Sulfur is one of the essential macronutrients required for plant growth. Since the expression of several sulfur-assimilatory genes is stimulated under the condition of sulfur deficiency (−S), transcriptional regulation of these genes is considered to be critical for the control of sulfur assimilation. In the last several years, the author and coworkers have been investigating molecular mechanisms of −S-inducible expression of high-affinity sulfate transporters, SULTR1;1 and SULTR1;2 in Arabidopsis thaliana. SULTR1;1 and SULTR1;2 facilitate sulfate uptake in roots. This review summarizes the recent progress about the transcriptional regulation of these sulfate transporters by focusing on three major topics. 1) Identification of a cis-acting element involved in the −S-inducible expression of SULTR1;1. 2) Cytokinin-dependent repression of SULTR expression and sulfate uptake. 3) Central transcription regulator SLIM1 controlling −S responsive gene expression including sulfur assimilation and metabolism.

Key words: Arabidopsis thaliana, Glucosinolate, Sulfur assimilation, Sulfate transporter, SULTR1;1, SULTR1;2, SLIM1.

Sulfur is one of the essential macronutrients required for plants. Plants use sulfate as the major sulfur source and synthesize sulfur-containing amino acids cysteine and methionine (Crawford et al. 2000; Leustek et al. 2000; Saito 2004). Animals, including humans, require sulfur-containing amino acids, mostly methionine, and proteins as dietary sulfur sources because of their inability to assimilate sulfate. Thus the plant ability to assimilate sulfate is essential for the global sulfur cycle in nature. In addition to amino acids and proteins, plants synthesize a variety of metabolites, which critical for biological processes and beneficial for humans (Crawford et al. 2000; Leustek et al. 2000; Saito 2004). Considering the essential roles of sulfur assimilation for both plant growth and quality, understanding the regulatory systems of plant sulfur assimilation is important for improvement of sulfur utilization capacity and nutritional values of plants. Improvement of sulfur utilization capacity would also contribute to plant remediation of excess sulfate found in particular environments.

In 1990’s, enzymes and sulfate transporters involved in the sulfur assimilatory pathway were identified by the efforts of many researchers (Leustek et al. 2000; Saito 2004). Sulfur assimilation starts from the uptake of external sulfate by the activity of sulfate transporters in roots. Sulfate taken up by plant roots are activated by ATP sulfulyrase and then reduced by two-step reactions catalyzed by APS reductase (APR) and sulfite reductase to produce sulfide. Then with cysteine synthase, sulfide reacts with \( O\)-acetyl-L-serine and turned into cysteine, the first organic form of sulfur in the plant sulfur assimilatory pathway. Glutathione (GSH), methionine and many kinds of sulfur containing compounds are produced from cysteine.

During the process of enzyme gene hunting for the enzymes in the sulfur assimilatory pathway in late 1990’s, Takahashi et al. (1997) found that mRNA levels of several sulfur assimilatory genes, sulfate transporter (SULTR), APR and serine acetyl transferase, were up-regulated by sulfur deficiency (−S) in A. thaliana. In 2000’s transcriptional regulation of sulfur assimilatory pathway responding to sulfur starvation has been extensively studied with the combination of omics-based approach (Hirai et al. 2003, 2004, 2005; Maruyama-Nakashita et al. 2003, 2005, 2006; Nikiforova et al. 2003) and the dissection of molecular machinaries.
underlying −S response of each gene responsible to −S, such as APR, β-subunit of β-conglycinin (a seed storage protein of soybean), NIT3 and SULTR (Awazuhara et al. 2002; Koprivova et al. 2000; Kutz et al. 2002, Maruyama-Nakashita et al. 2004ab; 2005; 2006; Ohkama et al. 2002). Recent transcriptomics studies suggested that activation of sulfate assimilation and repression of glucosinolate (GSL) production may occur in parallel in response to −S (Hirai et al. 2003; 2004; 2005; Maruyama-Nakashita et al. 2003; 2005; 2006; Nikiforova et al. 2003). The entire network of sulfur metabolism is coordinately regulated under −S. This review focuses on the recent progress of transcriptional regulation of sulfur assimilation, which has been lead by the study on high affinity sulfate transporters in *A. thaliana*.

**Identification of a cis-acting element involved in the −S-inducible expression of high affinity sulfate transporter, SULTR1;1**

The entire process of uptake, distribution and remobilization of sulfate has gradually been clarified by the recent reverse genetic approaches about SULTRs (Takahashi et al. 2006). Among the 12 members of SULTR in *A. thaliana*, two high-affinity sulfate transporters, SULTR1;1 and SULTR1;2, facilitate the initial uptake of sulfate in roots (Takahashi et al. 2000; Vidmar et al. 2000; Shibagaki et al. 2002; Yoshimoto et al. 2002). They are expressed in the epidermis and cortex of roots, and their transcripts accumulation is elevated under −S conditions to maximize sulfate uptake for efficient utilization of limited amount of sulfate in the soil (Takahashi et al. 2000; Vidmar et al. 2000; Shibagaki et al. 2002; Yoshimoto et al. 2002).

Inhibitor studies demonstrated that −S response of SULTR1;1 is controlled at the level of transcription and requires protein phosphatase activity (Figure 1; Maruyama-Nakashita et al. 2004a). It was likely that a specific sulfur-responsive cis-element was present in the SULTR1;1 promoter and elucidation of the element is important for understanding of the molecular mechanism of −S responsive gene expression. The promoter region of SULTR1;1 was dissected for deletion and gain-of-function analysis using a firefly luciferase gene as a reporter in transgenic *A. thaliana*. The 16-bp sulfur-responsive element from −2777 to −2762 of SULTR1;1 promoter was identified as a sufficient and necessary cis-acting element for the −S-responsive expression (Maruyama-Nakashita et al. 2005). The element was also found sufficient for repression by application of cysteine or glutathione (GSH). Base substitution analysis further identified the core sequence GGAGACA (Figure 1). This core sequence and its homologous sequence were found in the several −S-inducible gene promoters, including the −S responsive region of NIT3 (Kutz et al. 2002) and the β-subunit gene promoter of β-conglycinin (Awazuhara et al. 2002), suggesting that a common regulatory mechanism induces the expression of a gene set required for adaptation to the −S conditions.

−S responsive cis-acting element of SULTR1;1 contains an auxin response factor (ARF) binding sequence (GAGACA). However, cis-acting element of SULTR1;1 was not responsive to exogenous auxin. There are some reports suggesting that increase of auxin in sulfur-starved plant roots mediate the signals for the regulation of −S-responsive genes (Kutz et al. 2002; Nikiforova et al. 2003). Connection between −S and auxin signal is still controversial, but the identification of ARF-like transcription factor could bind to the cis-acting element and induce −S-responsive expression of SULTR1;1 may explain the relationship.

**Cytokinin-dependent repression of SULTR expression and sulfate uptake**

To setup a simple and traceable system for detection of SULTR expression, a GFP reporter system that generally displays the expression of SULTR1;2 was constructed (Figure 2; Maruyama-Nakashita et al. 2004b). In the PSULTR1;2-GFP plants, carrying a −2160 bp fragment of the 5′-region of SULTR1;2 fused to the GFP coding sequence, GFP accumulation showed typical sulfur responses that correlate with the changes in SULTR1;2 mRNA levels; accumulation of GFP was induced by −S, but was repressed in the presence of reduced sulfur compounds, cysteine and GSH (Figure 2). The significant correlation between the GFP accumulation and the SULTR1;2 expression ensured the authenticity of GFP expression in PSULTR1;2-GFP plants for monitoring promoter-dependent regulation of SULTR1;2 (Figure 2).

By monitoring SULTR1;2 expression using PSULTR1;2-GFP, it was demonstrated that cytokinin negatively regulates sulfate uptake in roots (Figure 3A; Maruyama-Nakashita et al. 2004b). Exogenous application of cytokinin repressed the uptake of sulfate, accompanied with a drastic reduction of SULTR1;1 and SULTR1;2 mRNA contents in roots (Figure 3B, C). The effect of cytokinin on sulfate uptake was less prominent in the cre1-1 mutant (Figure 3C), lacking the CRE1/WOL/
AHK4 cytokinin receptor (Inoue et al. 2001). However, −S inducibility of sulfate uptake is not altered by cytokinin or in the crel-1 mutant (Figure 3C; Maruyama-Nakashita et al. 2004b), suggesting that the cytokinin-dependent repression of sulfate uptake is independent from the sulfur-responsive regulation.

The repression of nutrient uptake in roots by cytokinin application has also been reported for the phosphate transporter, PT1 (Martin et al. 2000; Karthikeyan et al. 2002; Franco-Zorrilla et al. 2002), and for the nitrate uptake of Fagus sylvatica (Collier et al. 2003). Cytokinin and its receptor CRE1/WOL/AHK4 possibly play an essential role in regulation of nutrient uptake in general. The downstream components in cytokinin signal transduction, which directly regulate nutrient uptake, i.e. transcription factors, remains to be determined.

Central transcription regulator SLIM1 controlling −S responsive gene expression including sulfur assimilation and metabolism

The finding of the cis-regulatory element is indicative of the existence of trans-acting regulatory proteins for the induction of transcripts for high-affinity sulfate transporter. Recently, we identified SLIM1 transcription factor as the key regulatory proteins controlling the upstream signaling cascades of sulfur metabolism through a forward genetics approach (Maruyama-Nakashita et al. 2006). From the ethyl-methanesulfonate-mutagenized population of P SULTR1;2-GFP plants, seedlings showing reduced levels of GFP were screened under −S conditions. sulfur limitation 1, slim1, was obtained as a family of allelic mutants with recessive, single-gene segregations. The slim1 mutants mostly lacked the −S induced GFP accumulation and endogenous SULTR1;2 expression observed in the parental plants (Figure 4A). In addition, high-affinity sulfate uptake and the plant growth were reduced in the slim1 mutants under the −S condition (Figure 4B).

Mutations causing the −S-response-less phenotypes of slim1 mutants were identified in a putative EIL-family transcription factor, ETHYLENE-INSENSITIVE3-LIKE3 (EIL3). The −S-response-less phenotype of slim1 mutants could be restored by the expression of A. thaliana SLIM1 and its rice homologues, but not by other EIL proteins including EIN3 (Chao et al. 1997; Guo and Ecker 2004), suggesting the uniqueness of the SLIM1/EIL3 sub-group members in EIL family as sulfur response regulators.

SLIM1 was the first transcription factor identified as the regulatory protein that affects the whole sulfur assimilatory pathway in higher plants. Findings obtained from the SLIM1 analysis will lead to in-depth analysis of the downstream regulatory elements and further facilitate general improvement of sulfur use efficiencies and engineering of beneficial GSL in cruciferous plants.

Concluding remarks

This review documented the recent findings about transcriptional regulation of the high affinity sulfate transporters and sulfur assimilatory pathway in A. thaliana (Figure 5). Identification of SLIM1 working upstream of −S signal transduction pathway and the cis-acting element which directly controls the −S responsive expression of high affinity sulfate transporter may allow
us to take the reverse genetics approach to identify the regulatory components working between them, e.g. transcription factor that directly controls sulfate uptake or a molecule that may control GSL synthesis and degradation. The SLIM1 localization was in the vascular tissues, similarly to the case of CRE1/WOL/AHK4 existing in the procambium and pericycle in roots (Mähönen et al. 2000), contrary to the fact that the nutrient uptake is conducted at the root surface. This apparent contradiction of the localization of regulators...
Figure 5. Transcriptional control of sulfur assimilation and metabolism responding to −S

and downstream transporters might indicate the importance of the inter-organ signaling in the control of sulfate uptake as previously described by Lappartient et al. (1999), and poses an interesting question concerning cell-to-cell communication of the regulatory signals. Moreover, research on upstream signals that regulate SLIM1 function is needed to further understand how plants sense environmental and internal sulfur status and control sulfur metabolism of themselves. I believe that these basic researches will contribute to improve crop value and plant use in phytoremediation.

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