Chapter 8

Molecular Biology and Functional Genomics for Identification of Regulatory Networks of Plant Sulfate Uptake and Assimilatory Metabolism

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Summary

Uptake of sulfate from the environment is critical for sulfur metabolism as it controls the quantity of sulfur to be distributed through the metabolic pathways. Similar to the uptake systems for other nutrient ions, transport of sulfate can be resolved into high- and low-affinity phases. Sulfur limitation stimulates the high-affinity sulfate transport system that essentially facilitates the uptake of sulfate in roots. Apparently, the induction of sulfate uptake by sulfur limitation is driven by demands of sulfur. Recent molecular biological studies have unveiled some important aspects behind the regulatory cascades of plant sulfur response and sulfate assimilation. In this review, we describe the regulatory steps controlling

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sulfate uptake and assimilatory metabolism in *Arabidopsis thaliana*, and further discuss on the networks of metabolic and hormonal regulation based on recent findings that deal with transcriptome and metabolome data analyses.

I. Sulfate Transport Systems in Plants

A. Sulfate Transporter Gene Family

As described in recent reviews, *Arabidopsis* genome encodes 12 distinct sulfate transporter genes that are classified into 4 functional groups (Buchner et al., 2004b; Takahashi et al., 2006). All 12 isoforms of sulfate transporters from *Arabidopsis* are structurally similar to the one first identified from *Stylosanthes hamata* by functional complementation of a yeast sulfate transporter mutant (Smith et al., 1995). Two additional homologues representing the group 5 members (Buchner et al., 2004b) appear to have low sequence similarity to sulfate transporters, though the exact functions have been remained unverified. Likewise the case of other nutrient transporters, multiplicities of isoforms may reflect complexities of the whole-plant sulfate transport systems in vascular plants. In fact, the functionality and localization of sulfate transporters are diversified among the isoforms, which lead us to set out for physiological characterization and dissection of the functions of individual transport components using T-DNA insertion mutants of *Arabidopsis* (Takahashi et al., 2006). The following two sections summarize the roles of individual transporters that correspond to the uptake and internal transport systems that have been verified from the analysis of T-DNA insertion mutants. In addition to this review article, general features of plant sulfate transport systems have been described by Hawkesford in Chapter 2.

B. Uptake of Sulfate

The initial uptake of sulfate occurs at the root surface. Earlier physiological studies suggested the high-affinity kinetics (phase I) of sulfate uptake system predominates under sulfur limited condition (Clarkson et al., 1983; Deane-Drummond, 1987). The high-affinity sulfate transporters that correspond to this sulfur-limitation inducible transport system are represented by the Group 1 members (Takahashi et al., 2006). The first isolated cDNAs, *SHST1* and *SHST2*, from *Stylosanthes hamata* were able to complement the yeast sulfate transporter mutant, and exhibited saturable kinetics of sulfate uptake with micromolar K_m values (Smith et al., 1995). In addition, the *SHST1* and *SHST2* mRNAs accumulated in root tissues when plants were starved for sulfate. The *Arabidopsis SULTR1;1* and *SULTR1;2* showed similar characteristics (Takahashi, et al., 2000; Shibagaki et al., 2002; Yoshimoto et al., 2002). They were localized in the root hairs, epidermis and cortex of roots, and their transcripts were significantly accumulated during sulfur limitation. The uptake of sulfate and the overall sulfur status was significantly affected by deletion of *SULTR1;2*, suggesting this transporter plays a major role in facilitating the uptake of sulfate in *Arabidopsis* roots (Maruyama-Nakashita et al., 2003). In addition, induction of *SULTR1;1* mRNA in the *sultr1;2* mutant indicates compensatory and demand-driven regulation of this isoform. Presence of the inducible isoforms of high-affinity sulfate transporters appears to be common in various plant species (Howarth et al., 2003; Buchner et al., 2004a, 2004b), which may allow flexible adaptation to fluctuating sulfur conditions in the environment.

C. Internal Transport of Sulfate

Pathways that mediate internal translocation of sulfate are complicated and are not completely resolved. However, studies using the knockouts of *Arabidopsis* sulfate transporters suggested several potential components that facilitate distribution of sulfate through the vasculature. In *Arabidopsis*, a low-affinity sulfate transporter, SULTR2;1, is suggested to play a pivotal role in controlling transport of sulfate at xylem parenchyma cells (Takahashi et al., 1997, 2000;

Abbreviations: OAS – O-acetyl-L-serine; SULTR – sulfate transporter; SURE – sulfur responsive element

Awazuhara et al., 2005). *SULTR2;1* was strictly regulated by sulfur status, exhibiting drastic induction of its mRNA in the roots of sulfur-starved plants. In addition to regulation of its transcript levels, the activity of SULTR2;1 was modulated by the presence of a co-localizing component, SULTR3;5, in the pericycle and xylem parenchyma cells of roots (Kataoka et al., 2004a). For sufficient transport of sulfate from root tissues to shoots, release of sulfate pools from vacuoles was an additional rate-limiting step under sulfur-limited conditions. The tonoplast-localizing sulfate transporters that serve for this essential step were encoded by *SULTR4;1* and *SULTR4;2* in *Arabidopsis* (Kataoka et al., 2004b). Both were inducible by sulfur limitation, and T-DNA insertion mutants showed accumulation of sulfate in the vacuoles, leading to a significant decrease in distribution of the incorporated sulfate to shoots. Besides root-to-shoot transport, source-to-sink transport is suggested to be important for allocation of sulfur storage from old to young tissues (Bourgis et al., 1999; Herschbach et al., 2000). In *Arabidopsis*, the function of a phloem-localizing component of sulfate transport system has been evidenced from the analysis of knockout mutant. SULTR1;3, the third member of high-affinity sulfate transporters, was localized in the companion cells of transport phloem, and was essentially required for the movement of sulfate from cotyledons to shoot meristems and roots in *Arabidopsis* (Yoshimoto et al., 2003).

II. Regulation by Sulfur

A. Demand-Driven Regulation

The external supply of sulfate is primarily important for regulation of sulfate uptake and assimilation (Leustek et al., 2000; Saito, 2004; Takahashi et al., 2006). Particularly, high-affinity sulfate transporters that facilitate the initial uptake of sulfate in roots are strictly regulated by sulfate availabilities (Takahashi et al., 2000; Yoshimoto et al., 2002; Shibagaki et al., 2002; Maruyama-Nakashita et al., 2004a, 2004c). In addition to sulfate, metabolites of sulfur assimilatory pathways are known to have major impacts on gene expression of sulfate transporters. When sulfate is adequately or excessively supplied from the environment, plants may synthesize cysteine, methionine and glutathione, causing feedback regulation of the uptake of sulfate. On feeding experiments, excessive application of cysteine and glutathione led to a drastic decrease of sulfate uptake activity in roots (Smith et al., 1997; Vidmar et al., 2000). In *Arabidopsis*, decrease of sulfate uptake is attributable to repression of *SULTR1;1* and *SULTR1;2*, both being down-regulated significantly in the presence of thiols in the medium (Maruyama-Nakashita et al., 2004c, 2005). In addition to the high-affinity sulfate transporters that facilitate the initial sulfate uptake, transcripts of *SULTR2;1* low-affinity sulfate transporter was regulated under a similar scheme (Vidmar et al., 2000). A split-root experiment suggests repressive signals can be translocated from distant organs through phloem (Lappartient et al., 1999). These results suggest that sulfate uptake and vascular transport are both controlled in a demand-driven manner not only by a local signal but also by systemic requirement of sulfur (Lappartient et al., 1999; Herschbach et al., 2000).

B. The Action of a Cysteine Precursor

O-acetyl-L-serine (OAS), the precursor of cysteine synthesis, positively affects the expression of high-affinity sulfate transporters (Smith et al., 1997; Maruyama-Nakashita et al., 2004c). In barley, induction of *HVST1* sulfate transporter by OAS was accompanied with increase in sulfate uptake activities (Smith et al., 1997). In addition, transcripts for adenosine 5′-phophosulfate reductase, the pivotal enzyme that controls the flux of sulfur through the sulfate assimilation pathway (Vauclare et al., 2002), were induced by the addition of OAS to the medium after nitrogen starvation (Koprivova et al., 2000). From these findings, one may postulate that OAS could provide signals of sulfur limitation response. Identification of an OAS accumulating *Arabidopsis* mutant, *osh1*, which stimulates the activity of sulfur limitation-responsive promoter of β-conglycinin β-subunit, supports this regulatory model (Ohkama-Ohtsu et al., 2004). Furthermore, microarray studies and other expression analysis indicate that feeding of OAS gives a pattern of gene expression profile that mimics sulfur limitation (Hirai et al., 2003, 2004). However, a time-course measurements of

tissue OAS contents indicates that induction of sulfate transporter precedes accumulation of OAS under sulfur limitation (Hopkins et al., 2005). This contrasts with the hypothesis placing OAS as a signal mediator that triggers up-regulation of sulfur assimilatory pathways, questioning when and how the accumulation of OAS serves for regulation of gene expression in sulfur-starved plants. Excessive accumulation of OAS simply may imply disturbance of cysteine synthesis by limited supply of sulfide under prolonged sulfur starvation (Hopkins et al., 2005). Biochemical studies indicate that OAS has an ability to control the activity and structure of cysteine synthase complex (Wirtz et al., 2004). The enzyme complex is dissociated and associated reversibly by OAS and sulfide, respectively. The two different states provide production of cysteine by free OAS(thiol)lyase or synthesis of OAS by the cysteine synthase complex that is reconstituted under an excess of sulfide over OAS. Apparently, a switching mechanism exists for the control of cysteine synthesis. Verification of these conceptual models hypothesizing the OAS actions awaits further investigation.

III. The *cis*-Acting Element of Sulfur Response

Cumulative information from the molecular biological works on plant sulfur response provided us with a notion that numbers of sulfur-responsive genes are coordinately regulated for metabolisms and probably for stress mitigation. A basic question arose here whether all these genes are regulated by sulfur under the same mechanisms? Transcriptional regulation of yeast *MET* genes is a well characterized example of sulfur response (Thomas and Surdin-Kerjan, 1997). *MET* genes that encode the enzymes for assimilatory sulfur metabolism have common *cis*-acting elements within their 5′-regions that are capable of binding the components of transcription factors regulated by availability of *S*-adenosylmethionine. Unlike this highly organized regulatory system in yeast, higher plants must have developed more complicated mechanisms, as has been anticipated from several modes of metabolic and plant hormone-mediated regulation discussed further in this article.

For the plant sulfur response, a sulfur-responsive *cis*-acting element, SURE, is reported from *Arabidopsis*. SURE is a 7-bp sulfur-responsive element identified in the 5′-region of *Arabidopsis SULTR1;1* sulfate transporter gene (Maruyama-Nakashita et al., 2005). Measurements of reporter activities of a series of promoter deletion constructs in transgenic *Arabidopsis* plants indicated that the sulfur-responsive region can be delimited to a specific GGAGACA sequence between the -2773 and -2767 region of *SULTR1;1*. Under this element, the reporter activity was induced by sulfur limitation or by OAS treatment, but was repressed by cysteine and glutathione. The SURE sequence contained the core sequence of the auxin response factor (ARF) binding site (Ulmasov et al., 1999; Hagen and Guilfoyle, 2002). *SULTR1;1* is slightly induced by auxin treatment, but SURE itself exhibited no response to auxin. These observations suggest that transcriptional activation of *SULTR1;1* in response to sulfur deficiency could involve a transcription factor which may structurally resemble to ARF in DNA-binding mechanisms; however the specific SURE-binding candidate has not yet been identified. The data of microarray analysis of a time course series of sulfate deprivation response indicated that SUREs are present in numbers of sulfur-responsive genes that are co-activated with *SULTR1;1* (Maruyama-Nakashita et al., 2005). Similar sequences were found in the sulfur-responsive 5′-regions of NIT3 nitrilase (Kutz et al., 2002) and β-conglycinin β-subunit (Awazuhara et al., 2002), suggesting generality of SURE-mediated regulation in plant sulfur response. However, interestingly, *SULTR1;2* showed no consensus of SURE sequences within its 5′-region. Although it is induced by sulfur limitation and its significance in facilitating sulfate uptake is remarkable (Shibagaki et al., 2002; Yoshimoto et al., 2002; Maruyama-Nakashita et al., 2003), this sulfate transporter is suggested to be regulated in a different manner independent of the SURE-mediated regulation.

IV. Regulation by Nitrogen and Carbon

Sulfate is metabolized to a sulfur-containing amino acid, cysteine, using sulfide and OAS. Reduction of sulfate primarily occurs in the presence

of ATP and reducing cofactors that can be provided by photosynthesis or from the pentose phosphate cycle (Leustek et al., 2000; Saito, 2004). OAS, the other substrate for cysteine synthesis, originates from serine. In chloroplasts, OAS is synthesized from 3-phosphoglycerate through the enzymes catalyzing dephosphorylation and transamination reactions (Ho and Saito, 2001). This indicates supply of carbon skeletons of cysteine is tightly linked with carbon and nitrogen metabolisms. Reminiscent of these metabolic linkages, transcripts for sulfate transporters and adenosine 5′-phophosulfate reductases, the key steps facilitating the sulfate assimilatory pathway, were both regulated by supply of nitrogen and carbon (Kopriva et al., 1999; Vidmar et al., 1999; Koprivova et al., 2000; Hesse et al., 2003; Wang et al., 2003; Maruyama-Nakashita et al., 2004b). In the case of high-affinity sulfate transporters, the sulfur-deficiency response of *SULTR1;1* and *SULTR1;2* was strongly attenuated in *Arabidopsis* roots by depletion of nitrogen and carbon sources from the medium (Maruyama-Nakashita et al., 2004b). In barley roots, sulfate uptake activities were transiently induced by replenishment of nitrate or ammonium to nitrogen-starved plants, which correlated with the increase of *HVST1* transcripts (Vidmar et al., 1999). In addition to sulfate transporters, various nutrient transporters facilitating the uptake of essential elements in *Arabidopsis* roots were positively and coordinately regulated during the day time and by supply of carbon source (Lejay et al., 2003). Metabolites of glycolytic pathway are suggested to be involved in this general regulation, although the molecular mechanisms are unknown.

V. Plant Hormone Signals

A. Cytokinin in Plant Sulfur Response

In addition to metabolic regulation, recent studies suggested that sulfur assimilation may involve plant hormone signals for its own regulation. The most evident case indicating a linkage between the component of signal perception and downstream gene expression is the cytokinin-dependent signaling cascade that participates in negative regulation of sulfate uptake in *Arabidopsis* roots (Maruyama-Nakashita et al., 2004c). This regulatory pathway was initially found by screening of plant hormones that affect the expression of *SULTR1;2* promoter-GFP. Among the plant hormones tested, cytokinin treatment specifically repressed the accumulation of GFP under sulfur-limited conditions. Commensurate with repression of the signals of GFP reporter, *SULTR1;1* and *SULTR1;2* mRNAs were coordinately down-regulated by the addition of cytokinin. This regulatory pathway involved a two-component phospho-relay of a cytokinin receptor histidine kinase, CRE1/WOL/AHK4 (Inoue et al., 2001; Kakimoto, 2003). Accordingly, the *Arabidopsis cre1–1* mutant was unable to regulate the high-affinity sulfate transporters in response to cytokinin (Maruyama-Nakashita et al., 2004c). Currently, we consider that cytokinin and sulfur may work independently for the control of sulfate uptake. When sulfate is limiting, sulfur-specific signals activate the expression of high-affinity sulfate transporters for the acquisition of sulfur source. By contrast, cytokinin provides a negative signal attenuating the influx of sulfate, similar to the case in phosphate uptake that also involves CRE1 cytokinin receptor for its regulation (Martin et al., 2000; Franco-Zorrilla et al., 2002). It is reported that a reporter gene fusion construct of a sulfur limitation-responsive promoter of β-conglycinin β-subunit responds positively to cytokinin in parallel with the induction of adenosine 5′-phophosulfate reductase (Ohkama et al., 2002), which is opposite to the response of sulfate transporters (Maruyama-Nakashita et al., 2004c). Under cytokinin treatment, repression of *SULTR1;1* and *SULTR1;2* sulfate transporters may lead to a substantial decline of internal sulfur status, consequently stimulating the expression of sulfur-responsive genes. Alternatively, the regulatory mechanisms of cytokinin response may differ between these two groups that apparently exhibit similar responses to sulfur limitation.

B. Interaction of Auxin and Glucosinolate Biosynthesis

Plants alter their root architectures during nutrient deficient conditions. The mechanisms and the sites of root growth differ among the nutrients absorbed by plant roots; sulfur deficiency generally stimulates the growth of lateral roots both in length and numbers (Hell and Hillebrand, 2001).

In *Arabidopsis*, degradation of indole glucosinolates is suggested to be involved in this growth regulation (Kutz et al., 2002). Under sulfur deficiency, thioglucosidase releases the aglycon from indole glucosinolates, and indole acetonitrile formed spontaneously after this reaction will be catalyzed by nitrilase, generating indole acetic acid (IAA) as a product that may stimulate the root growth. The transcript of *NIT3* nitrilase was inducible by sulfur deprivation in *Arabidopsis* roots, providing a mechanism for root growth under sulfur deficiency (Kutz et al., 2002). In addition to these findings, microarray studies indicated that some auxin-responsive genes were positively regulated in sulfate-starved plants (Hirai et al., 2003; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003). However, direct evidence for increase of IAA content has not been shown for sulfate-starved plants, suggesting IAA derived from degradation of glucosinolates might locally affect the growth of lateral roots.

C. Jasmonic Acid Signaling

Jasmonic acid (JA) is a plant hormone synthesized by oxidation of linolenic acid constituting the membrane lipids. Several lines of evidence suggest significance of JA-signaling in mitigation of oxidative stresses and synthesis of defense chemicals, both being related with sulfur metabolism. Shortage of sulfur supply leads to a significant decrease of cellular glutathione contents, and therefore may cause oxidative stresses. Corollary of this, the expression of genes for JA synthesis was induced by sulfur limitation or by knockout of *SULTR1;2* sulfate transporter (Hirai et al., 2003; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003). Under sulfur deficiency, synthesis of glucosinolates can be down-regulated for recycling of sulfur, and induction of JA synthesis may occur in parallel. When provided with ample supply of sulfur, JA treatment caused induction of metabolic genes for glutathione synthesis (Xiang and Oliver, 1998). Recent microarray studies additionally indicate that both reduction of sulfate to thiols and synthesis of glucosinolates are induced by JA treatment (Jost et al., 2005; Sasaki-Sekimoto et al., 2005). These findings suggest that JA may act positively both for active synthesis of antioxidants and glucosinolate production, which appears to be contradictory to the case in sulfur-starved plants. Under sulfur deficiency, nutritional demand of sulfur recycling may presumably override the JA-derived signals that are required for the synthesis of glucosinolates acting as defense chemicals against pathogenic attack. In fact, a JA-deficient mutant lacking allene oxide synthase gene showed normal sulfur-limitation responses, as represented by induction of sulfate transporters and adenosine 5′-phophosulfate reductase, and repression of genes for glucosinolate synthesis under sulfur-deficient conditions (Yano et al., 2005). This supports the hypothesis suggesting divergence of sulfur limitation signals from the JA-mediated regulatory cascades. Findings of regulatory complexes of JA with sulfur metabolisms and antioxidant recycling systems suggest significance of sulfur fertilization on stressed environments that plants may encounter in nature.

VI. Prospects of Transcriptome and Metabolome Analyses for Novel Gene Findings

A. Co-Regulated Genes

In the functional genomics era, the use of microarrays has become a powerful tool to characterize the changes of transcriptomes at given conditions or genetic varieties. Most recently, the use of Affymetrix GeneChip arrays provided us a holistic view of transcript profiles that covers more than 70% of the coding genes in the *Arabidopsis* genome. Studies using this pre-manufactured array or other microarrays with equivalent qualities provided us unprecedented images of plant sulfur response that occurs on transcript regulation (Hirai et al., 2003, 2004, 2005; Maruyama-Nakashita et al., 2003, 2005; Nikiforova et al., 2003). The whole dataset of transcriptome appears to be a promiscuous mass, but with an appropriate experimental design and proper handling of data clusters, typical features of sulfur response can arise extractable.

In general, sulfur-deficiency responsive genes are co-regulated with high-affinity sulfate transporters in *Arabidopsis* roots (Maruyama-Nakashita et al., 2005, 2006). Appearance of their transcripts on a time course of sulfate deprivation allowed us to identify the presence of *cis*-acting

element, SURE, within their promoter regions, although some exceptions such as *SULTR1;2* and *SULTR2;1* were present in the same category. More recently, genetic analysis of sulfur limitation response-less *Arabidopsis* mutants identified a key transcription factor, SLIM1, which is necessary for the coordinate expression of genes for sulfur assimilation and secondary sulfur metabolisms (Maruyama-Nakashita et al., 2006). Transcriptome analysis of *slim1* mutant revealed that many of the sulfur metabolic pathways are regulated by SLIM1, suggesting its hub-like function within the regulatory cascade (Maruyama-Nakashita et al., 2006) (Fig. 1).

In addition to direct or specific effects of sulfate or related sulfur metabolites on transcript regulation of sulfate transport and metabolism, the array data indicated that supply of nitrogen, carbon and plant hormones may additionally influence sulfur metabolism (Wang et al., 2003; Hirai et al., 2004; Jost et al., 2005; Sasaki-Sekimoto et al., 2005). The effects of nitrogen and carbon on the array of sulfur-responsive genes are suggested to be the global response (Hirai et al., 2004). Under such conditions, sulfur-responsive genes may fluctuate with the entire metabolic systems, as represented by the changes of energy metabolism and/or photosynthesis, rather than with a specific cue of sulfur demand that directly controls the uptake of sulfate and assimilatory metabolism.

B. Approaches from Metabolomics

As for the metabolome analysis, comprehensiveness is rather obscure as compared with the transcriptome data, and the numbers of annotated metabolites are still limited (Hirai et al., 2004, 2005; Nikiforova et al., 2005). However, challenges to the untargeted metabolite analysis have made substantial progresses in determining the functions of metabolic enzymes and genetic diversities of glucosinolate synthesis in *Arabidopsis*. Data processing thorough a batch-learning self-organized map analysis, genes and metabolites of glucosinolate synthesis were co-clustered within the same group or in its vicinities, forming gene-to-gene or metabolite-to-metabolite networks (Hirai et al., 2004, 2005). This method has been shown to be useful for novel gene finding that eventually assisted functional identification of desulfoglucosinolate sulfotransferase (Hirai et al., 2005). Genetic diversities of glucosinolate compositions have been well characterized in *Arabidopsis*, as have been exemplified by polymorphisms of key metabolic enzymes between several different ecotypes (Halkier and Gerschenzon, 2006). More

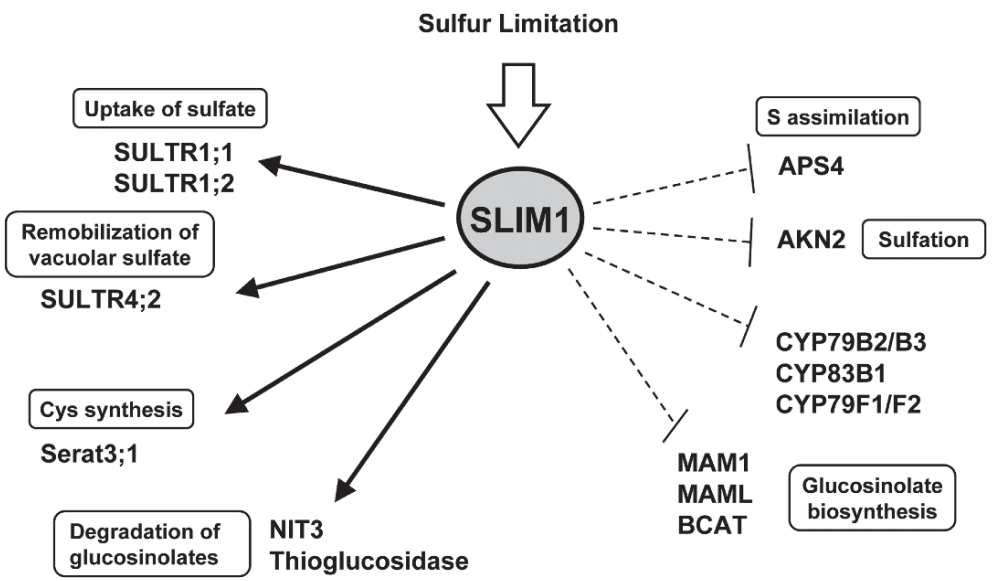

Fig. 1. SLIM1-mediate regulation of sulfate transport and metabolism.
AKN2, 5′adenylylsulfate kinase; APS4, ATP sulfurylase; BCAT, branched-chain amino acid aminotransferase; CYP, cytochrome P450; MAM, methyl(thio)alkylmalate synthase; NIT3, nirilase; Serat, serine acetyltransferase; SULTR, sulfate transporter.

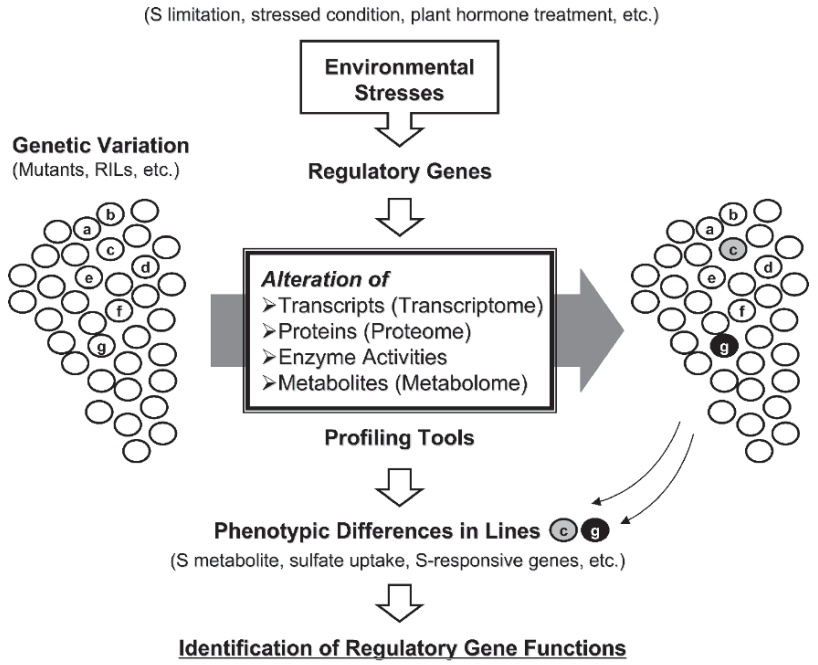

Fig. 2. Omics-based profiling of a large-scale genetic variation for functional identification of regulatory genes in plant metabolisms.

recently, quantitative trait loci representing these known differences of glucosinolate compositions among the *Arabidopsis* ecotypes were resolved by liquid chromatography mass spectrometry-based metabolic profiling of recombinant inbred line populations, suggesting a potential of this untargeted approach for gene finding (Keurentjes et al., 2006).

Approaches from the untargeted metabolomics were successful for identification of the enzymes for synthesis of secondary metabolites through quantitative detection of the accumulating final products. However, these would not be the case with nutrient assimilation and primary metabolisms where the metabolites can easily be distributed or metabolized in reversible pathway networks. Redundancies of the enzymes may also hamper identification of their specific functionalities. In addition, unlike the metabolic enzymes that may have direct impacts on modulating the contents of metabolites, the functions of regulatory genes are suggested to be rather conditional. In other words, positive or negative effects of regulatory genes on fluctuation of tissue metabolite levels might only be detectable during certain stressed conditions where these metabolites are necessarily accumulated. The untargeted or -omics-based approaches are still under way for identification of regulatory gene functions (Fig. 2). The use of genetic variations, comparative analysis of multiple environmental setups, throughputness of metabolite measurements or gene expression analyses, and statistics for interpretation of holistic data outputs, may become the prerequisites for findings of novel regulatory genes modulating the profiles of plant metabolic systems.

References

Awazuhara M, Fujiwara T, Hayashi H, Watanabe-Takahashi A, Takahashi H and Saito K (2005) The function of SULTR2;1 sulfate transporter during seed development in *Arabidopsis thaliana*. Physiol Plant 125: 95–105

Awazuhara M, Kim H, Goto DB, Matsu A, Hayashi H, Chino M, Kim S-G, Naito S and Fujiwara T (2002) A 235-bp region from a nutritionally regulated soybean seed-specific gene promoter can confer its sulfur and nitrogen response to a constitutive promoter in aerial tissues of *Arabidopsis thaliana*. Plant Sci 163: 75–82

Bourgis F, Roje S, Nuccio ML, Fisher DB., Tarczynski MC, Li C, Herschbach C, Rennenberg H, Pimenta MJ, Shen TL, Gage DA and Hanson AD (1999) *S*-methylmethio-

nine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. Plant Cell 11: 1485–1498

Buchner P, Stuiver CE, Westerman S, Wirtz M, Hell R, Hawkesford MJ and De Kok LJ (2004a) Regulation of sulfate uptake and expression of sulfate transporter genes in *Brassica oleracea* as affected by atmospheric H_2S and pedospheric sulfate nutrition. Plant Physiol 136: 3396–3408

Buchner P, Takahashi H and Hawkesford MJ (2004b) Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. J Exp Bot 55: 1765–1773

Clarkson DT, Smith FW and Vanden Berg PJ (1983) Regulation of sulfate transport in a tropical legume, *Macroptilium atropurpureum* cv. Siratro. J Exp Bot 34: 1463–1483

Deane-Drummond CE (1987) The regulation of sulfate uptake following growth of *Pisum sativum* L. seedlings in S nutrient limiting conditions. Interaction between nitrate and sulphate transport. Plant Sci 50: 27–35

Franco-Zorrilla JM, Martin AC, Solano R, Rubio V, Leyva A and Paz-Ares J (2002) Mutations at *CRE1* impair cytokinin-induced repression of phosphate starvation responses in *Arabidopsis*. Plant J 32: 353–360

Hagen G and Guilfoyle TJ (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49: 373–385

Halkier BA and Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57: 303–333

Hell R and Hillebrand H (2001) Plant concepts for mineral acquisition and allocation. Curr Opin Biotechnol 12: 161–168

Herschbach C, van Der Zalm E, Schneider A, Jouanin L, De Kok LJ and Rennenberg H (2000) Regulation of sulfur nutrition in wild-type and transgenic poplar over-expressing γ-glutamylcysteine synthetase in the cytosol as affected by atmospheric H_2S. Plant Physiol 124: 461–473

Hesse H, Trachsel N, Suter M, Kopriva S, von Ballmoos P, Rennenberg H and Brunold C (2003) Effect of glucose on assimilatory sulphate reduction in *Arabidopsis thaliana* roots. J Exp Bot 54: 1701–1709

Hirai MY, Fujiwara T, Awazuhara M, Kimura T, Noji M and Saito K (2003) Global expression profiling of sulfur-starved Arabidopsis by DNA macroarray reveals the role of O-acetyl-L-serine as a general regulator of gene expression in response to sulfur nutrition. Plant J 33: 651–663

Hirai MY, Klein M, Fujikawa Y, Yano M, Goodenowe DB,. Yamazaki Y, Kanaya S, Nakamura Y, Kitayama M, Suzuki H, Sakurai N, Shibata D, Tokuhisa J, Reichelt M, Gershenzon J, Papenbrock J and Saito K (2005) Elucidation of gene-to-gene and metabolite-to-gene networks in arabidopsis by integration of metabolomics and transcriptomics. J Biol Chem 280: 25590–25595

Hirai MY, Yano M, Goodenowe DB, Kanaya S, Kimura T, Awazuhara M, Arita M, Fujiwara T and Saito K (2004) Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 101: 10205–10210

Hopkins L, Parmar S, Blaszczyk A, Hesse H, Hoefgen R and Hawkesford MJ (2005) O-acetylserine and the regulation of expression of genes encoding components for sulfate uptake and assimilation in potato. Plant Physiol 138: 433–440

Ho C-L and Saito K (2001) Molecular biology of the plastidic phosphorylated serine biosynthetic pathway in *Arabidopsis thaliana*. Amino Acids 20: 243–259

Howarth JR, Fourcroy P, Davidian J-C, Smith FW and Hawkesford MJ (2003) Cloning of two contrasting high-affinity sulphate transporters from tomato induced by low sulphate and infection by the vascular pathogen *Verticillium dahlia*. Planta 218: 58–64

Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K and Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. Nature 409: 1060–1063

Jost R, Altschmied L, Bloem E, Bogs J, Gershenzon J, Hahnel U, Hansch R, Hartmann T, Kopriva S, Kruse C, Mendel RR, Papenbrock J, Reichelt M, Rennenberg H, Schnug E, Schmidt A, Textor S, Tokuhisa J, Wachter A, Wirtz M, Rausch T and Hell R (2005) Expression profiling of metabolic genes in response to methyl jasmonate reveals regulation of genes of primary and secondary sulfur-related pathways in *Arabidopsis thaliana*. Photosynth Res 86: 491–508

Kakimoto T (2003) Perception and signal transduction of cytokinins. Annu Rev Plant Biol 54: 605–627

Kataoka T, Hayashi N, Yamaya T and Takahashi H (2004a) Root-to-shoot transport of sulfate in Arabidopsis: evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. Plant Physiol 136: 4198–4204

Kataoka T, Watanabe-Takahashi A, Hayashi N, Ohnishi M, Mimura T, Buchner P, Hawkesford MJ, Yamaya T and Takahashi H (2004b) Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in Arabidopsis. Plant Cell 16: 2693–2704

Keurentjes JJ, Fu J, de Vos CH, Lommen A, Hall RD, Bino RJ, van der Plas LH, Jansen RC, Vreugdenhi D and Koornneef M (2006) The genetics of plant metabolism. Nat Genet 38: 842–849

Kopriva S, Muheim R, Koprivova A, Trachsel N, Catalano C, Suter M and Brunold C (1999). Light regulation of assimilatory sulphate reduction in *Arabidopsis thaliana*. Plant J 20: 37–44

Koprivova A, Suter M, Op den Camp R, Brunold C and Kopriva S (2000) Regulation of sulfur assimilation by nitrogen in Arabidopsis. Plant Physiol 122: 737–746

Kutz A, Muller A, Hennig P, Kaiser WM, Piotrowski M, Weiler EW (2002) A role for nitrilase 3 in the regulation of root morphology in sulphur-starving *Arabidopsis thaliana*. Plant J 30: 95–106

Lejay L, Gansel X, Cerezo M, Tillard P, Müller C, Krapp A, von Wirén N, Daniel-Vedele F and Gojon A (2003) Regulation of root ion transporters by photosynthesis: functional importance and relation with hexokinase. Plant Cell 15: 2218–2232

Lappartient AG, Vidmar JJ, Leustek T, Glass AD and Touraine B (1999) Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound. Plant J 18: 89–95

Leustek T, Martin MN, Bick JA and Davies JP (2000) Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. Annu Rev Plant Physiol Plant Mol Biol 51: 141–165

Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de la Pena A, Leyva A and Paz-Ares J (2000) Influence of cytokinins on the expression of phosphate starvation responsive genes in *Arabidopsis*. Plant J 24: 559–567

Maruyama-Nakashita A, Inoue E, Watanabe-Takahashi A, Yamaya T and Takahashi H (2003) Transcriptome profiling of sulfur-responsive genes in Arabidopsis reveals global effect on sulfur nutrition on multiple metabolic pathways. Plant Physiol 132: 597–605

Maruyama-Nakashita A, Nakamura Y, Tohge T, Saito K and Takahashi H (2006) *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism. Plant Cell 18: 3235–3251

Maruyama-Nakashita A, Nakamura Y, Watanabe-Takahashi A, Inoue E, Yamaya T and Takahashi H (2005) Identification of a novel *cis*-acting element conferring sulfur deficiency response in *Arabidopsis* roots. Plant J 42: 305–314

Maruyama-Nakashita A, Nakamura Y, Watanabe-Takahashi A, Yamaya T and Takahashi H (2004a) Induction of SULTR1;1 sulfate transporter in Arabidopsis roots involves protein phosphorylation/dephosphorylation circuit for transcriptional regulation. Plant Cell Physiol 45: 340–345

Maruyama-Nakashita A, Nakamura Y, Yamaya T and Takahashi H (2004b) Regulation of high-affinity sulphate transporters in plants: towards systematic analysis of sulphur signalling and regulation. J Exp Bot 55: 1843–1849

Maruyama-Nakashita A, Nakamura Y, Yamaya T and Takahashi H (2004c) A novel regulatory pathway of sulfate uptake in Arabidopsis roots: Implication of CRE1/WOL/AHK4-mediated cytokinin-dependent regulation. Plant J 38: 779–789

Nikiforova V, Freitag J, Kempa S, Adamik M, Hesse H and Hoefgen R (2003) Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. Plant J 33: 633–650

Nikiforova VJ, Kopka J, Tolstikov V, Fiehn O, Hopkins L, Hawkesford MJ, Hesse H and Hoefgen R (2005) Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of Arabidopsis plants. Plant Physiol 138: 304–318

Ohkama N, Takei K, Sakakibara H, Hayashi H, Yoneyama T and Fujiwara T (2002) Regulation of sulfur-responsive gene expression by exogenously applied cytokinins in *Arabidopsis thaliana*. Plant Cell Physiol 43: 1493–1501

Ohkama-Ohtsu N, Kasajima I, Fujiwara T and Naito S (2004) Isolation and characterization of an Arabidopsis mutant that overaccumulates O-acetyl-L-Ser. Plant Physiol 136: 3209–3222

Saito K (2004) Sulfur assimilatory metabolism. The long and smelling road. Plant Physiol 136: 2443–2450

Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakura N, Suzuki H, Hirai MY, Noji M, Saito K, Masuda T, Takamiya K, Shibata D and Ohta H (2005) Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. Plant J 44: 653–668

Shibagaki N, Rose A, Mcdermott JP, Fujiwara T, Hayashi H, Yoneyama T and Davies JP (2002) Selenate-resistant mutants of *Arabidopsis thaliana* identify *Sultr1;2*, a sulfate transporter required for efficient transport of sulfate into roots. Plant J 29: 475–486

Smith FW, Ealing PM, Hawkesford MJ and Clarkson DT (1995) Plant members of a family of sulfate transporters reveal functional subtypes. Proc Natl Acad Sci USA 92: 9373–9377

Smith FW, Hawkesford MJ, Ealing PM, Clarkson DT, Vanden Berg PJ, Belcher AR and Warrilow AGS (1997) Regulation of expression of a cDNA from barley roots encoding a high affinity sulfate transporter. Plant J 12: 875–884

Takahashi H, Watanabe-Takahashi A, Smith FW, Blake-Kalff M, Hawkesford MJ and Saito K (2000) The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. Plant J 23: 171–182

Takahashi H, Yamazaki M, Sasakura N, Watanabe A, Leustek T, de Almeida Engler J, Engler G, Van Montagu M and Saito K (1997) Regulation of sulfur assimilation in higher plants: a sulfate transporter induced in sulfate starved roots plays a central role in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 94: 11102–11107

Takahashi H, Yoshimoto N and Saito K (2006) Anionic nutrient transport in plants: the molecular bases of sulfate transporter gene family. In: Setlow JK (ed) Genetic Engineering, Principles and Methods, Vol 27, pp 67–80. Springer, New York

Thomas D and Surdin-Kerjan Y (1997) Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 61: 503–532

Ulmasov T, Hagen G and Guilfoyle TJ (1999) Dimerization and DNA binding of auxin response factors. Plant J 19: 309–319

Vauclare P, Kopriva S, Fell D, Suter M, Sticher L, von Ballmoos P, Krahenbuhl U, den Camp RO and Brunold C (2002) Flux control of sulphate assimilation in *Arabidopsis thaliana*: adenosine 5′-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols. Plant J 31: 729–740

Vidmar JJ, Schjoerring JK, Touraine B and Glass ADM (1999) Regulation of the *hvst1* gene encoding a high-affinity sulfate transporter from *Hordeum vulgare*. Plant Mol Biol 40: 883–892

Vidmar JJ, Tagmount A, Cathala N, Touraine B and Davidian J-CE (2000) Cloning and characterization of a root specific high-affinity sulfate transporter from *Arabidopsis thaliana*. FEBS Lett 475: 65–69

Wang R, Okamoto M, Xing X and Crawford NM (2003) Microarray analysis of the nitrate response in Arabidopsis roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. Plant Physiol 132: 556–567

Wirtz M, Droux M and Hell R (2004) *O*-acetylserine (thiol) lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana*. J Exp Bot 55: 1785–1798

Xiang C and Oliver DJ (1998) Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis. Plant Cell 10: 1539–1550

Yano M, Hirai MY, Kusano M, Kitayama M, Kanaya S and Saito K (2005) Studies for elucidation of jasmonate signaling in the response of Arabidopsis to sulfur deficiency. In: Saito K, De Kok LJ, Stulen I, Hawkesford MJ, Schnug E, Sirko A, Rennenberg H (eds) Sulfur Transport and Assimilation in Plants in the Postgenomic Era, pp 165–168. Backhuys Publishers, Leiden, The Netherlands

Yoshimoto N, Inoue E, Saito K, Yamaya T and Takahashi H (2003) Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in Arabidopsis. Plant Physiol 131: 1511–1517

Yoshimoto N, Takahashi H, Smith FW, Yamaya T and Saito K (2002) Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in *Arabidopsis* roots. Plant J 29: 465–473