Auxin Response Factors and Aux/IAA Proteins Potentially Control -S Responsive Expression of SULTR1;1

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Auxin Response Factors and Aux/IAA Proteins Potentially Control –S Responsive Expression of *SULTR1;1*

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Abstract Sulfur is an essential nutrient for plants and its deficiency (–S) severely affects plant growth. To acquire limited sulfur under –S, plants have evolved signal transduction pathways resulting in enhanced sulfate uptake and assimilation. The transcript level of the high affinity sulfate transporter *SULTR1;1* is dramatically induced by –S. The –S-induced expression of *SULTR1;1* is dependent on SLIM1 transcription factor which controls a broad range of –S responsive gene expression. Previously we identified the sulfur-responsive element of *SULTR1;1* (SURE11) which includes a 6 bp sequence, GAGACA, identical to the binding sequence of auxin response factors (ARFs). ARFs are a family of transcription factors that promote or repress the expression of auxin responsive genes. The function of ARFs is inhibited by the hetero-dimerization with Aux/IAA proteins that cannot bind to the sequence. Though *SULTR1;1* expression was not modulated by exogenously applied auxin, the identity between *SURE*-core sequence and the ARF binding sequence suggests that one of the ARF works to induce *SULTR1;1* expression under –S. In this paper, we attempt to predict which ARFs and Aux/IAA proteins potentially control –S-induced expression of *SULTR1;1* by using microarray data on *slim1* and the parental plants. Five ARF and seven Aux/IAA proteins were up- or down-regulated by –S in parental plants. Among them, none of the ARF modified its –S response in *slim1*, but five Aux/IAA proteins lost their –S response in *slim1*, including IAA13 and IAA28 whose positive function in sulfur assimilation had been reported. These results indicated that this method could be useful in predicting candidates for regulatory genes working in –S responsive gene expression.

Sulfur is an essential nutrient for plants and its deficiency severely affects the plant growth, crop yield and quality. Sulfate, the major form of sulfur that plants can utilize for the synthesis of cysteine and methionine, is taken up from plant roots by the activity of sulfate transporters. In Arabidopsis, two high-affinity sulfate transporters,

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SULTR1;1 and SULTR1;2, expressed in the epidermis and cortex of roots, play essential roles in the uptake of sulfate (Takahashi et al. 2000; Vidmar et al. 2000; Shibagaki et al. 2002; Yoshimoto et al. 2002, 2007; Maruyama-Nakashita et al. 2003). Gene expression of both *SULTR1;1* and *SULTR1;2* is dramatically induced by the depletion of sulfate (–S) in a promoter-dependent manner, thereby the sulfate uptake activity is induced by –S (Takahashi et al. 2000; Shibagaki et al. 2002; Yoshimoto et al. 2002; Maruyama-Nakashita et al. 2004a, b). However, the signal transduction pathways from perception of the sulfur status to the regulation of these transporters are not fully understood at the molecular level.

To uncover the regulatory mechanisms of sulfur response, the *cis*-acting element involved in the –S response was investigated. For *SULTR1;1*, deletion and gain-of-function analysis using the luciferase reporter gene in transgenic *Arabidopsis* revealed that the 16 bp sulfur responsive element (SURE11) from –2777 to –2761 of the promoter was sufficient and necessary for the –S-responsive expression, which was reversed when supplied with sulfate, cysteine and glutathione (GSH) (Maruyama-Nakashita et al. 2005). Base substitution analysis indicated the significance of a 7 bp sequence (GGAGACA) as a core element. The core sequences exist in the promoter regions of several –S-inducible genes, suggesting a common mechanism for –S regulation.

The core sequence of SURE11 includes the ARF binding sequence (GAGACA), which has previously been reported as an auxin response element (Ulmasov et al. 1997, 1999; Hagen and Guilfoyle 2002). However, SURE11 was not responsive to naphthalene acetic acid (NAA), indicating its specific function in the sulfur response (Maruyama-Nakashita et al. 2005). In *Arabidopsis*, 23 genes are reported as ARF family transcription factors, but their function in the regulation of auxin responsive gene expression has not been investigated for all the family members (Okushima et al. 2005). There is a possibility that an ARF-like transcription factor binds to the *SURE* core sequence and induces –S-dependent expression of *SULTR1;1*. Generally, ARF regulates the transcription of auxin responsive genes in a dimer form and the heterodimerization between Aux/IAA and ARF inhibits the access of ARF to the binding sequence by inhibiting the dimerization between ARFs (Guilfoyle and Hagen 2007). Though the ARF binding sequence in the SURE11 is not repeated, a similar regulatory circuit to control *SULTR1;1* expression by ARF and Aux/IAA proteins could exist.

Another regulatory protein of –S-inducible expression of *SULTR1;1* is SLIM1 transcription factor (Maruyama-Nakashita et al. 2006). SLIM1 controls both the activation of several sulfur assimilatory genes including sulfate transporters and degradation of glucosinolates, and the repression of glucosinolates synthetic genes under –S conditions (Maruyama-Nakashita et al. 2006). As SLIM1 controls both –S up-regulated and down-regulated gene expression, there should be other regulators connecting SLIM1 and each metabolic process. In this report, to isolate the transcription factors that directly control *SULTR1;1* expression, the ARF and Aux/IAA proteins whose expression is modulated by sulfate availability and SLIM1 were investigated using the microarray data obtained from *slim1* mutants (Maruyama-Nakashita et al. 2006).

In the Arabidopsis genome, 23 genes of ARF and 29 genes of Aux/IAA exist (Table 1). Among them, probes for 16 and 22 genes of ARF and Aux/IAA, respectively, exist on Affymetrix ATH1 GeneChip microarray (Table 1). The previous

Table 1 Selection of ARF and Aux/IAA proteins responsive to sulfur deficiency in a SLIM1-dependent manner

	ARF	AuX/IAA
Arabidopsis Genome	**23**	**29**
Genechip	**16**	**22**
Signals can be trusted	**13**	**18**
Sulfur responsive in parental plants (P<0.1)	**5**	**7**
Sulfur response is different between parental and *slim1* plants (P<0.1)	**0**	**5**

ARF and Aux/IAA proteins were selected whose transcript levels were influenced by sulfur deficiency (−S) and SLIM1 existence. The previous GeneChip data were used for the selection (Maruyama-Nakashita et al. 2006). $P_{SULTR1;2}$-*GFP* (parental), *slim1-1*, *slim1-2* plants were grown vertically on an agar medium containing 1500 μM or 15 μM of sulfate (+S, −S) for 10 days, and duplicated root RNA samples were used for the Affymetrix ATH-1 GeneChip analysis. "Signals can be trusted" means signals were present or marginal at least once over the 12 experiments. Among the genes whose signals can be trusted, −S-responsive genes were selected by comparing the gene expression between parental plants grown under +S and –S conditions. The genes whose responses to –S were modified in *slim1* mutants were selected by comparing the –S/+S ratio of transcript levels between parental and *slim1* plants. Student's *t*-test was performed and the genes showing probability values less than 0.1 were selected

Table 2 ARF and IAA proteins responsive to sulfur deficiency

ARF			Aux/IAA		
Affymetrix ID	AGI code	Gene name	Affymetrix ID	AGI Code	Gene name
247468_at	AT5G62000	ARF2	257766_at	AT3G23030	IAA2
256311_at	AT1G30330	ARF6	**257769_at**	**AT3G23050**	**IAA7**
254194_at	AT4G23980	ARF9	**255788_at**	**AT2G33310**	**IAA13**
251289_at	AT3G61830	ARF18	**246376_at**	**AT1G51950**	**IAA18**
256010_at	AT1G19220	ARF19	**246861_at**	**AT5G25890**	**IAA28**
			253423_at	**AT4G32280**	**IAA29**
			247906_at	AT5G57420	IAA33

Genes selected as "sulfur responsive in parental plants" in Table 1 were listed. The genes selected as "sulfur response is different between parental and *slim1* plants" in Table 1 were shown in *bold characters*

GeneChip experiment was performed using duplicated root RNA samples of $P_{SULTR1;2}$-*GFP* (parental line), *slim1-1*, *slim1-2* plants grown on an agar medium containing 1500 μM (+S) or 15 μM (−S) of sulfate for 10 days. Among the 12 experiments, signals for 13 and 18 genes of ARF and Aux/IAA, respectively, were detected as statistically trusted data whose signals were present or marginal at least once over the 12 experiments (Table 1). From these genes, −S responsive genes were selected by comparing the signals of parental plants grown under +S and –S conditions, which included five *ARF* genes, *ARF2*, *ARF6*, *ARF9*, *ARF18* and *ARF19* and seven Aux/IAA genes, *IAA2*, *IAA7*, *IAA13*, *IAA18*, *IAA28*, *IAA29* and *IAA33* (Tables 1 and 2; Fig. 1). Among the genes obtained, *ARF2*, *IAA13* and *IAA28* were reported as –S up-regulated genes (Nikiforova et al. 2003, 2005). Though the up-regulation of *ARF2* and *IAA13* by –S were consistent with the previous reports, the

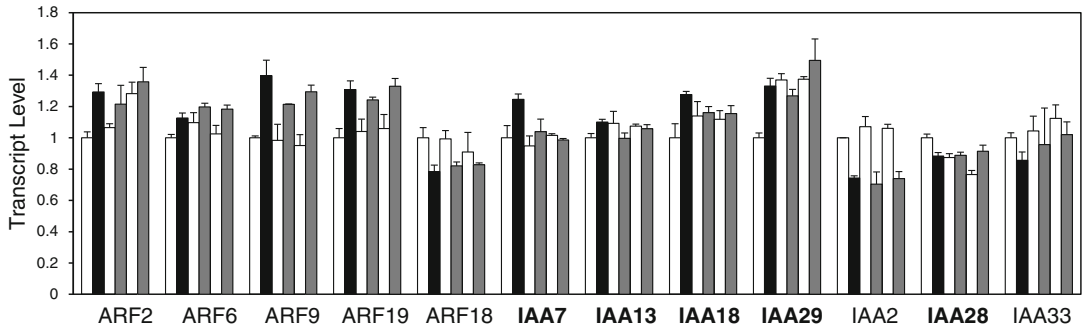

Fig. 1 Relative transcript levels of ARF and Aux/IAA proteins responsive to sulfur deficiency. Average values of relative transcript levels of the genes selected as "sulfur responsive in parental plants" in Table 1 were presented. Each bar graph represents relative transcript levels in parental plants grown under + S (*white*) or –S (*black*), those in *slim1-1* grown under + S (*white*) or –S (*gray*), those in *slim1-2* grown under + S (*white*) or –S (*gray*), from left to right. The relative transcript levels calculated are those of parental plants grown under + S as 1.0. *Error bars* are standard errors. The genes selected as "sulfur response is different between parental and *slim1* plants" in Table 1 are shown in *bold characters*

down-regulation of *IAA28* was not (Fig. 1, Nikiforova et al. 2003, 2005). This may be due to differences in culture conditions: here plants were grown vertically on an agar medium containing 1500 or 15 μM sulfate for 10 days, whereas Nikiforova et al. (2003, 2005) grew plants horizontally on an agar medium containing 750 and 65 μM sulfate. Another microarray analysis with –S treated roots demonstrated the down-regulation of *IAA28* by –S which also treated the plants on agar media (Winter et al. 2007; Iyer-Pascuzzi et al. 2011).

The genes whose response to –S were modified in *slim1* mutants were selected by comparing the –S/+S ratio of transcript levels in parental and *slim1* plants. From this, five genes of Aux/IAA proteins, IAA7, IAA13, IAA18, IAA28 and IAA29, were obtained as the –S responsive SLIM1-dependent genes (Tables 1 and 2, Fig. 1). Their expression in root epidermis suggested that they can directly control *SULTR1;1* expression by accessing the proteins binding to SURE11 (Arabidopsis eFP Browser, Winter et al. 2007; Birnbaum et al. 2003; Nawy et al. 2005). Among the IAA proteins selected, IAA13 and IAA28 demonstrated a positive function in sulfur assimilation (Falkenberg et al. 2008), indicating that this method could be useful in predicting candidates for regulatory genes working in –S responsive gene expression. As Aux/IAA proteins do not have DNA binding domains, they should affect the activity or the interaction capacity of the ARF-like transcription factors binded to SURE11.

There are some reports suggesting that an increase of auxin in sulfur-starved plants may mediate the signals for the regulation of –S-responsive genes (Kutz et al. 2002; Nikiforova et al. 2003; Kasajima et al. 2007). The transcript level of nitrilase 3 was increased two-fold by –S in parental plants but the same increase was not observed in *slim1* mutants (Maruyama-Nakashita et al. 2006). The transcript levels of five IAA proteins, *IAA7*, *IAA13*, *IAA18*, *IAA28* and *IAA29*, were modulated by auxin in a very similar way with –S treatment, such that *IAA7*, *IAA13*, *IAA18* and

IAA29 were up-regulated but *IAA28* was down-regulated by auxin (Arabidopsis eFP Browser, Winter et al. 2007; Goda et al. 2008). Though the transcript level of *SULTR1;1* was not influenced by auxin (Maruyama-Nakashita et al. 2005; Goda et al. 2008), auxin and its signal transduction can be an important component in plant adaptation to –S.

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