or 50µM of sulfate (−S, black bar) as described previously (Maruyama-Nakashita et al. 2005, 2015). Root tissues from 20 plantlets were pooled as one sample and used for determining the Luc mRNA levels by real-time PCR or for assaying the Luc activities. Error bars denote the standard error of the mean (SEM, n = 5). Asterisks indicate significant differences (Student’s t-test; *p < 0.01) between +S and −S conditions in each plant line.

Dice Real Time System (Takara) using the gene-specific primers for Luc, Luc-552F: 5′-GTCCCTTGGATGGACA AACGAAC-3′ and Luc-674R: 5′-GGATCTCTGGCAC TGCGAGAATCT-3′, and for UBQ2, UBQ2-144F: 5′- CCAAGGGCTAGAAAGGAAGGA-3′ and UBQ2- 372R: 5′-TGGAGAGGAAGTGAAACAT-3′, as reported previously (Maruyama-Nakashita et al. 2004a, 2004b). The results indicated that both PL2160 and PL2160ΔUTR plants express similar levels of Luc mRNA showing significantly increased accumulations under −S relative to +S conditions (Figure 2B). The −S/+S ratios of Luc mRNAs ranged from 2.10 to 11.34 in PL2160, and from 2.11 to 3.77 in PL2160ΔUTR, respectively, which were similar and equally significant between PL2160 and PL2160ΔUTR (Figure 2B). Luc activities were also determined using the roots of these transgenic lines grown under +S and −S conditions according to the methods described previously (Maruyama-Nakashita et al. 2005, 2015; Figure 2C). In contrast to the mRNA levels, the Luc activities were detected only in PL2160 but not in PL2160ΔUTR plants. The Luc activities were consistently higher under −S relative to +S in five independent PL2160 lines with a range of −S/+S ratios being 2.14 to 6.09. These trends of increase in Luc activities under −S well reflected the Luc mRNA accumulations in PL2160 transgenic lines (Figure 2B, C).

The differences shown between PL2160 and PL2160ΔUTR plants indicated that 5′UTR of SULTR1;2 was not necessary for the control of transcription. The Luc mRNA levels were consistently elevated under −S to a similar extent in PL2160 and PL2160ΔUTR, suggesting that the −S-induced expression of SULTR1;2 is controlled through the function of the −2160 to −155 region of the 5′-upstream sequence that may serve as an enhancer for transcriptional activation under −S (Figure 2B). The −S/+S ratios were similar between Luc mRNA levels and Luc activities in PL2160 plants (Figure 2B, C), implicating that 5′UTR of SULTR1;2 was not involved in the control of mRNA stability.

It was intriguing to find the absence of Luc activities in PL2160ΔUTR (Figure 2C), because the sequences starting from the position −7 bp of the first ATG to the end of the Luc coding sequence as well as the sequence context around that translational start codon, which has been reported to be important for translation of mRNAs (Luetteke et al. 1987; Lukaszewicz et al. 2000; Rangan et al. 2008), were identical between the two constructs (Figure 1). As there was the possibility that the first AUG appeared at −22 bp of the first ATG of Luc in PL2160ΔUTR (Figure 1), which has different frame from Luc, could recruit ribosome and inhibit the translation of Luc by the translational overlap (Jackson et al. 2010; von Arnim et al. 2014), 5′UTR sequence of Luc in PL2160ΔUTR plants were determined by 5′RACE as described previously (Maruyama-Nakashita et al. 2015). In brief, following the RNA preparation from roots of PL2160ΔUTR plants grown on the +S and −S media, reverse transcription and RT-PCR was carried out using SMART RACE cDNA Amplification Kit (Clontech-Takara Bio) and the primers, Universal Primer A mix (Short) and Luc-5′RACE-1 (5′-ACGACACCACGAGTATAGCTTGCAGAACAAC-3′), then the amplified fragments were sequenced. The determined 5′UTR did not contain the first AUG appeared at −22 bp of
Luc coding sequence, excluding the possibility that the translation of Luc is inhibited by the translational overlap with upstream open reading frame (Jackson et al. 2010; von Arnim et al. 2014). The 5′UTR of SULTR1;2 can be necessary for the recruitment of translational initiation factors to couple the 5′-cap structure with the 3′-poly(A) tail, which may help the pre-initiation complex to start translation (Kawaguchi and Bailey-Serres 2002; Sonenberg and Hinnebusch 2009; Wilkie et al. 2003). Given the assumption that transcription of Luc occurs in the same manner and efficiency determining Luc mRNA levels in PL2160 and PL2160ΔUTR, it is unlikely that SULTR1;2 5′UTR controls Luc mRNA stability. With regard to the absence of Luc activities in PL2160ΔUTR, we cannot exclude a possibility that the first ATG in the Luc coding sequence could have been read through and another ATG used for the translation of non-functional Luc proteins.

In summary, we demonstrated that the −S-responsive accumulation of Luc mRNA is controlled by the 5′-upstream non-transcribed region of SULTR1;2 independent of the function of 5′UTR. Since the sulfur-responsive element found in SULTR1;1 promoter region is not present in the 5′-upstream region of SULTR1;2, novel elements responsible for −S-responsive transcriptional regulation of SULTR1;2 probably exist in the −2160 to −155 region. Precise determination of these elements would reveal the transcriptional molecular machinery involved in regulation of −S-induced expression of sulfate uptake systems that are required for plant survival under sulfur deprived conditions.

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References

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