Chapter 4

Metabolism of Cysteine in Plants and Phototrophic Bacteria

Rüdiger Hell* and Markus Wirtz
Department of Molecular Biology of Plants, Heidelberg Institute of Plant Sciences, University of Heidelberg, Im Neuenheimer Feld 360, D-69120 Heidelberg, Germany

Summary

The sulfur amino acids cysteine and methionine function in many basic and essential processes of life. For cysteine this includes structural, catalytic, regulatory and metabolic functions. The special redox chemistry of sulfur and the thiol group in particular proved to be a versatile tool during evolution, not the least in electron transport processes in association with iron. Plants are primary producers and carry out assimilatory sulfate reduction to first synthesize cysteine that subsequently forms the backbone for methionine formation. This reaction sequence seems to be conserved in all phototrophic organisms. The position of cysteine biosynthesis between assimilation of inorganic sulfate and metabolization of organic

* Corresponding author, Phone: +49 6221 54 6284, Fax: +49 6221 54 5859, E-mail:rhell@hip.uni-hd.de

Rüdiger Hell et al. (eds.), Sulfur Metabolism in Phototrophic Organisms, 59–91.

sulfide makes it a prime target for coordination of both complex processes. It is thus a mediator between supply and demand in sulfur metabolism of a cell.

Much attention has been paid to cysteine biosynthesis in plants, while less is known about the pathway in algae, cyanobacteria and purple bacteria. Recent evidence indicates that the two enzymes of cysteine synthesis, serine acetyltransferase and *O*-acetylserine-(thiol)-lyase are highly conserved between these groups and, at least in plants, form a reversible protein complex. This so-called cysteine synthase complex has been suggested to act as sensor for sulfide in cells and to be part of a regulatory loop that maintains cysteine homeostasis between sulfate reduction and cysteine consumption. Kinetic studies of the properties of the enzymes together with structural modelling of the proteins in the cysteine synthase complex as well studies using transgenic plants strongly support this unique regulatory system in plants.

The degradation of cysteine is still an under-investigated subject. Possible alternative routes of thiol transfer and sulfide release are compiled here and discussed with respect to their putative functions in S-transfer reactions, cysteine degradation, detoxification reactions and iron-sulfur cluster biosynthesis.

I. Introduction

Cysteine biosynthesis in phototrophic organisms represents the process of integration of reduced sulfur produced by assimilatory sulfate reduction into the organic form of an amino acid. In contrast, some organisms including mammals and some fungi, first assimilate reduced sulfur into methionine and then synthesize cysteine via transsulfurylation (Hell, 1997; Wirtz and Droux, 2005; see chapter by Höfgen and Hesse, this book). Thus, primary producers in general first add reduced sulfur to activated serine, i.e. *O*-acetylserine, as acceptor. In a second step sulfide replaces acetate and cysteine is released. From cysteine all downstream products including methionine, cofactors (coenzyme A, biotin, thiamine, lipoic acid), iron-sulfur clusters and many other compounds are produced (Hell, 1997; Beinert, 2000). The importance of the fixation of reduced sulfur in phototrophic organisms is therefore comparable to the assimilation of reduced nitrogen by the glutamine synthetase/glutamate-oxo-glutarate-aminotransferase system. In plants, algae and most bacteria, as far as the latter have been investigated in this respect, the synthesis of cysteine occurs in a two-step reaction sequence catalyzed by two enzymes: Serine acetyltransferase (SAT; also termed serine transacetylase; EC 2.3.1.30) and *O*-acetylserine-(thiol)-lyase (OAS-TL; EC 2.5.1.47; common synonyms are *O*-acetylserine sulfhydrylase and cysteine synthase). The biochemical pathway of cysteine synthesis was first described in an enzymological sense by the landmark work of N. M. Kredich and coworkers using *Salmonella typhimurium* (Kredich and Tomkins, 1966). They designated the SAT encoding gene as *cysE* and the OAS-TL encoding genes *cysK* and *cysM* and discovered the protein association between SAT and OAS-TL, the cysteine synthase complex (CSC; Kredich et al., 1969).

Abbreviations: CAS – β-cyanoalanine synthase; CDes – cysteine desulfhydrase; IC50 – 50% inhibition constant; NAS – N-acetylserine, OAS – O-acetylserine; OAS-TL – O-acetylserine (thiol) lyase; PLP – pyridoxal 5′-phosphate; SAT - serine acetyltransferase

II. Cysteine Synthesis in Prokaryotes

Most prokaryotes use the two-step pathway encoded by *cysE*-like and *cysK*/*M*-like genes for cysteine biosynthesis according to genome databases, but exceptions exist, for instance in the genus Bacillus and probably also other Taxa. Even archaebacteria from habitats that are rich in reduced sulfur rely on these genes and have been shown to encode functional gene products similar to prokaryotes (Kitabatake et al., 2000).

A. The Enterobacteria Regulatory System

Cysteine synthesis is best investigated in *S. typhimurium* and *E. coli* with respect to structural components and regulation (Kredich, 1996) and forms the basis for investigation of the process in phototrophic organisms. Cysteine synthesis in enterobacteria is part of the cys-regulon that comprises structural genes for sulfate uptake, reduction and cysteine synthesis. Uptake of sulfate is mediated by a sulfate permease, which is also the case in cyanobacteria. It is a multisubunit ABC-type transport system that differs structurally

completely from the sulfate/proton cotransporters found in plants (see chapter by Hawkesford in this book). Interestingly, sequence similarity suggests a relationship to the sulfate transport systems (*SulP*) in the chloroplast envelope of the green alga *Chlamydomonas* and the moss *Marchantia* (Chen et al., 2003; Melis and Chen, 2005). Such sequences are, however, missing in the genomes of vascular plants according to databases and seem to have been lost during evolution of higher photoautotrophs. The assimilatory reduction pathway proceeds via 5′-adenosine-phosphosulfate (APS), 3′-phosphoadenosine-phosphosulfate (PAPS), sulfite and sulfide, most likely without any bound intermediate despite the instability and potential toxicity of these intermediates. The biochemical functions and evolution of the central enzyme in this pathway, assimilatory (P)APS reductase are reviewed in this book (see chapter by Kopriva et al.). SAT activity requires acetyl-coenzyme A (acetyl-CoA) and serine to form *O*-acetylserine (OAS) as energetically activated form of serine. OAS-TL activity is typically encoded by two genes: *cysK* and *cysM*. The *cysK* gene product uses OAS and sulfide to form cysteine (type A OAS-TL), while the *cysM* product also accepts thiosulfate as substrate (type B OAS-TL). The reaction between *O*-acetylserine and thiosulfate produces *S*-sulfocysteine, which is converted into cysteine by a so far uncharacterized mechanism. It has been proposed that the *cysM* gene is only effectively operating under anaerobic growth conditions (Kredich, 1996).

Since enterobacteria are usually well supplied with reduced sulfur or even sulfur amino acids from their environment, it is just consequent that the sensing of free cysteine concentrations is at the center of the cys-regulon, taking care that the energy consuming uptake and reduction of inorganic sulfate is only activated in situations where the cellular demand for cysteine exceeds external availability. The regulation is based on *cysE*, the only gene with constitutive expression in the regulon. The encoded SAT protein has exclusively been found in association with OAS-TL and is highly sensitive to feedback inhibition by cysteine (K_I = 1.1 μM; Kredich et al., 1969). Thus, in the presence of sufficient free cysteine in the cell SAT activity is shut down and hardly any OAS is produced. If cysteine or sulfide levels drop SAT begins to form OAS, the substrate for OAS-TL. However, lack of sulfide results in accumulation and chemical disproportionation of OAS to *N*-acetylserine (NAS). NAS, in turn, is an activator for CysB, a transcriptional activator of promoters of the cys-regulon genes. Activation of sulfate uptake and reduction brings back sulfide into the cell that is consumed by OAS-TL to form cysteine. As soon as cysteine levels reach sufficient concentrations, SAT is feedback inhibited and lack of OAS results in shut-down of the regulon. The cys-regulon represents a typical repressor/operator mechanism of prokaryotic gene regulation (reviewed by Kredich, 1996). It seems evident that phototrophic bacteria developed at least partly different regulatory mechanisms due to their lifestyle.

B. Phototrophic Bacteria

Cysteine synthesis in bacteria with oxygenic photosynthesis is much less investigated compared to enterobacteria. The cyanobacterium *Synechococcus* sp. PCC 7942 (formerly *Anacystis nidulans*) contains three genes involved in cysteine synthesis. Two are encoded on an endogenous plasmid and are transcriptionally regulated by sulfate availability. The gene *srpG* encodes a type A OAS-TL whereas *srpH* encodes SAT. Both genes show significant sequence homology to the corresponding *E. coli* genes and are able to functionally complement *E. coli cysK cysM* and *cysE* mutant strains that are auxotrophic for cysteine. DNA hybridization indicates a second copy of srpG in *Synechococcu*s sp. PCC 7942, possibly encoding for a type B OAS-TL enzyme (Nicholson et al., 1995; Nicholson and Laudenbach, 1995). Sulfate activation and reduction proceed by the same enzymes in cyanobacteria and enterobacteria (Niehaus et al., 1992). However, the only known regulatory protein in cyanobacterial sulfur metabolism, CysR, appears not be functionally equivalent to cysB in enterobacteria and thus its role in cysteine synthesis is much less defined. CysR expression itself is regulated by sulfur availability and the encoded protein is assumed to bind DNA due to a helix–loop–helix motif and similarity to other prokaryotic regulatory proteins. Inactivation of CysR results in loss of induction of numerous genes under sulfur limiting conditions. This includes the sulfate permease system (see chapter by Hawkesford, this book), a

periplasmic protein of unknown function and the *srpG* and *srpH* genes. Since CysR is not required for growth when sulfate or thiosulfate are the only sulfur sources, it may be involved in regulating growth on other sulfur-containing compounds such thiocyanate (Laudenbach et al., 1991).

An important aspect of sulfur availability for microorganisms in general from a genetic as well as an ecological point of view is the reduction of growth yield under conditions of sulfur limitation. If methionine and cysteine could be replaced in the organism's major proteins for sulfur-free amino acids, the saving in sulfur would potentially increase nutrient use efficiency and competitiveness. Sulfate permease, which is abundantly produced by sulfur-starved enterobacteria and cyanobacteria lacks Met and Cys residues. Moreover, the cyanobacterium *Calothrix* sp. PCC 7601 harbours sulfur-depleted versions of the photosynthesis-related protein phycocyanin and its auxiliary polypeptides that are specifically expressed under conditions of sulfur limitation. The elevated synthesis of these proteins positively affects the sulfur budget of these cells (Mazel and Marliere, 1989).

III. Cysteine Synthesis in Plants and Algae

A. Subcellular Compartmentation and Functional Genomic Organisation

Similar to prokaryotes, the synthesis of cysteine in plants and algae can be divided into three steps: (1) assimilatory sulfate reduction to reduced sulfide, (2) provision of carbon and nitrogen containing acceptor for sulfur, (3) fixation of reduced sulfur into the organic backbone to produce cysteine, the first stable form of bound reduced sulfur. In contrast to sulfide production and the synthesis of most amino acids in plants and algae, cysteine synthesis takes place not only in plastids but also in the cytosol and the mitochondria. Consequently, small nuclear gene families encode SAT and OAS-TL proteins that are imported into these compartments (Fig. 1). Plants and at least green algae appear to be similarly organized in this respect. *Euglena* differs fundamentally in its subcellular organization with sulfate reduction being confined to mitochondria, while little is known about the locations of cysteine synthesis. Because *Euglena* is a taxonomically distant, facultative photosynthetic organism, the reader is conferred to specialized literature (Saidha et al., 1988; Schiff et al., 1993).

The reasons for compartmentation of cysteine synthesis have been subject of speculation, but clear evidence as to its function is still missing. Lunn et al. (1990) suggested that endomembranes may be impermeable for cysteine transport, pointing out that each compartment with the capacity for protein biosynthesis would therefore require its own cysteine biosynthesis. While this hypothesis is intriguing, it seems awkward to assume that all amino acid acids can be transported into and inside the cell by broad-spectrum amino acid transporters, but not cysteine. Specialized functions in sulfur metabolism, such as assimilation in plastids, regulation in the cytosol and degradation in the mitochondria, have been suggested (Hell, 1997). However, mitochondria are the site of sulfur amino acid degradation in animals via sulfite oxidase, but this molybdoenzyme enzyme is located in the peroxisomes in plants (see chapter by Hänsch and Mendel, this book). A different possibility is provided by the chemical properties of sulfide, the endproduct of assimilation. Sulfide always exists in equilibrium between HS^- and H_2S at around neutral pH values. H_2S is believed to be membrane permeable and thus freely available to all compartments as substrate for OAS-TL (Fig. 1). The volatility of H_2S could require cysteine synthesis in the cytosol to capture this gas and avoid costly losses of reduced sulfur. In turn, mitochondria might need to be protected from H_2S in order to avoid interference with the electron transport chain or even discharge of the proton gradient by the HS^- equilibrium, similar to the toxicity of NH_3/NH_4^+. On the other hand, it should not be overlooked that mitochondria as well as chloroplasts require substantial amounts of elemental S in iron-sulfur clusters of their electron transport chains. This partially reduced oxidation state of sulfur is believed to be derived from cysteine via carrier-mediated steps (see Padmavathi et al., this book).

In contrast to such special functions the three subcellular locations could of course be the consequence of sheer redundancy. However, the targeted overexpression of an enzymatically inactive SAT in the cytosol of tobacco cells

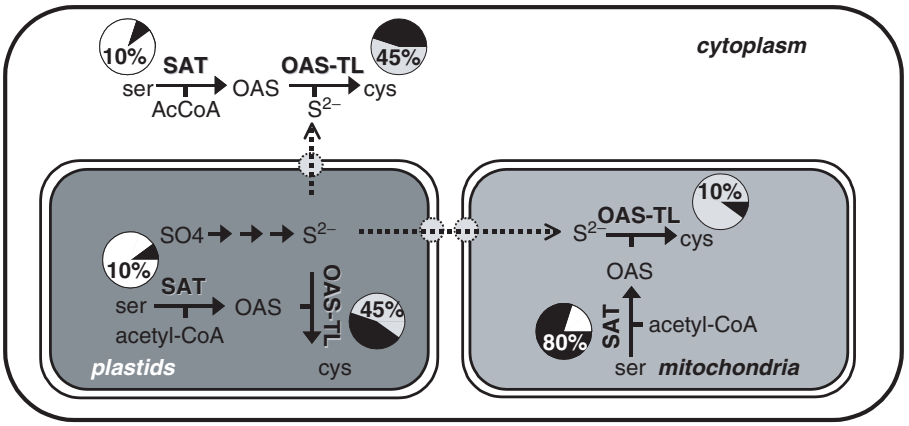

Fig. 1. Subcellular distribution of serine acetyltransferase and *O*-acetylserine(thiol)lyase activity in higher plants. The figure represents a schematic view on a higher plant cell. De novo cysteine synthesis takes place in the cytoplasm (white), the mitochondria (grey) and the plastids (dark grey). Important metabolites are depicted in the reaction pathways, reaction steps are shown as arrows. Enzymes are named above the reaction in bold and shadowed letters. In a subcellular compartment, the percentages of total SAT and OAS-TL activities are shown in white and grey pie chart diagrams, respectively. For better understanding the percentage of total activity is also indicated in the pie chart. The localisation of OAS-TL activity was revealed in spinach (Lunn et al., 1990) and confirmed in Datura innoxia (Kuske et al., 1996). Subcellular distribution of SAT was analysed in Pisum sativum (Ruffet et al., 1995). Transport of metabolites across membranes is indicated as dashed arrows.

resulted in strong changes in cysteine and glutathione contents, suggesting an important and specific role of cysteine synthesis for each compartment (Wirtz and Hell, 2007). In addition, the nearly ubiquitous and constitutive expression of mRNAs encoding SAT and OAS-TL as revealed by *Arabidopsis thaliana* gene expression profile databases (see https://www.genevestigator.ethz.ch/) supports a general requirement in all cell types under all conditions, rather than redundancy.

Contradictory to these observations, the absence of functional OAS-TL activity has been reported for mitochondria of spinach (*Spinacea oleracea* L.). Warrilow and Hawkesford (2000) showed in enzymological experiments that OAS-TL activity from spinach mitochondria in fact is the result of a side activity of β-cyanoalanine synthase. This enzyme has been found in cytosolic and/or mitochondrial compartments in several plant species; it catalyzes a partial backward reaction of OAS-TL (section V.E) and is, as OAS-TL, a structural member of the superfamily of β-substituting alanine synthases (bsas). *Arabidopsis thaliana* carries independent and true OAS-TL and β-cyanoalanine synthase enzymes in its mitochondria (Hatzfeld et al., 2000; Jost et al., 2000) and so far no other plant species has been reported with similar properties as spinach. Further genome analyses will allow to fully evaluate mitochondrial cysteine synthesis.

The role of the different compartments is further obscured by the relative distribution of enzymatic activities. These have been determined from cellular fractions for all three compartments of spinach and pea (Droux, 2003; Droux, 2004) and are complemented by a number of localization studies on either SAT or OAS-TL with other species (Lunn et al., 1990; Rolland et al., 1992; Ruffet et al., 1995; Kuske et al., 1996). In general, it appears that about 45% of OAS-TL activity is associated with plastids, 45% with the cytosol, and the remainder with mitochondria. In contrast, 80% of total SAT activity is found in mitochondria, 10% in plastids and 10% in the cytosol (Fig. 1). Thus, measurable maximal activities of the two consecutive steps of cysteine synthesis differ substantially with respect to the three subcellular locations. Unless substrate availability or unknown factors strongly affect these activities, the exchange of OAS and probably also cysteine has to be assumed between cytosol and the organelles.

B. Gene Families and Features of Gene Products

The model plant *Arabidopsis thaliana* is by far the best investigated higher organism with

respect to cysteine synthesis (Hell et al., 2002). The Arabidopsis SAT gene family consists of five members which have been named with respect to their location at the chromosomes as AtSAT1 (At1g55920, L34076), AtSAT2 (At2g17640, L78444,), AtSAT3 (At3g13110, U22964), AtSAT4 (At4g35640, AF331847) and AtSAT5 (At5g56760, U30298). Other nomenclatures from various descriptions of cDNAs confuse this organization. A unified nomenclature termed Serat for serine acetyltransferase has been suggested (Kawashima et al., 2005), but currently does not comprise all available sequences in the public databases. AtSAT2 and 4 were added very recently to the SAT gene family based upon their sequence homology and their availability to complement cysteine-auxotrophy of *E. coli* mutants lacking endogenous SAT activity (Kawashima et al., 2005). However, AtSAT2 and 4 are much less expressed than AtSAT1, 3 and 5 with respect to mRNA levels. K_M values of purified recombinant or native plant SATs range from 1 to 3 mM for serine and 0.01 to 0.3 mM for acetyl-CoA, corresponding to the assumed cellular concentrations of these molecules (Ruffet et al., 1994; Noji et al., 1998; Wirtz et al., 2000; Wirtz et al., 2001). To a very limited extent, also propionyl-CoA, but not buthionyl-CoA, may be accepted as a substrate (R. Hell, unpublished). In contrast, the affinities of AtSAT2 and AtSAT4 are 10 to 100-fold lower for both substrates. These properties cast some doubts on a significant contribution of these isoforms to cysteine synthesis (Kawashima et al., 2005).

The localization of all Arabidopsis SAT isoforms has been verified using SAT-GFP-fusion proteins. AtSAT2, 4 and 5 are located in the cytosol and AtSAT1 and AtSAT3 are targeted to plastids and mitochondria (Sun et al., 2004; Kawashima et al., 2005). The localization of AtSAT1 appears not entirely clear due to a report with variable development-dependent plastid or cytosolic localization (Noji et al., 1998). It should be mentioned that AtSAT1 protein was found in association with the nucleus, in nuclear proteome analysis (Bae et al., 2003). However, SAT proteins are of low abundance, as shown by biochemical purification of SAT from spinach chloroplasts (Ruffet et al., 1994). Therefore, immuno-localisation with isoform-specific antibodies might be required to confirm the localization of AtSATs.

Activities and isoforms of SATs have been described for subcellular compartments of plants of different taxonomy. This refers to a plastid SAT isoform from spinach (SAT56; (Noji et al., 2001b) as well as cDNAs encoding cytosolic (SAT7) and organelle-localized (SAT1) SATs from tobacco (Wirtz and Hell, 2003a). In pea and in spinach, cytosolic, chloroplastic and mitochondrial SAT activities were characterized (Droux, 2003; Droux, 2004).

With respect to algae, only a plastid-localized SAT and OAS-TL have been reported for *Chlamydomonas reinhardtii*. Both genes are strongly upregulated in response to sulfur deficiency (Ravina et al., 2002). The availability of complete genome sequences of algae of distant taxonomic origin including *C. reinhardtii* (greenalgae), *Thalassiosira pseudonana* (a diatom) *Cyanidioschyzon merolae* (unicellular red algae) will allow detailed comparisons of the evolutionary origin and compartmental organization of genes of cysteine synthesis. *C. reinhardtii* probably carries three SAT and four OAS-TL encoding genes. The effect of upregulation depends on the presence of an active *Sac1* gene (Ravina et al., 2002). The *sac* mutants of *C. reinhardtii* are described in detail in this book (see chapter Shibagaki and Grossman). Sac1 shows similarity to a Na^+/SO_4^{2-} cotransporters and may function as a sulfate sensor. Many, but not all transcripts related to sulfur assimilation depend on Sac1 for their expression (Zhang et al., 2004).

OAS-TL proteins are, in contrast to SATs, more variable in their enzymatic functions. OAS-TL has a considerable variability for the nucleophilic reactant (section IV.B) and may be involved in the synthesis of secondary products in some plant species (Murakoshi et al., 1986; Warrilow and Hawkesford, 2000; Maier, 2003). OAS-TLs belong to the pyridoxal-phosphate-dependent superfamily of β-substituting alanine synthases (bsas). This family also includes β-cyanoalanine synthase (CAS), based on high amino acid homology. CAS is believed to function in the detoxification of cyanide using cysteine as substrate and releasing β-cyano alanine and sulfide (section V.E). The OAS-TL gene family of Arabidopsis consists of 9 genes that encode for 8 functionally transcribed OAS-TL-like proteins (Jost et al., 2000). OAS-TL A (At4g14880), B (At2g43750) and C (At3g59760) are the most

expressed authentic OAS-TLs in this family and the proteins have been localized in the cytosol, the plastid and the mitochondria, respectively (Hell et al., 1994; Hesse and Hoefgen, 1998; Hesse et al., 1999; Wirtz et al., 2004). The combination of subcelluar localisation and biochemical studies of recombinant AtcysD1 (At3g04940), AtcysD2 (At5g28020) suggest that these gene products are authentic OAS-TL isoenzymes of low abundance and cytosolic localization. In contrast, AtcysC1 (At3g61440) is predicted to be localized in mitochondria and to act as a CAS *in vivo* (Hatzfeld et al., 2000; Yamaguchi et al., 2000). The functions of the remaining OAS-TL like proteins (At3g03630 and At5g28030) in plant sulfur metabolism are currently unknown.

With respect to function the careful, enzyomological analysis of the three major expressed OAS-TLs in Arabidopsis revealed that their affinities for sulfide are much higher than previously described (Wirtz and Hell, 2003b; Wirtz et al., 2004). The K_M values range from 3 to 6 μM and correspond to the assumed sulfide levels in plant and form the basis for the observed rates of cysteine synthesis *in planta* (Schmidt and Jäger, 1992). The true enzyme substrate is likely to be HS^-, which constitutes about 50% of total sulfide at pH around pH 7 (Wirtz et al., 2004). Although sulfide concentrations have apparently never been quantified exactly in plant tissues, they are generally believed to be in the low μM range (Hell et al., 2002). If this assumption holds true, sulfide availability would not limit rates of cysteine synthesis under sufficient sulfur supply. In contrast, the affinity of OAS-TL for the second substrate, OAS, was confirmed to be rather low. The K_M values for OAS are approximately 10 to 100 times higher than the concentrations found in whole leaf extracts from Arabidopsis and *Brassica oleracea* (Kim et al., 1999; Awazuhara et al., 2000; Wirtz et al., 2004). This strongly suggests that OAS rather than sulfide limits cysteine synthesis at sulfur sufficient conditions, which is an important feature of the cysteine synthase complex model of regulation (section VI.B).

Total extractable OAS-TL activities determined under substrate saturating conditions will hardly ever constitute a bottleneck for plant growth. However, *in vivo*, lowered availability of substrate concentrations or increased demand may well result in a shortage of cysteine production. It was suggested that OAS-TL activity can indeed limit rates of cysteine in various stress situation, when high amounts of cysteine are required, e.g. for synthesis phytochelatins in the presence of cadmium (Barroso et al., 1999; Dominguez-Solis et al., 2001; Romero et al., 2001; Dominguez-Solis et al., 2004). Accordingly, OAS-TL overexpression in plastids and cytosol supports the resistance to cadmium and sulfur containing pollutants, which are believed to act toxic via sulfide release (Harada et al., 2001; Noji et al., 2001a).

C. Gene Expression

SAT isoforms in *A. thaliana* show a semi-constitutive transcription pattern during development of the plant according to comprehensive microarray data (see https://www.genevestigator.ethz.ch/ for public database). This is more or less confirmed by individual expression analyses from other plant species, although much less data are available (Saito et al., 1997; Urano et al., 2000). RNAs of cytosolic, mitochondrial and plastidial SAT isoforms have been detected in all analyzed tissues of *A. thaliana*, indicating that cysteine synthesis in all these compartments is required in all tissues during development of higher plants. This also applies to developing seeds, which are capable to assimilate sulfate provided by maternal tissue into cysteine (Tabe and Droux, 2001; Tabe and Droux, 2002). While plastid AtSAT1 has been described as the most prominently transcribed isoform in leaves (Kawashima et al., 2005), this result is challenged by microarray and cell fractionation data. In *Pisum sativum* leaves, approximately 80% of total SAT activity was located in mitochondria and the residual SAT activity was distributed between cytosol and plastids (Ruffet et al., 1995). Species-specific differences in the localization of cysteine synthesis or the fact that the mRNA level of an expressed gene is not necessarily indicative for the amount of protein may be responsible for these discrepancies.

Transcriptional regulation in response to stress as indicated by mRNA contents of SAT isoforms in various species generally shows not more than threefold variation. Only the AtSAT4 gene is reported to be strongly up regulated during sulfur starvation and by treatment with cadmium in roots and leaves (Kawashima et al., 2005). Given

the uncertain enzymatic identity of the encoded gene product, it remains currently unclear whether AtSAT4 upregulation is related to cysteine deficiency or is part of a more general response of the plant to cope with stress in general.

Genes encoding OAS-TL-like proteins appear to be more or less ubiquitously expressed in all plant cell types analyzed so far, with little variation in the content of RNA, protein and extractable enzyme activity in response to external factors (Brunold, 1990; Hell et al., 1994; Dominguez-Solis et al., 2001). In fact, mRNA contents of an OAS-TL gene encoding OAS-TL A from Arabidopsis has been used as constitutive control in a gene expression study (Koprivova et al., 2000). However, several experiments strongly suggest the OAS-TL A gene to respond specifically to cadmium or salt exposure with up to a sevenfold increase in mRNA level (Barroso et al., 1999; Dominguez-Solis et al., 2001). Accordingly, overexpression of the corresponding gene conferred enhanced cadmium tolerance (Dominguez-Solis et al., 2004). Remarkably, OAS-TL but also SAT gene expression is elevated in leaf trichomes of Arabidopsis and, according to *in situ* hybridization data, responds to salt and cadmium treatments, presumably to support glutathione synthesis (Gutierrez-Alcala et al., 2000; Howarth et al., 2003).

IV. Structure of Proteins of Cysteine Synthesis in Bacteria and Plants

In their groundbreaking and pioneering work Nicolas Kredich and coworkers revealed in the late 1960s the presence of the CSC in the pathogenic enterobacterium *S. typhimurium*. By using size-exclusion chromatography they determined a total molecular weight of 309 kDa for the hetero-oligomeric CSC, of 160 kDa for the SAT homomer and of 68 kDa for the OAS-TL homomer (Becker et al., 1969; Kredich et al., 1969; Kredich and Becker, 1971). Although the CSC has been known for almost 40 years, the overall structure is still (2007) unresolved. Nonetheless, in the last decades progress has been made to understand the structure/function relationship inside the CSC by analysing separately the structure of the SAT and OAS-TL subunits by X-ray crystallography.

A. Serine Acetyltransferase

The first functional analysis of the SAT domain structure was performed using a eukaryotic SAT from the mitochondria of Arabidopsis. By using the yeast two hybrid system the N-terminal α-helical domain of SAT was identified as the SAT–SAT interaction domain, while the C-terminal domain was revealed to account for both, enzymatic activity and SAT-OAS-TL interaction inside the complex (Bogdanova and Hell, 1997). The idea of a bifunctional C-terminal SAT domain was further strengthened by the modelling of the C-terminus of plant SAT using bacterial acyltransferase structures as templates. These posses an unusual left-handed parallel β-helix (Vuorio et al., 1991; Vaara, 1992). By computational modelling of the SAT C-terminus to these acyltransferases, the C-terminus of SAT could be split into two sections: a left-handed parallel β-helix (LβH) domain, which carries the catalytically active site, and a C-terminal tail that could not be modelled due to low homology to the acyltransferases (Wirtz et al., 2001). Partial deletion of the C-terminal tail of prokaryotic SAT results in loss of interaction with OAS-TL, as shown by co-purification experiments using SAT and OAS-TL of *E. coli* (Mino et al., 1999; Mino et al., 2000b). In addition, several amino acids of the C-terminal tail of plant and bacterial SAT seem to be involved in the feedback inhibition of SAT by cysteine the end product of the pathway (Noji et al., 1998; Inoue et al., 1999; Wirtz and Hell, 2001). Although most features of the C-terminal tail of prokaryotic and eukaryotic SAT seems to be identical, the last C-terminal amino acids are more divergent in prokaryotic and eukaryotic SATs than the LβH structure (Fig. 2). The latter contains an imperfect tandem repeat of a hexapeptide sequence described as [LIV]-[GAED]-X2-[STAV]-X, which is responsible for the β-helical folding (Vaara, 1992). All analysed proteins containing this motive, including SAT, are placed into the superfamily of acyltransferases, whose members are known to act *in vivo* as trimers (Raetz and Roderick, 1995; Beaman et al., 1997). Thus; it was not surprising that bacterial SAT was revealed to be a dimer of trimer in its native uncomplexed form (Fig. 2A, Hindson et al., 2000). In 2004, the three-dimensional structure of SAT homomer from the prokaryotes *E. coli* (Pye

et al., 2004) and *Hamophilus influenzea* (Gorman and Shapiro, 2004) were resolved, whereby the predicted overall structural features of SAT could be unequivocally confirmed (Fig. 2B).

Consistently, in *E. coli* and *H. influenzae*, whose SATs share 71% identity at the amino acid sequence level, three SAT monomers form a trimer. The three clefts between the three subunits form a catalytic center (Fig. 2C), which suggests that active site residues from two subunits may contribute to form one joint catalytic center (Pye et al., 2004), see below. Two SAT trimers are arranged in the quaternary structure of a dimer (dimer of trimers) so that the dimer interface is constituted by parts of the N-terminal α-helices of all subunits. The α-helical domain of each subunit is formed by residues 1–140 in the *E. coli* SAT protein and comprises eight α-helices. Helix α1 and α2 as well as α3 and α4 run in an anti-parallel fashion and are connected by 2 β-turns; the residual α-helices are meandering with different angles. The repetition of helices α2–α4 by the molecular threefold axis forms a surface that interacts loosely with the cognate structure in the opposing trimer and thereby stabilizes the dimerization of trimers (Olsen et al., 2004). All of the residues involved in dimerization are found within a N-terminal extension of approximately 80 amino acids that is found only in a subset of SATs (13% of all SAT like proteins). This indicates that SATs fall into two subgroups: Group A, whose members posses the extension and

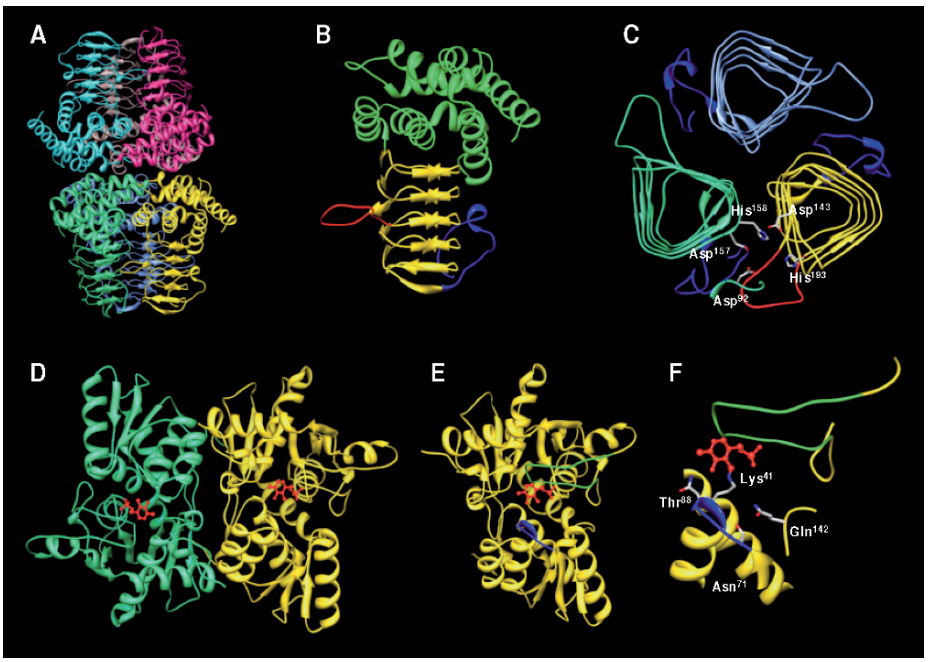

Fig. 2. Three-dimensional structures of prokaryotic SAT and OAS-TL. The figure depicts the overall homomeric quaternary structure of SAT (A) and OAS-TL (D). In both cases the monomers are shown in different colours in order to illustrate the sterical position of the monomers. Each SAT monomer (B) consists of an α-helical domain (green), a β-sheet domain (golden) that included a random coil loop (red) and the C-terminal tail (dark blue), which is responsible for CSC formation. Please note that the last 11 amino acid residues of the C-terminal tail are missing in the structural presentation, since they were not solved by X-ray crystallography. (C) Top view on an SAT trimer, where α-helical domains are deleted for better overview. The active site of SAT lies in between the β-sheet domain of two monomers, thus a SAT 'dimer of trimers' (A) contains six independent active sites. The most important residues for binding of substrates and the enzymatic activity are named and shown in sticks. Each OAS-TL monomer (E) of the native OAS-TL dimer (B) contains one PLP (red) that is bound via an internal Schiff base by Lys^{41}. The asparagine loop, which is important for substrate binding and induction of the global conformational change of OAS-TL during catalysis is shown in dark blue. The green colour indicates the β8a–β9a loop that seems to be important for interaction with SAT. Both structural elements are in close proximity to the active site of OAS-TL (F). Important residues for binding of substrates and the enzymatic activity are highlighted in F. The software UCSF Chimera V1.2422 (University of California, San Francisco) was used to visualize SAT (pdb: 1T3d) and OAS-TL (pdb: 1OAS) (*See Color Plates*).

form hexameric SAT, and group B, whose members lacks the extension and are likely to have a trimeric quaternary structure (Gorman and Shapiro, 2004). A proof for this hypothesis has not been provided.

Residues 141–262 of the *E. coli* SAT form the β-helical domain, which is a typical LβH comprising fourteen β-strands forming five coils of the helix. Apart form the final C-terminal tail, there is only one break from the β-helix; this is a loop from residue Gly^{184} to His^{193} that has a random coil topology. The His^{193} of this loop, with His^{158} and three aspartic acid residues (Asp^{92}, Asp^{143} and Asp^{157}), forms the core of the catalytically active site (Fig. 2C). While Asp^{92} and Asp^{157} stabilize the positive charge of the amino group of the substrate serine, the imidazole ring of His^{158} is supposed to form a classical 'catalytic triad' with the oxygen of the γ-hydroxyl group of serine and the Asp^{143} of the adjacent SAT subunit. Thus, six independent active sites exist in the hexameric SAT homomer (Olsen et al., 2004). In the 'catalytic triad', His^{158} acts as a base to activate the hydroxyl group of serine for a nucleophilic attack on the carbonyl carbon of the acetyl group of acetyl-CoA. This attack would result in an oxyanion tetrahedral intermediate, whose negative charge would be stabilized by the imidazole of His^{193}. The collapse of the oxyanion tetrahedral intermediate would result in the formation of the reaction products: OAS and CoA, whereby the same general base residue (His^{158}) acts a general acid and donates a proton of the sulfur atom of CoA. This mechanism would be in good agreement with reaction mechanisms of other acyltransferase, which belongs to the LβH subfamily of acyltransferases (Johnson et al., 2005). Elaborate solvent deuterium kinetic isotope effects measurements indicated that the rate limiting step of this reaction is the nucleophilic attack of the serine hydroxyl group on the thioester of acetyl-CoA (Johnson et al., 2004). Based on initial velocity studies and on the analysis of *H. influenzae* SAT structure in the presence of CoA (1SST) and cysteine, the kinetic mechanisms of SAT is supposed to be sequential equilibrium ordered (Fig. 3A), which would be in agreement with the kinetic mechanism revealed for all other LβH acyltransferases (Sugantino and Roderick, 2002; Hindson and Shaw, 2003; Johnson et al., 2004). The outcome of previous initial velocity studies using the *S. typhimurium* SAT suggested the occurrence of a bi bi ping pong – instead of a sequential – kinetic mechanism (Leu and Cook,

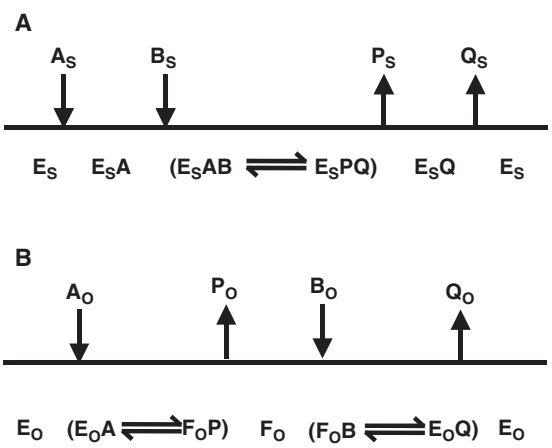

Fig. 3. Kinetics mechanism of serine acetyltransferase and *O*-acetylserine(thiol)lyase. The kinetic mechanisms for SAT (A) and OAS-TL (B) are sequential ordered and bi bi ping pong, respectively. (A) Acetyl-CoA (A_S) binds to SAT (E_S) before serine (B_S) is recruited. Afterwards the catalytic triad is formed, allowing a nucleophilic attack of serine on acetyl-CoA (E_SAB). The collapse of the oxyanion tetrahedral intermediate ($E_SAB \leftrightarrow E_SPQ$) results in the formation of the reaction products: OAS (P_S) and CoA (Q_S). (B) In the first-half reaction OAS-TL binds OAS (A_O) and releases the first product acetate (P_O). E_O represents the internal Schiff-base between PLP and Lys^{41} of OAS-TL, while F_O indicates the α-aminoacrylate external Schiff base conformation of OAS-TL. The second substrate sulfide (B_O) attacks in a nucleophilic manner on the α-aminoacrylate intermediate and the final product cysteine (Q_O) is released (second-half reaction).

1994). While almost all results of Leu and Cook (1994) could be confirmed, only the burst kinetic experiment, which gives rise to the hypothesis of a ping pong mechanism for SAT, was not reproducible (Johnson et al., 2004).

The sequential kinetic reaction mechanism of SAT is also consistent with the inhibition mechanism by cysteine (section VI.A), which affects not only one but both substrates. SAT of *E. coli* (1T3D) and *H. influenzae* (1SSQ) were crystallized in the presence of cysteine. In both crystals the cysteine was found in the binding pocket of serine, providing an elegant explanation for the competitive inhibition of cysteine for serine (Olsen et al., 2004; Pye et al., 2004). That the cysteine and the serine binding pockets overlap is also supported by kinetic and microcalorimetric data obtained using serine analogous (Hindson and Shaw, 2003). These results confirm that the cysteine feedback inhibitor binds to the active site of SAT. Beside the direct competition with serine, cysteine also reduced the affinity of SAT for acetyl-CoA, after binding to the enzyme (Kredich and Tomkins, 1966; Leu and Cook, 1994; Johnson et al., 2004). The molecular basis for the reduction of acetyl-CoA affinity is a displacement of the C-terminal tail of SAT in the presence of cysteine. Parts of the C-terminal tail compete with the pantentheinyl arm of acetyl-CoA for binding to the extended LβH loop in the cysteine bound SAT crystals. In the absence of cysteine, acetyl-CoA is able to displace the C-terminal tail in order to enter the binding pocket, while the C-terminal tail closed the acetyl-CoA binding site more efficiently when cysteine is bound to SAT (Olsen et al., 2004). The importance of the C-terminal tail for inhibition of SAT activity by cysteine was also confirmed by mutational analysis of bacterial and plant SATs C-terminal tails (Denk and Böck, 1987; Nakamori et al., 1998; Inoue et al., 1999; Takagi et al., 1999; Wirtz and Hell, 2003a). These results indicate that specific features of SAT domains have the same function in prokaryotic and eukaryotic SAT. Although no eukaryotic SAT has been crystallized up to now, computational modelling of eukaryotic SAT to bacterial SAT suggest that the overall structure of pro- and eukaryotic SATs are identical (Wirtz and Hell, 2006). The basis for this homology modelling is the high sequence identity between the two types of SAT, which is in the order of 34–44%.

B. O-acetylserine(thiol)lyase

The structures of OAS-TL from the prokaryotes *H. influenzae* and *S. typhimurium* have been determined with and without its substrates (Burkhard et al., 1998; Burkhard et al., 1999). The structure of the first plant OAS-TL was recently solved, demonstrating a high degree of conservation between plant and enterobacterial OAS-TL at the structural level (Bonner et al., 2005). The structure resolved from the plant OAS-TL crystal overall confirms the model of OAS-TL, which was derived from homology modelling of plant to bacterial OAS-TL. The sequence identity between the pro- and eukaryotic OAS-TL proteins is in the same range as for the respective SAT proteins. Taken together these results indicate that the same homology model approach is also suitable for plant SAT (Wirtz and Hell, 2006).

OAS-TL from plants and bacteria form stable homodimers with a molecular weight of 68 to 75 kDa according to their amino acid sequences. Crystallization of OAS-TL from bacteria revealed that the dimers interact only at the dimer interface and are oriented in such a way that the two active sites are facing each other (Fig. 2D). Each OAS-TL subunit carries a tightly bound pyridoxal 5′-phosphate (PLP) at the catalytic center. All active site residues are contributed from one subunit (Rabeh and Cook, 2004). The active site with the PLP is located within a cleft deep in the center of each monomer that is flanked by N- and C-terminal domains (Fig. 2E). This conformation may hamper the standard approach of expression of recombinant proteins with N- or C-terminal tags, resulting in less active or kinetically altered enzymes.

Lys^{41} of bacterial OAS-TL is responsible for the linkage to the PLP, whose presence is a prerequisite for an enzymatically active enzyme (Burkhard et al., 1999). Asn^{71}, which is located on the so-called asparagine loop, and Gln^{142} bind OAS and the resulting intermediates along the reaction pathway. Functional analysis of the plant OAS-TL reveals Thr^{74} and Ser^{75} as important active site residues (Fig. 2F). Most likely both residues are involved in stabilizing the transition state of the second-half reaction (see below). The stabilisation of the transition state has direct impact on the affinity for the second substrate, sulfide (Bonner et al., 2005). The conservation of the active site

residues strongly supports the idea of the same kinetic mechanism for OAS-TLs from prokaryotes and eukaryotes.

OAS-TL is one of the most extensively studied enzymes with respect to the reaction mechanism and the kinetic behaviour. The type of reaction kinetic of the enzyme is bi bi ping pong (Cook and Wedding, 1976; Tai et al., 1993): In this type of kinetic, after binding of the first substrate, OAS, the first product, acetate, is released, before the second substrate, sulfide, interacts with the reaction intermediate to give the second product, cysteine (Fig. 3B). Mechanistically, the overall β-substitution of the acetate group of OAS by sulfide can be subdivided into an elimination and an addition reaction, which represent the first-half and the second-half of the reaction, respectively. In short, the first-half reaction includes the binding of OAS and the formation of an α-aminoacrylate intermediate, whereby acetate is released. The second-half reaction is initiated by the attack of sulfide, in the form of HS^-, to the Cβ of α-aminoacrylate intermediate to give a cysteine external Schiff base. Then transimination results in the release of cysteine (Tai et al., 1995; Rabeh and Cook, 2004; Wirtz et al., 2004). The reaction is accompanied by a large conformational change of the protein after the binding of OAS. The binding of OAS in the active site induced a local rearrangement of the asparagine loop (Fig. 2E, F) that shifted the enzyme from the so called 'open' to the 'closed' conformation. Formation of the 'closed' conformation results in expel of bulk solvent from the reaction room and the closure of the active site, which is necessary for the second-half of the reaction, since the α-aminoacrylate is highly reactive (Burkhard et al., 1999). Although the sequence identity of the asparagine loop from prokaryotes and eukaryotes is very high, the triggering function of the asparagine loop for the overall conformational change is doubted in plant OAS-TLs (Bonner et al., 2005). The methods used for identification of chemical mechanisms and different conformational changes of OAS-TL during catalysis included fluorescence (Benci et al., 1997, 1999), phosphorescence (Strambini et al., 1996), and UV visible spectroscopy (Schnackerz et al., 1995) in combination with mutageneic (Rege et al., 1996; Tai et al., 1998) and crystallographic approaches (Burkhard et al., 1998; Burkhard et al., 1999). Most of this tremendously intensive study is comprehensively summarized by Rabeh and Cook (2004). In agreement with the high K_M of OAS-TL for OAS, the first-half reaction is limiting the overall β-substitution reaction (Woehl et al., 1996), while the second-half of reaction is irreversible (Tai et al., 1995). This biochemical phenotype of OAS-TL is important for cysteine regulation in higher plants. In fact cysteine synthesis must be limited by OAS production for proper regulation of the sulfur assimilation pathway by CSC formation (section VI.C, D) and the transcriptional control of OAS regulated genes. While OAS production by SAT activity is inhibited by cysteine, OAS-TL seems not to be regulated at all. However, sulfide is a competitive substrate inhibitor of bacterial OAS-TL, with a K_I of 50 μM (Cook and Wedding, 1976; Tai et al., 1993). The identification of an allosteric anion-binding site at the dimer interface, which can be occupied by sulfide, led to the hypothesis of an allosterically regulated OAS-TL, due to catabolism of cysteine in bacteria (Burkhard et al., 2000).

C. Cysteine Synthase Complex

The 3D-structure of the CSC is not known. Crystallography of free and complex associated plant SAT was attempted but not successful, due to instability of plant SAT protein (personal communication J. Jez Danforth Center, St. Louis). Moreover, no precise analytical data are available for the molecular weight of both bacterial and plant CSC. The stoichiometry of SAT and OAS-TL subunits in the complex can thus be hypothesized on a theoretical ground. The only estimate of the size of the complex arises from size exclusion chromatography, which revealed a molecular weight of 300 kDa for CSCs from native protein extracts of spinach and tobacco (Droux et al., 1992; Wirtz and Hell, 2007). The size of the bacterial CSC is in the same range (Kredich et al., 1969), indicating an overall similar structure in prokaryotes and eukaryotes. The uncomplexed bacterial SAT is known to be a 'dimer of trimers', while OAS-TL from plants and bacteria form dimeric homomers (see above). On the basis of this knowledge, one hexameric SAT homomer (Fig. 2A) is supposed to form the core of the CSC, which is the completed by the recruitment of two OAS-TL dimers (Fig. 2D). As a consequence,

SAT and OAS-TL subunits would exist in a 6: 4 (1.5: 1) stoichiometric ratio in the CSC. Fluorescence titration assays of CSC yielded an experimentally determined ratio of 1.43: 1 for SAT and OAS-TL, essentially supporting the theoretical stoichiometry (Campanini et al., 2005).

One interaction site of SAT with OAS-TL inside the complex is the C-terminal tail of SAT (Fig. 2B). This is proven by partial deletion of this tail in bacterial SATs, which resulted in the loss of SAT ability to interact with OAS-TL (Mino et al., 1999; Mino et al., 2000b). Co-crystallization of OAS-TL from *H. influenzae* and *A. thaliana* with the last 10 amino acid residues of the SAT C-terminal tail demonstrated that the binding site of this decapeptide is located at the active site of OAS-TL, where also OAS binds (Huang et al., 2005; Francois et al., 2006). The key active site residues Thr^{74}, Ser^{75} and Gln^{147} lock the decapeptide in the binding site via hydrogen bonds (numbering of residues according to AtOAS-TL A, section IV.B), which explains how complex formation downregulates OAS-TL activity (Francois et al., 2006). OAS would compete with the SAT C-terminal tail for binding at the catalytic center of OAS-TL. Assuming this as the only OAS binding site in the complex, such a competition provides also a mechanistic basis for the dissociation of the CSC by OAS. Kinetic characterization of the association of bacterial OAS-TL with the C-terminal tail of SAT showed that the binding affinity of this decapeptide was 250-fold lower than full-length SAT (Mino et al., 2000b). However, the largest part, and thus other potential interaction sites, of SAT were missing in this experiment. Although the C-terminal decapeptide of *H. influenzea* and *S. typhimurium* are highly divergent, the affinity for the C-terminal decapeptide of *H. influenzea* and *S. typhimurium* (K_D ~ 0.6 to 1 μM) were in the same range for OAS-TL of *H. influenzae*, which indicates a general binding mechanism in the γ-proteobacteria (Campanini et al., 2005). In contrast, the plant C-terminal decapeptide appeared to have high affinity for OAS-TL (K_D = 5–100 nM) (Kumaran and Jez, 2007), which is even more affine than reported for the full-length SAT from Arabidopsis cytosol and mitochondria. The K_D of both proteins for OAS-TL were independently determined by initial velocity and surface plasmon resonance studies and are in the range of 25 to 40 nM (Droux et al., 1998; Berkowitz et al., 2002). The different structural composition of pro- and eukaryotic decapeptides may be one reason for the variability in their affinity for their respective interaction partner. The only conserved residue in plant and bacterial SAT decapeptides is the very final Ile residue, which, at least in plants, is responsible for the molecular recognition of the decapeptide by OAS-TL (Francois et al., 2006).

The lower affinity of the bacterial decapeptide for OAS-TL compared to the full-length SAT raises the question of whether additional sites for interaction or recognition exist in SAT and OAS-TL. Initial protein–protein interaction analyses using OAS-TL mutants that were genetically modified in the highly conserved β8A-β9A surface loop (Fig. 2E) point towards an involvement of this loop in CSC formation (Bonner et al., 2005). The corresponding interaction site of the SAT protein has not been located; its identification would allow a more detailed analysis of the assembly of SAT and OAS-TL subunits inside the CSC.

The association of the SAT C-terminus with the catalytic site of OAS-TL would place the OAS-TL dimers at the distal ends of the SAT 'dimer of trimers'. If symmetry applies here, we may speculate that one of the three SAT C-termini of a trimer interacts with the active site of one subunit of an OAS-TL dimer. In principle, also two C-terminal tails could bind into the two active sites of the OAS-TL homodimer. Both, plant and bacterial C-terminal decapeptides bind to the OAS-TL dimer in a 2: 1 ratio. Nonetheless, one could not conclude from this experiment that two C-terminal tails of SAT bind one OAS-TL dimer, due to possible steric interferences of C-terminal tails in the SAT homohexamer. However, addition of a molar excess of the decapeptide to the CSC results in no detectable binding of the decapeptide, indicating that no active site of complex bound OAS-TL is available (Campanini et al., 2005). The latter fact could be the reason for the strong inactivation of OAS-TL inside the complex, which results in a more than 10-fold decrease of OAS-TL activity in bacterial and plant CSC (Kredich et al., 1969; Droux et al., 1998; Wirtz et al., 2001; Wirtz and Hell, 2006). The effective inactivation of OAS-TL demonstrates the inability of the CSC to channel substrates and is the basis for the regulatory function of the complex *in vivo* (Hell and Hillebrand, 2001).

Beside the effector driven dissociation of the CSC by OAS both plant and bacterial CSC are stabilized by sulfide (Kredich et al., 1969; Wirtz and Hell, 2006). The function of this stabilization *in vivo* could be the increase of OAS levels for enhanced cysteine and glutathione production. Enhanced glutathione production is needed during certain stress conditions like pathogen attack or heavy metal stress, when enough sulfide is present. Without the stabilizing effect of sulfide, OAS would dissociate the CSC, thereby down-regulating its own synthesis. A possible target for sulfide stabilization would be the active site of OAS-TL, where OAS and C-terminal tail of SAT bind. But no stable sulfide binding pocket was found in the active site of OAS-TL, which is consistent with the irreversibility of the second-half reaction (Tai et al., 1995). The identification of an allosteric anion-binding site at the dimer interface of bacterial OAS-TL provides an alternative target site for sulfide (Burkhard et al., 2000). The binding of sulfide to this site causes a conformational shift in the asparagine loop to a new 'inhibited' state of OAS-TL that differs from the 'open' or 'closed' conformation of the enzyme (section IV.B). It is conceivable that the 'inhibited' conformation of OAS-TL stabilizes the C-terminal tail of SAT in the active site of OAS-TL and promotes complex stability.

Due to the inactivity of OAS-TL inside the complex, the complex associated OAS-TL is believed to act as a regulatory subunit of SAT. In theory, association of SAT and OAS-TL activates SAT in order to produce OAS for cysteine synthesis (section VI.E). A trimeric SAT homomer contains three independent active sites, which can all be used to synthesize cysteine. Most likely all active sites can act separately, since in the form III crystal of *H. influenzae* SAT (pbf file: 1SST) all possible binding sites for acetyl-CoA are loaded with CoA in the presence of serine (Olsen et al., 2004). It is difficult to conceive how the binding of one OAS-TL dimer to one or two C-terminal tails at the distal end of a SAT trimer can result in an effective up-regulation of all active sites in the trimer. In agreement with this consideration, neither the affinity for acetyl-CoA and serine nor the maximal activity (V_{max}) of the bacterial SAT is altered by complex formation (Kredich et al., 1969; Cook and Wedding, 1978; Mino et al., 2000a). However, formation of CSC protects SAT from proteolysis and inactivation by low temperature in *in vitro* experiments (Mino et al., 2000a). Under *in vivo* conditions, both would result in higher SAT activity by CSC formation. Cytosolic SAT of *A. thaliana* was shown to be strongly upregulated by complex formation due to increase of maximal velocity (Bonner et al., 2005) and by increasing the affinity for acetyl-CoA and serine (Droux et al., 1998). The mitochondrial SAT from *A. thaliana* was also characterized in respect to their activation by complex formation. In the latter study only a slight but significant activation of mitochondrial SAT by complex formation was observed. This activation was based on a higher affinity of complex associated SAT for acetyl-CoA (Wirtz et al., 2001). Whether the slight increase of SAT activity by complex formation is sufficient to regulate OAS production under *in vivo* conditions needs to be confirmed. A molecular explanation for the increased acetyl-CoA affinity could be provided by the rearrangement of the C-terminal tail of SAT during binding to OAS-TL. Parts of the same tail were also shown to cover the binding pocket for acetyl-Co after the binding of cysteine, causing a reduction of the affinity of *H. influenzae* SAT for acetyl-CoA (Olsen et al., 2004; Johnson et al., 2005). Taken together, these results put a note of caution to the generally accepted assumption that SAT is activated by complex formation, due to increased affinity or V_{max} of the enzyme in the complex. At least in bacteria, other factors like temperature or proteases are necessary to prevent decreased activity of SAT upon complex formation. The moderate activation of mitochondrial AtSAT upon complex formation in *in vitro* experiments possibly suggests a similar scenario in the mitochondria of plants.

V. S-transfer Reactions and Degradation of Cysteine

Enzymatic degradation of cysteine was hypothesized in the 1940s to take place in bacteria and animals (Fromageot et al., 1940; Smythe, 1945; Fromageot, 1951). Forty years later enzymatically catalyzed degradation of cysteine came to focus also in higher plants as a result of the pioneering work of several research groups (Harrington and Smith, 1980; Rennenberg, 1983; Rennenberg et al.,

1987; Schmidt, 1987). Four major functions for cysteine catabolism in higher plants are discussed in the literature: (a) recycling of cysteine bound sulfur as a result of protein degradation, (b) deposition of elemental sulfur and release of sulfide in response to pathogen defense, (c) transfer of sulfur from cysteine to iron-sulfur clusters and (d) detoxification of cyanide by using cysteine as an acceptor. In contrast to synthesis of cysteine the current knowledge about degradation of cysteine is scarce. Anabolism and catabolism of cysteine must be separated in order to achieve coordination between the two pathways. This separation can be achieved by localization of the two pathways in different subcellular compartments of the plant cell, temporal delimitation of respective protein activities, or the use of different enantiomers of cysteine specifically for degradation (D-cysteine) and synthesis (L-cysteine) (Wirtz and Droux, 2005). Up to now it is unclear if the mandatory separation of cysteine synthesis and degradation is achieved by only one of these mechanisms, several or all of them.

The following reactions (Fig. 4) can be envisaged to occur in the catabolism of cysteine: (A) L-cysteine desulfhydrase reaction or L-cysteine lyase, (B) L-cystine lyase reaction, (C) cysteine desulfurase reaction, (D) D-cysteine desulfhydrase reaction, (E) β-cyanoalanine synthase reaction, (F) decomposition of cysteine by OAS-TL, (G) conversion of cysteine to mercaptopyuvate by transaminases or (H) amino acid oxidases. Since the formation of β-mercaptopyruvate has not been shown in plants so far (Schmidt, 2005) and the kinetic properties of the most abundant OAS-TLs in Arabidopsis does not allow a significant backward reaction (Wirtz et al., 2004), the last three possible reaction mechanisms will not be discussed in this review. Nevertheless, several candidate genes exist in higher plants that may be involved in the degradation of cysteine (Fig. 5).

A. L-cysteine Desulfhydrase or L-cysteine Lyase

Although other cysteine consuming activities have been shown in higher plants (see below), the enzymes that are the most likely candidate for the degradation of cysteine in higher plants are L-cysteine desulfhydrase (EC 4.4.1.15, L-CDes or L-cysteine lyase, Fig. 4A) or L-cystine lyases. By feeding ^{35}S-labeled L-cysteine (which contains *per se* cystine) to cultured tobacco cells Harrington and Smith (1980) observed that the initial step in cysteine (or cystine) degradation yielded pyruvate and sulfide in a 1:1 ratio and possibly ammonium. The catabolic reaction was linear with respect to time and amount of protein and had a pH optimum of 8 in crude extracts. A L-CDes with a pH optimum of 8 has also been shown in *Streptococcus anginosus* (Yoshida et al., 2002). Preliminary kinetic data for the tobacco L-CDes indicated that the K_M is approximately 0.2 mM cysteine. Pre-incubation of the tobacco cells with cysteine results in 15–20-fold increase of the extractable degradative activity, while intracellular cysteine concentrations increases (Harrington and Smith, 1980). In contrast, S fertilization of field grown *Brassica napus* also significantly increase the contents of total S, glutathione and cysteine, but decreases L-CDes activity. In the same study the activity of L-CDes in *B. napus* was found to be induced by infection with *Pyrenopeziza brassicae* that increased cysteine and glutathione contents as well (Bloem et al., 2004). The latter finding gives rises to the hypothesis that L-CDes are involved in defence against pathogens by releasing toxic sulfide. Taken together these results indicate that L-CDes activity is not only regulated by intracellular cysteine levels, but also by unknown endogenous and environmental signals.

So far an enzyme with exclusive L-CDes activity has not been isolated from higher plants, although several candidate genes for L-cysteine desulfhydrases were identified in the genome of *Arabidopsis thaliana* (Fig. 5). Like in higher plants the activity of the L-CDes in the enterobacterium *Salmonella typhimurium* is inducible by pre-incubation with cysteine. The L-CDes of *S. typhimurium* was purified to quasi homogeneity after induction with cysteine. The native protein has a molecular weight of 229 kDa, suggesting a hexa-homoligomeric structure composed of 37 kDa subunits, which contain one PLP per subunit (Kredich et al., 1972). Substrate saturation experiments using the purified enzyme demonstrated positive cooperativity with a Hill-coefficient of 1.9 and a K_M for L-cysteine of 0.17 and 0.21 mM (Kredich et al., 1973). Both the inducibility of the enzymatic activity by cysteine and the biochemical characteristics of the enzyme were similar in *S. typhimurium*

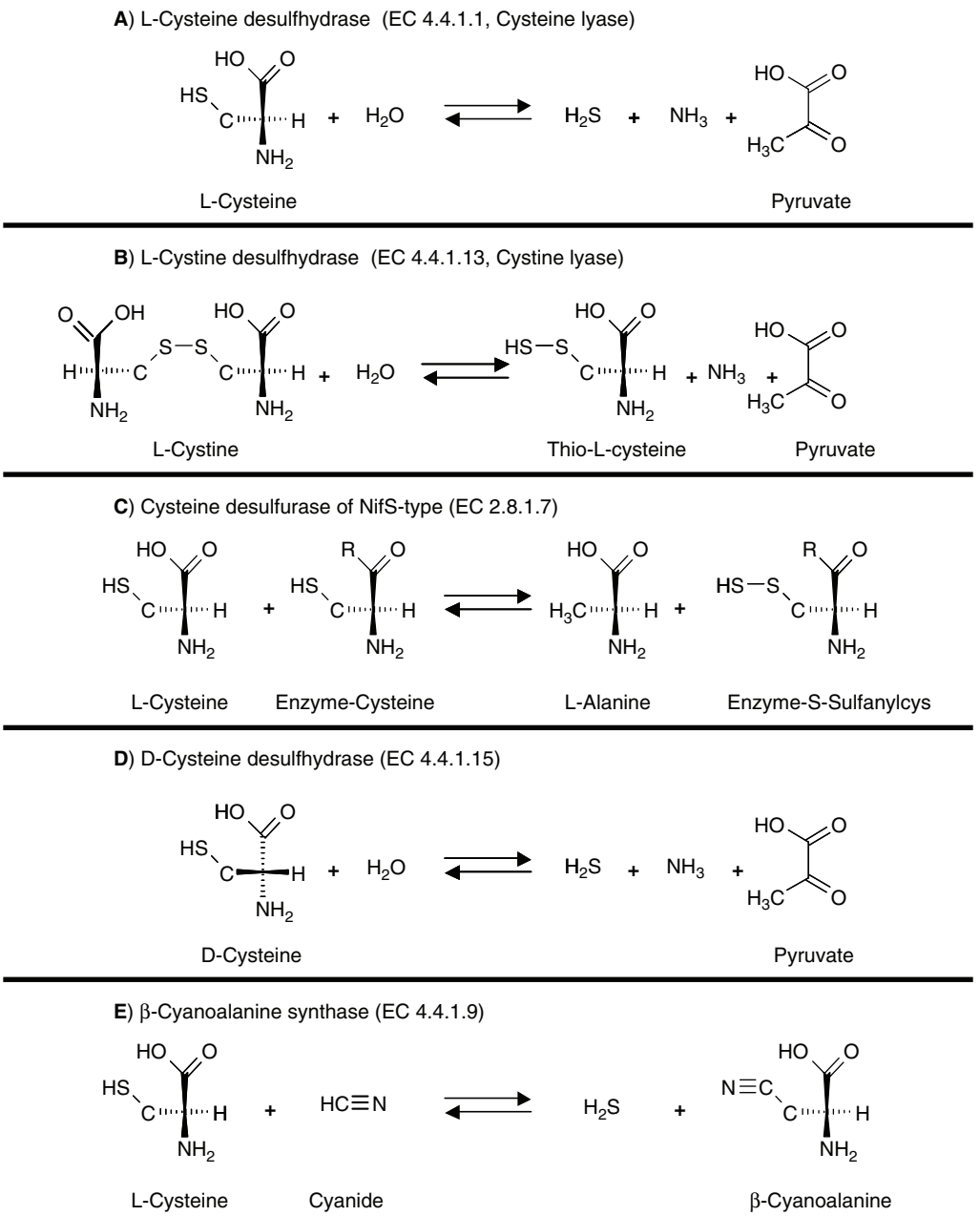

Fig. 4. Cysteine consuming reactions. The figure depicts the candidate reactions for degradation of cysteine. Note that L-cysteine desulfhydrase (A), L-cystine desulfhydrase (B), Cysteine desulfurase (C) and β-cyanoalanine synthase (E) degrade the L-enantiomer of cysteine, while D-cysteine desulfhydrase (D) uses the D-enatiomer of cysteine as substrate. The chemical structures of substrate and products are shown according to IUPAC nomenclature.

and tobacco indicating a conserved regulation of cysteine degradation in pro- and eukaryotes.

B. L-cystine Desulfhydrase or L-cystine Lyase

L-cystine is the oxidized form of cysteine in which two L-cysteine molecules are linked together via a disulfide bridge. L-cystine was identified in all analyzed higher plants, but the exact degree of oxidized cysteine is hard to quantify, due to oxidation of cysteine during extraction. The L-cysteine pool is kept usually in a reduced state by the reduction/oxidation buffer glutathione, which is present in high excess of L-cysteine. Nevertheless L-cystine can accumulate during oxidative stress

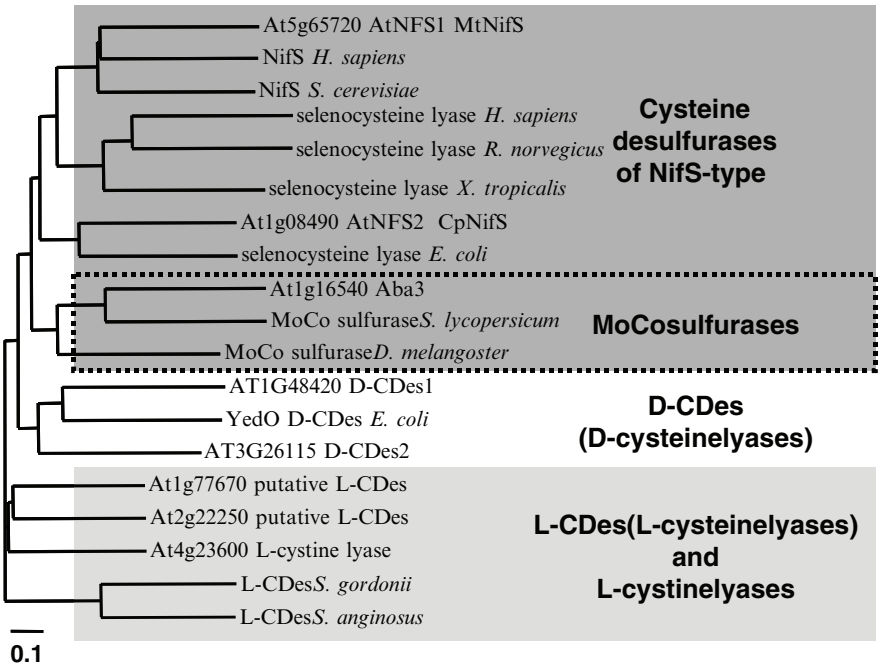

Fig. 5. Sequence relationships of cysteine degrading enzymes. The phylogenetic tree was created with Vector NTI 9 (Invitrogen, Darmstadt) using the full length proteins. Annotation of enzymes is according to their description in the respective GenBank™ accession: AtCpNifS: Q93WX6, AtMtNifS: O49543, NifS *H. sapiens*: Q9Y697, NifS *S. cerevisiae*: NP_009912, selenocysteine lyase *H. sapiens*: NP_057594 *R. norvegicus*: XP_343628, *X. tropicalis*: NP_001011164, *E. coli*: NP_416195, Aba3: Q9C5X8, Molybdenum cofactor (MoCo) sulfurases *S. lycopersicum*: Q8LGM7, *D. melangoster*: Q9VRA2, D-CDes1:NP_175275, D-CDes2: ABM06014, YedO: NP_416429, At1g77670: ABI49465, At2g22250: AAD23617, At4g23600: BAF01453, L-CDes *S. gordonii*: BAC20222, *S. angionosus*: BAC00815. The Arabidopsis proteins are functionally characterized (section V) or indicated as putative. MoCo sulfurases and NifS proteins from prokaryotes and eukaryotes cluster together and form the group of cysteine desulfurases of NifS type (dark grey). D-cysteine desulfhydrases (white) share higher sequence similarity with cysteine desulfurases than with L-cysteine desulfhydrases (grey).

conditions like high light, cold stress or pathogen attack. Recently, Jones and coworkers revealed that CORI3, which is annotated as a tyrosine aminotransferase-like protein, acts *in vitro* as a L-cystine lyase (EC 4.4.1.13, Fig. 4B, Jones et al., 2003). Interestingly CORI3 is jasmonic acid and salt stress-inducible, which may indicate regulation of cysteine/cystine degradation by stress and hormone signalling (Gong et al., 2001; Sasaki et al., 2001). In animals and bacteria cystathionine γ-lyase is known to act as a L-CDes and L-cystine lyase (Smacchi and Gobbetti, 1998). Usually, cystathionine γ-lyase catalyses the cleavage of cystathionine, which results in the production of cysteine and the byproducts ammonium and α-ketobutyrate. Nevertheless, the enzyme can also use L-cysteine and L-cystine as substrates. For a detailed description of all possible substrates and reaction mechanisms of this multifunctional enzyme look up the enzyme-database BRENDA (http://www.brenda.uni-koeln.de/). Biochemical characterization of the plant cystathionine β-lyase indicates that it have not this wide substrate specificity (Staton and Mazelis, 1991).

C. Cysteine Desulfurase of NifS-type

The cysteine desulfurases of the NifS-type (EC 2.8.1.7, Fig. 4C) identified in Arabidopsis differ in their subcellular localization and developmental expression patterns. At1g16540 (Aba3) is located in the cytosol (Heidenreich et al., 2005), while At5g65720 (MtNifS, AtNFS1) and At1g08490 (CpNifS, AtNFS2) are transported to mitochondria and plastids, respectively. MtNifS and CpNifC provide elemental sulfur for iron-sulfur cluster formation in the respective organelle, while Aba3 acts as a molybdenum cofactor sulfurase.

Plants with reduced CpNifS expression exhibited chlorosis, a disorganized chloroplast structure, stunted growth and eventually became necrotic and died before seed set (Van Hoewyk et al., 2007), demonstrating that cysteine desulfurase activity in plastids is essential for iron-sulfur cluster formation and optimal plant growth (Ye et al., 2005; Ye et al., 2006). MtNifS is responsible for desulfuration of cysteine in order to provide elemental sulfur for iron-sulfur cluster formation in mitochondria (Frazzon et al., 2007), which is believed to provide iron-sulfur clusters for cytosolic and nuclear iron-sulfur proteins (Balk and Lobreaux, 2005). Very recently the AtSufE protein was resolved to be an essential activator of both plastidic and mitochondrial desulfurases in Arabidopsis (Xu and Moller, 2006). The stringent regulation of MtNifS and CpNifS activity by the presence AtSufE allows an optimal coordination between cysteine degradation for iron-sulfur cluster formation and cysteine synthesis by OAS-TL in mitochondria and plastids. So far it is unknown how the coordination between cysteine degradation for molybdenium cofactor synthesis and cysteine synthesis in the cytosol is achieved. An elegant way would be allosteric inhibition of Aba3 activity by the molybdenum cofactor itself, but a detailed biochemical analysis of Aba3 in this respect is missing to date.

D. D-cysteine Desulfhydrase

In contrast to the former described cysteine degrading activities the D-cysteine desulfhydrase (D-CDes, EC 4.4.1.15, Fig. 4D) uses D-cysteine instead of L-cysteine, which provides an elegant explanation for the presence of cysteine producing and degrading activities in the same sub-cellular compartment. The authors are not aware of any report that unequivocally demonstrate the presence of D-cysteine as a natural occurring metabolite of higher plants. A racemase that converts L-cysteine to D-cysteine has also not been isolated from higher plants (Schmidt, 1986), but protein bound L-cysteine could be converted to D-cysteine non enzymatically by the base-catalysed racemization reaction (Friedman, 1999). In addition, higher plants are suggested to take up D-amino acids from the soil, where they are secreted by microorganisms (Aldag et al., 1971). Although D-cysteine was not isolated from plants so far, D-amino acids in general are believed to be principle components of gymnosperms as well as of monocotyledonous and dicotyledonous angiosperms (Bruckner and Westhauser, 2003). Green plants and algae are capable of degrading D-cysteine specifically by D-CDes activity (reviewed in Schmidt, 1986). The L-CDes and D-CDes activities were easily distinguishable in cucurbit and tobacco, since the enzymes catalysing both reactions differ in their response to inhibitors, their subcellular localization and had different pH optima. In both plants species the extractable D-CDes activity was more than one order of magnitude higher than the L-CDES activity (Rennenberg et al., 1987).

Recently, two proteins catalysing the D-CDes reaction *in vitro* were identified in Arabidopsis due to their similarity with the D-CDes of *E. coli* (YedO, Fig. 5). Expression analysis on transcript level suggests that D-CDes1 (At1g48420) and D-CDes2 (At3g26115) might have specific roles during development of Arabidopsis. While D-CDes1 catalyses specifically the desulfuration of D-cysteine, D-CDes2 accepts L-cysteine beside D-cysteine as substrate (Riemenschneider et al., 2005a; Riemenschneider et al., 2005b). *In silico* analysis of transit peptides from both proteins indicate a mitochondrial localization, which was proven for D-CDes1 independently by immunological detection and GFP-fusion constructs. Consistent with its sulfur remobilizing function D-CDes activity was identified to be highest in senescent plant leaves. Neither D-CDes1 nor D-CDes2 transcript levels could explain the significant increase of C-Des activity during senescence (Riemenschneider et al., 2005a). The latter results indicate a post-translational control of the D-CDes proteins or the existence of further D-CDes enzymes, which are not identified.

E. β-cyanoalanine Synthase

β-Cyanoalanine synthase (CAS; EC 4.4.1.9, Fig. 4E) catalyzes the formation of the non-protein amino acid β-cyanoalanine from cysteine and cyanide, thereby playing a pivotal role in cyanide detoxification (Floss et al., 1965; Meyers and Ahmad, 1991). CAS activity has been detected in bacteria (Dunnill and Fowden, 1965), insects (Meyers and Ahmad, 1991) and plants (Blumenthal et al., 1968; Hendrickson and Conn, 1969). In plants,

ethylene synthesis by 1-aminocyclopropane-1-carboxylic acid oxidase results in the production of cyanide (Peiser et al., 1984). Inhibition of CAS activity by 2-aminoxyacetic acid revealed that detoxification of cyanide is dependant on CAS activity in higher plants. However, several-hundred fold increases of cyanide production during ripening of apple and avocado fruits are not accomplished by a severe induction of extractable CAS activity (Yip and Yang, 1988). Cyanide toxicity is caused by binding of cyanide to the iron atom of the enzyme cytochrome c oxidase in the fourth complex in the mitochondrial membrane. This denatures the enzyme, and the final transport of electrons from cytochrome c oxidase to oxygen cannot be completed. As a result, the electron transport chain is disrupted, meaning that the cell can no longer aerobically produce ATP for energy. Consequently CAS activity is located exclusively in mitochondria of insects (Meyers and Ahmad, 1991). Several lines of evidence indicate that in higher plants CAS is also exclusively localized in mitochondria. Firstly, CAS activity was purified to homogeneity for the first time from mitochondrial fractions of blue lupine (Hendrickson and Conn, 1969), secondly, the identified CAS proteins of spinach and Arabidopsis are both located in the mitochondrial lumen (Warrilow and Hawkesford, 1998; Hatzfeld et al., 2000). Thirdly, the background activity of CAS that was identified in the cytosol of cocklebur (*Xanthium pennsylvanicum*) seeds is attributed to cytosolic OAS-TL (Maruyama et al., 1998). CAS and OAS-TL are structurally very similar proteins, which are able to catalyse both: β-cyanoalanine and cysteine synthesis to a certain extend. For that reason it is hard to distinguish if a protein acts *in vivo* as a CAS or an OAS-TL. Only a careful biochemical characterisation of kinetic constants for both reactions allows a categorization of a certain enzyme to act as CAS or OAS-TL under *in vivo* conditions (Warrilow and Hawkesford, 2000; Yamaguchi et al., 2000). By using these careful biochemical characterization in combination with immunological detection, the putative isoforms of OAS-TL from spinach and potato were identified as CAS (Warrilow and Hawkesford, 1998; Maruyama et al., 2000). Most likely a feature that specifies an authentic OASTL is the ability to interact physically with SAT protein in the CSC. While two-hybrid system and co-purification experiments show the associated SAT and OAS-TL enzymes (Bogdanova and Hell, 1997; Droux et al., 1998; Wirtz et al., 2001; Hell et al., 2002; Droux, 2003), such an interaction makes no sense for a CAS.

However, in *A. thaliana* both proteins are present in mitochondria (Hatzfeld et al., 2000; Jost et al., 2000; Wirtz et al., 2004). The presence of both enzymatic activities in the mitochondria would allow an efficient detoxification of cyanide via cysteine by consumption of serine, which is produced in mitochondria in high amounts by photorespiration (Fig. 6). In contrast to potato and spinach, Arabidopsis produces cyanogenic glucosinolates, which are part of the defence system against herbivores in all Brassicaceae. It is conceivable that the enhanced cyanide metabolism in the Brassicaceae requires a more efficient mitochondrial cyanide detoxification system consisting of CAS and OAS-TL in mitochondria itself.

In Arabidopsis CAS is 37 kDa protein (trivial name: *At*CysC1), which is encoded by the gene At3g61440. The transcript levels of At3g61440 are almost constant in seeds, rosettes, roots and the inflorescence, with the exception that in pollen only small amounts of transcript could be detected (data from https://www.genevestigator.ethz.ch). In agreement with the finding that enhanced ethylene formation does not induce extractable CAS activity, the transcript level of At3g61440 is kept constant in senescent leaves. Recombinant AtCysC1 has a specific activity of 62 µmol sulfide min^{-1} mg^{-1} protein and K_M values of 2,5 mM and 60 µM for cysteine and cyanide, respectively (Hatzfeld et al., 2000). The spinach CAS has similar biochemical properties (Warrilow and Hawkesford, 2000). It seems doubtful that the low affinities of the recombinant CAS proteins for cyanide allows keeping the cyanide concentration at cellular levels around 0.2 µM, which is far below the K_i of cyanide (10–20 µM) to inhibit respiration (Yip and Yang, 1988).

VI. Regulation of Cysteine Flux in Plants

Regulation in any metabolic pathway is a mechanism to adjust supply and demand. Major triggers for regulation are plant development, environmental factors like stress and nutrient supply.

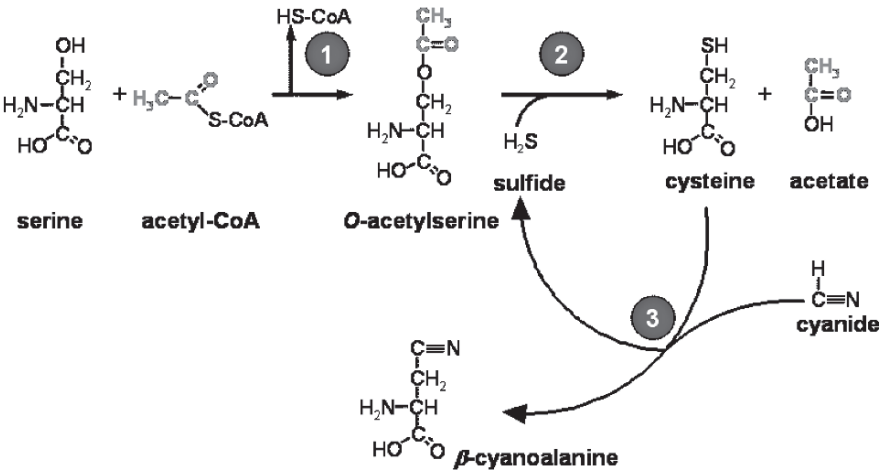

Fig. 6. Detoxification of cyanide in mitochondria of *Arabidopsis thaliana*. Serine acetyltransferase (1) and *O*-acetylserine (thiol)lyase (2) catalyze the synthesis of cysteine in a two step process. The fate of the acetyl-moiety during catalysis is shown in grey. In mitochondria β-cyanoalanine synthase (3) uses cysteine as an acceptor to detoxify cyanide. During the detoxification of cyanide sulfide is released, which can be fixed by OAS-TL to reconstitute the acceptor for cyanide.

The spatial component includes root-to-shoot and source-sink communication, i.e. the supply of sulfate to roots, its transport along the plant axis, the demand by developing seeds or locally stressed organs and transport of reduced sulfur as nutrient or signal within the plant. In all cases the cell, independent of its position in the plant body, has to be able to sense supply and demand of metabolites and to respond adequately. Searches for long-distance signals as well as cellular signal transduction have been in the focus of plant sulfur research. Green algae and in particular *Chlamydomonas* have been and still are model organisms in the latter respect (see chapter by Shibagaki and Grossman, this book). Work on Arabidopsis suggested that cysteine synthesis plays an integral role in the regulation of primary sulfur metabolism. In particular the properties of the cysteine synthase protein complex gave rise to theories about a new metabolite sensing mechanism (Hell and Hillebrand, 2001; Droux, 2003). Moreover, the reaction intermediate of cysteine synthesis, OAS, forms a direct connection of sulfate assimilation with nitrate assimilation and carbon metabolism (Kopriva et al., 2002; Hesse et al., 2004). Network analyses using microarrays confirm these assumptions (see Takahashi and Saito, this book). The regulation of flux into and through cysteine synthesis therefore is of central importance for growth and fitness of the plant. It is noteworthy that this position of cysteine synthesis, although based on totally different regulation, parallels the early regulatory system in enterobacteria.

A. Allosteric Regulation of Cysteine Synthesis by Feedback Inhibition

Feedback inhibition of SAT by cysteine is an important feature for the enterobacteria regulatory system and is also found in several SATs from plants. Unfortunately, no information on feedback sensitivity of SAT from phototrophic bacteria is available. For higher plants an elegant model of flux control of cysteine synthesis was suggested (Saito, 2000), based on the observation that in *A. thaliana* the cytosolic SAT isoform is strongly feedback inhibited by cysteine, whereas the plastid and mitochondrial SAT isoforms are mostly insensitive (Noji et al., 1998). 50% (IC_{50}) inhibition of maximal activity was determined for the cytosolic SATs from watermelon (*Citrullus vulgaris*) at 1.8 µM and from *A. thaliana* at 2.9 µM cysteine (Saito et al., 1995; Noji et al., 1998). In this model cytosolic SAT activity is considered to particularly important for the control of OAS concentrations, since is potentially involved in the expression control of sulfur metabolism genes in response to sulfate availability. In the organelles feedback-insensitive SAT isoforms would reside to allow independent cysteine formation and this has indeed been reported (Noji et al., 1998; Saito,

2000). An independent analysis of recombinant SAT3 from Arabidopsis mitochondria however, reported an intermediate sensitivity for IC_{50} of 50–70 µM cysteine (Wirtz and Hell, 2003a).

Furthermore, the degree of SAT feedback-sensitivity varies considerably between plant species and subcellular compartment. A plastid SAT from spinach (SAT56) showed an IC_{50} of 7.6 µM cysteine (Noji et al., 2001b). One organelle-localized (SAT1) SAT from tobacco (*Nicotiana tabacum*) showed an IC_{50} value of about 50 µM cysteine and another organelle-localized SAT was not inhibited at all at cysteine concentrations up to 600 µM (SAT4; Wirtz and Hell, 2003a). In contrast, a tobacco cytosolic SAT (SAT7) was indeed inhibited by 50% at about 50 µM cysteine. In pea (*Pisum sativum*) the protein fraction with cytosolic SAT activity was not inhibited by cysteine at all, whereas the SAT activities in isolated chloroplasts and mitochondria displayed IC_{50} values of 33 and 283 µM, respectively (Droux, 2003). A note of caution is added to these data: they were mostly determined using non-purified or recombinant enzymes with N-terminal fusions. Furthermore, these inhibition assays were obviously performed with SAT proteins in the absence of OAS-TL. Whether the degree of feedback inhibition by cysteine changes with the association or dissocitation of the cysteine synthase complex is not known.

Allosteric regulation of cytosolic SAT as primary mechanism of flux control is difficult to interpret in view of a number of *in vivo* experiments. The abiotic defense response of plants that is induced by heavy metals or xenobiotics in order to form glutathione in the cytosol results in an increased flux and accumulation of cysteine which would inhibit SAT (Rüegsegger and Brunold, 1992; Farago and Brunold, 1994). Similarly, fumigation of plants with H_2S results in enhanced levels of cysteine and is even sufficient to cover the sulfur demand of the plant (De Kok et al., 2002). Over-expression of feedback-sensitive SAT from *E. coli* and *A. thaliana* in transgenic plants promotes elevated cysteine levels (Blaszczyk et al., 1999; Harms et al., 2000; Noji and Saito, 2002; Wirtz and Hell, 2003a). Any accumulation of cysteine in a compartment with feedback-sensitive SAT could only be explained, if the subcellular cysteine pools were separated, but the distribution of cysteine within cells is evidently not known to date.

B. Application of Transgenic Plants

Transcriptional regulation of the sulfate assimilation pathway is most pronounced at the level of sulfate transporter gene expression (Maruyama-Nakashita et al., 2003; Maruyama-Nakashita et al., 2004; Maruyama-Nakashita et al., 2006). Deficiency of sulfate rapidly induces the expression of genes encoding sulfate transporter proteins of the plasmalemma. The genes encoding activation and reduction steps are expressed in most cells at a basal level, but can also be modulated by sulfate supply and demand. However, the mRNA changes are smaller and often even less effected at the protein and activity levels is observed when they are compared to sulfate transport (Logan et al., 1996; Takahashi et al., 1997; Bork and Hell, 2000; Koprivova et al., 2000). As stated above, the response of genes related to cysteine synthesis to sulfate limitation and their corresponding enzyme activity changes are rather small (Hell et al., 1994; Hesse et al., 1999). Thus, at least with respect to sulfate supply, the classical parameter for sulfur research, the importance of transcriptional control seems to decrease from expression of sulfate transporter genes to cysteine synthesis (Hell, 2003). It should be cautioned that other environmental factors such as light and nitrogen nutrition or stress may act differently on the transcription of these genes.

This observation nevertheless suggests that cysteine synthesis is a flux control point for primary sulfur metabolism. If cysteine synthesis is enhanced, a feedback to sulfate uptake and activation would be expected that provides sufficient sulfide and OAS for cysteine synthesis. Understanding of regulation of cysteine synthesis itself is therefore the potential key to the control of assimilatory sulfur metabolism in general.

Modification of the activities of SAT and OAS-TL in the cytosol, plastid and mitochondria are one way to test the regulatory function of cysteine synthesis. Mutants with decreased activities of one of the protein isoforms due to insertional inactivation have not been reported so far. Over-expression approaches in Arabidopsis, tobacco and potato using both proteins basically confirm the limiting role of SAT (Sirko et al., 2004). The expectation of such an approach is an enhanced formation of OAS as far as the substrates serine and acetyl-CoA are available. Total measurable

SAT activity in leaf extracts of wildtype plants of many tested species is about 400 times lower than OAS-TL activity (Droux, 2003). OAS will therefore even in overexpressor lines accumulate only to a moderate extent as long as sufficient sulfide is available, but cysteine and glutathione will show increased steady-state levels. Consequently, the over-expression of OAS-TL in plastids or the cytosol had only minor effects on the contents of cysteine and glutathione, although remarkable effects on stress tolerance were reported (Dominguez-Solis et al., 2001; Harada et al., 2001; Noji et al., 2001a; Youssefian et al., 2001; Kawashima et al., 2004). In line with the above observations isolated chloroplasts from wildtype and transgenic lines with OAS-TL overexpression in this compartment, when supplied with external OAS, showed enhanced cysteine and glutathione formation (Saito et al., 1994). However, assuming strongly increased consumption of cysteine and particularly glutathione under the stress conditions applied in these reports, the improved tolerance of transgenic lines suggests a successfully increased flux rate *in vivo*, even so OAS-TL was already present in the wildtype plants at high activity.

According to the expectation, expression of either bacterial or plant SAT in the cytosol or plastids achieved significantly higher steady-state concentrations of cysteine and glutathione, depending on whether cysteine feedback-sensitive or insensitive SAT proteins were applied. Where determined, OAS contents accumulated too much lower extent than thiols. Unfortunately, these transgenics plants were less intensively tested for their stress tolerance properties (Sirko et al., 2004; Wawrzynski et al., 2006). No reports have included cysteine degrading activity in SAT overexpressors. SAT overexpression studies all indicate that the substrates for OAS-TL, the final step of cysteine synthesis, are rate limiting (Blaszczyk et al., 1999; Harms et al., 2000; Noji and Saito, 2002; Wirtz and Hell, 2003a, 2007).

In addition to these data, an independent experiment using an SAT protein that had been mutated and enzymatically inactivated in its catalytic center, provided astonishing changes in the sulfur composition of the transformed plants (Wirtz and Hell, 2007). This SAT protein from Arabidopsis was still able to interact with OAS-TL, including endogenous OAS-TL, when expressed in the cytosol of tobacco leaves. The tobacco SAT was apparently at least partially out-competed by strongly accumulating mutated SAT, presumably resulting in down-regulation of cysteine synthesis in the cytosol and up-regulation in the chloroplasts and mitochondria. This conclusion by the authors of the study was supported by up to 25 times enhanced cysteine steady-state levels compared to wildtype, twofold increased OAS concentrations and a significant overall accumulation of total sulfur content in the transgenic lines. This was the largest reported enhancement of flux into cysteine so far that strongly suggests that the formation of the CSC together with exchange mechanisms between cellular compartments or lack thereof are of great importance for the regulation of cysteine synthesis (Wirtz and Hell, 2007).

C. Effectors and Properties of the Cysteine Synthase Complex

The availability of substrates is of general importance for any rate of product formation, if the concentrations run below binding constants and Michaelis-Menten constants of the respective protein. In case of cysteine synthesis serine is unlikely to limit, but acetyl-CoA may, at least in some compartments or metabolic situations, become critical for maximal activity of SAT in the CSC (Wirtz and Droux, 2005). As outlined before, the K_M values of OAS-TL for OAS are rather high, giving rise to the assumption that OAS prevents free OAS-TL from maximal rates of cysteine formation under most circumstances. While substrate affinities of bacterial and at least Arabidopsis OAS-TL proteins for sulfide are in the low micromolar range (Wirtz et al., 2004), the true cellular sulfide concentrations are neither known under sufficient nor limiting sulfate supply. Since the integration of sulfide is the decisive step of the entire assimilatory pathway, it seems reasonable to assume that its availability is critical to maintain cysteine synthesis and thus growth. The product cysteine, as discussed before, may control the rate of its formation by feedback inhibition of SAT, but depending on the compartment and the properties of the present SAT isoform.

These considerations suggest OAS and sulfide as potentially most important factors for the flux rate of cysteine synthesis. In fact, both metabolites are not only intermediates of the pathway

but have additional functions: OAS seems to be involved in the control of sulfur-related gene expression and OAS as well as sulfide exert allosteric properties on the association of SAT and OAS-TL in the CSC. OAS has long been known to destabilize the CSC in enterobacteria and plants (Kredich et al., 1969; Droux et al., 1998), but only recently its cellular concentrations could be reliably determined. Data from several authors using different tissues from Arabidopsis (leaf, silique, cell culture), soybean (cotyledon) and potato (*in vitro* shoots) revealed overall concentrations of 0.3 to 6 µM after extraction of whole samples, if cells were supplied with sufficient sulfate. Independent of the large differences in the time courses monitored, the typical response to sulfate limitation consisted in an increase of OAS levels to values between 4 and 60 µM. Unfortunately, virtually nothing is known about subcellular differences and possible transport of OAS between subcellular compartments in higher plants (Kim et al., 1999; Awazuhara et al., 2000; Wirtz and Hell, 2003a; Hopkins et al., 2005).

These changes of OAS concentrations in response to sulfate limitation suggest a function in signal transduction that is corroborated by feeding studies. Addition of OAS to barley roots (Smith et al., 1997), *Zea mays* cell cultures (Clarkson et al., 1999) and *Arabidopsis* plants (Koprivova et al., 2000; Kopriva et al., 2002) resulted in the increase of mRNA levels and activities of sulfate transporters, ATP sulfurylase and APS reduktase. External concentrations of 1 mM OAS over-ruled the repressing effect of sufficient sulfate in the growth medium. It may be concluded from these findings that OAS is directly or indirectly involved in the control of sulfate uptake and assimilation. This role applies to a large number of genes and not just primary reactions. OAS feeding induced the expression of the nitrilase gene 3 that is involved in glucosinolate degradation in *Arabidopsis* (Kutz et al., 2002). DNA microarray analysis comparing sulfate deficiency and OAS feeding in *Arabidopsis* plants revealed more than hundred up-regulated mRNAs of which about 40% were identical between the sulfate deficiency response and the effect of OAS feeding (Hirai et al., 2003, Takahashi and Saito, this book).

Interesting evidence for the contribution of OAS to sulfur deficiency signalling is provided by identification of a promoter fragment present in the *Arabidopsis Nit3* gene (Kutz et al., 2002) and the soybean β-conglycinin gene (Kim et al., 1999; Ohkama et al., 2002). This element alone in combination with activation elements was sufficient to confer sulfate deficiency and OAS responsiveness to reporter genes. This strongly suggests that OAS is an intermediate in cysteine synthesis and in addition a potential element of signal transduction to regulate sulfur homeostasis in a plant cell. However, detailed analysis of the promoter of sulfate transporter 1;1 (*Sultr1;1*) from Arabidopsis identified a 16 basepair element ('SURE') that was alone sufficient to confer sulfate deficiency response and repression by cysteine and glutathione, but not activation by OAS (Maruyama-Nakashita et al., 2005).

D. Biochemical and Allosteric Properties of the Cysteine Synthase Complex

The CSC is very unusual for a metabolic protein complex because its function in bacteria and plants is obviously different from substrate channeling of OAS (Cook and Wedding, 1978; Droux et al., 1998). It seems now evident that the docking of the C-terminus of SAT to the catalytic cleft of OAS-TL strongly hinders access of OAS and thus results in diffusion of the intermediate into the surrounding solution (Huang et al., 2005; Francois et al., 2006, section III.B). This finding elegantly explains why OAS can dissociate the CSC (via competition with the C-terminus for the catalytic cleft) and why free OAS-TL dimers are required (to catch released OAS and free sulfide). The function of OAS-TL in the CSC was explained as regulatory subunit that acts by stabilisation and activation of SAT (Droux et al., 1998, Fig. 7).

It is indeed remarkable that SAT activity seems to rely on the association with OAS-TL. SAT activity has never been detected in plant or bacterial protein preparations without associated OAS-TL protein. Release of SAT from the complex *in vitro* results in rapid loss of activity (Droux et al., 1998; Wirtz et al., 2001) and to achieve maximal SAT activity for cysteine production a 400-fold excess of OAS-TL activity is required (Ruffet et al., 1994; Droux et al., 1998). This correlates well with the reported 354-fold and 306-fold excess of OAS-TL to SAT activity in chloroplasts of spinach and pea (Ruffet et al., 1994; Ruffet et al., 1995). However,

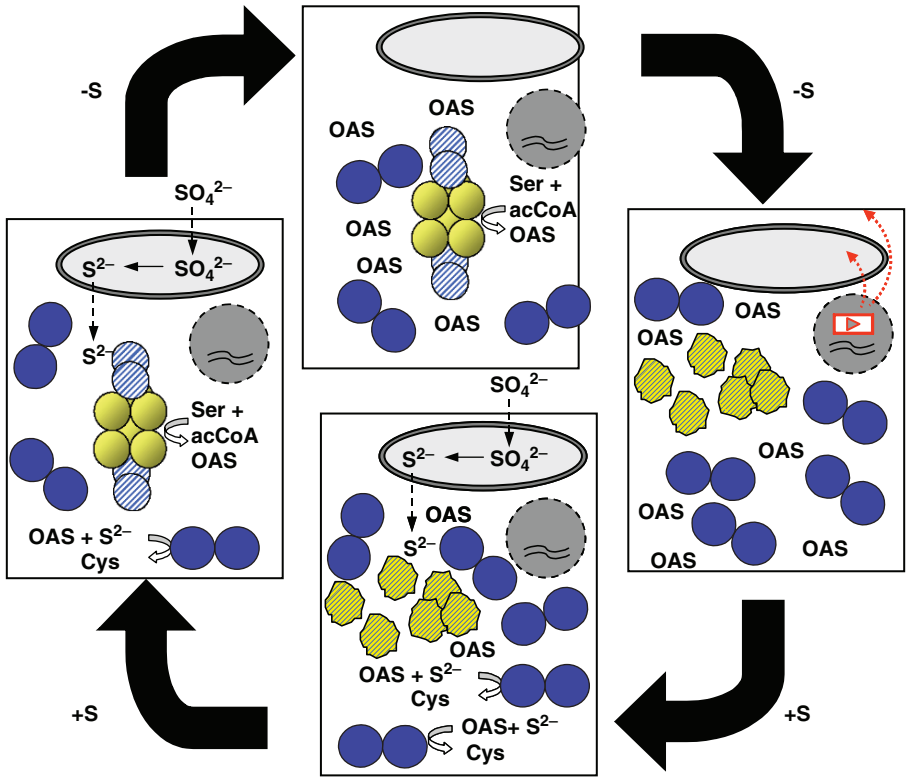

Fig. 7. Hypothesis for the function of the cysteine synthase complex *in planta*. The figure depicts the theoretical model for the sensor and regulatory function of the cytosolic CSC by *O*-acetylserine (OAS) dependant dissociation of the CSC. Under normal sulfur supply (left panel) sulfate (SO_4^{2-}) enters the cell and is reduced to sulfide (S^{2-}) in the plastids (light grey circles). Sulfide leaks as H_2S out or is actively transported across the plastid membranes (dashed arrows) into the cytoplasm. In the cytoplasm sulfide is used by free *O*-acetylserine(thiol)lyase dimers (OAS-TL, dark circles) to synthesize cysteine. The precursor of cysteine is OAS, which is produced by serine acetyltransferase (SAT, light grey circles). Under these conditions SAT is active, since it is associated with OAS-TL in the CSC. OAS releases the CSC since OAS-TL inside the complex (dashed circles) is inactive. At sulfur deficient conditions OAS accumulates, since sulfide is not available for conversion of OAS to cysteine by free OAS-TL (upper panel). Prolonged sulfur deficiency raises OAS levels above a certain threshold and causes the CSC to dissociate (right panel). Dissociation of the CSC results in inactivation of SAT (dented circles) to prevent consumption of acetyl-CoA (acCoA). The high OAS level, the dissociated CSC or a combination of those is the trigger for transcriptional activation of sulfur metabolism related genes in the nucleus (dashed dark circle). The activation of the sulfate transporting and reducing system makes sulfide become available (lower panel). As a result the OAS concentration decreases, since OAS is converted to cysteine by OAS-TL. When the OAS level falls below the threshold for dissociation the CSC, SAT and OAS- TL reassociate to form the CSC. SAT is activated and synthesizes OAS with a rate adapted to the current sulfate supply (left panel).

since the CSC self-assembles even *in vitro* from its constituents in the absence of OAS, it may be suspected that any protein extraction from living cells goes along with a dilution and thus strongly decreases OAS concentrations. This could result in assembly of the CSC from SAT and OAS-TL proteins that under native *in vivo* conditions might have been unbound.

The relevance of OAS-promoted dissociation is supported by precise concentration-dependent dissociation kinetics. While the dissociation of the *S. typhimurium* complex and a plant complex mixed from *Arabidopsis* and spinach subunits by millimolar concentrations of OAS had long been known (Kredich et al., 1969; Droux et al., 1998), the CSC from *Arabidopsis* mitochondria was quantitatively analyzed using special protein interaction techniques (Berkowitz et al., 2002). An equilibrium dissociation constant of 57 µM OAS was observed as well as a dissociation rate constant of 77 µM OAS with a cooperative Hill-constant. These findings suggest that the equilibrium of CSC association/dissociation can be effectively shifted almost like a switch (Berkowitz

et al., 2002). Since fluctuations of cellular OAS concentrations in this range have been observed (see above) in response to sulfate limitation, the equilibrium of CSC formation could control the rate of cysteine synthesis due to the complex-dependent activity of SAT.

Moreover, stabilization of the complex has already been reported, when 1 mM sulfide was added to the CSC from *S. typhimurium*. This treatment completely prevented the dissociation caused by OAS (Kredich et al., 1969). Again this effect was corroborated for a plant CSC. In the presence of 1 mM OAS and completely dissociated recombinant CSC from *Arabidopsis* mitochondria, the addition of increasing concentrations of sulfide resulted in association of SAT and OAS-TL with about 30 μM sulfide causing 50% decrease of OAS-TL activity due to complex association (Wirtz and Hell, 2006). Although the quantification of sulfide stabilization needs refinement, these *in vitro* data strongly suggest a physiological relevance for the regulation of CSC association and flux control of cysteine synthesis.

E. Metabolite Sensing and Regulation by the Cysteine Synthase Complex

The described evidence was integrated into a model that positions the CSC as a sensor in the regulation of cellular cysteine homeostasis (Droux et al., 1998; Hell, 1998; Hell and Hillebrand, 2001; Hell et al., 2002). The roots are the actual sites of nutrient perception, but decrease of external followed by internal availability of sulfate is important for growth and viability of all cells. Therefore, a basic cellular model might apply to source as well as sink cell. Sulfate deficiency will first result in lack of sulfide and then of cysteine and glutathione until protein biosynthesis stalls. Sulfide is a robust, fully reduced sulfur compound with little toxicity potential due to its very low concentrations. APS and sulfite seem less likely as sensing metabolites because of the lability of APS and high reduction potential of sulfite. In analogy to sulfate assimilation, a reduced nitrogen compound such as ammonia or glutamine has been suspected as sensing metabolite (Crawford and Forde, 2002). This regulatory model is based on the fully associated CSC during sufficient sulfate supply, meaning that all available SAT is bound to OAS-TL but leaving an excess of free and active OAS-TL dimers (Fig. 7). SAT is active and produces OAS that diffuses into the solution because of blocked catalytic cleft of bound OAS-TL. Instead, free OAS-TL dimers catalyze the formation of cysteine from OAS and sulfide. Under this condition OAS concentrations, including the cytosol, are below the dissociation threshold for complex dissociation. Accordingly, sulfide may stabilize the complex to sustain OAS formation and sulfate transport and assimilation genes are partially repressed.

If sulfate limitation in the environment of the cell results in a limitation of sulfide, OAS concentrations will start to increase, because a critical substrate for cysteine synthesis in missing. The first consequence is OAS accumulation above the dissociation threshold concentration of about 50–80 μM and dissociation of the CSC in the absence of sulfide. Free SAT rapidly loses activity and avoids further consumption of acetyl-CoA. Second, increased OAS concentrations may trigger the de-repression of genes of sulfate transport, *ATP* sufurylase and *APS* reductase. The affinity and capacity for sulfate uptake at the plasmalemma increases and helps to import sulfate if at all available. Sulfide is produced by assimilation and can immediately react with OAS catalyzed by active OAS-TL dimers. When OAS concentrations fall again below the dissociation threshold, the complex can re-assemble including active SAT. The production of OAS starts again at a rate that corresponds to the sulfide status of the cell. Re-adjusted cysteine or glutathione concentrations may repress the uptake and assimilation genes. It should be noted that this model describes the CSC as part of a sensor system that regulates the flux of primary sulfur metabolism in a cell, whereas cysteine is synthesized by free OAS-TL dimers (Fig. 7).

This working model places the CSC in the signal transduction line of sulfate as a macronutrient. Very little is known so far about the upstream events the mediate between sulfate deficiency and transcriptional and growth responses (Schachtman and Shin, 2007). Microarray analyses have revealed numerous downstream events including interaction with nitrogen assimilation (Hirai et al., 2003; Nikiforova et al., 2004; Nikiforova et al., 2005), but only recently the first transcription factor (SLIM1) has been described that seems to control at least part of the genes of the typical sulfate deficiency response (Maruyama-Nakashita

et al., 2006). If the CSC model proves to be correct, this excludes by no means the possibility of additional sensors, e.g. for sulfate or glutathione. However, no sensor for any plant nutrient has been described so far (Schachtman and Shin, 2007).

However, a number of open questions put a note of caution to the model. The mechanism of SAT inactivation outside the complex is entirely unknown (section IV.C). So far no post-translational modification has been reported that might account for activity changes, although the phosphorylation of a soybean cytosolic SAT was shown to result in decreased feedback sensitivity for cysteine (Liu et al., 2006). Particularly critical is the lack of correlation between OAS fluctuations, sulfate transport capacity and sulfate transporter kinetics (Hopkins et al., 2005). While OAS concentrations increase early on after transfer of single cells to sulfate deficient medium and approximately in parallel to the expression of a sulfate transporter (Wirtz et al., 2004), the induction of sulfate transporter genes precedes OAS accumulation in long-term experiments (Buchner et al., 2004; Hopkins et al., 2005). The apparently continuous accumulation of OAS can at present only be explained by subcellular compartmentation that removes OAS from the CSC complexes in cytosol, plastids and mitochondria.

On the other hand, the cysteine synthase model agrees with many physiological situations of sulfur metabolism. Optimal sulfur nutrition via H_2S would stabilize the complex to maximize OAS production for cysteine synthesis. This was indeed observed in whole plant experiments (De Kok et al., 2002). Long-term feeding of OAS potentially results in partial complex dissociation, but at the same time the induced sulfate assimilation genes generate more sulfide that stabilizes the complex, allowing for elevated cysteine levels (Neuenschwander et al., 1991). The over-expression of SAT in transgenic plants would produce more complex-bound SAT and consequently more OAS and cysteine formation as was observed (Blaszczyk et al., 1999; Harms et al., 2000; Noji and Saito, 2002; Wirtz and Hell, 2003a). The model is compatible with allosteric regulation by cysteine feedback inhibition of SAT as well (Saito, 2000). This mechanism would act downstream of sulfide and OAS, while the sensing mechanism serves as a trigger for upstream regulation.

References

Aldag R, Young J and Yamamoto M (1971) An enzymatic chromatographic procedure for the determination of D-amino acids in plant and soil extracts. Phytochemistry 10: 267–274

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

Bae MS, Cho EJ, Choi E-Y and Park OK (2003) Analysis of the *Arabidopsis* nuclear proteome and its response to cold stress. Plant J 36: 652–663

Balk J and Lobreaux S (2005) Biogenesis of iron-sulfur proteins in plants. Trends Plant Sci 10: 324–331

Barroso C, Romero LC, Cejudo FJ, Vega JM and 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

Beaman TW, Binder DA, Blanchard JS and Roderick SL (1997) Three-dimensional structure of tetrahydrodipicolinate N-succinyltransferase. Biochemistry 36: 489–494

Becker MA, Kredich NM and Tomkins GM (1969) The purification and characterization of *O*-acetylserine sulfhydrylase-A from *Salmonella typhimurium*. J Biol Chem 244: 2418–2427

Beinert H (2000) A tribute to sulfur. Eur J Biochem 267: 5657–5664

Benci S, Vaccari S, Mozzarelli A and Cook PF (1997) Time-resolved fluorescence of *O*-acetylserine sulfhydrylase catalytic intermediates. Biochemistry 36: 15419–15427

Benci S, Vaccari S, Mozzarelli A and Cook PF (1999) Time-resolved fluorescence of *O*-acetylserine sulfhydrylase. Biochim Biophys Acta 1429: 317–330

Berkowitz O, Wirtz M, Wolf A, Kuhlmann J and Hell R (2002) Use of biomolecular interaction analysis to elucidate the regulatory mechanism of the cysteine synthase complex from *Arabidopsis thaliana*. J Biol Chem 277: 30629–30634

Blaszczyk A, Brodzik R and Sirko A (1999) Increased resistance to oxidative stress in transgenic tobacco plants overexpressing bacterial serine acetyltransferase. Plant J 20: 237–243

Bloem E, Riemenschneider A, Volker J, Papenbrock J, Schmidt A, Salac I, Haneklaus S and Schnug E (2004) Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. J Exp Bot 55: 2305–2312

Blumenthal SG, Hendrickson HR, Abrol YP and Conn EE (1968) Cyanide metabolism in higher plants. 3. The biosynthesis of β-cyanolanine. J Biol Chem 243: 5302–5307

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

Bonner ER, Cahoon RE, Knapke SM and Jez JM (2005) Molecular basis of cysteine biosynthesis in plants: structural and functional analysis of *O*-acetylserine sulfhydrylase from *Arabidopsis thaliana*. J Biol Chem 280: 38803–38813

Bork C and Hell R (2000) Expression patterns of the sulfite reductase gene from *Arabidopsis thaliana*. In: Sulfur nutrition and sulfur assimilation in higher plants: molecular, biochemical and physiological aspects, Brunold C, Davidian, J-C, De Kok, L, Rennenberg H, Stulen I (eds), pp 299–301. P. Haupt, Bern

Bruckner H and Westhauser T (2003) Chromatographic determination of L- and D-amino acids in plants. Amino Acids 24: 43–55

Brunold C (1990) Reduction of sulfate to sulfide. In: Sulfur nutrition and sulfur assimilation in higher plants, Rennenberg H, Brunold C, De Kok LJ, Stulen E (eds), pp 13–32. SPB Academic

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

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

Burkhard P, Tai CH, Jansonius JN and Cook PF (2000) Identification of an allosteric anion-binding site on *O*-acetylserine sulfhydrylase: structure of the enzyme with chloride bound. J Mol Biol 303: 279–286

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

Campanini B, Speroni F, Salsi E, Cook PF, Roderick SL, Huang B, Bettati S and Mozzarelli A (2005) Interaction of serine acetyltransferase with *O*-acetylserine sulfhydrylase active site: evidence from fluorescence spectroscopy. Protein Sci 14: 2115–2124

Chen HC, Yokthongwattana K, Newton AJ and Melis A (2003) *SulP*, a nuclear gene encoding a putative chloroplast-targeted sulfate permease in *Chlamydomonas reinhardtii*. Planta 218: 98–106

Clarkson DT, Eugénio D and Sara A (1999) Uptake and assimilation of sulphate by sulphur deficient *Zea mays* cells: the role of *O*-acetyl-L-serine in the interaction between nitrogen and sulphur assimilatory pathways. Plant Physiol Biochem 37: 283–290

Cook PF and Wedding RT (1976) A reaction mechanism from steady state kinetic studies for *O*-acetylserine sulfhydrylase from *Salmonella typhimurium* LT-2. J Biol Chem 251: 2023–2029

Cook PF and Wedding RT (1978) Cysteine synthetase from *Salmonella typhimurium* LT-2. Aggregation, kinetic behavior, and effect of modifiers. J Biol Chem 253: 7874–7879

Crawford NM and Forde BG (2002) Molecular and developmental biology of inorganic nitrogen nutrition. The Arabidopsis Book: 1–25

De Kok LJ, Stuiver CEE, Westerman S and Stulen I (2002) Elevated levels of hydrogen sulfide in the plant environment: nutrient or toxin. In: Air pollution and plant biotechnology. Prospects for phytomonitoring and phytoremediation, Omasa K, Saji H, Youssefian S and Kondo N (eds), pp 201–219. Springer, Tokyo

Denk D and Böck A (1987) L-Cysteine biosynthesis in *Escherichia coli*: nucleotide sequence and expression of the serine acetyltransferase (*cysE*) gene from the wild-type and a cysteine-excreting mutant. J Gen Microbiol 133: 515–525

Dominguez-Solis JR, Gutierrez-Alcala G, Vega JM, Romero LC and 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

Dominguez-Solis JR, Lopez-Martin MC, Ager FJ, Ynsa MD, Romero LC and Gotor C (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. Plant Biotech J 2: 469–476

Droux M (2003) Plant serine acetyltransferase: new insights for regulation of sulphur metabolism in plant cells. Plant Physiol Biochem 41: 619–627

Droux M (2004) Sulfur assimilation and the role of sulfur in plant metabolism: a survey. Photosynth Res 79: 331–348

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

Droux M, Ruffet ML, Douce R and 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

Dunnill PM and Fowden L (1965) Enzymatic formation of β-cyanoalanine from cyanide by *Escherichia coli* extracts. Nature 208: 1206–1207

Farago S and Brunold C (1994) Regulation of thiol contents in maize roots by intermediates and effectors of glutathione synthesis. J Plant Physiol 144: 433–437

Floss HG, Hadwiger L and Conn EE (1965) Enzymatic formation of β-cyanoalanine from cyanide. Nature 208: 1207–1208

Francois JA, Kumaran S and Jez JM (2006) Structural basis for interaction of *O*-acetylserine sulfhydrylase and serine acetyltransferase in the *Arabidopsis* cysteine synthase complex. Plant Cell 18: 3647–3655

Frazzon AP, Ramirez MV, Warek U, Balk J, Frazzon J, Dean DR and Winkel BS (2007) Functional analysis of *Arabidopsis* genes involved in mitochondrial iron-sulfur cluster assembly. Plant Mol Biol 64: 225–240

Friedman M (1999) Chemistry, nutrition, and microbiology of D-amino acids. J Agric Food Chem 47: 3457–3479

Fromageot C (1951). In: The enzymes, Sumner J and Myrbäck K (eds), pp 1237–1243. Academic, New York

Fromageot C, Wookey E and Chaix P (1940) Enzymologia 9: 198–214

Gong Z, Koiwa H, Cushman MA, Ray A, Bufford D, Koreeda S, Matsumoto TK, Zhu J, Cushman JC, Bressan RA and Hasegawa PM (2001) Genes that are uniquely stress regulated in salt overly sensitive (*sos*) mutants. Plant Physiol 126: 363–375

Gorman J and Shapiro L (2004) Structure of serine acetyltransferase from *Haemophilus influenzae* Rd. Acta Crystallogr D Biol Crystallogr 60: 1600–1605

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

Harada E, Choi EJ, Tsuchisaka A, Obata H and Sano H (2001) Transgenic tobacco plants expressing a rice cysteine synthase gene are tolerant to toxic levels of cadmium. J Plant Physiol 158: 655–661

Harms K, von Ballmoos P, Brunold C, Hofgen R and Hesse H (2000) Expression of a bacterial serine acetyltransferase in transgenic potato plants leads to increased levels of cysteine and glutathione. Plant J 22: 335–343

Harrington H and Smith I (1980) Cysteine metabolism in cultured tobacco cells. Plant Physiol 65: 151–155

Hatzfeld Y, Maruyama A, Schmidt A, Noji M, Ishizawa K and Saito K (2000) β-Cyanoalanine synthase is a mitochondrial cysteine synthase-like protein in spinach and *Arabidopsis*. Plant Physiol 123: 1163–1172

Heidenreich T, Wollers S, Mendel RR and Bittner F (2005) Characterization of the NifS-like domain of ABA3 from *Arabidopsis thaliana* provides insight into the mechanism of molybdenum cofactor sulfuration. J Biol Chem 280: 4213–4218

Hell R (1997) Molecular physiology of plant sulfur metabolism. Planta 202: 138–148

Hell R (1998). Molekulare Physiologie des Primärstoffwechsels von Schwefel in Pflanzen. Shaker, Aachen

Hell R (2003) Metabolic regulation of cysteine synthesis and sulfur assimilation. In: Sulfate transport and assimilation in plants, Davidian JC, Grill D, De Kok LJ, Stulen I, Hawkesford MJ, Schnug E and Rennenberg H (eds), pp 21–31. Backhuys Publishers, Leiden

Hell R, Bork C, Bogdanova N, Frolov I and 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

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

Hell R, Jost R, Berkowitz O and Wirtz M (2002) Molecular and biochemical analysis of the enzymes of cysteine biosynthesis in the plant *Arabidopsis thaliana*. Amino Acids 22: 245–257

Hendrickson HR and Conn EE (1969) Cyanide metabolism in higher plants. IV. Purification and properties of the β-cyanolanine synthase of blue lupine. J Biol Chem 244: 2632–2640

Hesse H and Hoefgen R (1998) Isolation of cDNAs encoding cytosolic (Accession No. AF044172) and plastidic (Accession No. AF044173) cysteine synthase isoforms from *Solanum tuberosum* (PGR98–057). Plant Physiol 116: 1604

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

Hesse H, Nikiforova V, Gakiere B and Hoefgen R (2004) Molecular analysis and control of cysteine biosynthesis: integration of nitrogen and sulphur metabolism. J Exp Bot 55: 1283–1292

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

Hindson VJ and Shaw WV (2003) Random-order ternary complex reaction mechanism of serine acetyltransferase from *Escherichia coli*. Biochemistry 42: 3113–3119

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

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

Howarth JR, Dominguez-Solis JR, Gutierrez-Alcala G, Wray JL, Romero LC and Gotor C (2003) The serine acetyltransferase gene family in *Arabidopsis thaliana* and the regulation of its expression by cadmium. Plant Mol Biol 51: 589–598

Huang B, Vetting MW and Roderick SL (2005) The active site of *O*-acetylserine sulfhydrylase is the anchor point for bienzyme complex formation with serine acetyltransferase. J Bacteriol 187: 3201–3205

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

Johnson C, Roderick S and Cook P (2005) The serine acetyltransferase reaction: acetyl transfer from an acylpantothenyl donor to an alcohol. Arch Biochem Biophys 433: 85–95

Johnson CM, Huang B, Roderick SL and Cook PF (2004) Chemical mechanism of the serine acetyltransferase from *Haemophilus influenzae*. Biochemistry 43: 15534–15539

Jones PR, Manabe T, Awazuhara M and Saito K (2003) A new member of plant CS-lyases. A cystine lyase from *Arabidopsis thaliana*. J Biol Chem 278: 10291–10296

Jost R, Berkowitz O, Wirtz M, Hopkins L, Hawkesford MJ and 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

Kawashima CG, Berkowitz O, Hell R, Noji M and Saito K (2005) Characterization and expression analysis of a serine acetyltransferase gene family involved in a key step of the sulfur assimilation pathway in *Arabidopsis*. Plant Physiol 137: 220–230

Kawashima CG, Noji M, Nakamura M, Ogra Y, Suzuki KT and Saito K (2004) Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. Biotechnol Lett 26: 153–157

Kim H, Hirai MY, Hayashi H, Chino M, Naito S and 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

Kitabatake M, So MW, Tumbula DL and Soll D (2000) Cysteine biosynthesis pathway in the archaeon *Methanosarcina barkeri* encoded by acquired bacterial genes? J Bacteriol 182: 143–145

Kopriva S, Suter M, von Ballmoos P, Hesse H, Krahenbuhl U, Rennenberg H and Brunold C (2002) Interaction of sulfate assimilation with carbon and nitrogen metabolism in *Lemna minor*. Plant Physiol 130: 1406–1413

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

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

Kredich NM and Becker MA (1971) Cysteine biosynthesis: serine transacetylase and *O*-acetylserine sulfhydrylase (*Salmonella typhimurium*). In: Methods in enzymology, pp 459–471. Academic Press LTD, London, UK

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

Kredich NM, Foote LJ and Keenan BS (1973) The stoichiometry and kinetics of the inducible cysteine desulfhydrase from *Salmonella typhimurium*. J Biol Chem 248: 6187–6196

Kredich NM, Keenan BS and Foote LJ (1972) The purification and subunit structure of cysteine desulfhydrase from *Salmonella typhimurium*. J Biol Chem 247: 7157–7162

Kredich NM and Tomkins GM (1966) The enzymic synthesis of L-cysteine in *Escherichia coli* and *Salmonella typhimurium*. J Biol Chem 241: 4955–4965

Kumaran S and Jez JM (2007) Thermodynamics of the interaction between O-acetylserine sulfhydrylase and the C-terminus of serine acetyltransferase. Biochemistry 46: 5586–5594

Kuske CR, Hill KK, Guzman E and 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

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

Laudenbach DE, Ehrhardt D, Green L and Grossman A (1991) Isolation and characterization of a sulfur-regulated gene encoding a periplasmically localized protein with sequence similarity to rhodanese. J Bacteriol 173: 2751–2760

Leu LS and Cook PF (1994) Kinetic mechanism of serine transacetylase from *Salmonella typhimurium*. Biochemistry 33: 2667–2671

Liu F, Yoo B-C, Lee J-Y, Pan W and Harmon AC (2006) Calcium-regulated phosphorylation of soybean serine acetyltransferase in response to oxidative stress. J Biol Chem 281: 27405–27415

Logan HM, Cathala N, Grignon C and Davidian JC (1996) Cloning of a cDNA encoded by a member of the *Arabidopsis thaliana* ATP sulfurylase multigene family. Expression studies in yeast and in relation to plant sulfur nutrition. J Biol Chem 271: 12227–12233

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

Maier TH (2003) Semisynthetic production of unnatural L-α-amino acids by metabolic engineering of the cysteine-biosynthetic pathway. Nat Biotechnol 21: 422–427

Maruyama A, Ishizawa K and Takagi T (2000) Purification and characterization of β-cyanoalanine synthase and cysteine synthases from potato tubers: are β-cyanoalanine synthase and mitochondrial cysteine synthase same enzyme? Plant Cell Physiol 41: 200–208

Maruyama A, Ishizawa K, Takagi T and Esashi Y (1998) Cytosolic β-cyanoalanine synthase activity attributed to cysteine synthases in cocklebur seeds. Purification and characterization of cytosolic cysteine synthases. Plant Cell Physiol 39: 671–680

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

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

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

Maruyama-Nakashita A, Nakamura Y, Yamaya T and Takahashi H (2004) A novel regulatory pathway of sulfate uptake in *Arabidopsis* roots: implication of CRE1/WOL/

AHK4-mediated cytokinin-dependent regulation. Plant J 38: 779–789

Mazel D and Marliere P (1989) Adaptive eradication of methionine and cysteine from cyanobacterial light-harvesting proteins. Nature 341: 245–248

Melis A and Chen H-C (2005) Chloroplast sulfate transport in green algae – genes, proteins and effects. Photosynth Res 86: 299–307

Meyers DM and Ahmad S (1991) Link between L-3-cyanoalanine synthase activity and differential cyanide sensitivity of insects. Biochim Biophys Acta 1075: 195–197

Mino K, Yamanoue T, Sakiyama T, Eisaki N, Matsuyama A and Nakanishi K (1999) Purification and characterization of serine acetyltransferase from *Escherichia coli* partially truncated at the C-terminal region. Biosci Biotechnol Biochem 63: 168–179.

Mino K, Yamanoue T, Sakiyama T, Eisaki N, Matsuyama A and Nakanishi K (2000a) Effects of bienzyme complex formation of cysteine synthetase from *Escherichia coli* on some properties and kinetics. Biosci Biotechnol Biochem 64: 1628–1640

Mino K, Hiraoka K, Imamura K, Sakiyama T, Eisaki N, Matsuyama A and Nakanishi K (2000b) Characteristics of serine acetyltransferase from *Escherichia coli* deleting different lengths of amino acid residues from the C-terminus. Biosci Biotechnol Biochem 64: 1874–1880.

Murakoshi I, Kaneko M, Koide C and Ikegami F (1986) Enzymatic synthesis of the neuroexcitatory amino acid quisqualic acid by the cysteine synthase. Phytochemistry 25: 2759–2763

Nakamori S, Kobayashi SI, Kobayashi C and Takagi H (1998) Overproduction of L-cysteine and L-cystine by *Escherichia coli* strains with a genetically altered serine acetyltransferase. Appl Environ Microbiol 64: 1607–1611

Neuenschwander U, Suter M and Brunold C (1991) Regulation of sulfate assimilation by light and O-acetyl-L-serine in *Lemna minor* L. Plant Physiol. 97: 253–258

Nicholson ML, Gaasenbeek M and Laudenbach DE (1995) Two enzymes together capable of cysteine biosynthesis are encoded on a cyanobacterial plasmid. Mol Gen Genet 247: 623–632

Nicholson ML and Laudenbach DE (1995) Genes encoded on a cyanobacterial plasmid are transcriptionally regulated by sulfur availability and CysR. J Bacteriol 177: 2143–2150

Niehaus A, Gisselmann G and Schwenn JD (1992) Primary structure of the *Synechococcus* PCC 7942 PAPS reductase gene. Plant Mol Biol 20: 1179–1183

Nikiforova VJ, Daub CO, Hesse H, Willmitzer L and Hoefgen R (2005) Integrative gene-metabolite network with implemented causality deciphers informational fluxes of sulphur stress response. J Exp Bot 56: 1887–1896

Nikiforova VJ, Gakiere B, Kempa S, Adamik M, Willmitzer L, Hesse H and Hoefgen R (2004) Towards dissecting nutrient metabolism in plants: a systems biology case study on sulphur metabolism. J Exp Bot 55: 1861–1870

Noji M, Inoue K, Kimura N, Gouda A and 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

Noji M and Saito K (2002) Molecular and biochemical analysis of serine acetyltransferase and cysteine synthase towards sulfur metabolic engineering in plants. Amino Acids 22: 231–243

Noji M, Saito M, Nakamura M, Aono M, Saji H and Saito K (2001a) Cysteine synthase overexpression in tobacco confers tolerance to sulfur-containing environmental pollutants. Plant Physiol 126: 973–980

Noji M, Takagi Y, Kimura N, Inoue K, Saito M, Horikoshi M, Saito F, Takahashi H and Saito K (2001b) Serine acetyltransferase involved in cysteine biosynthesis from spinach: molecular cloning, characterization and expression analysis of cDNA encoding a plastidic isoform. Plant Cell Physiol 42: 627–634

Ohkama N, Goto DB, Fujiwara T and Naito S (2002) Differential tissue-specific response to sulfate and methionine of a soybean seed storage protein promoter region in transgenic Arabidopsis. Plant Cell Physiol 43: 1266–1275

Olsen L, Huang B, Vetting M and Roderick S (2004) Structure of serine acetyltransferase in complexes with CoA and its cysteine feedback inhibitor. Biochemistry 43: 6013–6019

Peiser GD, Wang TT, Hoffman NE, Yang SF, Liu HW and Walsh CT (1984) Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylic acid during its conversion to ethylene. Proc Natl Acad Sci U S A 81: 3059–3063

Pye VE, Tingey AP, Robson RL and Moody PC (2004) The structure and mechanism of serine acetyltransferase from *Escherichia coli*. J Biol Chem 279: 40729–40736

Rabeh WM and Cook PF (2004) Structure and mechanism of *O*-acetylserine sulfhydrylase. J Biol Chem 279: 26803–26806

Raetz CR and Roderick SL (1995) A left-handed parallel beta helix in the structure of UDP-N-acetylglucosamine acyltransferase. Science 270: 997–1000

Ravina CG, Chang CI, Tsakraklides GP, McDermott JP, Vega JM, Leustek T, Gotor C and Davies JP (2002) The *sac* mutants of *Chlamydomonas reinhardtii* reveal transcriptional and posttranscriptional control of cysteine biosynthesis. Plant Physiol 130: 2076–2084

Rege VD, Kredich NM, Tai CH, Karsten WE, Schnackerz KD and Cook PF (1996) A change in the internal aldimine lysine (K42) in O-acetylserine sulfhydrylase to alanine indicates its importance in transimination and as a general base catalyst. Biochemistry 35: 13485–13493

Rennenberg H (1983) Cysteine desulfhydrase activity in cucurbit plants: Stimulation by preincubation with L- or D-cysteine. Phytochemistry 22: 1557–1560

Rennenberg H, Arabatzis N and Grundel I (1987) Cysteine desulphydrase activity in higher plants: Evidence for the action of L- and D-cysteine specific enzymes. Phytochemistry 26: 1583–1589

Riemenschneider A, Bonacina E, Schmidt A and Papenbrock J (2005b) Isolation and characterization of a second D-cysteine desulfhydrase-like protein from *Arabidopsis*. In: Sulfur transport and assimilation in plants in the post genomic era, Saito K, De Kok LJ, Stulen I, Hawkesford MJ, Schnug E, Sirko A and Rennenberg H (eds), pp 103–106. Backhuys Publishers, Leiden

Riemenschneider A, Wegele R, Schmidt A and Papenbrock J (2005a) Isolation and characterization of a D-cysteine desulfhydrase protein from *Arabidopsis thaliana*. FEBS J 272: 1291–1304

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

Romero LC, Dominguez-Solis JR, Gutierrez-Alcala G and Gotor C (2001) Salt regulation of *O*-acetylserine (thiol)lyase in *Arabidopsis thaliana* and increased tolerance in yeast. Plant Physiol Biochem 39: 643–647

Rüegsegger A and Brunold C (1992) Effect of cadmium on γ-glutamylcysteine synthesis in maize seedlings. Plant Physiol 99: 428–433

Ruffet ML, Droux M and 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 ML, Lebrun M, Droux M and 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

Saidha T, Na SQ, Li JY and Schiff JA (1988) A sulphate metabolizing centre in *Euglena* mitochondria. Biochem J 253: 533–539

Saito K (2000) Regulation of sulfate transport and synthesis of sulfur-containing amino acids. Curr Opin Plant Biol 3: 188–195

Saito K, Inoue K, Fukushima R and Noji M (1997) Genomic structure and expression analyses of serine acetyltransferase gene in *Citrullus vulgaris* (watermelon). Gene 189: 57–63

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

Saito K, Yokoyama H, Noji M and 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

Sasaki Y, Asamizu E, Shibata D, Nakamura Y, Kaneko T, Awai K, Amagai M, Kuwata C, Tsugane T, Masuda T, Shimada H, Takamiya K, Ohta H and Tabata S (2001) Monitoring of methyl jasmonate-responsive genes in Arabidopsis by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. DNA Res 8: 153–161

Schachtman DP and Shin R (2007) Nutrient sensing and signaling: NPKS. Annu Rev Plant Biol 58: 47–69

Schiff S, Stern AI, Saidha T and Li J (1993) Some molecular aspects of sulfate metabolism in photosynthetic organisms. In: Sulfur nutrition and assimilation in higher plants. Regulatory, agricultural and environmental aspects, De Kok LJ, Stulen I, Rennenberg H, Brunold C and Rauser WC (eds), SBP Academic, The Hague

Schmidt A (1986) Regulation of sulfur metabolism in plants. Progr. Bot. 48: 133–150

Schmidt A (1987) D-cysteine desulfhydrase from spinach. Methods Enzymol 143: 449–453

Schmidt A (2005) Metabolic background of H_2S release from plants. In: Sino-German workshop on aspects of sulfur nutrition of plants, De Kok LJ and Schnug E (eds), pp 121–129

Schmidt A and Jäger K (1992) Open questions about sulfur metabolism in plants. Annu Rev Plant Physiol Plant Mol Bio 43: 325–349

Schnackerz KD, Tai CH, Simmons JW, 3rd, Jacobson TM, Rao GS and Cook PF (1995) Identification and spectral characterization of the external aldimine of the O-acetylserine sulfhydrylase reaction. Biochemistry 34: 12152–12160

Sirko A, Blaszczyk A and Liszewska F (2004) Overproduction of SAT and/or OASTL in transgenic plants: a survey of effects. J Exp Bot 55: 1881–1888

Smacchi E and Gobbetti M (1998) Purification and characterization of cystathionine γ-lyase from *Lactobacillus fermentum* DT41. FEMS Microbiol Lett 166: 197–202

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

Smythe CV (1945). Adv Enzymol 5: 237–247

Staton AL and Mazelis M (1991) The C-S lyases of higher plants: homogenous β-cystathionase of spinach leaves. Arch Biochem Biophys 290: 46–50

Strambini G, Cioni P and Cook P (1996) Tryptophan luminescence as a probe of enzyme conformation along the O-acetylserine sulfhydrylase reaction pathway. Biochemistry 35: 8392–8400

Sugantino M and Roderick SL (2002) Crystal structure of Vat(D): an acetyltransferase that inactivates streptogramin group A antibiotics. Biochemistry 41: 2209–2216

Sun Q, Emanuelsson O and van Wijk KJ (2004) Analysis of curated and predicted plastid subproteomes of Arabidopsis. Subcellular compartmentalization leads to distinctive proteome properties. Plant Physiol 135: 723–734

Tabe LM and Droux M (2001) Sulfur assimilation in developing lupin cotyledons could contribute significantly to

the accumulation of organic sulfur reserves in the seed. Plant Physiol 126: 176–187

Tabe LM and Droux M (2002) Limits to sulfur accumulation in transgenic lupin seeds expressing a foreign sulfur-rich protein. Plant Physiol 128: 1137–1148

Tai CH, Nalabolu SR, Jacobson TM, Minter DE and Cook PF (1993) Kinetic mechanisms of the A and B isozymes of *O*-acetylserine sulfhydrylase from *Salmonella typhimurium* LT-2 using the natural and alternative reactants. Biochemistry 32: 6433–6442

Tai C, Nalabolu S, Simmons J, Jacobson T and Cook P (1995) Acid-base chemical mechanism of *O*-acetylserine sulfhydrylases-A and -B from pH studies. Biochemistry 34: 12311–12322

Tai CH, Yoon MY, Kim SK, Rege VD, Nalabolu SR, Kredich NM, Schnackerz KD and Cook PF (1998) Cysteine 42 is important for maintaining an integral active site for O-acetylserine sulfhydrylase resulting in the stabilization of the α-aminoacrylate intermediate. Biochemistry 37: 10597–10604

Takagi H, Kobayashi C, Kobayashi S and Nakamori S (1999) PCR random mutagenesis into *Escherichia coli* serine acetyltransferase: isolation of the mutant enzymes that cause overproduction of L-cysteine and L-cystine due to the desensitization to feedback inhibition. FEBS Lett 452: 323–327

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

Urano Y, Manabe T, Noji M and Saito K (2000) Molecular cloning and functional characterization of cDNAs encoding cysteine synthase and serine acetyltransferase that may be responsible for high cellular cysteine content in *Allium tuberosum*. Gene 257: 269–277

Vaara M (1992) Eight bacterial proteins, including UDP-N-acetylglucosamine acyltransferase (LpxA) and three other transferases of *Escherichia coli*, consist of a six-residue periodicity theme. FEMS Microbiol Lett 76: 249–254

Van Hoewyk D, Abdel-Ghany SE, Cohu CM, Herbert SK, Kugrens P, Pilon M and Pilon-Smits EA (2007) Chloroplast iron-sulfur cluster protein maturation requires the essential cysteine desulfurase CpNifS. Proc Natl Acad Sci U S A 104: 5686–5691

Vuorio R, Hirvas L and Vaara M (1991) The Ssc protein of enteric bacteria has significant homology to the acyltransferase Lpxa of lipid A biosynthesis, and to three acetyltransferases. FEBS Lett 292: 90–94

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

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

Wawrzynski A, Kopera E, Wawrzynska A, Kaminska J, Bal W and Sirko A (2006) Effects of simultaneous expression of heterologous genes involved in phytochelatin biosynthesis on thiol content and cadmium accumulation in tobacco plants. J Exp Bot 57: 2173–2182

Wirtz M and Hell R (2001) Recombinant production of cysteine and glutathione by expression of feedback-insensitive serine acetyltransferase in *Escherichia coli*. In Nachwachsende Rohstoffe für die Chemie – 7. Symposium Dresden, pp 722–728. Landwirtschaftsverlag, Münster

Wirtz M and Hell R (2003a) Production of cysteine for bacterial and plant biotechnology: Application of cysteine feedback-insensitive isoforms of serine acetyltransferase. Amino Acids 24: 195–203

Wirtz M and Hell R (2003b) Comparative biochemical characterization of OAS-TL isoforms from *Arabidopsis thaliana*. Backhuys Publishers, Leiden

Wirtz M and Droux M (2005) Synthesis of the sulfur amino acids: cysteine and methionine. Photosynth Res 86: 345–362

Wirtz M and Hell R (2006) Functional analysis of the cysteine synthase protein complex from plants: structural, biochemical and regulatory properties. J Plant Physiol 163: 273–286

Wirtz M and Hell R (2007) Dominant-negative modification reveals the regulatory function of the multimeric cysteine synthase protein complex in transgenic tobacco. Plant Cell 19: 625–639

Wirtz M, Berkowitz O and Hell R (2000) Analysis of plant cysteine synthesis *in vivo* using *E. coli* as a host. In: Sulfur nutrition and sulfur assimilation in higher plants: molecular, biochemical and physiological aspects, Brunold C, Davidian J-C, De Kok L, Rennenberg H, Stulen I (eds), pp 297–298. P. Haupt, Bern

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

Wirtz M, Berkowitz O, Droux M and 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

Woehl EU, Tai CH, Dunn MF and Cook PF (1996) Formation of the α-aminoacrylate immediate limits the overall reaction catalyzed by *O*-acetylserine sulfhydrylase. Biochemistry 35: 4776–4783

Xu XM and Moller SG (2006) AtSufE is an essential activator of plastidic and mitochondrial desulfurases in *Arabidopsis*. EMBO J 25: 900–909

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

Ye H, Abdel-Ghany SE, Anderson TD, Pilon-Smits EA and Pilon M (2006) CpSufE activates the cysteine desulfurase CpNifS for chloroplastic Fe-S cluster formation. J Biol Chem 281: 8958–8969

Ye H, Garifullina GF, Abdel-Ghany SE, Zhang L, Pilon-Smits EA and Pilon M (2005) The chloroplast NifS-like protein of *Arabidopsis thaliana* is required for iron-sulfur cluster formation in ferredoxin. Planta 220: 602–608

Yip WK and Yang SF (1988) Cyanide metabolism in relation to ethylene production in plant tissues. Plant Physiol 88: 473–476

Yoshida Y, Nakano Y, Amano A, Yoshimura M, Fukamachi H, Oho T and Koga T (2002) *lcd* from *Streptococcus anginosus* encodes a C-S lyase with α,α-elimination activity that degrades L-cysteine. Microbiology 148: 3961–3970

Youssefian S, Nakamura M, Orudgev E and Kondo N (2001) Increased cysteine biosynthesis capacity of transgenic tobacco overexpressing an *O*-acetylserine(thiol) lyase modifies plant responses to oxidative stress. Plant Physiol 126: 1001–1011

Zhang Z, Shrager J, Jain M, Chang C-W, Vallon O and Grossman AR (2004) Insights into the survival of *Chlamydomonas reinhardtii* during sulfur starvation based on microarray analysis of gene expression. Eukaryotic Cell 3: 1331–1348