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Molecular and biochemical analysis of the enzymes of cysteine biosynthesis in the plant *Arabidopsis thaliana*

Review Article

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Summary. Among the amino acids produced by plants cysteine plays a special role as a mediator between assimilatory sulfate reduction and provision of reduced sulfur for cell metabolism. Part of this characteristic feature is the presence of cysteine synthesis in plastids, mitochondria and cytosol. Plants are the major source of reduced sulfur for human and animal nutrition. Cysteine biosynthesis deserves special attention, since reduced sulfur is channelled from cysteine into many sulfur-containing compounds in food and feed. Recent investigations are reviewed that focus on structure and regulation of cysteine synthesis in the model plant *Arabidopsis thaliana*. These data indicate that cysteine synthesis is not just an intermediate reaction step but that it is part of a regulatory network that mediates between inorganic sulfur supply and the demand for reduced sulfur during plant growth and in response to environmental changes.

Keywords: Sulfur amino acids – Plant biotechnology – Metabolic regulation

Abbreviations: Bsas, β-substituted alanine synthase; CAS, β-cyanoalanine synthase; OAS, O-acetylserine; OAS-TL, O-acetylserine (thiol) lyase; SAT, serine acetyltransferase

Sulfate assimilation and cysteine synthesis

The synthesis of cysteine represents the incorporation of reduced inorganic sulfur into the first stable organic compound. Its significance for autotrophic organisms is comparable to the fixation of ammonia by glutamine synthetase. Cysteine biosynthesis takes place in a two-step process: serine and acetyl-coenzyme A are condensated by serine acetyltransferase (SAT; EC 2.3.1.30) to form O-acetylserine (OAS). This intermediate reacts with sulfide to form cysteine by the action of OAS (thiol) lyase (OAS-TL; also termed cysteine

synthase; EC 4.2.99.8). Cysteine is the only sulfide donor for all reduced sulfur containing cell constituents in plants. It plays an essential role in both structural and catalytic respect in proteins and together with iron is indispensable for electron transfer reactions in photosynthesis and respiration. The reactivity of their nucleophilic thiol group makes cysteine, methionine, glutathione, coenzyme A and vitamins such as biotin, lipoic acid and thiamine very versatile molecules in intermediary metabolism. The biochemical position of cysteine synthesis is thus located at the end of sulfate assimilation reaction sequence and at the beginning of numerous downstream biosynthetic pathways. Assimilatory sulfate reduction begins after sulfate uptake with the activation of sulfate by ATP and is followed by two reduction steps that together transfer 8 electrons to form free sulfide. The catalysing enzymes are ATP sulfurylase, adenosine 5′-phosphosulfate reductase and sulfite reductase (see Hawkesford and Wray, 2000; Saito, 2000; Leustek et al., 2000; Hoefgen et al., 2001, for recent reviews). The sulfate assimilation pathway has only recently been entirely elucidated and the use of *Arabidopsis thaliana* as a model was instrumental for cloning of all the genes of the pathway leading from sulfate uptake to cysteine and methionine synthesis. *Arabidopsis* is consequently the best investigated plant with respect to sulfur metabolism at the molecular level. The following sections will thus focus on cysteine synthesis in this plant and review recent progress in cellular, genomic, structural and regulatory aspects of cysteine synthesis.

Cellular organisation

Cell fractionation studies from several plant species indicate that SAT and OAS-TL activities are present in plastids (chloroplasts as well as non-green plastids), the cytosol and mitochondria (Lunn et al., 1990; Rolland et al., 1992; Kuske et al., 1996). Although there is hardly any evidence for the subcellular localization of the *Arabidopsis* cysteine synthesis enzymes based on activity assays, there is evidence obtained by fusion proteins or tagging for three SAT isoforms (Noji et al., 1998) and the mitochondrial OAS-TL (Hesse et al., 1999), suggesting that the principal distribution of isoforms may be similar in plant species.

However, the relative distribution of SAT and OAS-TL activities between the three compartments appears to be quite uneven. Pea leaves are the only source in which both enzyme activities have been localized simultaneously (Ruffet et al., 1995): About 90% of SAT and 25% OAS-TL activity were found in sediments after differential centrifugation, i.e. in organelles, and the rest in the supernatants representing the cytosol. Within the sediment again about 90% of SAT activity was attributed to mitochondria but more than 80% of OAS-TL activity was found in chloroplasts. While OAS-TL activity is in excess in all compartments on a per protein basis, its ratio to SAT activity varies between 4 in mitochondria and 200 to 300 in cytosol and chloroplasts, respectively. These conditions may undergo developmental changes, since the localization of one SAT isoform was

reported to vary between plastids and cytosol depending on leaf age in *Arabidopsis* (Noji et al., 1998).

In the few cases where isoforms for the same enzymatic reaction are distributed between more than two subcellular compartments, the individual functions could be assigned, e.g. for aspartate amino transferase (Schultz et al., 1998). In contrast, the roles of the three cysteine synthesis pools are currently unclear. It has been suggested that cysteine may be impermeable for plant endomembranes (Lunn et al., 1990), but this seems unlikely since all other proteinogenic amino acids are supposed to be synthesized in plastids and exported into the cytoplasm. Transport of cysteine across the plasmalemma is well known and an amino acid permease with preference for cysteine has recently been cloned from *Vicia faba* (Miranda et al., 2001). An alternative function for cytosolic and mitochondrial cysteine synthesis could be either recycling of reduced sulfur from catabolic sources or capturing of sulfide that potentially leaks out of plastids in form of membrane-permeable H_2S. It is noteworthy in this context that so far only evidence for the last step of methionine synthesis in the cytosol is available. Subcellular localization experiments showed no association of methionine synthase activity with plastids (Wallsgrove et al., 1983) and all plant cDNAs encoding methionine synthase lack any indication of a transit peptide (Eichel et al., 1995; Hesse et al., 2001). If these observations hold true, one would have to conclude that methionine or an equivalent of it must be imported into plastids. Taken together, almost nothing is known to date about communication, cooperation and contribution of the different cellular sites for cysteine synthesis.

Serine acetyltransferase

The analysis by the Arabidopsis Genome Initiative (2000) reveals five SAT genes (Fig. 1). Each localizes to one of the five *Arabidopsis* chromosomes and for practical reasons it is suggested to classify the *Arabidopsis* SATs with respect to their chromosomal location, in order to avoid confusion rising from the often redundant names of the published cDNA clones. In this nomenclature the gene of SAT1 (AT1g55920) is located on chromosome 1 and corresponds to the cDNAs SAT1 (Murillo et al., 1995; EMBL accession number L42212), SAT5 (= SAT3, Ruffet et al., 1995; Z34888) and was later named SAT B (Bogdanova and Hell, 1997) and SAT-p (plastid localization according to Noji et al., 1998). The corresponding cDNA of gene *SAT2* (AT2g17640) has been cloned as SAT106 (Howarth et al., 1999, cited in EMBL accession AF112303). Gene *SAT3* (AT3g13110) is represented by cDNAs SAT1–6 (Bogdanova et al., 1995; X82888), SAT-A (Hell and Bogdanova, 1995), SAT-1 (Roberts et al., 1996; U22964) and was additionally labelled SAT-m (mitochondrial localization, Noji et al., 1998). The cDNA SAT-52 that corresponds to the *SAT5* gene (AT5g56760) was first cloned by Howarth et al. (1997; U30298) and later termed SAT C (Bogdanova and Hell, 1997) and SAT-c (cytosolic isoform, Noji et al., 1998). The *SAT4* gene (AT4g35640) is the only family member that has not been verified by a

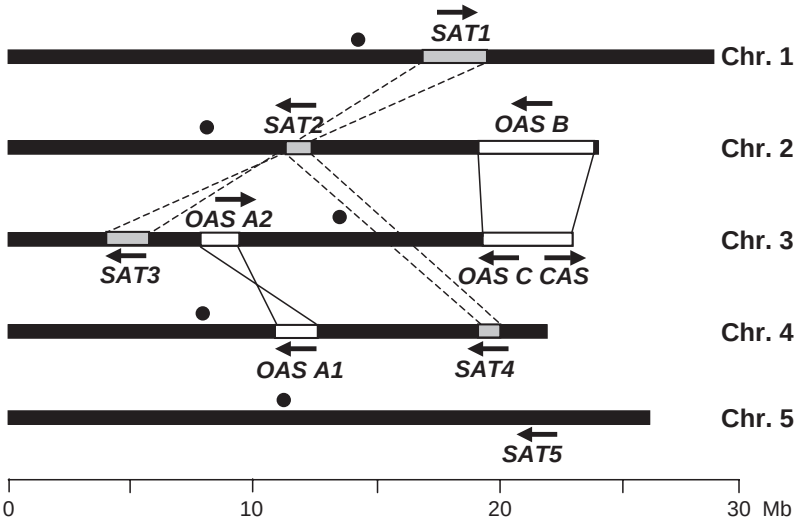

Fig. 1. Chromosomal localization of cysteine synthesis genes encoding SAT and OAS-TL. Circles indicate centromer regions; open and grey boxes represent duplicated chromosomal segments and arrows describe the orientation of the genes according to the Arabidopsis Genome Initiative (2000). Relative chromosome sizes are shown in megabases (*Mb*)

functional assay, e.g. by complementation using a SAT deficient *cysE* mutant strain from *E. coli*. Consequently, no cDNA has been cloned and to date no expressed sequence tag for the gene is available in the database. Little is known about the *SAT2* gene product that is only identified by the complementation test of cDNA SAT106 (Acc. AF112303). The presence of introns is quite variable among the *Arabidopsis* SAT genes, ranging from none (*SAT1* and *SAT3*), to one (*SAT5*), and to 7 or 8 introns (*SAT4* and *SAT2*). Also the putative promoter regions of the five genes are quite divergent.

Expression studies using Northern hybridisation of the *SAT1*, *SAT3* and *SAT5* genes indicate semi-constitutive patterns in leaf and root as may be expected from a housekeeping gene. Sulfate deprivation results in no more than two-fold increases of mRNA levels of the *SAT3* and *SAT5* genes but not of *SAT1* (Bogdanova et al., 1995, Roberts et al., 1996, Takahashi et al., 1997). A strong expression of the *SAT1* gene was observed by in-situ hybridization in leaf trichomes. The mRNA contents in this specialized cell type were down regulated by salt stress (see also OAS-TL section; Gutierrez-Alcala et al., 2000).

Precise kinetic data on SAT enzymatic properties are still scarce, due to the low abundance, the notorious lability of the protein and its tight interaction with OAS-TL. In all cases studied so far except one (Kuske et al., 1994) SAT from plant tissue was associated with OAS-TL and no free SAT activity was found (Nakamura et al., 1987; Nakamura and Tamura, 1990; Droux et al., 1992). Only one study analysed a homogeneous and native SAT fraction: spinach SAT had been enriched 300.000-fold from the stroma of

chloroplasts (Ruffet et al., 1994). This protein displayed a molecular mass of 33 kD and apparent K_m values of 2.4 mM for serine and 0.35 mM for acetyl-coenzyme A. In the absence of OAS-TL the SAT activity was very labile in this study. Interestingly, a 400-fold excess of OAS-TL activity was required to achieve the maximum rate of SAT activity. This ratio fits well to the observed activity relations in isolated chloroplasts (Ruffet et al., 1994 and 1995) and suggests that interaction of the protein with OAS-TL is important for catalytic activity. Most other recent studies refer to purified recombinant SATs from *Arabidopsis*. Droux et al. (1998) analysed the putative plastid SAT1 protein very carefully and reported quite similar K_m values of 2.8 mM for serine and 0.9 mM for acetyl-coenzyme A for the free protein. The same protein had increased affinities for both substrates (K_m of 1.0 mM for serine and 0.34 mM for acetyl-coenzyme A) when associated with OAS-TL, similar to what has been described for SAT from *Salmonella typhimurium* (Kredich et al., 1969). Comparable values were also obtained for *SAT1*, *SAT3* and *SAT5* in the presence of bacterial or plant OAS-TL (Wirtz et al., 2001; Noji et al., 1998).

The feedback inhibition of bacterial SAT by cysteine (K_i = 1.1 μM) is generally regarded as a crucial control element of cysteine regulation (reviewed by Kredich, 1996). The cytosolic SAT5 protein from *Arabidopsis* showed almost comparable sensitivity to cysteine with an apparent non-competitive K_i of 11 μM for serine and a competitive K_i of 7 μM for acetyl-coenzyme A. Domains responsible for inhibition have been mapped to the amino- and carboxy-terminal ends of SAT using domain swapping and site-directed mutagenesis (Inoue et al., 1999). Interestingly, the putative plastid and mitochondrial SAT isoforms were not inhibited at all under these conditions. This combination of subcellular localization and feedback-sensitivity of *SAT1*, *SAT3* and *SAT5* was integrated into an elegant model that explains the signal transduction between external sulfate supply, activation of cytosolic SAT and de-repression of nuclear- encoded sulfate transporter genes (Saito, 2000). In this hypothesis the sensitivity of the cytosolic isoforms constitutes a specific mechanism of control for the formation of the reaction intermediate OAS that had previously been shown to trigger the induction of genes of sulfate transporters in the plasmalemma of root cells (Smith et al., 1997; Hawkesford and Smith, 1997).

The quaternary structure of plant SAT was only determined in association with OAS-TL and assumed to form a homotetramer that interacts with two homodimers of OAS-TL. Similar to *S. typhimurium*, the plant cysteine synthase complex was estimated to have an apparent molecular mass of 310 to 350 kD. However, recent analysis of SAT from *E. coli* in the absence of OAS-TL indicated a dimer of trimers (Hindson et al., 2000). No three-dimensional structure of any SAT is available, but a computational model of the carboxy-terminus of *SAT3* could be generated, because SAT belongs to a family of acyl- and acetyltransferases with a repeated hexapeptide motif annotated in PROSITE. The model showed a prism-like arrangement of parallel β-sheets (Wirtz et al., 2001) that might support the homotrimer arrangement described for the bacterial enzyme. The carboxy-terminal domain was bifunctional with respect to protein-protein interaction with OAS-TL and transferase activity

as demonstrated by deletion analysis and site-directed mutagenesis. Using the yeast two-hybrid system, the *SAT3* protein was shown to contain a second protein interaction domain for SAT homomer formation. Disruption of the SAT/OAS-TL interaction additionally resulted in total loss of catalytic SAT activity, suggesting that complex formation with OAS-TL is a prerequisite for SAT function (Bogdanova et al., 1997; Wirtz et al., 2001).

O-acetylserine (thiol) lyase

The cellular compartmentation of cysteine synthesis is reflected by at least 11 database entries for full-length cDNAs assigned as OAS-TLs. The analysis of the encoded proteins based on primary sequence information is difficult. First, OAS-TLs belong to a large superfamily of pyridoxal-phosphate enzymes, in particular to the subfamily of β-substituted alanine synthases (Bsas; Hatzfeld et al., 2000). Second, OAS-TLs are capable of catalysing a partial backward reaction that results in the release of sulfide (cysteine + cyanide $\rightarrow$ β-cyanoalanine + sulfide). The same reaction is catalyzed by β-cyanoalanine synthase (CAS; EC 4.4.1.9), a structurally very similar enzyme that has been characterized in several plant species. It is thought to act in detoxification of cyanide that is generated e.g. during ethylene biosynthesis. The enzymology is further complicated because CAS can catalyse the cysteine synthesis reaction as well. OAS-TL and CAS enzymes can be distinguished by high specific activities for their preferred reaction, a significant substrate inhibition of CAS, but not OAS-TL, by cyanide, and a pH optimum of pH 10 for CAS (Warrilow and Hawkesford, 1998, 2000). Using this biochemical distinction it was demonstrated that a cDNA from spinach (CS-C; D37963) that had originally been assigned as the mitochondrial OAS-TL (Saito et al., 1994) in fact encodes a CAS-type enzyme. According to these studies spinach leaves would contain no mitochondrial OAS-TL (Warrilow and Hawkesford, 1998, 2000). The same cannot be excluded for other plant species as well; in earlier localization experiments only a small percentage of total OAS-TL activity was found in mitochondria and the OAS-TL reactions had been determined without regard to CAS properties (Lunn et al., 1990; Rolland et al., 1992; Kuske et al., 1994).

Genomic analysis of the most strongly expressed OAS-TLs in *Arabidopsis* (Hell et al., 1994; Hesse et al., 1999) revealed that four closely related genes are probably responsible for most of the OAS-TL activity (Fig. 1). According to the Arabidopsis Genome Initiative (2000) the OAS-TLs are not linked to the SAT genes. The encoded proteins OAS-TL A1, OAS-TL B and OAS-TL C were shown to be enzymatically "true" cysteine synthesizing enzymes as compared to CAS kinetics (Jost et al., 2000). The *OAS A1* (At4g14880) and *OAS A2* (At3g22460) genes encode cytosolic isoforms, whereas the *OAS B* (At2g43750) and *OAS C* (At3g59760) genes encode the plastid and mitochondrial enzymes, respectively. Exon-intron structures and splice site junctions are highly conserved among these two gene pairs and to a lower extent between all four genes. In contrast, the putative promoter areas differ

considerably, suggesting specific regulatory patterns of the isogenes (Jost et al., 2000). The *OAS A1*/*OAS A2* and *OAS B*/*OAS C* gene pairs are probably results of segmental duplications within the genome as suggested by the *Arabidopsis* Genome Initiative (2000). According to these analyses, the *OAS A* precursor gene has been duplicated from chromosome 4 to chromosome 3 or vice versa due to an illegitimate recombination event. As a consequence of functional redundancy the *OAS A2* gene then apparently developed into a pseudogene that is transcribed but not translated into a functional OAS-TL protein (Jost et al., 2000). A similar evolutionary event might have occurred in the case of the OAS B gene on chromosome 2 and OAS C gene on chromosome 3. The *OAS C* gene then turned into a mitochondrial OAS-TL, probably by acquisition of 5′ sequences to form a corresponding transit peptide. The *OAS C* gene product has been shown to be a true OAS-TL with mitochondrial localisation (Jost et al., 2000; Hesse et al., 1999). An additional intrachromosomal duplication might have created the *CAS* gene (At3g61440), since it is located next to *OAS C* on chromosome 3. It encodes a mitochondrial CAS protein as demonstrated by Hatzfeld et al. (2000; ARAthBsas3;1; AJ010505) and by Yamaguchi et al. (2000; AtcysC1, AB024282), Whether the presence of a mitochondrial OAS-TL is unique for *Arabidopsis* or the Brassicaceae family in general remains to further analyses.

Evolutionary analysis separated these *OAS* genes clearly from a CAS-like group of genes (Jost et al., 2000). Interestingly, two cDNAs encoding cytosolic proteins (AtcysD1, AB024284, and cysD2, AB024283) are associated with the CAS group (Jost et al., 2000), although they appear to be even more specific for the OAS-TL reaction than OAS-TL A1, B and C (Hatzfeld et al., 2000, Yamaguchi et al., 2000). Such a clear substrate preference is unusual for the β-substituted alanine synthases in general and the recombinant CYSC1/ ARAthBsas3;1, CYSD1/ ARAthBsas4;1 and CYSD2 proteins display surprisingly different affinities for the substrates OAS and sulfide (Hatzfeld et al., 2000, Yamaguchi et al., 2000). The exact function of these two proteins as well as another OAS-TL-like cDNA (CS26; Nakamura et al., 1997; AB003041) is thus not entirely clear. With respect to substrate specificity it should be noted that purified OAS-TL-like proteins are also capable of catalysing other nucleophilic elimination reactions between OAS and other coumpounds such as pyrazole and nitriles (Ikegami and Murakoshi, 1993).

Gene expression analysis revealed semi-constitutive presence of OAS-TL mRNAs as was found for SAT isoforms. Moderate increases of mRNA accumulation of 2- to 3-fold were reported in response to sulfate starvation (Hell et al., 1994; Hesse et al., 1999; Barroso et al., 1995). Similar effects were observed in response to varied nitrogen and carbon supply, indicating that there might be some integration of metabolic events between the C, N and S assimilation pathways (Barroso et al., 1995; Hesse et al., 1999). Abiotic stress like salt and heavy metals can induce the mRNA of cytosolic OAS-TL A in leaves (cDNA Atcys-3A (X84097); Barroso et al., 1999; Dominguez-Solis et al., 2001) up to 7-fold. The stress-response appears to be mediated by abcisic acid. Moreover, the cytosolic isoform is strongly expressed in leaf trichomes

together with *SAT1* as shown by in-situ hybridisation and repressed by salt stress in these cells (Gutierrez-Alcala et al., 2000).

Catalytic properties of OAS-TLs (EC 4.2.99.8) have been determined from a range of plant species (see Ikegami and Murakoshi, 1993, for review). The affinity for OAS is reported in a range from 1 to 7 mM and from 20 to 60 μM for sulfide. Precise assays are difficult because OAS undergoes rapid chemical transition to N-acetylserine at pH values above pH 7.8. The exact pH optimum of the enzyme is thus not known. Furthermore, the equilibrium between S^{2-}, HS^- and H_2S is pH-dependent as well. It seems likely that HS^- is the actual substrate of the reaction. The affinities for both substrates appear rather high. OAS concentrations required for half-maximal reaction velocity would be in the range of quite abundant amino acids like glutamate or serine but apparently are considerably lower in plants, even under consideration of subcellular volumes (Bowsher and Towbin, 2001; Awazuhara et al., 1999; Kim et al., 1999). Sulfide concentrations in plants on the other hand are essentially not known. However, considering the potential toxicity of sulfide in a living cell they are expected to be very low. Unless unknown effectors modulate the properties of OAS-TL in vivo it has to be concluded that the enzyme works far below its maximum rate in the cell.

Active OAS-TL in plants and bacteria consists of a dimer with a molecular weight between 57 and 72 kD. The three-dimensional structure of the cysK encoded OAS-TL from *Salmonella typhimurium* has been resolved (Burkhard et al., 1998). Due to the high overall amino acid homology between plant and bacterial OAS-TLs it seems reasonable to assume similar tertiary structures. The monomer of the *Salmonella* enzyme has two domains that each consists of a central β-sheet surrounded by several α-helices. A β-sheet at the N-terminus interacts in a parallel orientation with a C-terminal β-sheet, thus covering the pyridoxal-phosphate cofactor inside the polypeptide. The interaction surfaces between the dimers are rather large and include N- as well as C-terminal parts of the monomers. This arrangement results in a tight composition of the dimer and hampers any mapping of catalytic or interaction domains by deletion analysis (O. Berkowitz, R. Hell, unpublished). The binding of the substrate OAS results in a drastic conformational change of the dimer that reduces the accessibility of the catalytic center to allow only binding of small molecules like sulfide. (Burkhard et al., 1999).

The cysteine synthase complex

The cysteine synthase complex has first been discovered in enterobacteria and had then been termed cysteine synthetase complex (see Kredich, 1996, for review). The plant cysteine synthase complex has been demonstrated for several plant species including *Arabidopsis* (Nakamura et al., 1987; Nakamura and Tamura, 1990; Droux et al., 1992; Saito et al., 1995; Bogdanova and Hell, 1997) with the exception of *Datura* (Kuske et al., 1994). The function of this metabolic protein complex has long been unclear. Work by Cook and Wedding (1977) and by Droux et al. (1998) demonstrated that the reaction

intermediate OAS is not channelled from the SAT subunit to the OAS-TL subunit in order to provide an efficient flux of substrate. In fact, OAS diffuses readily out of the complex, presumably because OAS-TL has strongly reduced affinity to OAS and sulfide when bound to SAT. As a consequence SAT apparently is only active in the complex and inactive without OAS-TL, whereas OAS-TL is almost inactive in the complex but quite active as a free homodimer (Droux et al., 1998). These results are supported by the organization of protein-protein interaction domains of SAT (Bogdanova and Hell, 1997) and the positive correlation of SAT catalytic activity with the ability to form the complex with OAS-TL (Wirtz et al., 2001). It was furthermore found that OAS could dissociate the cysteine synthase complex, at least in vitro (Kredich et al., 1969; Droux et al., 1998). Another important observation was the de-repressive effect of OAS on sulfate transporter genes as demonstrated by feeding-experiments (Smith et al., 1997; Hawkesford et al., 1997).

These findings have been combined in a hypothesis that explains the function of the cysteine synthase complex as a metabolic sensor of the sulfide status of a plant cell (Fig. 2; Hell, 1998; Hell and Hillebrand, 2001). Under conditions of sufficient sulfate supply the complex is associated, SAT forms OAS that diffuses out of the complex and reacts with sulfide through catalysis of free OAS-TL dimers. When sulfate and consequently sulfide becomes limiting the latter reaction stops and OAS accumulates. Upon a certain threshold the accumulated OAS exerts two functions: 1) OAS dissociates the complex, reducing SAT activity and thus further consumption of acetyl-coenzyme A; 2) OAS triggers the de-repression of some genes encoding

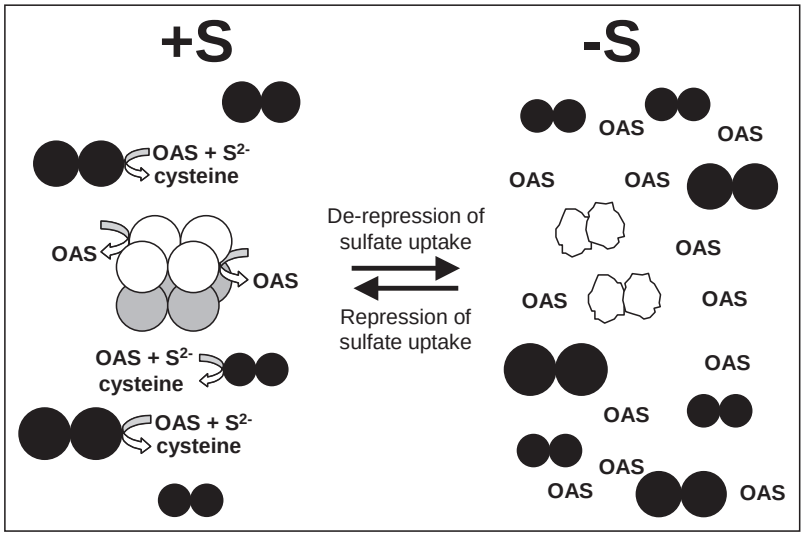

Fig. 2. Regulatory model of the cysteine synthase complex. Reversible protein-protein interaction is based on changes of OAS concentrations as a consequence of sulfur supply (*+S/−S*) of the cell. Open symbols represent SAT, dark symbols stand for OAS-TL, in the active or inactive states of the enzymes

sulfate transporters. Due to the increased affinity for sulfate uptake the process becomes reversible: sulfate is imported and reduced to sulfide that reacts with OAS via OAS-TL dimers. OAS concentrations would decline until the complex can associate again and the genes of high and low affinity sulfate transporters would be at least partially repressed. SAT can resume its activity in the complex at a rate that is adjusted to the availability of sulfide. The equilibrium of complex association and dissociation would determine the rate of OAS and thus of cysteine formation. At the same time, this equilibrium would be adjusted to the available sulfide concentration and coupled to the import of sulfate at the plasmalemma. According to this hypothesis the cysteine synthase complex functions as a metabolic sensor and part of a control system of primary sulfur metabolism at the cellular level (Hell and Hillebrand, 2001). While such a system could control the metabolite flux upstream of cysteine, the feedback inhibition of SAT might form a downstream mechanism of product control (Saito, 2000). Both regulatory models are confined to the cellular level and might form the integrating basis of source-sink interaction in a whole plant concept of sulfur metabolism.

References

Arabidopsis Genome and Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408: 796–815

Awazuhara M, Hirai M Y, Hayashi H, Chino M, Naito S, Fujiwara T (2000) O-Acetyl-L-serine content in rosette leaves of *Arabidopsis thaliana* affected by S and N nutrition. In: Brunold C (ed) Sulfur nutrition and sulfur assimilation in higher plants. P Haupt, Bern, pp 331–333

Barroso C, Vega JM, Gotor C (1995) A new member of the cytosolic O-acetylserine (thiol) lyase gene family in *Arabidopsis thaliana*. FEBS Lett 363: 1–5

Barroso C, Romero LC, Cejudo J, Vega JM, Gotor C (1999) Salt-specific regulation of the cytosolic O-acetylserine(thiol)lyase gene from *Arabidopsis thaliana* is dependent on abscisic acid. Plant Mol Biol 40: 729–736

Bogdanova N, Hell R (1997) Cysteine synthesis in plants: protein-protein interactions of serine acetyltransferase from *Arabidopsis thaliana*. Plant J 11: 251–262

Bogdanova N, Bork C, Hell R (1995) Cysteine biosynthesis in plants: Isolation and functional characterization of a cDNA encoding serine acetyltransferase from *Arabidopsis thaliana*. FEBS Lett 358: 43–47

Bowsher CG, Tobin AK (2001) Compartmentation of metabolism within mitochondria and plastids. J Exp Bot 52: 513–527

Burkhard P, Rao GS, Hohenester E, Schnackerz KD, Cook PF, Jansonius JN (1998) Three-dimensional structure of O-acetylserine sulfhydrylase from *Salmonella typhimurium*. J Mol Biol 283: 121–133

Burkhard P, Tai C, Ristroph CM, Cook PF, Jensonius JN (1999) Ligand binding induces a large conformational change in O-acetylserine sulfhydrylase from Salmonella typhimurium. J Mol Biol 291: 941–953

Cook PF, Wedding RT (1977) Initial kinetic characterization of the multienzyme complex, cysteine synthetase. Arch Biochem Biophys 178: 293–302

Dominguez-Solis JR, Gutierrez-Alcala G, Vega JM, Romero LC, Gotor C (2001) The cytosolic O-acetylserine(thiol)lyase gene is regulated by heavy metals and can function in cadmium tolerance. J Biol Chem 276: 9297–9302

Droux M, Martin J, Sajus P, Douce R (1992) Purification and characterization of O-acetylserine (thiol) lyase from spinach chloroplasts. Arch Biochem Biophys 295: 379–390

Droux M, Ruffet M-L, Douce R, Job D (1998) Interactions between serine acetyltransferase and O-acetylserine (thiol) lyase in higher plants. Structural and kinetic properties of the free and bound enzymes. Eur J Biochem 255: 235–245

Eichel J, Gonzales JC, Hotze M, Matthews RG, Schröder J (1995) Vitamin-B_{12}-independent methionine synthase from a higher plant (*Catharanthus roseus*). Molecular characterization, regulation, heterologous expression, and enzyme properties. Eur J Biochem 230: 1053–1058

Gutierrez-Alcala G, Gotor C, Meyer AJ, Fricker M, Vega JM, Romero LC (2000) Glutathione biosynthesis in *Arabidopsis* trichome cells. Proc Natl Acad Sci USA 97: 11108–11113

Hatzfeld Y, Maruyama A, Schmidt A, Noji M, Ishizawa K, Saito K (2000) β-Cyanoalanine synthase is a mitochondrial cysteine synthase-like protein in spinach and *Arabidopsis thaliana*. Plant Physiol 123: 1163–1171

Hawkesford MJ, Smith FW (1997) Molecular biology of plant sulphate transporters. In: Cram WJ, De Kok LJ, Stulen I, Brunold C, Rennenberg H (eds) Sulphur metabolism in higher plants. Molecular, ecophysiological and nutritional aspects. Backhuys Publ, Leiden, pp 13–26

Hawkesford MJ, Wray JL (2000) Molecular genetics of sulphate assimilation. Adv Bot Res 33: 159–223

Hell R (1998) Molecular physiology of primary sulfur metabolism in plants. Shaker Publ, Aachen

Hell R, Bogdanova N (1995) Characterization of a full-length cDNA encoding serine acetyltransferase from *Arabidopsis thaliana*. Plant Physiol 109: 1498

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

Hell R, Bork C, Bogdanova N, Frolov I, Hauschild R (1994) Isolation and characterization of two cDNAs encoding for compartment specific isoforms of O-acetylserine (thiol) lyase from *Arabidopsis thaliana*. FEBS Lett 351: 257–262

Hesse H, Lipke J, Altmann T, Höfgen R (1999) Molecular cloning and expression analyses of mitochondrial and plastidic isoforms of cysteine synthase (O-acetylserine(thiol)lyase) from *Arabidopsis thaliana*. Amino Acids 16: 113–131

Hesse H, Kreft O, Maimann S, Zeh M, Willmitzer L, Höfgen R (2001) Approaches towards understanding methionine biosynthesis in higher plants. Amino Acids 20: 281–289

Hindson VJ, Moody PCE, Rowe AJ, Shaw WV (2000) Serine acetyltransferase from *Escherichia coli* is a dimer of trimers. J Biol Chem 275: 461–466

Höfgen R, Kreft O, Willmitzer L, Hesse H (2001) Manipulation of thiol contents in plants. Amino Acids 20: 291–299

Howarth JR, Roberts MA Wray JL (1997) Cysteine biosynthesis in higher plants: a new member of the *Arabidopsis thaliana* serine acetyltransferase small gene-family obtained by functional complementation of an *Escherichia coli* cysteine auxotroph. Biochim Biophys Acta 1350: 123–127

Ikegami F, Murakoshi I (1993) Heterocyclic β-substituted alanines. In: Lea PJ (ed) Enzymes of secondary metabolism, vol 9. Academic Press, London, pp 133–151

Inoue K, Noji M, Saito K (1999) Determination of the sites required for the allosteric inhibition of serine acetyltransferase by L-cysteine in plants. FEBS Lett 266: 220–227

Jost R, Berkowitz O, Wirtz M, Smith L, Hawkesford MJ, Hell R (2000) Genomic and functional characterization of the *oas* gene family encoding O-acetylserine (thiol) lyases, enzymes catalyzing the final step in cysteine biosynthesis in *Arabidopsis thaliana*. Gene 253: 237–247

Kim H, Hirai MY, Hayashi H, Chino M, Naito S, Fujiwara T (1999) Role of O-acetyl-l-serine in the coordinated regulation of the expression of a soybean seed storage-protein gene by sulfur and nitrogen nutrition. Planta 209: 282–289

Kredich NM (1996) Biosynthesis of cysteine. In: Neidhardt FC, Curtiss R, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umberger E (eds) *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology. ASM Press, Washington DC, pp 514–527

Kredich NM, Becker MA, Tomkins GM (1969) Purification and characterization of cysteine synthetase, a bifunctional protein complex, from *Salmonella typhimurium*. J Biol Chem 244: 2428–2439

Kuske CR, Thicknor LO, Guzman E, Gurley LE, Valdez JG, Thompson ME, Jackson PE (1994) Purification and characterization of O-acetylserine sulfhydrylase isoenzymes from *Datura innoxia*. J Biol Chem 269: 6223–6232

Kuske CR, Hill KK, Guzman E, Jackson PJ (1996) Subcellular location of O-acetylserine sulfhydrylase isoenzymes in cell cultures and plant tissues of *Datura innoxia* mill. Plant Physiol 112: 659–667

Leustek T, Martin MN, Bick J-A, Davies JP (2000) Pathways and regulation of sulfur metabolism revealed through molecular studies. Annu Rev Plant Physiol Plant Mol Biol 51: 141–166

Lunn JE, Droux M, Martin J, Douce R (1990) Localization of ATP-sulfurylase and O-acetylserine(thiol)lyase in spinach leaves. Plant Physiol 94: 1345–1352

Miranda M, Borisjuk L, Tewes A, Heim U, Sauer N, Wobus U, Weber H (2001) Amino acid permeases in developing seeds of *Vicia faba* L.: expression precedes storage protein synthesis and is regulated by amino acid supply. Plant J 28: 61–71

Murillo M, Foglia R, Diller A, Lee S, Leustek T (1995) Serine acetyltransferase from *Arabidopsis thaliana* can functionally complement the cysteine requirement of a *cysE* mutant strain of *Escherichia coli*. Cell Mol Biol Res 41: 425–433

Nakamura K, Tamura G (1990) Isolation of serine acetyltransferase complexed with cysteine synthase from *Allium tuberosum*. Agr Biol Chem 54: 649–656

Nakamura K, Hayama A, Masada M, Fukushima K, Tamura G (1987) Measurement of serine acetyltransferase activity in crude plant extracts by a coupled assay system using cysteine synthase. Plant Cell Physiol 28: 885–891

Nakamura T, Koizumi N, Sano H (1997) Isolation of a novel cysteine synthase cDNA (AB003041) from *Arabidopsis thaliana*. Plant Physiol 114: 747

Noji M, Inoue K, Kimura N, Gouda A, Saito K (1998) Isoform-dependent differences in feedback regulation and subcellular localization of serine-acetyltransferase involved in cysteine biosynthesis from *Arabidopsis thaliana*. J Biol Chem 273: 32739–32745

Roberts MA, Wray JL (1996) Cloning and characterisation of an *Arabidopsis thaliana* cDNA clone encoding an organellar isoform of serine acetyltransferase. Plant Mol Biol 30: 1041–1049

Rolland N, Droux M, Douce R (1992) Subcellular distribution of O-acetylserine (thiol)lyase in cauliflower (*Brassica oleracea* L.) inflorescence. Plant Physiol 98: 927–935

Ruffet M-L, Droux M, Douce R (1994) Purification and kinetic properties of serine acetyltransferase free of O-acetylserine(thiol)lyase from spinach chloroplasts. Plant Physiol 104: 597–604

Ruffet M-L, Lebrun M, Droux M, Douce R (1995) Subcellular distribution of serine acetyltransferase from *Pisum sativum* and characterization of an *Arabidopsis thaliana* putative cytosolic isoform. Eur J Biochem 227: 500–509

Saito K, Tatsuguchi K, Takagi Y, Murakoshi I (1994) Isolation and characterization of cDNA that encodes a putative mitochondrion-localized isoform of cysteine synthase (O-acetylserine(thiol)-lyase) from *Spinacia oleracea*. J Biol Chem 269: 28187–28192

Saito K, Yokoyama H, Noji M, Murakoshi I (1995) Molecular cloning and characterization of a plant serine acetyltransferase playing a regulatory role in cysteine biosynthesis from watermelon. J Biol Chem 270: 16321–16326

Schultz CJ, Coruzzi GM (1995) The aspartate aminotransferase gene family of *Arabidopsis* encodes isoenzymes localized to three distinct subcellular compartments. Plant J 7: 61–75

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

Takahashi H, Yamazaki M, Sasakura N, Watanabe A, Leustek T, de Almeida Engler J, Engler G, van Montagu M, 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 Adad Sci USA 94: 11102–11107

Takahashi H, Watanabe-Takahashi A, Smith FW, Blake-Kalff M, Hawkesford MJ, 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

Wallsgrove RM, Lea PJ, Miflin BJ (1983) Intracellular localization of aspartate kinase and the enzymes of threonine and methionine biosynthesis in green leaves. Plant Physiol 71: 780–784

Warrilow AGS, Hawkesford MJ (1998) Separation subcellular location and influence of sulphur nutrition on isoforms of cysteine synthase in spinach. J Exp Bot 49: 1625–1636

Warrilow AGS, Hawkesford MJ (2000) Cysteine synthase (O-acetylserine (thiol) lyase) specificities classify the mitochondrial isoform as a cyanoalanine synthase. J Exp Bot 51: 985–993

Wirtz M, Berkowitz O, Droux M, Hell R (2001) The cysteine synthase complex from plants: mitochondrial serine acetyltransferase from *Arabidopsis thaliana* carries a bifunctional domain for catalysis and protein-protein interaction. Eur J Biochem 268: 686–693

Yamaguchi Y, Nakamura T, Kusano T, Sano H (2000) Three *Arabidopsis* genes encoding proteins with differential activities for cysteine synthase and β-cyanoalanine synthase. Plant Cell Physiol 41: 465–476

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