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Review

Synthesis of the sulfur amino acids: cysteine and methionine

Markus Wirtz[1,*] & Michel Droux[2]
[1]*Heidelberg Institute of Plant Sciences (HIP), University of Heidelberg, Im Neuenheimer Feld 360, 69120 Heidelberg, Germany;* [2]*UMR 2847 CNRS/Bayer Cropscience, Bayer Cropscience, 69263 Lyon, France;* **Author for correspondence (e-mail: mwirtz@hip.uni-hd.de; fax: +49-6221-545859)*

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Abstract

This review will assess new features reported for the molecular and biochemical aspects of cysteine and methionine biosynthesis in *Arabidopsis thaliana* with regards to early published data from other taxa including crop plants and bacteria (*Escherichia coli* as a model). By contrast to bacteria and fungi, plant cells present a complex organization, in which the sulfur network takes place in multiple sites. Particularly, the impact of sulfur amino-acid biosynthesis compartmentalization will be addressed in respect to localization of sulfur reduction. To this end, the review will focus on regulation of sulfate reduction by synthesis of cysteine through the cysteine synthase complex and the synthesis of methionine and its derivatives. Finally, regulatory aspects of sulfur amino-acid biosynthesis will be explored with regards to interlacing processes such as photosynthesis, carbon and nitrogen assimilation.

Abbreviations: acetyl-CoA – acetyl-coenzyme A; AdoHCys – *S*-adenosylhomocysteine; AdoMet – *S*-adenosylmethionine; APR – adenosine-5′-phosphosulfate-reductase; C – carbon; CAS – β-cyanoalanine synthase; CBL – cystathionine β-lyase; CGS – cystathionine γ-synthase; CSC – cysteine synthase complex; Cys – cysteine; GSH – glutathione; Met – methionine; N – nitrogen; OAS – *O*-acetylserine; OAS-TL – *O*-acetylserine(thiol)lyase; ROS – reactive oxygen species; SAT – serine acetyltransferase; SMM – *S*-methylmethionine

Introduction

The sulfur-containing amino acids, cysteine (Cys) and methionine (Met) are essential for the entire biological kingdom because of their prominent tasks in primary and secondary metabolism (Ravanel et al. 1998b; Leustek et al. 2000; Saito 2000, 2004; Droux 2004;). The lowest oxidation state (−2) of sulfur in Cys and Met is the fundamental chemical and physical state of these organosulfur compounds that account for their biochemical functions (Giles et al. 2003). In proteins, Cys residues are important for stabilization of tertiary and quaternary protein conformation through disulfide bridges. In addition, sulfur atoms in thiol groups of protein bound Cys provide sites for catalytic activities (Gilbert 1990). Furthermore, protein associated and free thiols are responsible for binding of metals and react with nucleophilic drugs and reactive oxygen (Giles et al. 2001; Cooper et al. 2002). The conversion of free thiol groups to disulfide bridges and *vice versa* constitutes a dynamic reactive system that is the basis for redox switches in protein. These redox switches are involved in modulation of essential metabolic functions and in regulation of metabolism in plants

cells in response to the environment (Schürmann and Jacquot 2000; Paget and Buttner 2003).

Besides its role in proteins, Cys participates in the synthesis of essential bio-molecules like antioxidants, vitamins and co-factors (Noctor et al. 1998; Leustek et al. 2000; Droux 2004; Saito 2004). The catalytic mechanism of these bio-molecules (such as glutathione, thiamine, lipoic acid, biotin, coenzyme A for example) is based on the reactivity of the thiol group that is a result of the relative weakness of the carbon–sulfur bond in this group.

Like Cys, free as well as protein bound Met has ubiquitous functions in plants. It plays a role in the initiation of mRNA translation and is the precursor of essential bio-molecules trough *S*-adenosylmethionine (AdoMet) (Ravanel et al. 1998b; Leustek et al. 2000; Lu 2000). AdoMet serves as a carbon skeleton donor for synthesis of polyamines, vitamins, co-factors, osmo-protectants and hormones such as ethylene (Droux 2004; Fontecave et al. 2004; Hesse et al. 2004). For that reason Met itself is an essential metabolite for primary and secondary metabolism of all life forms. In proteins, the role of Met is associated with its hydrophobic properties for protein folding and determination of the half-span of proteins (Giglione et al. 2003). In addition, oxidation of exposed protein Met residues in presence of chemicals or oxidants results in alteration of protein folding and catalysis (Levine et al. 2000; Hoshi and Heinemann 2001).

Plants, bacteria and fungi can assimilate inorganic sulfur as sulfate (oxidation state +6) for reduction to sulfide leading to the synthesis of sulfur-containing amino-acids (Kredich 1996; Marzluf 1997; Thomas and Surdin-Kerjan 1997; Leustek et al. 2000). In contrast animals and humans lack the capability to reduce sulfate. As a consequence humans and most animals rely on their diet for provision of reduced sulfur in terms of Cys and Met (Tabe and Higgins 1998; Zhao et al. 2000). Thus, plants are the most important source of essential sulfur amino acids for humans and animals, which constitutes general economic interest of sulfur amino acid biosynthesis in higher plants (Ravanel et al. 1998b; Zhao et al. 1999; Höfgen et al. 2001).

Cysteine and methionine synthesis: A complex organization in plant cells

Updates for the assimilation and reduction of sulfate were reported recently (Droux 2004; Saito 2004). In short, after incorporation from the soil through roots specific transporters, sulfate is distributed into plant-cells through a family of transporters needed to cross all the cells barriers (Buchner et al. 2004a). Minor part of sulfate is used for sulfation of proteins in the cytosol, whereas most part of sulfate is activated and reduced into a three-step mechanism to sulfide exclusively in plastids before its incorporation into a serine derivative leading to the first organic sulfur amino acid, cysteine (Leustek et al. 2000; Droux 2004; Saito 2004).

Cysteine biosynthesis

The synthesis of cysteine can be subdivided into three steps in plants and bacteria: (1) the assimilatory sulfate reduction which provides reduced sulfur in terms of sulfide, (2) the synthesis of the carbon (C) and nitrogen (N) containing the backbone for cysteine, and (3) the incorporation of reduced sulfur into the organic backbone. The released cysteine represents the first form of reduced organic sulfur in plants cells. As in bacteria, the synthesis of the C/N-backbone of cysteine is catalyzed by serine acetyltransferase (SAT, EC 2.3.1.30), which transfers an acetyl-moiety from acetyl-Coenzyme A (acetyl-CoA) to serine, leading to *O*-acetylserine (OAS) formation (Droux 2004; Hell et al. 2002; Johnson et al. 2005). Then *O*-acetylserine(thiol)lyase (OAS-TL, EC 2.5.1.47) converts OAS into cysteine in the presence of sulfide (Droux 2004; Hell 1997). SAT and OAS-TL form a multienzyme complex called cysteine synthase complex (CSC; Droux 2003; Hell 1998; Kredich et al. 1969), in which protein–protein interactions regulate activities of both enzymes (see section Regulation). In contrast to sulfide production and to the synthesis of most amino acids in higher plants, cysteine synthesis takes place not only in plastids where it is linked to sulfate reduction, but also in the cytosol and the mitochondria (Figure 1; Droux 2003; Lunn et al. 1990; Rolland et al. 1992; Ruffet et al. 1995; Wirtz et al. 2004).

In the model plant *Arabidopsis thaliana* the SAT gene family (*AtSerat*) consists of five members based upon their sequence homology and the availability to complement cysteine-auxotrophy of *Escherichia coli* mutants lacking endogenous SAT activity (Hell et al. 2002). With respect to their location at the chromosomes (*AtSAT*x) or from the grouping in the

phylogenetic tree recently reported (*Serat*x.y), this family is defined as *AtSAT*1 (*Serat*2.1: At1g55920, L34076), *AtSAT*2 (*Serat*3.1: At2g17640, L78444), *AtSAT*3 (*Serat*2.2: At3g13110, U22964), *AtSAT*4 (Serat3.2: At4g35640, AF331847) and *AtSAT*5 (*Serat*1.1: At5g56760, U30298) (Bogdanova et al. 1995; Ruffet et al. 1995; Howarth et al. 1997; Noji et al. 1998; Howarth et al. 2003; Kawashima et al. 2005). *AtSAT*2 and *AtSAT*4 are 10–100 times less transcribed, differently regulated at the transcriptional level and have unfavorable biochemical properties for catalysis of SAT reaction in comparison to the major expressed SAT isoenzymes: *AtSAT*1, 3 and 5. The former are less characterized and have been added very recently to the SAT gene family. Their contribution to net cysteine synthesis is still questionable; therefore this review will focus on regulatory aspects of *AtSAT*1, 3 and 5.

The localization of all *Arabidopsis* SAT isoforms was analyzed using SAT-GFP-fusion proteins in the homologous system. These studies indicate that *AtSAT*2, 4 and 5 are located in the cytosol, whereas *AtSAT*1 and *AtSAT*3 are targeted to the plastids and the mitochondria (Noji et al. 1998; Kawashima et al. 2005). The localization of *AtSAT*1 seems to switch from plastidic to cytosolic localization depending on the leaf age of the *Arabidopsis* plants (Noji et al. 1998). In one report, proteomics studies of *Arabidopsis* nuclei isolated by sub-cellular fragmentation identified *AtSAT*1 within this compartment, which may suggest new proteins functions for this isoform (Bae et al. 2003). In addition, improved *in-silico* analyses of the primary sequence indicate that *AtSAT*1 and *AtSAT*4 are of cytosolic and plastid localization, respectively (see PPDB: http://ppdb.tc.cornell.edu/; Sun et al. 2004). These inconsistencies in localization of SAT proteins point out that immuno-localization using specific antibodies would be necessary to confirm the SAT localization in *Arabidopsis* cell-compartments.

Biochemical characterizations of plant SATs were performed independently in different laboratories using crude plant extracts, isolated proteins from enriched plant cell compartments and recombinant proteins (Chronis and Krishnan 2004; Noji et al. 1998; Roughan 1997; Ruffet et al. 1994, 1995; Urano et al. 2000; Wirtz et al. 2000, 2001). Taken together these results strongly indicate limitation of SAT activity rather by acetyl-CoA than by serine supply at least in plastids, in which sulfate reduction takes place.

All SAT isoforms show a semi-constitutive transcription pattern during development of the model plant *A. thaliana* (Hell et al. 2002; Howarth et al. 2003; Kawashima et al. 2005). Only in one report the plastids SAT is significantly upregulated at the transcriptional level in *Arabidopsis* leaves but not in roots (Takahashi et al. 1997). Noteworthy the transcriptional regulation of SAT in other plant taxa is barely analysed, although the enzymes catalyses the crucial step in cysteine biosynthesis. Noteworthy, in enteric bacteria SAT is the only constitutively expressed gene of the sulfate assimilation pathway (Kredich 1996; Mosulen et al. 2003). Quantitative RT-PCR analysis identifies the plastid *AtSAT*1, and to less extent *AtSAT*3 and *AtSAT*5, as the prominently transcribed gene in leaves (Kawashima et al. 2005). By contrast, in *Pisum sativum* approximately 80% of total SAT activity is located in mitochondria, whereas residual SAT activity is equally distributed between cytosol and plastids (Ruffet et al. 1995; Lappartient et al. 2000). This discrepancy may be explained by species-specific differences in localization of cysteine synthesis or the fact that mRNA levels of genes do not reflect in any case the amount of transcribed protein, for instance due to post-transcriptional regulatory mechanisms. High SAT activity in plastids would make sense in terms of OAS supply for net synthesis of cysteine, since sulfide is produced only in plastids. In contrast substrates for the SAT reaction are mainly produced in mitochondria by photorespiration and oxidative decarboxylation of pyruvate, which would favor high SAT activity in mitochondria. The conclusion of the available results is that the compartmentalization of OAS net synthesis is still undiscovered, although OAS synthesis is one key regulatory step in synthesis of cysteine (see Regulation). In addition, transport of OAS between compartments in a plant cell could be accomplished by amino acid transporters that are known to have broad substrate specifity, making the regulation of cysteine biosynthesis more complex (Boorer et al. 1996; Fischer et al. 1995).

The second enzyme involved in the synthesis of cysteine, OAS-TL, has broad substrate specificity for the nucleophilic reactant and may be involved in some plant species in the synthesis of secondary products (Maier 2003; Murakoshi et al. 1986; Noji et al. 1993; Warrilow and Hawkesford 1998, 2000, 2002). OAS-TL belongs to the super family of

β-substituting alanine synthases, including also the β-cyanoalanine synthase (EC 4.4.1.9, CAS), which share high homology at nucleotide and amino acid level (Droux 2004; Hatzfeld et al. 2000; Jost et al. 2000; Wirtz et al. 2004). CAS catalyzes detoxification of cyanide with cysteine, forming β-cyanoalanine and sulfide (Warrilow and Hawkesford 2002). Since OAS-TL catalyses to some extend the reaction of CAS as a side-reaction and *verse visa*, it is difficult to distinguish between authentic OAS-TL and CAS only by sequence homology without careful biochemical characterization (Warrilow and Hawkesford 1998, 2000). A property that defines an authentic OAS-TL seems to be its ability to make protein interactions with SAT as was demonstrated for plant OAS-TL using the two-hybrid system and by co-purification experiments of the associated enzyme in the CSC (Bogdanova and Hell 1997; Droux et al. 1998; Hell et al. 2002; Wirtz et al. 2000, 2001). At present the β-substituting alanine synthases family of *Arabidopsis* consists of 8 OAS-TL like proteins (Jost et al. 2000). The genes encoding for isoforms of OAS-TL-like proteins are more or less ubiquitously expressed in all plant organ cell types analyzed so far with little variation of content of RNA, protein and extractable enzyme activity in response to external factors (Brunold 1990; Dominguez-Solis et al. 2001; Hell et al. 1994). OAS-TL A (At4g14880), B (At2g43750) and C (At3g59760) are considered as authentic OAS-TLs, which are located in cytosol, plastid and mitochondria of *Arabidopsis* cells (Hell et al. 1994; Hesse and Hoefgen 1998; Hesse et al. 1999; Wirtz et al. 2004). In *Spinacia oleracea* and *Datura inoxia* OAS-TL activity is associated to 45% with plastids, with an additional 45% in the cytosol, whereas the remaining activity is present in mitochondria (Kuske et al. 1996; Lunn et al. 1990). Biochemical analysis of AtcysD1 (At3g04940) and AtcysD2 (At5g28020) indicate that both encode for additional authentic OAS-TLs, which are located in the cytosol (Hatzfeld et al. 2000; Yamaguchi et al. 2000). In contrast, AtcysC1 (At3g61440) is predicted to be localized in mitochondria and supposed to act at *in vivo* condition as CAS (Hatzfeld et al. 2000; Yamaguchi et al. 2000). The remaining OAS-TL-like proteins (At3g03630 and At5g28030) are less characterized; hence their function in plant sulfur metabolism is unknown. Moreover there are single reports from large-scale proteomic approaches that indicates new localization of authentic OAS-TLs isoforms in sub-compartments of plastids (Friso et al. 2004; Sun et al. 2004). These results need to be verified by targeted analysis of the corresponding proteins, to rule out the possibility of false positive signals by cross contamination with other compartments.

As in bacteria, authentic OAS-TL is a homodimer containing pyridoxal-5′-phosphate as cofactor. It catalyzes the substitution of the acetyl group in the β-position of the activated serine substrate, OAS, in presence of the nucleophile sulfide (Rabeh and Cook 2004). Rapid-scanning and fluorescence stopped-flow experiments using the bacterial OAS-TL showed that conversion of OAS to the α-aminoacrylate intermediate and acetate, is rate-limiting for the overall reaction (Woehl et al. 1996). The K_m-value (Michaelis constant) for OAS of bacterial and plant OAS-TLs are revealed to be in the low millimolar range (Wirtz and Hell 2003a, Wirtz et al. 2004). This is approximately 100-times higher than the concentration calculated for OAS in leaves of non-stressed *Arabidopsis* (Awazuhara et al. 2000; Kim et al. 1999). In contrast, the affinity of OAS-TLs for sulfide is in the low micromolar range (Wirtz et al. 2004). All together, these data indicate that OAS rather than sulfide limits cysteine synthesis at sufficient sulfur supply, which is one prerequisite for the regulatory component of the CS model (see section Regulation). Nevertheless, OAS-TL activity can become a limiting step at various stress situations wherever high cysteine synthesis is required (Barroso et al. 1999; Dominguez-Solis et al. 2001, 2004; Romero et al. 2001). Accordingly, it is not surprising that OAS-TL over expression in plastids and cytosol confers resistance to sulfide dioxide and cadmium (Harada et al. 2001; Noji et al. 2001).

Methionine biosynthesis

Methionine belongs to the aspartate-derived amino acids (Amir et al. 2002). Biosynthesis of this sulfur-containing amino acid is tripartite with the sulfur atom coming from cysteine, the nitrogen/carbon skeleton from a phosphorylated-homoserine, and the methyl group from folates (Figure 1) (Ravanel et al. 1998b; Droux 2004; Hesse et al. 2004). Most of the methionine synthesized in plants cell is converted into adenosylmethionine

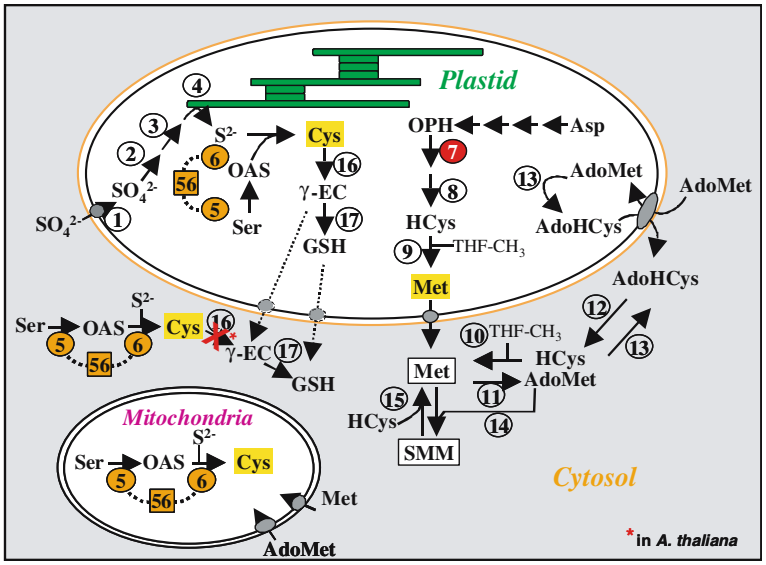

Figure 1. Compartmentalization of sulfur amino acids biosynthesis in plants cells. The scheme summarizes recent findings and the complexity of the sulfur network with regards to compartmentalization of cysteine and methionine synthesis. 1: Sulfate transport; 2: sulfate activation (ATP sulfurylase); 3: sulfate reduction (APS reductase); 4: ferredoxin linked sulfite reduction (sulfite reductase); 5: serine activation (serine acetyltransferase); 6: sulfide condensation to activated serine (*O*-acetylserine (thiol) lyase); 56: cysteine synthase complex (CSC); 7: cystathionine γ-synthase (CGS1); 8: cystathionine β-lyase, 9: plastid Atms3 (methionine synthase); 10: cytosol Atms1–2 (methionine synthase); 11: AdoMet synthethase, 12: *S*-adenosyl-homocysteine hydrolase; 13: *S*-adoMet dependent methylase; 14: *S*-AdoMet-methionine methyltransferase; 15: *S*-methylmethionine-homocysteine methyltransferase; 16: GSH precursor synthesis (γ-glutamyl-cysteine syntethase, GSH1), 17: glutathione syntethase (GSH2); Asp: aspartate; AdoHCys: *S*-adenosylhomocysteine; AdoMet: *S*-adenosyl-methionine; Cys: cysteine; γ-GC: γ-glutamylcysteine; GSH: glutathione; HCys: homocysteine; OAS: *O*-acetylserine; OPH, *O*-phosphohomoserine; THF: tetrahydrofolate polyglutamate; S^{2-}: sulfide; Ser: serine; SMM: *S*-methylmethionine. Dashed arrows indicate possible transport across plastid membranes.

(AdoMet), a sulfonium compound of absolute importance for metabolism (Ravanel et al. 1998b; Droux 2004; Hesse et al. 2004).

The transfer of the sulfur atom from cysteine to homocysteine through the transsulfuration pathway with cystathionine as intermediate occurs only in plant plastids and has been extensively studies in *Arabidopsis* (Ravanel et al. 1996, 1998a, b; Droux 2004; Hesse et al. 2004). In this pathway, reactions are catalyzed respectively by cystathionine γ-synthase (CGS1, EC 4.2.99.9; At3g01120) and cystathionine β-lyase (CBL, EC 4.4.1.8, At3g57050) (Ravanel et al. 1998b). In contrast to bacteria, the nitrogen/carbon precursor for the synthesis of homocysteine is *O*-phosphohomoserine, a common intermediate with the synthesis of threonine (see Hesse et al. 2004). In addition, the mature plant CGS1 exhibits at its N-terminus an additional module of about 120 amino-acids. This particular extension plays a role in its regulation (see section Regulation; Onouchi et al. 2004). Biochemical and kinetic parameters for CGS from diverse sources were extensively analyzed (review in Ravanel et al. 1998b; Clausen et al. 1999). These kinetic values for both substrates led to estimation that CGS reaction would proceed only to 1–2% of its maximal rate in plant cells (Ravanel et al. 1998a). The structure, substrate specificity and ping-pong mechanism was recently confirmed through the resolution and analysis of the *Nicotiana tabaccum* CGS (Steegborn et al. 1999). Physiochemical properties of the second enzyme of the transsulfuration pathway (CBL) were relatively similar to those of *Escherichia coli* (Ravanel et al. 1996, 1998b). Interestingly, while the bacterial enzyme degrades cystine and cystathionine equally, the reaction with cystine accounted for only 16% with the plant enzyme (Ravanel et al. 1996). Such observations suggest major differences in the amino acids around the pyridoxal phosphate site between the bacterial and plant CBL, as confirmed through resolved structures from both sources (Clausen et al. 1996; Breitinger et al. 2001).

In the last step, Met synthesis involves the methylation of the thiol group of HCys in a

reaction catalyzed by a vitamine B12 independent methionine synthase (MS, EC 2.1.1.14). The enzyme requires a methyl donor, a polyglutamyl derivative of THF, N_5-methyltetrahydropteroyl polyglutamate, containing three glutamate residues, phosphate and zinc for activity (Ravanel et al. 1998b; Eckermann et al. 2000; Droux, 2004; Hesse et al., 2004). Multiple forms of MS could be evidenced in plants, and in the genome of *A. thaliana* (Eckermann et al. 2000; Droux 2004; Ravanel et al. 2004). Physico-biochemical investigations performed with the *Arabidopsis thaliana* methionine synthase (MS) family: the cytosolic, Atms1, (At5g17920) and its compartment homologue Atms2 (At3g03780); the plastid localized Atms3 (At5g20980) (Ravanel et al. 2004) revealed that the plastid isoform showed a stronger affinity for the polyglutamate derivatives of folates than the cytosolic isoforms (Ravanel et al. 2004). Immunoblot analyses of purified cell-fractions and transient expression of GFP fusion proteins in *Arabidopsis* protoplasts confirmed that Atms1 and Atms2 are linked to the cytosolic compartment while Atms3 is clearly associated to the soluble fraction of the plastid (Figure 3). The plastid localization of MS was corroborated by an independent study using a proteomic approach for characterization of chloroplast-associated proteins (Friso et al. 2004; Sun et al. 2004). These new findings clarified the long-term discussions on the irrefutable evidence of only a cytosolic site for methionine synthesis (Hesse et al. 2004).

Cytosolic methionine synthase is linked to AdoMet synthesis since this function, up to now, was only associated within this compartment (Ravanel et al. 1998; Droux 2004; Hesse et al. 2004). In addition the role of the cytosolic MS is important for the recycling of homocysteine resulting from hydrolysis of *S*-adenosylhomocysteine (AdoHCys), produced from cytosolic AdoMet-dependent methylation (Kloor and Osswald 2004). It should be noted that AdoHCys is a strong inhibitor of methylation steps and should be transformed into HCys. Furthermore, accumulation of the hydrolytic product (Hcys) from the reaction catalyzed by SAH hydrolase is reversible (Kloor et al. 1998; Kloor and Osswald 2004). Thus, HCys should be eliminated through its degradation, transport in others compartment, or directly transformed into methionine, the later step being catalyzed by the cytosolic methionine synthase (Ravanel et al. 1998b, 2004). If the recycling of AdoHcys occurs in the cytosol, relative information for such reactions within the plastid was reported. In particular essential AdoMet-dependent reactions may occur to sustain plastid-specific biosynthetic pathways, such as siroheme synthesis (Leustek et al. 1997; Droux 2004). To this end, evidence for a counter-exchange transporter of AdoHCys from the plastid to the cytosol with cytosolic synthesized AdoMet was recently described (Ravanel et al. 2004). In addition to this complex cell-compartmentalization, plants are able to produce a compound unique SMM (*S*-methylmethionine) through an irreversible reaction catalyzed by an *S*-adenosylmethionine:methionine dependent *S*-methyltransferase (EC 2.1.1.12) which involves transfer of the methyl transfer from AdoMet to Met (Ranocha et al. 2001). SMM is converted back to two molecules of Met in the presence of HCys by an enzyme called SMM-homocysteine-*S*-methyltransferase (EC 2.1.1.10) (Ranocha et al. 2001; Kocsis et al. 2003). This mechanism presents a second system to remove the excess of HCys produced in the cytosol when folate derivatives are too low for methionine synthesis. SMM is present in millimolar concentration in plants and constituted storage for sulfur and methyl groups, as well a system to maintain the cell AdoMet level constant as was demonstrated, in mutant plants with inactivated methionine *S*-methyltransferase (Kocsis et al. 2003).

Regulation of cysteine and of methionine synthesis

Our current knowledge on the regulation of the plant sulfur network leading to Cys and to Met synthesis at the level of the complex cellular compartment structure has been integrated in Figure 1. The figure considers all described enzyme activities reported in relation to the fate of Cys with synthesis of GSH and the fate of Met and its derivatives. While the uptake and reduction of sulfur is mainly controlled at the transcriptional level, Cys synthesis appears to be less controlled at the transcriptional, but more on the post-translational level (Figures 2 and 3). In addition, Met controls its own synthesis through its derivatives AdoMet at the level of CGS (Figure 3).

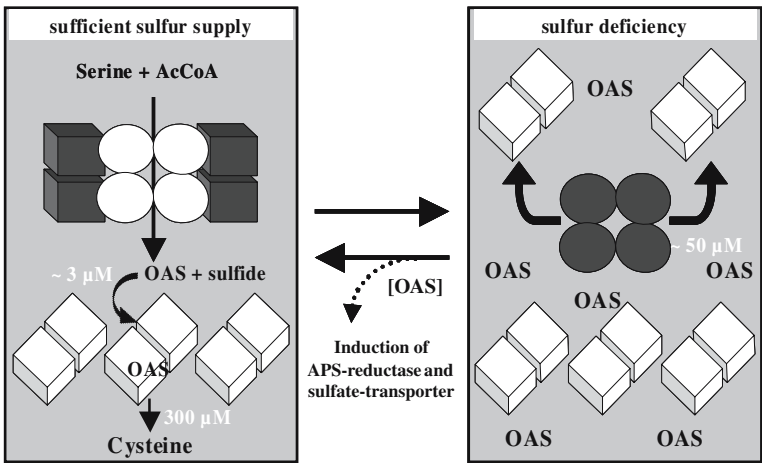

Figure 2. Regulation of cysteine synthesis flux through the cysteine synthase complex. The CSC refers to the multimeric enzymatic complex composed of SAT and OAS-TL. Reversible protein–protein interaction in CSC is based on changes in cellular OAS and sulfide concentration as a consequence of sulfur supply of the cell. As result of this interaction, activities of the corresponding enzymes that composed the cysteine synthase complex are regulated. Active SAT and OAS-TL are shown as white balls and cubes, respectively, whereas black filling indicates less efficient enzymes in catalysis. Values in white letters refer to concentration of *O*-acetylserine (OAS), respectively, for half-maximal binding to OAS-TL, activity of OAS-TL or dissociation of the cysteine synthase complex.

On the regulation at the plant level

The root is the major uptake organ of sulfur in terms of sulfate, whereas most sulfate reduction and assimilation takes place in leaves (Hell 1997; Saito 2000). Since Cys synthesis is the terminal step in sulfur assimilation, it is dependant of sufficient sulfate supply from the roots. Communication between leaf and root is therefore essential to maintain sulfur homeostasis for a balanced Cys, GSH and Met synthesis in higher plants. The major transport forms of Cys and Met in the phloem are GSH and *S*-methylmethionine (SMM), respectively (Bourgis et al. 1999). Several lines of evidence favor the hypothesis of demand-driven control of sulfate uptake and reduction in roots by leaves via phloem transported GSH (Herschbach and Rennenberg 1994; Lappartient and Touraine 1996, 1997; Lappartient et al. 1999; Vauclare et al. 2002).

In contrast to the demand driven control hypothesis transgenic poplar plants with significantly enhanced GSH contents in leaves show no variation of APR transcript levels in roots, but surprisingly these roots still react on exogenous applied GSH (Hartmann et al. 2004). In *Brassica oleracea* sulfate concentration in the root itself rather than a demand-driven control by thiols from leaves appears to be the signal to regulate sulfate uptake capacities in response to sulfate deficiency (Buchner et al. 2004b). Moreover feeding of OAS induces high affinity sulfate transporter and APR expression in roots of higher plants (Koprivova et al. 2000; Smith et al. 1997, Figure 3). OAS is assumed to be not a transport metabolite of the phloem, since it is chemically instable (Flavin and Slaughter 1965). Taken together these findings strongly indicate an additional internal control of net sulfate uptake by the root itself due to sulfate, OAS or a combination of both metabolites, towards the feedback regulation by leaves via demand-driven control. Since OAS is the product of SAT activity, SAT appears to be one of the key regulators not only in Cys synthesis but also in sulfur homeostasis of the entire plant.

Plant serine acetyltransferase as a limiting factor

SAT is approximately 400-times less abundant on the activity level than OAS-TL in leaves of all analyzed higher plants so far, including spinach, tobacco and *A. thaliana*; (Ruffet et al. 1994; Droux, 2003; Wirtz and Hell, unpublished data). Consequently, over-expression of OAS-TL derived from bacteria or plants in plastids or in the cytosol of tobacco results only in minor increases of Cys and GSH at normal sulfur supply (Sirko et al. 2004). By contrast, feeding of OAS to chloroplasts isolated from these transgenic plants enhances Cys and GSH accumulation (Saito et al. 1994). In

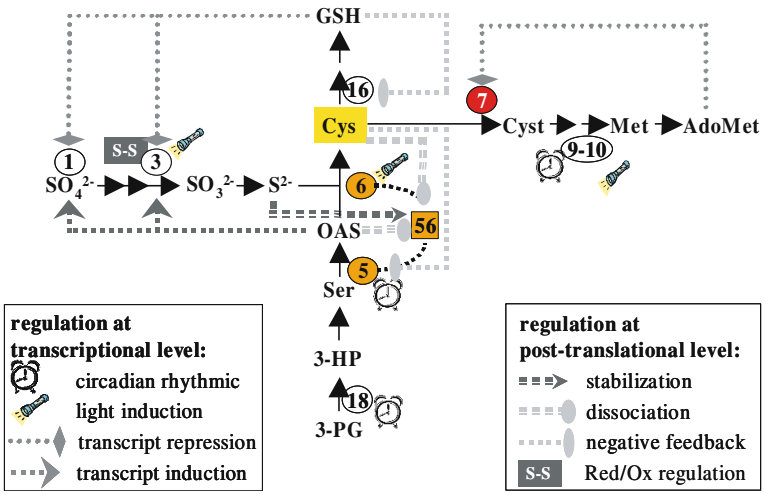

Figure 3. Schematic overview for regulatory mechanisms controlling sulfur amino acid biosynthesis in plant cells. Enzymes involved in sulfur metabolism are regulated at transcriptional and post-translational level by internal and environmental factors. Regulatory mechanisms are indicated by symbols or arrows as explained in legends. Solid arrows represent reactions in sulfur metabolism catalyzed by enzymes. Corresponding enzymes are denoted by numbers encircled. 1: Sulfate transporters; 3: APR (reduction of sulfate); 5: SAT (synthesis of OAS); 6: OAS-TL (synthesis of cysteine); 56: cysteine synthase complex (CSC); 7: cystathionine γ-synthase (CGS1); 9–10: methionine synthase; 16: γ-glutamyl-cysteine syntethase (synthesis of GSH precursor); 18: phosphoglycerate dehydrogenase (serine synthesis in plastids). Please note that several enzyme activities are encoded by isoenzymes that are located in more than one compartment and not all isoenzymes are subject to the depicted regulation. In addition, tissue specific-dependent protein expression and transport of signal molecules must be taken into account, especially in case of sulfate transporters and APR (see text for further explanation).

summary, these physiological approaches confirm *in vitro* results suggesting that OAS-TL activity is not a limiting factor in the synthesis of Cys (see Cysteine biosynthesis section). Furthermore, the production of OAS is limiting OAS-TL activity compared to sulfide formation in plastids at regular sulfur supply (Noji and Saito 2002; Saito 2004). The provision of OAS-TL substrates is regulated very strictly in order to avoid Cys accumulation to toxic acting levels. Consequently, SAT activity seems to become an efficient trigger for net Cys synthesis *in planta*. This is also indicated by over-expression of SAT and APR in higher plants (Blaszczyk et al. 1999; Harms et al. 2000; Tsakraklides et al. 2002; Wirtz and Hell 2003b).

The mostly constitutive transcription pattern of SAT in all analyzed higher plants so far emphasizes the importance of post-translational control at the level of this enzyme activity. Like bacteria higher plants posses SATs that are strongly inhibited by Cys, the end product of sulfate assimilation pathway (Figure 3). The concentration of Cys for 50% inhibition of plant SAT activity is in the range of 10 μM, a value similar to that reported for prokaryotic SAT (Saito et al. 1995). Moreover, in *Arabidopsis*, a compartment specific inhibition was observed with Cys controlling activity of cytosolic *AtSAT5*, while mitochondria and plastid localized SATs were found to be Cys insensitive (Noji et al. 1998). In contrast, subcellular feedback inhibition patterns of compartment specific SAT fraction from pea indicate that only SAT associated with the sulfate assimilation pathway in plastids is regulated by Cys (Lappartient et al. 2000). Additionally, a plastid localized Cys sensitive SAT from spinach was also characterized (Noji et al. 2001b). The latter results demonstrate that feedback inhibition of SAT is not an exclusive feature of cytosolic SAT in higher plants as assumed by the results obtained for SATs from *Arabidopsis* and added a note of caution to assign results obtained in *Arabidopsis* as universally valid for all higher plants.

The C-terminal domain of plant and bacterial SAT was identified to be responsible for Cys inhibition by direct mutagenesis of SAT in combination with domain swapping experiments (Inoue et al. 1999). Interestingly, the C-terminal domain of SAT is also responsible for heterologous protein interaction between SAT and OAS-TL (see next section), but the impact of CSC

formation on feedback inhibition of SAT by Cys is still unaddressed. In addition, the plastids CSC from pea leaves was reported to be dissociated in presence of physiological level of Cys (Droux 2003). Although, was not yet confirmed with homogenous preparation of CSC, this finding supposed an additional feedback regulation of Cys on SAT activity via CSC dissociation (Figure 2).

OAS-one key metabolite in regulation of sulfur assimilation

Resolution of the multimeric CSC trough 3D crystallography is not yet available for bacteria and plants. The structures of the bacterial subunits of the complex were resolved and extensively studies (see Rabeh and Cook 2004; Johnson et al. 2005 and references therein for a full aspect). Free OAS-TL is a homodimeric protein that undergoes a large conformational change upon OAS binding (Burkhard et al. 1998, 1999). Inside the complex affinity of OAS-TL for its substrates declined, which forms the basis of OAS-TL inactivity inside the complex (Droux et al. 1998). If OAS dependent dissociation of CSC is mediated by the conformational change of OAS-TL inside the complex upon OAS binding is speculative, but the declined affinity of OAS-TL inside the complex would be an explanation for the higher OAS concentration needed for complex dissociation in comparison to binding of OAS to free OAS-TL homodimers (see above). Primary structures of SAT from higher plants and enteric bacteria are defined by an N-terminal domain composed of α-helices and a C-terminal domain containing a hexapeptide repeat signature that forms a left-handed parallel β-helix (Bogdanova and Hell 1997). The latter carries the SAT/OAS-TL interaction site as well as the catalytic reaction center, providing the structural basis for regulation of SAT activity by CSC formation (Wirtz et al. 2001). In contrast, the N-terminal domain of SAT is responsible for SAT/SAT interaction as demonstrated by deletion analysis of plant SAT and 3D-structure of SAT from bacteria (Bogdanova and Hell 1997; Pye et al. 2004). Moreover analytical ultracentrifugation of the bacterial SAT demonstrates that the enzyme is a dimer of homotrimers in absence of OAS-TL (Hindson et al. 2000). This particular organization was confirmed recently through X-ray crystallographic structure determination of native bacterial SAT in complex with substrates or Cys (Olsen et al. 2004; Pye et al. 2004). In this aspect it is noteworthy that CSC from enteric bacteria and higher plants is assumed to consist of homo-tetrameric SAT and two OAS-TL dimers on the basis of gel filtration experiments (Droux et al. 1998; Kredich et al. 1969). An explanation for the discrepancy between hexameric structure of SAT in absence of OAS-TL and the proposed tetrameric structure of SAT inside the CSC would be reconstitution of SAT monomers prior to OAS-TL binding, but this appears to be curious. For that reason stoichiometry of SAT and OAS-TL subunits inside CSC is questionable and should be confirmed independently of gel filtration experiments.

Although the CSC is investigated since 1966 in prokaryotes, its function for regulation of Cys synthesis is still controversially discussed. Substrate channeling of OAS from SAT to OAS-TL inside CSC can be excluded, because OAS-TL is inactivated by complex formation in pro- and eukaryotes (Becker et al. 1969; Droux et al. 1998). Like in higher plants bacterial CSC is dissociated by OAS, but dissociation of bacterial CSC does not result in regulation of SAT-activity in *in vitro* assays (Kredich et al. 1969). Nevertheless, CSC formation increases the stability of prokaryotic SAT against cold inactivation and proteolysis *in vitro* (Mino et al. 2001). Physiological importance of the latter finding remains unclear, since specific degradation of SAT in response to Cys availability is not observed neither in pro- nor in eukaryotic cells. In contrast, SAT of higher plants is activated by CSC formation *in vitro*, as demonstrated by biochemical analysis of CSC containing cytosolic *Arabidopsis* SAT and plastidic spinach OAS-TL (Droux et al. 1998). Regulation of SAT-activity by homologous CSC formation of subunits from diverse subcellular compartments of higher plants is not confirmed. Different regulations of pro- and eukaryotic SAT by CSC formation are most likely explainable by different requirements of enteric bacteria and higher plants in terms of Cys supply. In contrast to enteric bacteria higher plants needs Cys synthesis during their entire development, whereas enteric bacteria are mainly supplied with Cys by their natural environment.

The most prominent hypothesis to explain regulatory mechanism of CSC in higher plants is the CSC-model that is proposed independently by

Droux and Hell in (1988) (Droux et al. 1998; Hell 1998). According to this model CSC senses sulfur availability inside a cell by present OAS concentration and adapts SAT activity to sulfur supply (Figure 2). At normal sulfur supply SAT is associated with OAS-TL in CSC and synthesizes OAS that is metabolized to Cys by a large excess of free OAS-TL (Droux et al. 1998; Ruffet et al. 1994). At sulfur deficiency OAS concentration rises immediately in plant cells as a result of sulfide limitation (Kim et al. 1999; Wirtz et al. 2004). Fluctuation of OAS steady state levels *in planta* seems to be sufficient to dissociate efficiently the CSC, as shown by protein interaction studies (Berkowitz et al. 2002; Wirtz et al. 2004). Dissociation of CSC inactivates SAT to prevent burnout of acetyl-CoA reservoirs and over-accumulation of OAS that is chemically instable. To allow OAS-dependent reversible SAT/OAS-TL interaction and thereby adjustment of SAT activity to available sulfide, free OAS-TL has to bind OAS at concentrations below complex dissociation constants. By contrast, catalysis of Cys has to be dependent on high K_M^{OAS} of free OAS-TL, because regulatory mechanism of CSC and free OAS-TL depends on limitation of Cys synthesis by OAS (previous section). Indeed the binding constant of free OAS-TL for OAS (K_D^{OAS}) was found to be less than 3 μM, whereas K_M^{OAS} of free OAS-TL is >300 μM (Wirtz et al. 2004). The CSC-dissociation constant was revealed to be 50–80 μM OAS (Berkowitz et al. 2002; Wirtz et al. 2004), which is almost perfectly in between binding of OAS (3 μM) to free OAS-TL and conversion to Cys (300 μM). Consequently, OAS acts in three ways on CSC and free OAS-TL: Firstly, it binds to free OAS-TL at low concentration, secondly, it can dissociate CSC at intermediate concentration in the absence of sulfide and thirdly, it is metabolized efficiently to Cys at high concentration (Figure 2).

Sulfide limitation at the cellular level is abolished by induction of APR expression as a result of elevated OAS concentration (Kopriva et al. 2002; Neuenschwander et al. 1991). Furthermore, high OAS concentration in roots induces the high affinity sulfate transporter system, which is accompanied by an increase in net sulfate uptake (Smith et al. 1997; Clarkson et al. 1999). As a consequence, during availability of sulfide, OAS is metabolized to Cys, lowering its cellular concentration. Moreover, sulfide was shown to counteract the dissociation of CSC in presence of OAS and thus resulting in a stabilization of CSC (Droux et al. 1998; Kredich et al. 1969). So, counteractions of both metabolites on CSC stability results in optimal SAT activity strictly depending on sulfur availability, thus modulating the flux of both compounds in cells.

OAS is further assumed to be one signal molecule in the global response to sulfur and nitrogen deficiency (Hirai et al. 2004). On the basis of its position at the branching point of carbon, nitrogen and sulfur metabolism, such kind of global regulation was assumed since the end of 1970, as OAS was demonstrated in higher plants (Smith 1977; Reuveny et al. 1980). The finding, that modulation of the sulfur flux is achieved by regulation of SAT activity, agrees with the model of a limitation in the entry of the N/C backbone in the pathway. In this aspect it is noteworthy that regulation of the entire flux by limitation of first or branching steps in a pathway due to substrate availability was also reported for other amino acid biosynthetic pathways (Amir et al. 2002; Droux 2004; Hesse et al. 2004). Recently, characterization of an *Arabidopsis* mutant with an increased level of OAS was reported (Ohkama-Ohtsu et al. 2004). As expected, genes for several sulfate transporters and APS reductase were up-regulated in this mutant. Surprisingly the level of Cys and GSH was unchanged in the OAS over-accumulating mutant as compared to the wild type, reinforcing the idea of additional signals besides OAS to coordinate flux of sulfate, nitrogen and carbon into Cys (Kopriva and Rennenberg 2004).

AdoMet-feedback regulator of methionine synthesis

Activities of enzymes of the transsulfuration sequence, CGS1 and CBL as the methionine synthase (MS) are not allosterically regulated by methionine (Ravanel et al. 1998, 2004; Droux 2004; Hesse et al. 2004). However, the capacity of the CGS1 was found to be strongly modulated by addition of methionine into plant cells media (Chiba et al. 1999; Onouchi et al. 2004). CGS is feedback-regulated at the level of its mRNA stability (Monato et al. 2002). Insights in the regulation of the methionine network was performed with a full set of transgenic approaches targeting the steps of the transsulfuration pathway, including the cytosolic methionine synthase, and also the enzyme in competition for the *O*-phosphohomo-

serine substrate, threonine synthase (Droux 2004; Hesse et al. 2004). Conclusion of theses studies revealed that CGS was the unique enzyme controlling the flux of sulfur and of the carbon–nitrogen backbone toward Met synthesis. By contrast to metabolite regulation, methionine synthase expression is found differentially expressed in plant organs during development (Eckermann et al. 2000). Interestingly, under conditions of salt stress, the expression of the MS mRNA was increased but the protein content remained constant (Eichel et al. 1995; Eckermann et al. 2000; Figure 3).

Most extensive developments on the molecular regulation of the methionine network were investigated by characterization of *Arabidopsis thaliana* mutant lines resistant to the toxic analogue of methionine, ethionine. These mutant lines called *mto1*(1–7) were characterized by elevated levels of CGS mRNA and of CGS activity as compared to wild type (Onouchi et al. 2004). Genetic analysis described a mutation within the conserved exon1 coding region (MTO1 region) where a single nucleotide changes resulted in one amino acid modification (Chiba et al. 1999, 2003; Amir et al. 2002). This important motif acts in *cis* to stabilize the CGS mRNA being resistant to degradation (Suzuki et al. 2001; Ominato et al. 2002; Onouchi et al. 2004). Translation of the protein is necessary for the regulation and stability of the mRNA as was confirmed by an experiment using the *in vitro* translation system of wheat germ extract (Chiba et al. 2003; Lambien et al. 2003). Finally, since AdoMet was revealed as the crucial metabolite involved in this regulation, suggesting that the Met dependent regulation was the result of its transformation into AdoMet in the cytosol (Onouchi et al. 2004). Interestingly, the MTO1 domain showed no features of known AdoMet binding domains. From this observation, it may be speculated that AdoMet regulation takes place through a protein intermediate. From the recent investigation using wheat germ extract, one could conclude that this intermediate is constitutively expressed (Chiba et al. 2003; Onouchi et al. 2004). In summary, in agreement with the proposal for the cytosol as the site for AdoMet synthesis, the regulation of plastid capacity for methionine biosynthesis could be assumed under the control of SMM to Met ratio (see above and Kocsis et al. 2003).

Cross talk of photosynthesis and sulfur amino acid synthesis

In chloroplasts, not only Cys, but also GSH, Met and its derivatives are linked to photosynthesis at different levels. Their syntheses depend on light through the photosynthetic electron transport chain leading to production of ATP, carbohydrates from CO_2 fixation and reducing power in terms of reduced ferredoxin and NADH + H^+. On the other hand Cys and Met are mandatory for synthesis of proteins in plastids and present precursors for the synthesis of essential intermediate cofactors of photosynthesis like sirohemes or iron sulfur clusters. Thus, limitation in any of these sulfur-containing compounds would indirectly affect the photosynthetic processes. In plastids, homeostasis of these complex interlacing metabolisms relies on a redox buffer consisting of GSH and related processes to cope with reactive oxygen species (ROS) formed during photosynthesis and stresses. The combination of photosynthesis with sulfate and nitrate assimilation in chloroplasts is exclusive in plant cells. Consequently, the plastid is the sole compartment, in which carbon, nitrogen and sulfur flux can be adjusted. The interface of these pathways is provided by synthesis of cysteine that contains C, N and S as the first stable organic thiol-containing compound. In agreement with its relevance for coordination of C, N and S metabolism, cysteine synthesis is limited by SAT activity, which catalyses the formation of the C/N containing backbone of cysteine. Indeed *AtSAT*1, the plastid localized SAT in *Arabidopsis* shows a circadian rhythm with peaking at the beginning of the light phase. Products of photosynthesis provide the precursor for synthesis of OAS in plastids by production of 3-phosphoglycerate. Phosphoglycerate dehydrogenase (EC 1.1.1.95) catalyzes the conversion of 3-phosphoglycerate to 3-hydroxy-pyruvate, thereby linking carbohydrate metabolism to cysteine synthesis (Figure 1). Interestingly, transcription of phosphoglycerate dehydrogenase and *AtSAT*1 is regulated by circadian rhythmic in the same manner resulting in efficient OAS production during onset of light in plastids (Takahashi et al. 1997; Harmer et al. 2000). The increase in SAT is accompanied by moderate induction of plastidic OAS-TL at the transcriptional level in response to light. It should be noted that the mitochondrial and cytosolic

SATs and OAS-TL are constitutively expressed in *Arabidopsis* (Hell et al. 1997; Takahashi et al. 1997; Hesse et al. 1999), emphasizing a prominent role of plastids in regulation of Cys synthesis. In addition, control for the expression of sulfate transporters and APR through a circadian clock with maximum in the light phase was also evidenced (Harmer et al. 2000; Kopriva et al. 1999). All these indications implicate coordination between sulfate reduction, Cys synthesis and photosynthesis through light at least for cysteine-linked functions localized within plastids. Accordingly, elimination of this fine tuning network by constitutive over-expression of APR activity in plastids resulted in a slightly chlorotic and stunted phenotype in *Arabidopsis* (Tsakraklides et al. 2002). Nevertheless it remains unclear, if the chlorotic phenotype of APR over-expresssing plants is caused by sulfide toxicity or deregulation of cysteine synthesis in chloroplasts. Indices for the latter hypothesis are provided by over-expression of prokaryotic OAS-TL in plastids of tobacco that also results in chlorosis, whereas over-expression of the same enzyme in the cytosol has no effect (Sirko et al. 2004). It is worth noting that the expression of eukaryotic OAS-TL in plastids of tobacco has also no effect on chlorophyll content or growth rate (Saito et al. 1994). Misregulation of endogenous plastidic SAT or OAS-TL enzymes by heterologous CSC formation could be an explanation for the observed discrepancies (see previous section). The reason for chlorosis in transgenic plants with altered Cys synthesis capacities in plastids is most likely a result of deregulated glutathione levels in chloroplasts. GSH synthesis is a two-step process, whereby both steps are dependent on ATP hydrolysis. (Figure 1). Particularly in the light Cys availability limits synthesis of GSH, since glycine accumulates as a result of photorespiration (Noctor et al. 1997). Although GSH1 is feedback inhibited by GSH, in all transgenic approaches significant up regulation of Cys in plastids or cytosol is accompanied by elevated steady state levels of GSH (Blaszczyk et al. 1999; Noji and Saito 2002; Wirtz and Hell 2003b). This indicates a crosstalk of Cys or OAS synthesis capacities between cytosol and plastids at least in *Arabidopsis*, since GSH1 of *Arabidopsis* was shown to be exclusively localized in plastids (Wachter et al. 2005). In contrast, biochemical analyses of spinach and pea reveal GSH1 activity in plastids and the cytosol (Hell and Bergmann 1990), implying significant difference in compartmentalization of sulfur metabolism in various plant species.

The two sulfur containing amino acids are essential for synthesis of highly abundant proteins involved in the photosynthetic process (e.g. RuBisCO, E.C. 4.1.1.39 and D1-protein). Moreover, some proteins functions are linked to additional prosthetic groups such as siroheme, iron–sulfur clusters or depend on S-containing cofactors like thiamin and biotin. Most of the sulfur atoms and methyl groups in these cofactors are derived essentially from Cys and AdoMet, indicating that a balanced provision of protein-S and S-cofactors in the chloroplast is necessary to maintain photosynthesis (Leustek et al. 2000; Droux 2004). Accordingly, the ratio protein-S/protein-N remains nearly constant under S-deficiency in the chloroplast, but is significantly reduced in the non-chloroplastic part of the cell (Willenbrink 1967). Up to now, this aspect is not explored and it remains unclear whether the balance in provision of chloroplast protein-S and S-cofactors includes regulation of compartment-specific cysteine and methionine synthesis capacities. The first indications for this kind of regulation are provided by analysis of SAT mRNA levels in response to short-term sulfur deficiency, showing that only plastid localized SAT is up-regulated in *Arabidopsis*, whereas cytosolic and mitochondrial SAT are not affected (Takahashi et al. 1997).

The first visible phenotype of long-term sulfur starvation in cyanobacteria, green algae and higher plants is paling due to reduction of chlorophyll content (Collier and Grossman 1992; Davies et al. 1996; Terry 1976). In higher plants reduction of chlorophyll by sulfur deficiency appears first in young leaves, in contrast to nitrogen deficiency, which causes paling of source leaves. This implicates that down regulation of photosynthesis by different mineral stresses is specifically regulated rather than a pleiotropic effect (Hell and Hillebrand 2001). At least partially the pale phenotype of sulfur deficiency is based upon reduced Cys synthesis, caused by sulfide limitation in higher plants. As a result of diminished *de novo* synthesis of Cys turnover of chlorophyll binding protein of Photosystem II (D1-protein) is significantly shifted to degradation (Dannehl et al. 1996; Godde and Hefer 1994). In

particular the availability of amino acids including cysteine can contribute to limit the synthesis of D1-protein in plastids, since D1-protein assembles co-translational into Photosystem II, whereby regulation at the post-translational level is diminished (Zhang et al. 1999). By contrast, in *Chlamydomonas reinhardtii* specific down regulation of photosynthesis in response to sulfur limitation is achieved by SAC1. Since SAC1 is mandatory for the process, down regulation of photosynthesis in *Chlamydomonas* spec. seems to be highly controlled and not the result of a general decrease in protein synthesis due to sulfur limitation (Davies et al. 1996). Extensive database searches revealed no *Sac*1 homologues in *Arabidopsis*, indicating that specific regulation of Photosystem II by SAC1 in response to sulfur limitation is a feature restricted to green algae.

Concluding remarks

During the last decade, understanding of the molecular, biochemical and physiological mechanisms controlling expression and activities of enzymes involved in sulfur metabolism was drastically improved using *Arabidopsis thaliana* as a model plant. In this aspect it should be pointed out, that not all results obtained using dicotyledonous plants such as Brassicceae, that are known for its complex secondary sulfur metabolism could be assigned to other plant taxa, including important graminaceous crop plants like corn and wheat.

The combination of new technologies like DNA array, high-throughput metabolomics and proteomics with genetic approaches such as engineered transgenic and T-DNA knockout plants reveals transcriptional and post-translational regulation of the sulfur network leading to Cys and Met synthesis. While most individual genes and proteins involved in sulfur metabolism are now well described, regulation of sulfur metabolism with respect to nitrogen to carbon fixation is starting to be understood at the level of gene–protein, protein–metabolite and gene–metabolite interactions. The difficulty in the interpretations of these interlacing pathways is based upon the complexicity of the plant-system. First, the multicompartment structure of the plant cell has to be considered. The plant plastid was identified as the exclusive compartment that contains all potential for net synthesis of sulfur containing amino acids from oxidized sulfate, regardless compartmentalization of OAS and Cys synthesis is far from being entirely discovered. Second, plant cells form multitissue structures challenging sophisticated tasks during development. Communication between these multi-tissue structures is poorly understood and needed exploration to integrate signaling pathways in the sulfur network. Finally, regulation of sulfur metabolism in plants with respect to environmental conditions, like abiotic stresses and interactions with organisms/pathogens is still poorly studied. All these points have to be analyzed in more detail to develop new strategies for improvement of nutritional value and stress resistance of crops plants.

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