Sulfur Assimilatory Metabolism. The Long and Smelling Road[1]

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Sulfur represents the ninth and least abundant essential macronutrient in plants, preceded by carbon, oxygen, hydrogen, nitrogen, potassium, calcium, magnesium, and phosphorus. The dry matter of sulfur in plants is only about one-fifteenth of that of nitrogen. Both sulfur and nitrogen are necessary to be assimilated into organic metabolites. While nitrogen is mainly used for structural macromolecules, sulfur plays critical roles in the catalytic or electrochemical functions of the biomolecules in cells.

WHY IS SULFUR IMPORTANT? BECAUSE SULFUR NOT ONLY SERVES AS A STRUCTURAL COMPONENT BUT ALSO PLAYS CHARACTERISTIC FUNCTIONS IN CELLS

Sulfur is found in amino acids (Cys and Met), oligopeptides (glutathione [GSH] and phytochelatins), vitamins and cofactors (biotin, thiamine, CoA, and S-adenosyl-Met), and a variety of secondary products (glucosinolates in Cruciferae and allyl Cys sulfoxides in *Allium*; Leustek, 2002). The thiol (sulfhydryl) group of Cys in proteins takes the job of maintaining protein structure by forming disulfide bonds between two Cys residues via oxidation. The thiol of Cys and GSH is often involved in the redox cycle by two thiol ↔ disulfide conversions. This interchange is versatile for redox control and mitigation against oxidative stress in nearly all aerobic organisms including plants (Leustek and Saito, 1999).

The nucleophilicity of the thiol group, and in particular GSH, plays a role in detoxification of xenobiotics by direct conjugation with sulfhydryl group mediated by GSH S-transferase. Phytochelatins, a polymerized version of GSH, are involved in detoxification of heavy metals by serving as chelating ligands through thiol groups. Sulfur-containing secondary products often have a characteristic smell and are regarded not only as defense compounds against herbivores and pathogenic organisms but also as signaling molecules for fundamental cellular functions (for example, Matsubayashi et al., 2002).

SULFUR NUTRITION IMPACTS AGRICULTURE, FOOD QUALITY, AND NUTRA- AND PHARMACEUTICALS

Sulfur assimilation in plants plays a key role in the sulfur cycle in nature. The inorganic sulfur in the environment, sulfate ion in soil and sulfur dioxide gas in the air, is fixed into Cys by the sulfur assimilation pathway in plants (Saito, 2000). Thereafter, Cys is converted to Met. Animals, however, do not have the assimilatory mechanisms for inorganic sulfur; they require Met as an essential amino acid for their source of sulfur nutrient.

Sulfur deficiency in agricultural areas in the world has been recently observed because emissions of sulfur air pollutants in acid rain have been diminished from industrialized areas. Fertilization of sulfur is required in these areas; otherwise, low crop quality and producibility are seen. In seed proteins, the levels of the sulfur-containing amino acids, Cys and Met, are low from the nutritional point of view for animals. Enhancing Met levels via genetic engineering has been shown to increase the nutritive value of seed crops for animals (Molvig et al., 1997). Recently, increased attention has been paid to the nutra- and pharmaceutical value of sulfur-containing plant products. Epidemiologic and experimental studies have suggested that sulfur-containing phytochemicals from cruciferous plants such as isothiocyanates may prevent cancer (Talalay and Fahey, 2001).

SULFUR IS ASSIMILATED THROUGH MULTIPLE STEPS: AN OVERVIEW

Figure 1 is an overview of the major pathways for plant sulfur metabolism. Sulfate (SO_4^{2-}) is the most oxidative and thus stable form of sulfur present in the soil. Uptake of sulfur into roots from the soil is almost exclusively via sulfate uptake. The form of sulfur found in xylem and phloem sap is also primarily sulfate, thus translocation of sulfur throughout the plant is mostly via unmetabolized sulfate. Subsequently, sulfate is subjected to activation to adenosine 5′-phosphosulfate
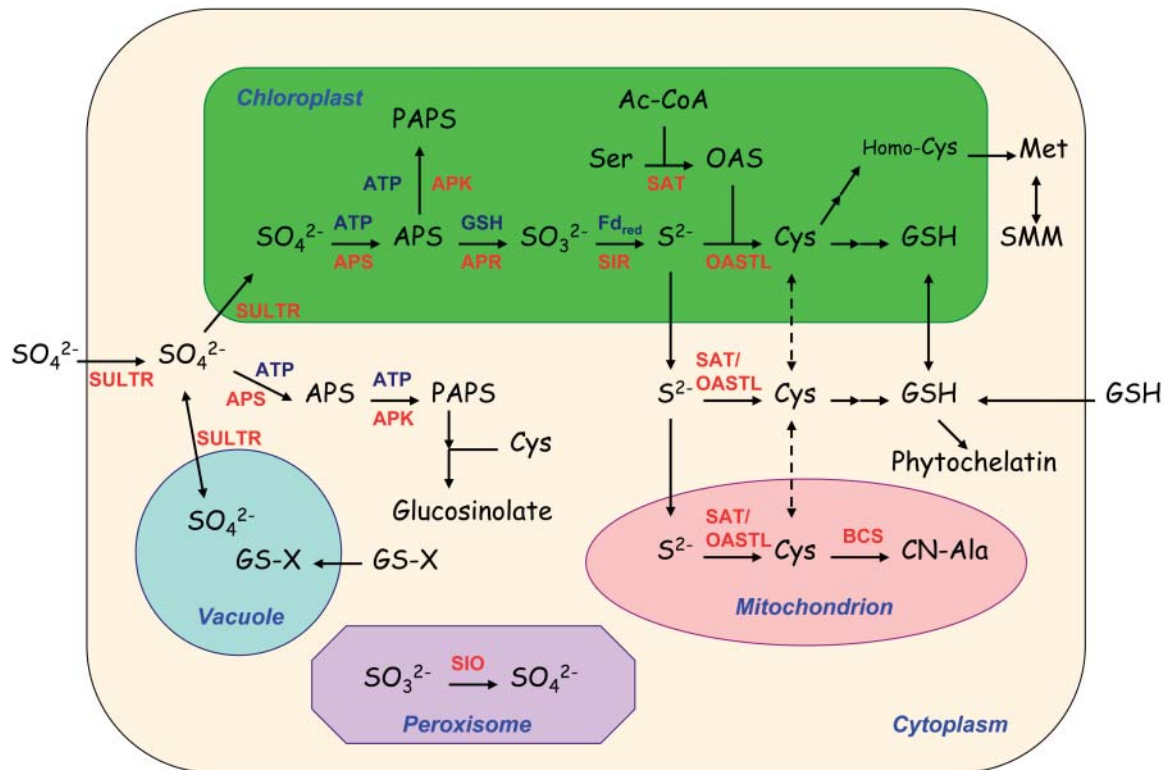
Figure 1. Sulfur assimilatory metabolism in the subcellular compartments of plant cells. Black indicates name of metabolites: Ac-CoA, acetyl-CoA; APS, adenosine 5′-phosphosulfate; CN-Ala, β-cyano-Ala; GSH, reduced glutathione; GS-X, glutathione conjugate; OAS, O-acetyl-Ser; PAPS, 3′-phosphoadenosine-5′-phosphosulfate (3′-phosphoadenylylsulfate); SMM, S-methyl-Met. Blue indicates names of cofactors: GSH, reduced glutathione; Fd$_{red}$, reduced ferredoxin. Red indicates the name of proteins: APK, APS kinase; APR, adenosine 5′-phosphosulfate reductase; APS, ATP sulfurylase; BCS, β-cyano-Ala synthase; OASTL, OAS(thiol)-lyase; SAT, Ser acetyltransferase; SIO, sulfite oxidase; SIR, sulfite reductase; SULTR, sulfate transporter.

(5′-adenylylsulfate [APS]) for further conversion. The major assimilatory pathway is reduction of APS to sulfite (SO_3^{2-}) and then sulfide (S^{2-}). The overall reduction from sulfate to sulfide requires one ATP and eight electrons. Sulfide is then coupled with O-acetyl-Ser (OAS) that is formed from Ser, yielding Cys. A relatively minor branch point in this pathway is from APS to 3′-phosphoadenosine-5′-phosphosulfate (PAPS), which is restricted for sulfation.

Cys is the central compound for production of a variety of metabolites containing reduced sulfur, such as Met, GSH, phytochelatins, and glucosinolates. Although the turnover rate of Cys is high, the cellular concentration of free Cys is maintained at a low level (approximately 20 μM), compared with high levels of GSH (approximately up to 10 mM; Leustek et al., 2000; Saito, 2000).

SULFATE UPTAKE AND TRANSLOCATION IS MEDIATED BY SPECIALIZED MEMBRANE PROTEINS

As mentioned above, sulfate is taken up into roots from the soil and is then distributed throughout the plant. Multiple transport steps through different membranes are involved: plasma membrane transporters in the root present in the outermost cell layers for initial uptake; plasma membrane transporters of vascular tissues for long-distance translocation and of leaf mesophyll cells for assimilation coupled with photosynthesis; and inside cells, transporters associated with organelle transport, in particular, plastids and vacuoles (Hawkesford, 2003).

Plasma membrane sulfate transporters are classified as proton/sulfate cotransporters that mediate active sulfate uptake driven by the transmembrane proton gradient. Thus, the uptake mediated by this transporter is pH dependent, and the proton gradient is generated by the plasma membrane proton ATPase. The sulfate transporter possesses 12 membrane-spanning domains and belongs to a large family of cation/solute cotransporters (Saito, 2000; Hawkesford, 2003).

A number of genes encoding sulfate transporters have been reported including 14 genes in Arabidopsis (Yoshimoto et al., 2002; Hawkesford, 2003). These are classified into five subfamilies, named SULTR1 to 5, according to their deduced amino acid sequences. This classification also indicates the distinct functions and catalytic properties of sulfate transporters belonging to the particular subfamily. The members in SULTR1 are high-affinity transporters. SULTR1;1 and SULTR1;2 of

Arabidopsis are inducible by sulfate depletion, localized to root epidermal cells, and thus responsible for initial uptake of sulfate from the rhizosphere (Takahashi et al., 2000; Shibagaki et al., 2002; Yoshimoto et al., 2002). A high-affinity transporter, SULTR1;3, is localized to the phloem and mediates long-distance translocation from source organs (root) to sinks (leaves and shoots; Yoshimoto et al., 2003). Low-affinity transporters belonging to the SULTR2 and SULTR3 subfamilies are localized to vascular tissues and are presumably involved in the uptake of sulfate from plant apoplasts into vascular cells. The transporters of SULTR4 subfamily were initially thought to be involved in plastid uptake; however, these are responsible for efflux of sulfate from the vacuole to the cytoplasm (Kataoka et al., 2004). Although some of transporter genes may be redundant in function, a complex array of transporters differing with regard to localization, transport kinetics, and inducible expression contribute to facilitate effective translocation of sulfate in the intact plant and specific cell compartments.

Besides proton/sulfate cotransporters, anion channels, ABC proteins, and oxaloacetate/sulfate transporters may mediate sulfate transport in plant cells (Leustek, 2002). Molecular identification of these additional transport systems still remains an open question.

SULFATE NEEDS TO BE ACTIVATED FOR ASSIMILATION

For assimilation, sulfate must be activated to APS, in which sulfate is linked by an anhydride bond to a phosphate residue by consumption of ATP and concomitant release of pyrophosphate. This reaction is catalyzed by ATP sulfurylase and is the sole entry step for the metabolism of sulfate. Subsequently, APS is further reduced by APS reductase (see later) or phosphorylated by APS kinase. APS kinase catalyzes the formation of PAPS. PAPS serves as a source of activated sulfate for sulfotransferases that catalyzes sulfation of a variety of compounds such as flavonoids, glucosinolates, and jasmonates. Since the reaction equilibrium of ATP sulfurylase favors the reverse direction, i.e. the formation of ATP and sulfate, the products of the forward reaction, i.e. APS and pyrophosphate, must be further metabolized immediately by the enzymes APS reductase, APS kinase, and pyrophosphatase.

ATP sulfurylase activity is found in chloroplasts and the cytosol. However, all four ATP sulfurylase genes in Arabidopsis likely encode plastidic forms. The cytosolic isoform is presumably produced from one of the four genes by using a different translational start codon (Hatzfeld et al., 2000a). Since further reduction of APS to sulfide takes place exclusively in plastids, the role of cytosolic ATP sulfurylase is not clear. APS formed in the cytosol may not be directly involved in assimilation but may participate in sulfation after being converted to PAPS (Rotte and Leustek, 2000).

UNIQUE MECHANISMS OPERATE FOR REDUCTION OF ACTIVATED SULFATE

The sulfate residue of APS is reduced to sulfite by APS reductase that has been previously referred to as APS sulfotransferase (Suter et al., 2000). APS reductase is a unique enzyme not found in enterobacteria, where APS needs to be further activated to PAPS for reduction to sulfite. APS reductase possesses a transit peptide that allows translocation of the mature protein to plastids. In vivo APS reductase is present as a homodimer most probably linked by a disulfide bond of the conserved Cys residue (Kopriva and Koprivova, 2004). The mature APS reductase consists of two distinct domains. The N-terminal domain resembles PAPS reductase, while the C-terminal domain exhibits homology to thioredoxin and acts as a glutaredoxin using reduced GSH as the electron donor (Bick et al., 1998).

APS reductase catalyzes a thiol-dependent two-electron reduction of APS to sulfite. The enzyme bound S-sulfo intermediate is presumed to be involved in the reduction of APS (Weber et al., 2000). A reduced Cys residue in APS reductase reacts with APS to form an enzyme-(Cys)-S-SO_3^- intermediate with the concomitant release of AMP, and then this intermediate is reduced with GSH to liberate SO_3^{2-} and the enzyme-(Cys)-S-SG. The active enzyme is regenerated by reduction of the enzyme-(Cys)-S-SG with a second molecule of GSH. In some lower plants such as the moss Physcomitrella patens, in addition to an APS reductase-dependent pathway, PAPS can be reduced to sulfite by PAPS reductase as in bacteria (Kopriva and Koprivova, 2004).

SULFITE IS REDUCED TO SULFIDE BY FERREDOXIN

Sulfite reductase catalyzes the transfer of six electrons from ferredoxin to sulfite to produce sulfide, S^{2-}. The sulfite reductase found in plant cells consists of a homooligomer containing a siroheme and an iron-sulfur cluster per subunit. Sulfite reductase is localized in plastids of both photosynthetic and nonphotosynthetic tissues. Electrons are supplied to ferredoxin from PSI in photosynthetic cells and from NADPH in nonphotosynthetic cells. The proper combination of different isoforms of ferredoxin, ferredoxin-NADP$^+$ reductase, and sulfite reductase is critical for efficient sulfite reduction (Yonekura-Sakakibara et al., 2000).

Sulfite is directly utilized as the sulfur donor for the formation of UDP-sulfoquinovose (6-deoxy-6-sulfo-Glc) from UDP-Glc (Sanda et al., 2001). UDP-sulfoquinovose is the precursor of the sulfolipid, sulfoquinovosyl diacylglycerol, present in photosynthetic membranes representing one of the few naturally occurring sulfonic acid (R-CH_2-SO_3^-) derivatives. A molybdenum enzyme, sulfite oxidase, catalyzes oxidation of sulfite to sulfate in peroxisomes (Eilers et al., 2001). This enzyme is widely distributed in

higher plants and is likely responsible for detoxification of sulfite rather than for chloroplast-based sulfur assimilation.

SULFIDE INCORPORATION INTO Cys REQUIRES Ser

Incorporation of sulfide into the β-position of amino acids is the terminal step of sulfur assimilation, leading to the formation of Cys. Two enzymes, Ser acetyltransferase and OAS(thiol)-lyase (Cys synthase), are committed for this step. These two enzymes are found in three major compartments of plant cells (Saito, 2000, and references therein), e.g. the cytosol, chloroplast, and mitochondrion, in contrast to specific localization of the enzymes of sulfate reduction in plastids.

Ser acetyltransferase catalyzes the formation of OAS from Ser and acetyl-CoA. Since this reaction is located at the connection between nitrogen and sulfur metabolism, Ser acetyltransferase plays a regulatory function in sulfur assimilation. Five genes encoding Ser acetyltransferases are found in the Arabidopsis genome (Hell et al., 2002; Kawashima et al., 2004). Three of these isoforms seem to play major roles in OAS formation, judging from their kinetic properties under normal growth conditions (Noji et al., 1998). These three isoforms are localized in the cytosol, plastid, and mitochondrion. The activity of one isoform is regulated by allosteric feedback inhibition by L-Cys, the terminal product of this pathway, at physiological Cys concentrations (2–10 μM). Two other isoforms are insensitive to Cys, indicating the presence of isoform-specific control mechanisms by Cys (Noji et al., 1998). Two minor forms of Ser acetyltransferase in Arabidopsis are induced under stress conditions, i.e. sulfur deficiency and heavy metal stress, and thus may play a role in adaptive responses to stresses (Kawashima et al., 2004).

Cys IS THE PIVOTAL COMPOUND IN SULFUR ASSIMILATION

Cys is the pivotal sulfur-containing compound regarded as the terminal metabolite of sulfur assimilation and the starting point for production of Met, GSH, and a variety of other sulfur metabolites. OAS(thiol)-lyase, catalyzing the formation of Cys from OAS and hydrogen sulfide, is in the same three subcellular compartments as Ser acetyltransferase. The catalytic activity of OAS(thiol)-lyase requires pyridoxal phosphate as a cofactor. OAS(thiol)-lyase belongs to a large family of enzymes catalyzing the reaction of β-substitution of amino acids, including β-cyano-Ala synthase. In fact, cytosolic and plastidic activities of β-cyano-Ala synthase are ascribed to the side reactions of OAS(thiol)-lyase. However, in mitochondria, the specific β-cyano-Ala synthase distinct from OAS(thiol)-lyase is responsible for β-cyano-Ala formation (Hatzfeld et al., 2000b). Even in the Arabidopsis genome nine sequences similar to authentic OAS(thiol)-lyase are found, and thus new functions for the biosynthesis of secondary products may be revealed when these genes are further characterized. The mechanisms and genes for degradation/recycling of Cys are not yet well understood. However, cystine-lyase catalyzing the first step of cystine breakdown has been recently characterized in Arabidopsis (Jones et al., 2003).

MULTIPLE REGULATORY CIRCUITS ARE LOCATED BETWEEN Cys, OAS, AND SULFIDE

Cys formation is controlled through a multiple regulatory circuit involving Ser acetyltransferase and OAS(thiol)-lyase. OAS is not only a rate-limiting metabolite of the Cys biosynthetic pathway (Saito et al., 1994) but also a positive regulatory factor for gene expression (Leustek et al., 2000; Saito, 2000; Leustek, 2002).

A unique regulatory mechanism operates through the formation of an enzyme complex involving Ser acetyltransferase and OAS(thiol)-lyase (Fig. 2). The OAS(thiol)-lyase concentration is far in excess of Ser acetyltransferase concentration (approximately 300-fold), indicating that only a fraction of the OAS(thiol)-lyase forms a complex with Ser acetyltransferase (Droux et al., 1998). The bound form of OAS(thiol)-lyase, which showed a dramatically decreased catalytic activity, acts as a regulatory subunit to positively modulate the activity of Ser acetyltransferase in the enzyme complex. The large amount of free OAS(thiol)-lyase is responsible for the actual catalytic function of Cys formation. The stabilization of the complex is controlled inversely by OAS and sulfide. OAS accumulation stimulated by sulfur deficiency promotes dissociation of the complex to attenuate the activity of Ser acetyltransferase, resulting in reduced OAS formation. In turn, upon increased sulfur supply, accumulated sulfide promotes formation of the complex, leading to stimulated OAS formation to fulfill Cys synthesis. This system allows the coordination of OAS synthesis from Ser and sulfate reduction for the efficient production of Cys.

Furthermore, OAS formation is controlled by isoform-specific inhibition of Ser acetyltransferase activity by L-Cys, the end product of this pathway (Fig. 2). This inhibition is specific to L-Cys; D-Cys, Met, nor GSH exhibits an inhibitory activity. The importance of this feedback regulation is supported by the following observations: (1) overexpression of a feedback-insensitive Ser acetyltransferase gene results in elevated levels of Cys in bacteria and plants (Noji and Saito, 2002; Wirtz and Hell, 2003); and (2) higher levels of Cys were found in Chinese chive (*Allium tuberosum*), in which a cytosolic Ser acetyltransferase was inhibited by a higher concentration of Cys than other plants (Urano et al., 2000).
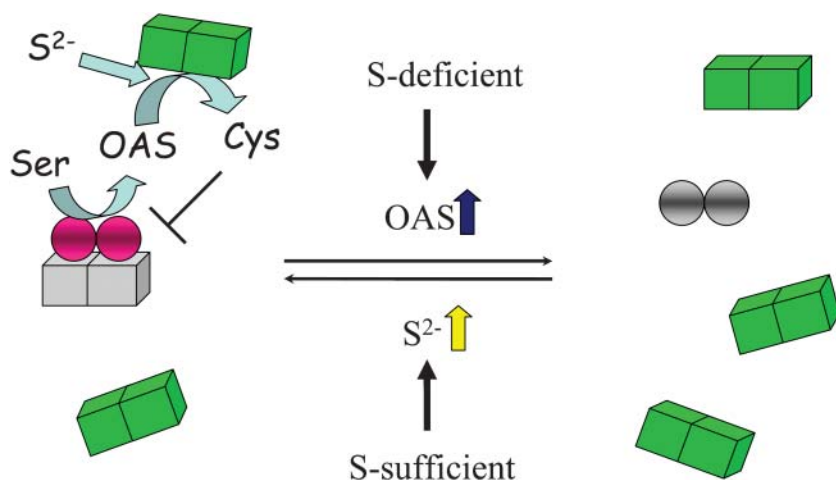
Figure 2. Regulatory circuit in the protein complex of Ser acetyltransferase (spheres) and OAS(thiol)-lyase (boxes). Concentrations of OAS(thiol)-lyase are in excess of Ser acetyltransferase. The bound OAS(thiol)-lyase positively modulates the activity of Ser acetyltransferase in the protein complex. The free form of OAS(thiol)-lyase actually catalyzes the formation of Cys that negatively acts on Ser acetyltransferase. Sulfur deficiency causes the increase of OAS eventually resulting in dissociation of the complex. The free form of Ser acetyltransferase has only limited activity. In turn, the increased sulfur supply accumulates sulfide to promote the formation of the complex.

GSH AND *S*-METHYL-Met REPRESENT MAJOR TRANSPORTED ORGANIC SULFUR-CONTAINING METABOLITES

GSH represents a major thiol-containing metabolite often present in millimolar concentrations, thus far exceeding Cys levels. Two enzymes, γ-glutamyl-Cys synthethase and GSH synthethase, are responsible for synthesis of GSH from Cys, Glu, and Gly with the consumption of two molecules of ATP. The activities of both enzymes have been found in the cytosol and in chloroplasts. Feedback inhibition of γ-glutamyl-Cys synthethase activity by GSH and the availability of Cys are the regulatory factors for GSH synthesis (Noctor et al., 2002). Transport of GSH and its derivatives is mediated by a recently characterized proton cotransporter (Zhang et al., 2004). However, uptake of GSH conjugates with xenobiotics into vacuole is mediated by ATP-energized transport via ABC proteins (Foyer et al., 2001).

S-Methyl-Met is also found to be a dominant transported form in the phloem. However, *S*-methyl-Met is not indispensable in sulfur transport, but the cyclic reaction of Met ↔ *S*-methyl-Met presumably contributes to short-term control of *S*-adenosyl-Met levels (Kocsis et al., 2003).

MULTILAYER REGULATORY MECHANISMS OPERATE IN SULFUR ASSIMILATION

It is well known that sulfate uptake and assimilation activity is induced (derepressed) under conditions of sulfur starvation or high demand for sulfur metabolites. An example of this is the enhanced phytochelatin synthesis in response to cadmium stress. This induction correlates with the inducible accumulation of steady-state mRNAs of a particular set of genes that encode sulfate transporters, notably high-affinity forms, and APS reductase, but not all genes in the sulfate assimilation pathway (Saito, 2000; Leustek, 2002, and references therein). Though precise molec-

ular mechanisms have not been identified as yet, it appears that OAS and thiol metabolites (GSH and Cys) operate oppositely with respect to induction of these genes (Fig. 3). Sulfur starvation causes an increase in OAS levels, which in turn induces the expression of genes encoding sulfate transporters and APS reductase, thereby overriding the repressive effect of sulfur-sufficient nutritional conditions (Smith et al., 1997; Koprivova et al., 2000). Addition of OAS mimics sulfur-deficiency stress, not only for sulfur-related gene expression but also for global cellular changes in terms of the transcriptome and metabolome. Similar sets of genes and metabolites are modulated by sulfur deficiency and OAS supplementation (Hirai et al., 2003, 2004). In contrast to OAS's positive effect, GSH acts as a negative regulator (repressor) of sulfur metabolism. GSH is thought to be a phloem-translocated signal molecule that represses the genes of sulfur assimilation (Lappartient et al., 1999). More generally, thiols such as Cys and GSH act negatively on gene expression (Vauclare et al., 2002). All these observations indicate that OAS and thiols are positive and negative regulators, respectively, on gene expression responding to sulfur starvation. However, it does not necessarily follow that these metabolites directly act on gene expression as shown for OAS in bacteria, but could involve multiple and complex regulatory pathways (Hartmann et al., 2004).

TRANSCRIPTOME AND METABOLOME STUDIES SHED LIGHT ON GLOBAL RESPONSES TO SULFUR NUTRITIONAL STRESS

What does control the activity of sulfur assimilation? A number of factors, such as nutrition, development, environmental factors, and stress appear to be involved in the control. Nutritional stresses of sulfur and nitrogen most notably modulate sulfur assimilation. Upon sulfur and nitrogen deficiency, sulfur is remobilized less efficiently than nitrogen, resulting in chlorosis of young leaves by sulfur deficiency, while
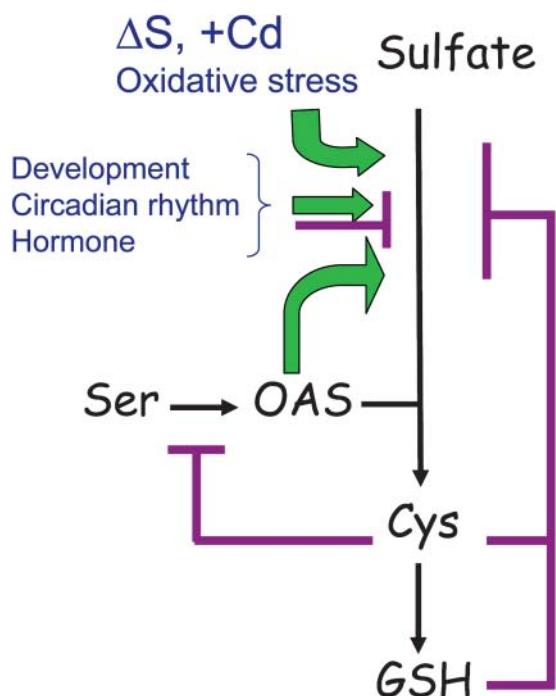
Figure 3. Positive and negative regulation of sulfur assimilatory metabolism. Sulfur starvation and increased demand of sulfur metabolites induce assimilatory metabolism. OAS also acts as a positive factor for induction. Plant development, circadian rhythms, and hormones influence sulfur metabolism either positively or negatively. As negative factors, Cys and GSH regulate specific steps of sulfur metabolism. Arrow indicates the positive effect, and bar indicates the negative effect.

the first appearance of nitrogen deficiency is in old leaves (Hawkesford, 2000).

Analyses of transcriptome (Hirai et al., 2003, 2004; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003; Wang et al., 2003) and metabolome (Hirai et al., 2004) in Arabidopsis indicate the presence of global multiple networks responding to nutritional deficient stress. Long-term sulfur deficiency results in similar global changes in the transcriptome and metabolome, as does nitrogen deficiency (Hirai et al., 2004). These are general responses common to sulfur and nitrogen deficiencies. These general nutritional responses differ in leaves and roots. Signaling pathways involving methyl jasmonate and auxin seem to be involved in the sulfur stress response (see below). Primary and secondary metabolic pathways, involving amino acids, carbohydrates, and glucosinolates, are modulated in response to sulfur deficiency stress. Nontargeted metabolome analysis indicates that the levels of glucosinolates from the array of approximately 3,000 putative metabolites decrease rapidly upon sulfur deficiency concomitant with a transient increase of isothiocyanates (Hirai et al., 2004; M. Hirai and K. Saito, unpublished data). Glucosinolates contain two or three sulfur atoms per molecule, and thus are regarded as storage and possibly mobilizing forms of assimilated sulfur in response to acute sulfur defi-

ciency. Since integrated -omics studies have just started, further investigation will provide more detailed information on holistic networks of sulfur metabolism.

HORMONAL CONTROL IS INVOLVED IN REGULATION OF ASSIMILATION

Some plant hormones are indicated to control gene expression related to sulfur metabolism. Several studies on transcriptome analysis suggest that methyl jasmonate and auxin are involved as signals of sulfur-deficiency stress (Hirai et al., 2003; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003). Indeed, methyl jasmonate induces a cluster of sulfur assimilation genes but not sulfate transporters, as well as well-known jasmonate-inducible genes (Y. Sasaki-Sekimoto and H. Ohta, unpublished data). Auxin signaling is presumably involved, at least as an example, through activation of nitrilase leading to changes in root morphology (Kutz et al., 2002). Cytokinin signaling also appears to be involved in gene expression related to sulfur metabolism. Cytokinins have been shown to down-regulate the expression of high-affinity sulfate transporter genes in Arabidopsis roots (Maruyama-Nakashita et al., 2004). By contrast, a sulfur-deficiency responsive promoter of soybean (*Glycine max*) β-conglycinin responds positively to cytokinin (Ohkama et al., 2002). Since cellular cytokinin levels do not significantly change in response to sulfur status, further investigations are needed on the signal perception and downstream gene expression events.

OTHER FACTORS SUCH AS DEVELOPMENT, THE CIRCADIAN CLOCK, AND ABIOTIC STRESSES ALSO INFLUENCE SULFUR ASSIMILATION

Sulfur assimilation is highly active in growing tissues where high levels of Cys and Met are required for protein synthesis. Indeed, gene expression of plastidic ATP sulfurylase and APS reductase is high in young leaves of Arabidopsis (Rotte and Leustek, 2000). Specialized cells such as trichomes exhibit high activities of enzymes for synthesis of Cys and GSH (Gutiérez-Alcalá et al., 2000), presumably due to the demand for high production of GSH and phytochelatins for detoxification of heavy metals (Choi et al., 2001). Developing seeds seem to assimilate sulfur directly from sulfate inside the seed, rather than translocating Cys or GSH from other tissues (Tabe and Droux, 2001).

Some sulfur assimilation genes, but not all, are regulated by circadian rhythm (Kopriva et al., 1999; Harmer et al., 2000). The expression of sulfate transporters, APS reductase, Ser acetyltransferase, and 3-phosphoglycerate dehydrogenase, the first enzyme of the plastidic Ser biosynthetic pathway, are at a peak just before the onset of the light period. In leaves, ATP, electrons, and 3-phosphoglycerate are generated as soon as the onset of light by photosynthesis, and these are ready for use in APS formation, sulfite reduction, and Ser formation, respectively. The coordinate

circadian control of a cluster of genes involved in sulfur assimilation and related pathways seems to ensure the efficient production of Cys by the preparation of sulfate and key enzymes just before the generation of reductants and substrates from photosynthesis.

Abiotic stresses such as heavy metals and oxidative stress affect sulfur assimilation. Once plants are exposed to heavy metals such as cadmium, phytochelatins (γ-Glu-Cys)$_n$-Gly are synthesized from GSH, which consequently consumes Cys. In fact, heavy metal stress promotes the expression of sulfur assimilation and transporter genes (Dominguez-Solis et al., 2001; Nocito et al., 2002).

To mitigate oxidative stress, GSH functions as a direct antioxidant and also as a reducing agent for other antioxidants such as ascorbic acid. Since Cys availability is a limiting factor for GSH synthesis, sulfur assimilation is also controlled by the cellular oxidative state. Most notably, an isoform of APS reductase is activated by oxidation of two hydrogen sulfides of Cys in the enzyme into a disulfide bond by oxidized glutathione (Bick et al., 2001). This is a mechanism that enzymes of sulfur assimilation and subsequent GSH synthesis are posttranslationally modified and thus promptly activated by after consumption of reduced GSH by oxidative stress mitigation.

CONCLUSIONS AND FUTURE DIRECTIONS

During the past few years, remarkable progress has been made on our basic understanding of the mechanisms, genes and proteins, and regulation of sulfur assimilation, profited from the development of molecular tools particularly in Arabidopsis. Most individual genes and proteins involved in sulfur metabolism have been characterized by modern technology. However, the holistic networks of entire gene-protein-metabolite related to sulfur metabolism have been poorly understood. In addition, the complex signaling pathway of sulfur nutritional stress remains a largely open question. Future research will focus on more global aspects of sulfur metabolism and signaling in terms of transcriptome, proteome, and metabolome, mainly with a model plant Arabidopsis. The basic knowledge obtained through fundamental studies can be directly applied to development of crops to meet the requirements of agricultural productivity, nutra- and pharmaceutical functionality, and environmental sustainability.

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