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REVIEW ARTICLE

Latest news about the sulfurtransferase protein family of higher plants

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Abstract Sulfurtransferases/rhodaneses (Str) comprise a group of enzymes widely distributed in all phyla which catalyze in vitro the transfer of a sulfur atom from suitable sulfur donors to nucleophilic sulfur acceptors. The best characterized Str is bovine rhodanese (EC 2.8.1.1) which catalyses in vitro the transfer of a sulfane sulfur atom from thiosulfate to cyanide, leading to the formation of sulfite and thiocyanate. Plants as well as other organisms contain many proteins carrying a typical rhodanese pattern or domain forming multi-protein families (MPF). Despite the presence of Str activities in many living organisms, the physiological role of the members of this MPF has not been established unambiguously. While in mammals these proteins are involved in the elimination of toxic cyanogenic compounds, their ubiquity suggests additional physiological functions. In plants, Str are localized in the cytoplasm, mitochondria, plastids, and nucleus. Str probably also transfer reduced sulfur onto substrates as large as peptides or proteins. Several studies in different organisms demonstrate a protein–protein interaction with members of the thioredoxin MPF indicating a role of Str in maintenance of the cellular redox homeostasis. The increased expression of several members of the Str MPF in various stress conditions could be a response to oxidative stress. In summary, data indicate that Str are involved in various essential metabolic reactions.

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Abbreviations

Acc. no.	Accession number
AR	Arsenate reductase
CAS	β-Cyano-L-alanine synthase
Cys	Cysteine
E. coli	*Escherichia coli*
3-MP	3-Mercaptopyruvate
3-MP Str	3-Mercaptopyruvate Str
Str	Sulfurtransferase(s)
TS	Thiosulfate
TS Str	Thiosulfate sulfurtransferase

Introduction

Sulfurtransferase/rhodanese (Str) enzymes catalyze the transfer of a sulfur atom from suitable sulfur donors to nucleophilic sulfur acceptors. The most studied and best characterized Str is bovine rhodanese which catalyzes in vitro the transfer of a sulfane sulfur atom from thiosulfate (TS) to cyanide, leading to the formation of sulfite and thiocyanate. Rhodanese, from the German word for thiocyanate, "Rhodanid", is a widespread enzyme. Rhodanese activity has been detected in all major phyla (http://www.ncbi.nlm.nih.gov/cgi-bin/COG, COG0607). Bovine liver rhodanese has been the object of numerous functional investigations (Westley 1973, 1981). According to the generally accepted mechanism, during catalysis the enzyme cycles between two distinct forms, the free enzyme (Rhod), and a covalent enzyme–sulfur intermediate (Rhod-S):

$$S_2O_3^{2-} + \text{Rhod} \rightarrow SO_3^{2-} + \text{Rhod} - S$$
$$\text{Rhod} - S + CN^- \rightarrow \text{Rhod} + SCN^-.$$

The original crystal structure of bovine liver rhodanese (Ploegman et al. 1978; Russell et al. 1978) and other independent crystallographic investigations (Gliubich et al. 1996) have shown that the Rhod-S intermediate is characterized by a persulfide bond at the sulfhydryl group of the essential cysteine (Cys) residue 247. The shape and properties of the proteins surface in the proximity of the active site are considered essential for substrate binding (Luo and Horowitz 1994). In this respect the alignment of the known amino acid sequences of rhodanese enzymes indicates a high overall sequence homology. The tertiary structure of bovine rhodanese is composed of two domains which are characterized by very similar three-dimensional folds in spite of a negligible overall sequence homology. The structural similarity of the two rhodanese domains has been considered as the prototype of divergent evolution from a common ancestor protein which, after gene duplication and under the constraint of tertiary structure conservation, led to the almost complete obliteration of sequence similarity between the N- and the C-terminal halves (Ploegman et al. 1978; Bordo et al. 2000).

It was shown that rhodanese domains are structural modules found as one-domain proteins, as tandemly repeated modules in which the C-terminal domain only bears the properly structured active site, or as members of multi-domain proteins. More than 8,000 sequences containing a rhodanese domain (PF00581) have been classified so far. In *Escherichia coli* (*E. coli*) several genes encoding proteins consisting of (or containing) a rhodanese domain bearing the potentially catalytic Cys have been identified. After the crystal structure of one of these proteins, the 12-kDa GlpE protein, has been solved, it can be considered to be the prototype structure for the ubiquitous one-domain rhodanese module (Ray et al. 2000; Spallarossa et al. 2001).

In many prokaryotes and eukaryotes 3-mercaptopyruvate (3-MP) Str activity was discovered. Both Str proteins, 3-MP Str and rhodanese, isolated from the same organism (here: rat) accepted 3-MP and TS but the ratios of their respective enzyme activities differed. The purified as well as the recombinant rat 3-MP Str revealed K_m values for 3-MP in the low millimolar range. This review focuses on enzymes in the categories EC 2.8.1.1, EC 2.8.1.2, and EC 2.8.1.3 (http://us.expasy.org/enzyme/).

General description of plant sulfurtransferases

Members of this protein family were described quite early in the biochemical history. Already in 1938 rhodanese activity was found to be present in plants (Gemeinhardt 1938), however, closer investigations were undertaken much later. Str activity was compared in crude extracts of cyanogenic and non-cyanogenic plants (Chew 1973; Kakes and Hakvoort 1992). Diurnal variation of cyanogenic glucosides, thiocyanate and rhodanese activity was measured in the cyanogenic plant, *Manihot esculenta* (Okolie and Obasi 1993). In 1984 Schmidt et al. started first attempts to purify 3-MP Str (Schmidt 1984) and TS Str (Schmidt et al. 1984) from photosynthetic organisms. Three different fractions containing Str activity were analyzed from the green alga *Chlorella fusca* (Schmidt et al. 1984). In *Chlamydomonas reinhardtii* TS reductase and rhodanese activities were postulated to be catalyzed by one enzyme (Prieto et al. 1997).

Sequencing of the complete *Arabidopsis* genome accelerated the analysis of plant Str on the molecular level. Independently, three groups isolated and analyzed two Str from *Arabidopsis* and called them either TS Str (Hatzfeld and Saito 2000) or 3-MP Str (Nakamura et al. 2000; Papenbrock and Schmidt 2000a, b). Both sequences coding for Str1 and Str2 from *Arabidopsis* (AtStr1, AtStr2) evolved probably by gene duplication. Database mining revealed the existence of 20 different Str or Str-like proteins of different length in *Arabidopsis* (Bauer and Papenbrock 2002; Bartels et al. 2007b; Table 1). Next to *Arabidopsis*, the first sequence from plant Str DNA sequences was published from *Datisca glomerata*, a Datiscaceae living in symbiosis with nitrogen-fixing bacteria (Okubara and Berry 1999). Then a full length Str sequence from wheat was annotated in the database (Accession number (Acc. no.) AAK64575) which shows 62% identity and 78% similarity to AtStr1. Meanwhile, members of the rhodanese family were identified in various plants by the use of databases (e.g. http://smart.embl-heidelberg.de/). In comparison to *Arabidopsis* (AtStr1) the *Brassica napus* Str shows an identity of 75.1% and a similarity of 81.4% in an alignment (http://www.ebi.ac.uk/Tools/emboss/align/), *Brassica oleracea* 76.9%/86.5%, *Arabidopsis* (AtStr.2) 68.6%/76%, *Populus trichocarpa* 70.8%/81.5%, *Vitis vinifera* 71.4%/82.8%, *Dactisca glomerata* 69.5%/79.7%, *Oryza sativa* 61.1%/72.2%, *Zea mays* 50.0%/63.5%, *Physcomitrella patens* 42.1%/55%, *Ostreococcus lucimarinus* 34%/47.5%, *Ostreococcus tauri* 35.4%/49.1%, *Chlamydomonas reinhardtii* 32.6%/49.2%, and *Picea sitchensis* 53.9%/68.4%. A phylogram (http://www.ebi.ac.uk/Tools/clustalw2/) estimated with these sequences shows three main groups (Fig. 1). Group 1 consists of two subgroups, namely Liliopsida (1a) and eudicotyledons (1b), group 2 comprises green algae and the moss *Physcomitrella patens*, and group 3 consists of only *Picea sitchensis*, belonging to the Coniferophyta reflecting

Table 1 Overview of the 20 members of the sulfurtransferase/rhodanese multi-protein family in *Arabidopsis thaliana*

	AGI ID	Amino acids	Localization (pred/exp)	Reference for localization	Putative in vivo or in vitro enzyme activity	References
Group I						
1	At1g79230	322	MT (exp)	Bauer et al. (2004) Heazlewood et al. (2004) Nakamura et al. (2000)	TS Str 3-MP Str	Papenbrock and Schmidt (2000a, b) Hatzfeld and Saito (2000) Nakamura et al. (2000)
2	At1g16460	318	Cyt (exp)	Bauer et al. (2004) Hatzfeld and Saito (2000) Nakamura et al. (2000)	TS Str 3-MP Str	
Group II						
3	At5g23060	387	MT (exp)	Heazlewood et al. (2004)	Similar to unknown protein	Db annotation
4	At4g01050	457	CP, thylakoid membrane (exp)	Peltier et al. (2004)	Hydroxyproline-rich glycoprotein	Db annotation
4a	At3g25480	264	CP, thylakoid membrane (exp)	Peltier et al. (2004)	Hypothetical protein	Db annotation
Group III						
5	At5g03455	132	Nucleus (pred/exp)	Landrieu et al. (2004)	Dual-specificity tyrosine phosphatase Acr2 AR	Landrieu et al. (2004) Duan et al. (2005)
6	At1g09280	581	Cyt (pred/exp)	http://www.expasy.ch	Unknown protein	Db annotation
7	At2g40760	522	MT (pred)	http://www.expasy.ch	Unknown protein	Db annotation
8	At1g17850	366	CP/ER (pred)	http://www.expasy.ch	Contains rhodanese-like PF00581 domain	Db annotation
Group IV						
9	At2g42220	234	CP, thylakoid membrane (exp)	Peltier et al. (2004) Bartels (2006)	*Datura innoxia* homolog Cd^{2+} induced	Louie et al. (2003)
10	At3g08920	214	MT (pred/preliminary exp)	Bartels (2006)	Unknown protein	Db annotation
11	At4g24750	260	CP/Per (pred/preliminary exp)	Bartels (2006)	Putative protein	Db annotation
Group V						
12	At5g19370	309	CP/MT (pred)	http://www.expasy.ch	Putative peptidyl-prolyl *cis–trans* isomerase	Db annotation Zhao et al. (2003)
13	At5g55130	464	CP/Cyt (pred)	http://www.expasy.ch	Molybdopterin synthase sulfurylase	Db annotation
Group VI						
14	At4g27700	237	CP (exp)	Bauer et al. (2004) Peltier et al. (2004)	Hypothetical protein	Db annotation
15	At4g35770	182	CP, thylakoid membrane (exp)	Bauer et al. (2004)	AtSen1, senescence association, dark-induced, MoCo synthesis TS Str	Oh et al. (1996) Schenk et al. (2005) Papenbrock, unpublished

Table 1 continued

	AGI ID	Amino acids	Localization (pred/exp)	Reference for localization	Putative in vivo or in vitro enzyme activity	References
16	At5g66040	120	CP (exp)	Bauer et al. 2004	Senescence-associated protein sen1-like protein; ketoconazole resistance protein-like TS Str	Db annotation Bauer and Papenbrock (2002)
17	At2g17850	150	Nu (pred)	http://www.expasy.ch	Putative senescence-associated rhodanese protein; similarity to Ntdin homology to defense and stress associated *Cucurbita* proteins	Yang et al. (2003) Walz et al. (2004)
17a	At2g21045	169	MT/Cyt (pred)	http://www.expasy.ch	Senescence-associated protein	Db annotation
18	At5g66170	136	Cyt (exp)	Bauer et al. (2004)	Senescence-associated protein sen1-like protein TS Str	Db annotation Bauer and Papenbrock (2002)

The protein name, gene identification, number of amino acids, predicted or experimentally shown localization, and remarks on different aspects are summarized including the respective references. The programs Predotar, PSORT, and TargetP were used for the localization prediction (http://www.expasy.ch/tools)

aa amino acids, *CP* chloroplast, *db* database, *ER* endoplasmatic reticulum, *exp* experimental, *ID* identification, *MT* mitochondrial, *pred* predicted

the taxonomy and the average number of sequences in each taxon.

The multi-protein Str family in *Arabidopsis*

Since in December 2000 the complete sequence of *Arabidopsis* was published (The Arabidopsis Genome Initiative 2000) a comprehensive characterization of the multi-protein family (MPF) could be started. In addition to the 18 Str identified previously (Bauer and Papenbrock 2002), recent data mining of *Arabidopsis* databases revealed the appearance of two more sequences containing one typical rhodanese domain. Therefore, the phylogenetic tree of AtStr had to be expanded by two members, AtStr4a and AtStr17a. All AtStr proteins have been classified in to six groups according to their amino acid sequence homologies (Bartels et al. 2007b; Table 1). Rhodanese domains are visualized within the Str protein sequences (Acc. No. SM00450) (http://smart.embl-heidelberg.de/). In Fig. 2 schematic diagrams of representative Str proteins occurring in *Arabidopsis* and containing rhodanese domain(s) are shown.

Localization of Str in *Arabidopsis* cells

The knowledge about the localization of a protein in the organism and in the cell often helps to elucidate its function. Compartmentalization plays an important role in regulation and communication of cellular processes, especially in plants (Papenbrock and Grimm 2001). In contrast to cells of heterotrophic eukaryotes, plant cells contain, besides mitochondria, one additional type of semi-autonomous organelles enveloped by two membranes, the plastids. The bacterial Str analyzed so far are localized either in the periplasm or in the cytoplasm. The GlpE protein from *E. coli* is a cytoplasmic protein (Ray et al. 2000). A rhodanese-like protein from *Synechococcus* sp. strain PCC 7942 is localized in the periplasm (Laudenbach et al. 1991). In mammalian cells 3-MP Str was found both in mitochondria and in cytoplasm whereas the subcellular localization of rhodanese proteins was exclusively in the mitochondria (Westley 1973; Jarabak and Westley 1978; Nagahara et al. 1998). A targeting sequence for the rat rhodanese was elucidated which forms an amphipathic α-helix. However, after the protein has been transported into the mitochondrial matrix space the targeting sequence is not cleaved (Waltner and Weiner 1995). Rat 3-MP Str contains also a putative targeting signal; the retention in the cytoplasm might be controlled by posttranslational modification, such as phosphorylation/dephosphorylation (Nagahara et al. 1999).

Different regions of the N-terminal parts of AtStr1 and AtStr2 were fused to the green fluorescent protein (GFP)

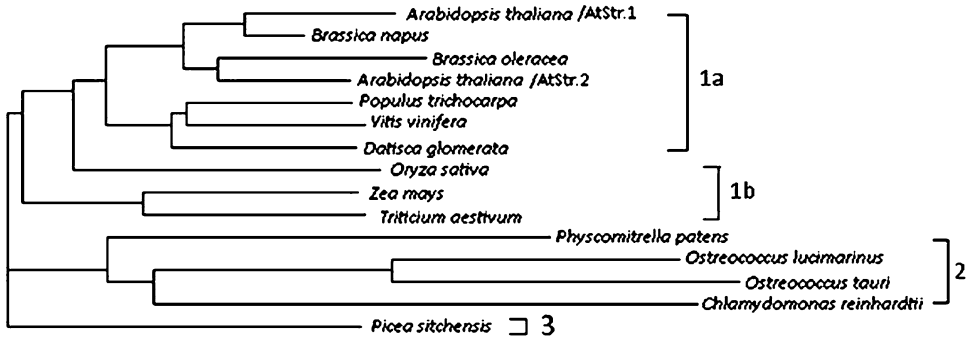

Fig. 1 The phylogram was obtained using the following protein sequences of two-domain Str in the Clustalw2 program (http://www.ebi.ac.uk/Tools/clustalw2/, method: neighbor-joining): *Arabidopsis thaliana* (L.) Heynh. (AtStr1: O64530; AtStr2: Q9S7Y9), *Brassica napus* L. (Papenbrock unpublished), *Brassica oleracea* L. (B2D2I1), *Populus trichocarpa* Torr. & A. Gray (A9PCY9), *Vitis vinifera* L. (A5B8K7), *Dactisca glomerata* K. Presl Baill. (Q9ZPK0), *Oryza sativa* (LOC_Os12g41500, http://rice.plantbiology.msu.edu/), *Zea mays* L. (B4FPY3), *Triticium aestivum* L. (Q94C43), *Physcomitrella patens* (Hedw.) Bruch & Schimp. (A9SGZ1), *Ostreococcus lucimarinus* strain CCE9901 (A4S6Y9), *Ostreococcus tauri* C. Courties & M.-J. Chrétiennot-Dinet (Q00VJ9), *Chlamydomonas reinhardtii* P.A. Dangeard (A8JB06), and *Picea sitchensis* (Bong.) Carr. (9NWI8)

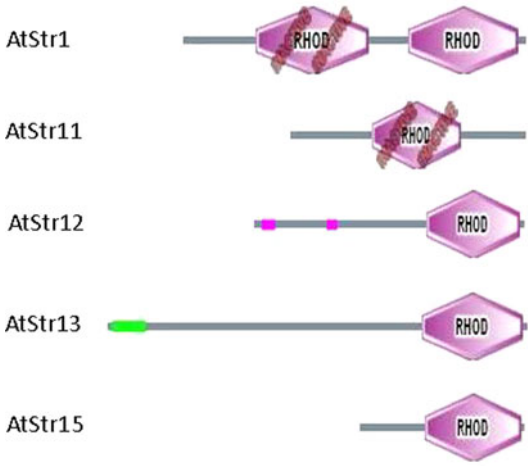

Fig. 2 Schematic diagrams of representative Str proteins containing rhodanese domain(s) occurring in *Arabidopsis*. The accession number for the rhodanese domain in SMART is SM00450 (http://smart.embl-heidelberg.de/)

(Nakamura et al. 2000). The results demonstrated a subcellular localization for AtStr1 and AtStr2 in the cytoplasm and in mitochondria, in agreement with immunoblots done in parallel. Some regions of AtStr1 fused to the green fluorescent protein were found to target not only in mitochondria, but also in the chloroplasts, suggesting that the regions of the targeting sequence recognized by protein import systems of mitochondria and chloroplasts are not identical (Nakamura et al. 2000). Other groups using the same methodology demonstrated the localization of AtStr1 in mitochondria and of AtStr2 in the cytoplasm (Hatzfeld and Saito 2000; Bauer et al. 2004).

AtStr14 and AtStr16 are localized in the chloroplasts, whereas AtStr18 remains in the cytoplasm (Bauer et al. 2004). The localization of AtStr15 protein is unusual. It was not clear if the protein was outside the chloroplast, on the chloroplast envelope or attached to the thylakoid membrane. A higher magnification indicated an association of AtStr15 with the thylakoid membrane confirmed by immune gold labeling approaches (Bauer et al. 2004). In a modified three-phase partitioning approach to isolate thylakoid membrane proteins, 4 out of 242 proteins contained a rhodanese domain (At4g01050, AtStr4; At3g25480, AtStr4a with one membrane spanning region; At2g42220, AtStr9; At4g27700, AtStr14) (Peltier et al. 2004). These results are in agreement with predictions by programs and with experimental results obtained with GFP fusions (Bauer et al. 2004; Table 1). In summary, for 14 out of 20 AtStr the localization was experimentally determined (for some proteins still preliminary) by different means (e.g. Bauer et al. 2004).

Pattern and profiles in *Arabidopsis* Str

The most important and unifying amino acid in all members of the Str MPF is a Cys residue in the C-terminal domain surrounded by a certain environment to form the active site. In addition, all proteins in the Str family are unified by well-defined highly conserved sequence domains. Because of the ubiquitous distribution of Str in eubacteria, archaea, and eukaryotes a large number of Str sequences are available. The search algorithms for typical conserved sequence domains were defined already some time ago and could be proven and improved in many approaches. In PROSITE two patterns were developed for the rhodanese family: consensus pattern 1 (FY)-x(3)-H-(LIV)-P-G-A–x(2)-(LIVF) (Acc. No. PS00380) and consensus pattern 2 (AV)-x(2)-(FY)-(DEAP)-G-(GSA)-(WF)-

x-E-(FYW) (Acc. No. PS00683). They are based on highly conserved regions, one is located in the N-terminal region, the other at the C-terminal extremity of the protein (http://www.expasy.ch). Only the rhodanese C-terminal pattern can be identified in all Str-like proteins.

In InterPro two entries were developed (http://www.ebi.ac.uk/interpro/): IPR001307 recognizes only TS Str (rhodanese-like, matches 1475 proteins, 12th November 2009), whereas in the pattern defined in IPR001763 (Rhodanese/Cdc25 fold, matches 11334 proteins, 12th November 2009) two rhodanese domains can be identified. This entry comprises similarities to the catalytic domain of Cdc25 phosphatase, the non-catalytic domains of eukaryotic dual-specificity MAP-kinase phosphatases, the non-catalytic domains of yeast PTP-type MAP-kinase phosphatases, the non-catalytic domains of yeast Ubp4, Ubp5, Ubp7, the non-catalytic domains of mammalian Ubp-Y, the *Drosophila* heat shock protein HSP-67BB, several bacterial cold-shock and phage-shock proteins, plant senescence-associated proteins, and catalytic and non-catalytic domains of rhodanese.

In two-domain Str the N- and C-terminal domains are connected by a linker. In plant Str the linker sequence is exceptionally longer than in sequences from other species (Burow et al. 2002). The one-domain Str from bacteria are fully active and show high similarity to the C-terminal domain of two-domain Str. These facts raise the question whether each domain of the two-domain AtStr might form an active Str enzyme by itself: Enzymatic activity of the AtStr1 resides in the C-terminal domain but is boosted by the N-terminal domain and the linker peptide in the full length enzyme (Burow et al. 2002). In *Arabidopsis* a number of proteins with one rhodanese domain were identified (Bauer and Papenbrock 2002; Bartels et al. 2007b). At least three of the small one-domain Str-like proteins similar to the one-domain Str from bacteria showed high TS Str activity (Bauer and Papenbrock 2002; Bartels 2006).

Besides many similarities, such as the conserved Cys residue, the characterized two-domain plant Str differ in their structure from the two-domain mammalian TS and 3-MP Str. The number and positions of Cys residues, and consequently maybe also the function in plant Str, are different from known Str sequences in other organisms (Burow et al. 2002). In spectroscopic analyses of recombinant AtStr1C332S it could be shown that the reported loss of Str activity in this mutant (Burow et al. 2002) is due not only to the loss of the persulfuration site but also due to conformational changes of the whole protein structure (Bartels et al. 2007a). Of the five Cys residues in AtStr1 a second Cys (C339) close to the catalytic C332 was suggested to be involved in catalysis. The Str activity of the AtStr1C339V mutant was shown to be reduced to 25% using TS as sulfur donor substrate and slightly but significantly using 3-MP (Burow et al. 2002). The substitution of C339 by valine did not alter the conformation of the protein as shown by spectroscopic analyses (Bartels et al. 2007a), thus supporting a catalytic rather than a structural role of C339. The Cys residue might act in recognizing and binding of the acceptor molecule in close vicinity to the active site. Determination of the three-dimensional structure of the AtStr1C339V mutant might clarify the role of C339 unambiguously.

The activity of a AtStr1 derivative with a shortened linker sequence was reduced by more than 60% in comparison to the wild-type activity, probably because of a drastically reduced protein stability (Burow et al. 2002). The mutant was demonstrated to be far more prone to proteolytic digestion by trypsin than the wild-type AtStr1 (Bartels et al. 2007a). The interdomain linker connecting both domains may have a role in positioning of the two domains to each other to provide an appropriate conformation for substrate binding. The plant-specific elongation of the interdomain linker sequence in AtStr1 was suggested to provide an extended hydrophobic environment surrounding the substrate binding site, enabling the protein to bind substrates as large as proteins. If this is true, the plant two-domain Str might act in the regulation of other proteins by the direct transfer of sulfane sulfur (Burow et al. 2002; Bartels et al. 2007a).

Analysis of three-dimensional Str structures

The tertiary structure of bovine rhodanese is composed of two domains which, in spite of a negligible sequence homology, are characterized by very similar three-dimensional folds connected by a loop at the surface of the molecule. Each domain displays α/β topology, with a central parallel five-stranded β-sheet surrounded by α-helices on both sides. The structural similarity of the two rhodanese domains has been considered as the prototype of divergent evolution from a common ancestor protein, which, after gene duplication and under the constraint of tertiary structure conservation, led to the almost complete obliteration of sequence similarity between the N- and the C-terminal halves (Russell et al. 1978; Bordo et al. 2000).

The *Azotobacter* RhdA protein was crystallized and its structure solved (Bordo et al. 2000). In spite of a strong similarity of an overall conserved protein in comparison to bovine rhodanese, the crystallographic investigations show that the process of substrate recognition in *Azotobacter* RhdA is based on a widely mutated active site environment. The activity is essentially dependent on the main-chain conformation of the active site loop and on the effect of an ensuing positive electrostatic field on the pK_a of the

catalytic residue C230. Side-chains from the active site loop, as well as from the surrounding regions, are supposed to be important for the process of substrate selectivity (Bordo et al. 2000). The crystal structure of 3-MP Str from *E. coli*, the SseA protein, displays conformational variation of the rhodanese active site loop, hosting the catalytic Cys residue. This structure may support a new sulfur transfer mechanism involving C237 as the nucleophilic species and H66, R102, and R262 as residues assisting catalysis (Spallarossa et al. 2004).

The 3-MP Str from *Leishmania major* is a crescent-shaped molecule comprising three domains. The N-terminal and central domains are similar to the TS Str and create the active site containing a persulfurated catalytic C253 and an inhibitory sulfite coordinated by R74 and R185. A serine protease-like triad, comprising D61, H75, and S255, is near C253 and represents a conserved feature that distinguishes 3-MP Str from TS Str. During catalysis, S255 may polarize the carbonyl group of 3-MP to assist thiophilic attack, whereas R74 and R185 bind the carboxylate group. The *L. major* 3-MP Str is unusual with an 80-amino acid C-terminal domain, bearing remarkable structural similarity to the FK506-binding protein class of peptidylprolyl *cis*/*trans*-isomerase. This domain may be involved in mediating protein folding and Str–protein interactions (Alphey et al. 2003).

The three-dimensional structure of the *Arabidopsis* protein encoded by At5g66040 (AtStr16) was determined by nuclear magnetic resonance (NMR) spectroscopy. AtStr16 contains a central β-sheet flanked on both sides by α-helices. The striking distinctive feature of the AtStr16 structure is an extra β-hairpin connecting the $\beta1\alpha1\beta2\alpha2$ $\beta3\beta4$ and $\alpha3\beta7\alpha4\beta8\alpha5\beta9$ which may play an important role in binding a specific substrate (Cornilescu et al. 2006). The three-dimensional structure of the rhodanese homology domain of At4g01050 (amino acid 175–295) (AtStr4) from *Arabidopsis* was also determined. Conventional sequence alignment did not display significant homology with proteins of known structure or function. But more sensitive algorithms provided evidence that this sequence may have a rhodanese fold. Structural analyses by NMR show a small α/β domain with a central five-stranded β-sheet surrounded by four α-helices (Pantoja-Uceda et al. 2005). For *Arabidopsis* AtStr proteins more work to analyze the three-dimensional structure by NMR or X-ray crystallography has to be done, especially elucidation of the two-domain AtStr including the substrate or a substrate analog.

Determination of in vitro activities of Str

A number of molecules can serve as sulfur donors in the Str reaction, such as TS, thiosulfonates, persulfides, and 3-MP. Several compounds, such as cyanide, thiols, and dithiols, can act as acceptors (Fig. 3). The term sulfane sulfur designates sulfur atoms that are bonded covalently in chains only to other sulfur atoms. Examples are the outer sulfur of TS ($^{-}SO_3S^{-}$) and thiosulfonate ions (RSO_3S^{-}), the internal chain sulfurs of organic and inorganic polysulfides (RSS_nSR), where R represents an anion or organic group, persulfides (RSS^{-}), polythionates ($^{-}O_3SS_nSO_3^{-}$), and elemental sulfur (S_8) (Wood 1987).

Most scientists use the enzyme assay described by Sörbo (1955). In the meantime, a number of alternative assays were successfully developed: the analysis of thiocyanate by capillary electrophoresis has been introduced (Glatz et al. 1999), a continuous assay was described based on the continuous determination of the sulfite product (Cannella et al. 1984), and recently the very sensitive ^{1}H NMR assay was developed (Melino et al. 2003). Some ideas exist about the nature of enzymes which synthesize the substrates for Str, such as 3-MP and TS. To understand the function of an enzyme in the metabolic network of the cell the enzymes around the protein of interest have to be identified. For example, in the case of 3-MP Str in plants one has to postulate enzymes which catalyze the synthesis of 3-MP (Fig. 4). In animals 3-MP is presumably formed by transamination of Cys; Cys transaminase could catalyze the formation of L-glutamate and 3-MP from 2-oxoglutarate and L-Cys. Theoretically, 3-MP could also be synthesized from L-Cys by parallel release of H_2O_2 and ammonium catalyzed by an amino acid oxidase or by a Cys dehydrogenase releasing 3-MP, ammonium, and protons. In any case 3-MP is a derivative of Cys and production of 3-MP might also be involved in regulation of the Cys pool. Up to now, most of the metabolic pathways suggested still remain hypothetical and more work needs to be done (Westley 1973; White 1982; Huang et al. 1998).

Besides a high homology of several short motifs in Str even the positions of single amino acids are conserved in all species investigated. It was assumed that these amino acids are relevant for the specificity of substrate binding.

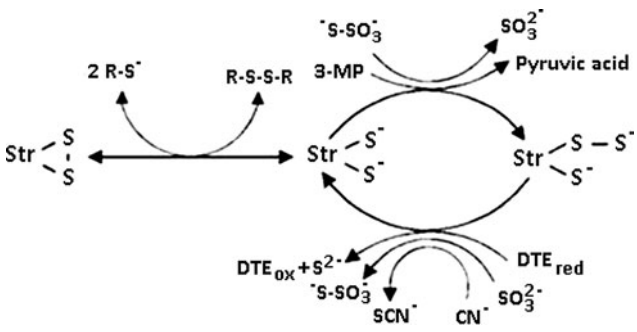

Fig. 3 Reaction scheme for 3-MP Str and TS Str. *3-MP* 3-mercaptopyruvate, *DTE* dithioerythritol (modified from Papenbrock and Schmidt 2000a)

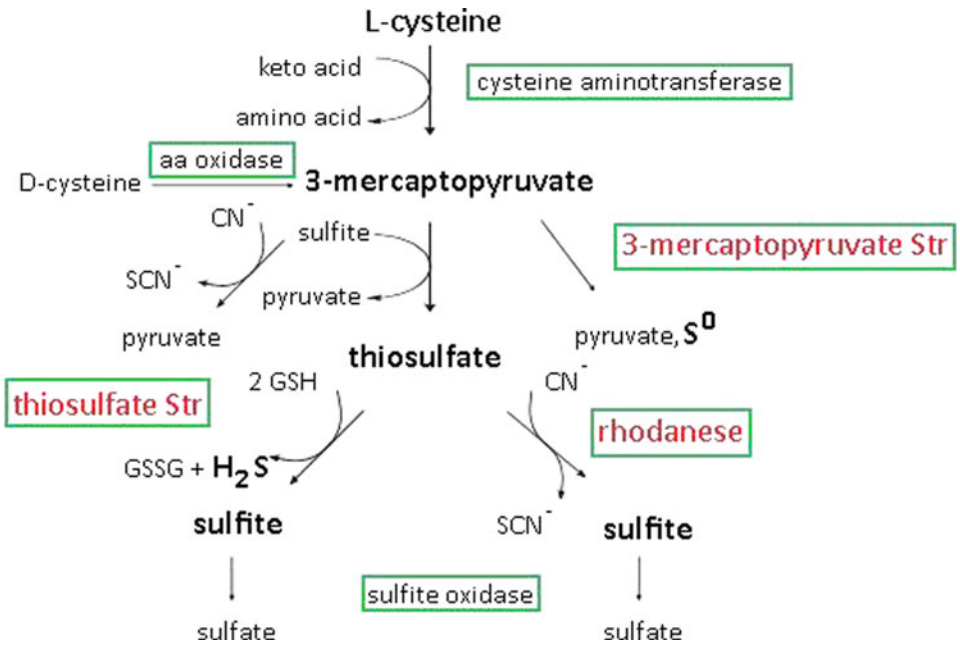

Fig. 4 Putative reactions catalyzed by Str

The role of non-Cys residues with respect to either 3-MP or TS specificity was investigated by mutagenesis studies (Luo and Horowitz 1994; Nagahara et al. 1995; Nagahara and Nishino 1996). A number of amino acids are indispensable for substrate binding and substrate specificity for 3-MP or TS, respectively. In both Str enzymes, 3-MP Str and rhodanese, from rat at least two amino acids in the active site are conserved, both enzymes accept 3-MP and TS but the ratio of their respective enzyme activities differs. C247 represents the catalytic site (formation of a persulfide) and R187 the substrate binding site. R187 and R196 of rat 3-MP Str are critical residues in determining substrate specificity for 3-MP, while R185, R247, and K248 of rat rhodanese are critical residues in determining substrate specificity for TS.

The purified as well as the recombinant rat 3-MP Str reveal K_m values for 3-MP in the low millimolar range. This K_m of the rat 3-MP Str for 3-MP is quite high, but also the k_{cat} is high; therefore 3-MP Str could be active at low physiological concentrations of 3-MP. 3-MP Str deficiency in the inherited human disease mercaptolactate-Cys disulfidurea results in alternative metabolism of 3-MP (Nagahara et al. 1995; Nagahara and Nishino 1996). K_m values for 3-MP of 0.2 mM have been reported for 3-MP Str from *L. major* using a thiol as the sulfur acceptor substrate (Williams et al. 2003). The 3-MP Str from *E. coli* (SseA) has a high affinity for 3-MP with a K_d value of 5 µM (Colnaghi et al. 2001). These observations support a role for 3-MP as the in vivo substrate for these 3-MP Str.

The reaction mechanism of rhodanese follows a ping-pong pattern (Westley and Heyse 1971). The reaction mechanism for 3-MP Str was reported to follow a sequential pattern based on the results of steady state kinetics (Jarabak and Westley 1978; Nagahara and Nishino 1996). It is still an open question whether the donor and acceptor substrates enter the active site in order.

Also the formation of selenium-substituted rhodanese by reaction with selenite and glutathione was shown. A selenium-bound rhodanese could be used as the selenium donor by reaction with selenite and glutathione in the in vitro selenophosphate synthetase assay. Selenophosphate is the active selenium-donor compound required by bacteria and mammals for the specific synthesis of SeCys-tRNA, the precursor of seleno-Cys in selenoenzymes (Ogasawara et al. 2001).

The two-domain AtStr resemble the 3-MP Str from mammals. In both proteins the residues which are important for binding 3-MP are conserved while putative residues necessary for TS binding have been replaced during evolution (Nakamura et al. 2000; Papenbrock and Schmidt 2000a, b). Due to the fact that AtStr1 has to activated by a thiol, such as 2-mercaptoethanol (Fig. 3), it was speculated that reduced sulfur is bound to both Cys residues, C332 and C339, and both sulfur atoms are involved in the reaction mechanism. The replacement of each Cys residue resulted in mutant forms which differed significantly in their stability, specific Str activities, and kinetic parameters which were determined for 3-MP as well as for TS as sulfur substrates: mutation of the putative active site Cys (C332) essentially abolished activity; for C339 a crucial role at least for the turnover of TS could be delineated (Burow et al. 2002; Bartels et al. 2007a). The function of C339 awaits further clarification in the future. The comparison of the kinetic parameters revealed that the specific activity of

AtStr1 was much higher when 3-MP was used as sulfur donor in comparison to TS. However, the K_m value for TS was much lower and finally the k_{cat}/K_m value was higher than with 3-MP. Both substrates could be metabolized naturally, but the physiological levels of 3-MP are rather low in comparison to the K_m determined in vitro (Papenbrock and Schmidt 2000a); the kinetic data may therefore indicate that better substrates still need to be found (Burow et al. 2002).

Mobilization of the sulfur of Cys as persulfide is the first step of sulfur transfer into thiamine, molybdopterin, 4-thiouridine, biotin, and lipoic acid, but the pathways diverge completely. For the first three compounds, one or several proteinic persulfides are involved, ending in the nucleophilic attack of a sulfur, persulfide, sulfide, or thiocarboxylate on a carbonyl equivalent (Schievelbein et al. 1969). ThiI, a protein originally suggested to be involved in thiamine biosynthesis (Thi operon), is an enzyme common to the biosynthetic pathways leading to both thiamine and 4-thiouridine in tRNA. The *E. coli* enzyme contains a C-terminal extension displaying sequence similarity to rhodanese. The C456 of ThiI aligns with the active site Cys residue of rhodanese, mutation impaired Str activity, and the generation of 4-thiouridine in tRNA. Only the ThiI proteins from *E. coli* and *H. influenza* possess the sequence of limited similarity to rhodanese (Donadio et al. 1990; Palenchar et al. 2000).

One of the Str (AtStr12) is annotated as peptidyl-prolyl *cis–trans* isomerase-like protein. However, to our knowledge no experimental evidence for this enzyme activity neither in vitro nor in vivo has been demonstrated.

Recently, a reaction chain consisting of three enzymes was shown to be involved in sulfide oxidation in animals. A membrane-bound sulfide: quinone oxidoreductase converts sulfide to persulfides and transfers the electrons to the ubiquinone pool. Subsequently, a sulfur dioxygenase in the mitochondrial matrix oxidizes one persulfide molecule to sulfite, consuming molecular oxygen. The final reaction is catalyzed by a Str, which adds a second persulfide from the sulfide: quinone oxidoreductase to sulfite, resulting in the final product TS. This role in sulfide oxidation is an additional physiological function of the mitochondrial Str in animals (Hildebrandt and Grieshaber 2008). The plant Str might be involved in a similar reaction chain producing TS in its reverse reaction. However, so far the other partners have not been identified in plant mitochondria.

The labile sulfane sulfur atom has been shown to have effects in biochemical systems which suggests that it may have several regulatory functions (Toohey 1989; Wróbel et al. 2009). This conclusion is supported by evidence that sulfane sulfur is generated by partially known metabolic pathways, that carrier proteins for stabilizing and transporting are widely distributed, and that it is effective in vitro at very low concentration in regulating the activities of many enzymes. Its properties of a very high potency and short half-life are consistent with a role as a finely tuned regulator (Toohey 1989).

A role in redox homeostasis: interaction of Str and thioredoxins

Several lines of evidence support the hypothesis that Str are involved in the maintenance of redox homeostasis by interacting with thioredoxins. First, results came from enzyme activity measurements in different species. The recombinant mammalian rhodanese catalyzes the direct oxidation of reduced thioredoxin evidently by reactive oxygen species. It was suggested that at least one Str isoform is involved in the detoxification of intramitochondrial oxygen-free radicals (Nandi et al. 2000). Kinetic analysis revealed that catalysis by purified GlpE from *E. coli* occurs by way of an enzyme–sulfur intermediate utilizing a double-displacement mechanism requiring an active site Cys. The K_m for TS was determined to 78 mM, the K_m for cyanide to 17 mM. When thioredoxin was used as acceptor the K_m was only 34 μM when TS was near its K_m, suggesting that thioredoxin or related dithiol proteins could be physiological substrates for Str (Ray et al. 2000).

Recently, the rat Str was suggested to be involved in the maintenance of the redox homeostasis by interacting with thioredoxin. Oxidative stress decreased 3-MP Str activity and increased the amount of Cys, a precursor of thioredoxin or glutathione. Furthermore, these cellular reductants restore the activity. Thus, the redox state regulates 3-MP Str activity at the enzymatic level, and on the other hand, 3-MP Str controls redox to maintain cellular redox homeostasis. As an intermediate the very stable formation of Cys-sulfenate was shown having even a lower redox potential than glutathione (Nagahara and Katayama 2005). In the same rat system Cys32 of *E. coli* thioredoxin reacted with two redox-active Cys of rat 3-MP Str by forming an intersubunit disulfide bond and a sulfenyl Cys247. A consecutively formed disulfide bond between thioredoxin and 3-MP Str must be cleaved for the activation. *E. coli* C32S thioredoxin, however, did not activate 3-MP Str. Reduced thioredoxin turns on a redox switch for the enzymatic activation of 3-MP Str which contributes to the maintenance of cellular redox homeostasis in rat cells (Nagahara et al. 2007).

Also in mammalian cells interaction of Str and thioredoxin plays an important role in the balance of the metabolism. The organo-sulfane sulfur compound, sodium 2-propenyl TS, was found to induce inhibition of Str activity in tumor cells. The activity of the enzyme was

restored by thioredoxin in a concentration- and time-dependent manner. The results suggest an involvement of the thioredoxin–thioredoxin reductase system in cancer cell cytotoxicity by organo-sulfane sulfur compounds and highlight the correlation between apoptosis induced by these compounds and the damage to the mitochondrial enzymes involved in the repair of the Fe–S cluster and in the detoxification system (Sabelli et al. 2008).

The increasing knowledge about redox regulation in plants shows more and more its importance in maintenance of the metabolism. In recent years the efforts to identify the major players in redox regulation by different methods are tremendous, including the characterization of thioredoxins. In a proteomic affinity approach an interaction of thioredoxin with 50 mitochondrial proteins was found, among them AtStr1 (Balmer et al. 2004). In a comparative proteomic approach cytosolic thioredoxin h3 from *Arabidopsis* was used in three methods for the identification of interacting proteins (Marchand et al. 2006). All together 73 interacting proteins could be identified, however, none of the cytoplasmic Str. All together more than 40 thioredoxins and thioredoxin-like proteins were identified in *Arabidopsis* (Meyer et al. 2005). Since a long time it is known that plants possess two thioredoxin systems, a cytoplasmic system including several thioredoxins and an NADPH-dependent thioredoxin reductase, and a specific chloroplastic system characterized by a ferredoxin-dependent thioredoxin reductase. Recently, also a functional plant mitochondrial system could be identified consisting of two thioredoxins and two NADPH-dependent thioredoxin reductases with dual-targeting (Laloi et al. 2001). As was discussed above also Str are localized in these compartments.

Spectroscopic studies using AtStr1 and mutants thereof suggest also a larger peptide or protein as substrate (Bartels et al. 2007a). Based on these enumerated results an investigation to analyze the interaction of several *Arabidopsis* thioredoxins with a number of Str by bimolecular fluorescence complementation (BiFC) (Bracha-Drori et al. 2004; Walter et al. 2004) was started. Specific interactions among a mitochondrial thioredoxin with mitochondrial AtStr1, among a plastidic thioredoxin with plastidic AtStr16, and a cytosolic thioredoxin with cytosolic AtStr2 was demonstrated (Holtgrefe and Papenbrock, unpublished results). It is still an open question how the specificity of the interactions among thioredoxins and target proteins can be explained. More information about the redox interactome is needed. In the background of published results one can assume that Str might act as a thioredoxin peroxidase with the formation of a sulfenate at the active site Cys. Therefore, plant Str might play a role in the control of redox homeostasis in the different subcellular compartments (Papenbrock et al. 2009).

For several Str the *in planta* function is still not clear

Despite the presence of Str activity in many living organisms, the physiological role of these enzymes has not yet been established unambiguously. While in mammals Str may be involved in the elimination of toxic cyanogenic compounds (Vennesland et al. 1982; Nagahara et al. 1999) its ubiquity suggests additional physiological functions. It has been proposed that Str detoxify oxygen radicals, e.g. by acting as a thioredoxin oxidase in mitochondria (Nandi et al. 2000), are involved in sulfate assimilation (Donadio et al. 1990), transport-specific sulfur compounds (Laudenbach et al. 1991), and may act as a sulfur invertase in the formation of prosthetic groups in Fe–S cluster proteins, such as ferredoxin (Bonomi et al. 1977; Pagani et al. 1984).

The biosynthesis of several vitamins, enzymes, and cofactors includes a step of sulfur transfer and the incorporation of sulfur into the respective substrate molecule. This could be carried out by Str as was shown for the biosynthesis of thiamine and thiouridine in *E. coli* (Palenchar et al. 2000) and for the synthesis of the molybdenum cofactor in *E. coli* and humans (Leimkuehler and Rajagopalan 2001; Matthies et al. 2004). Especially one-domain Str proteins have been associated with specific stress conditions (Bordo and Bork 2002). In general, Str might further activate or deactivate distinct proteins by direct transfer of sulfane sulfur and thus fulfill a regulatory role in the organism (Toohey 1989).

One of the *Arabidopsis* proteins containing one rhodanese domain is involved in the biosynthesis of the molybdenum cofactor synthesis (AtStr13, Fig. 2). Four plant enzymes depend on molybdenum: nitrate reductase, sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. To gain biological activity and fulfill its function in enzymes, molybdenum has to be complexed by a pterin compound thus forming the molybdenum cofactor. Molybdenum cofactor synthesis protein 3 (AtStr13/Cnx5) is essential during biosynthesis of the molybdenum cofactor. After molybdopterin synthase has transferred two sulfur atoms to precursor Z, it has to be resulfurated to reactivate the enzyme for the next reaction cycle of precursor Z conversion. This resulfuration is catalyzed by AtStr13/Cnx5 involving an adenylation of molybdopterin synthase followed by sulfur transfer (Matthies et al. 2004). AtStr13/Cnx5 is a two-domain protein consisting of an N-terminal domain responsible for adenylating molybdopterin synthase and a C-terminal rhodanese domain where the sulfur is bound to a conserved Cys in the form of persulfide (Matthies et al. 2004; Mendel 2007).

The detoxification of cyanide was suggested as the main function of Str in plants. The widespread occurrence of ethylene synthesis suggests that this pathway is the

principle source of endogenous cyanide in many plants although cyanide can be produced by other metabolic processes, e.g. hydrolysis of cyanogenic glycosides and in amino acid oxidase reactions (Kende 1993). Ethylene is produced during ripening of fruits and also during senescence processes in leaves (Abeles et al. 1992). During ethylene biosynthesis, one molecule of cyanide is produced for each molecule of ethylene by the oxidation of 1-aminocyclopropane-1-carboxylic acid in the presence of iron and oxygen (Blumenthal et al. 1968; Manning 1988). Cyanide is highly toxic for the cell, especially as a potent inhibitor of metalloenzymes, and needs to be detoxified immediately. In plants, at least two protein families could act in its detoxification, Str and β-cyano-L-alanine synthase (CAS, EC 4.4.1.9). Str catalyze the formation of the less toxic thiocyanate. In mammals this compound is mainly excreted in urine (Nagahara et al. 1999); for plants thiocyanate-degrading enzymes are unknown although they have to be postulated. CAS catalyzes the formation of β-cyano-L-alanine from Cys in the presence of cyanide (Blumenthal et al. 1968). β-cyano-L-alanine is further metabolized by the gene product of *NIT4* acting as nitrilase or hydratase to asparagine and aspartic acid, respectively, or can be conjugated to γ-glutamyl-β-cyano-L-alanine (Piotrowski et al. 2001). A number of experiments have been done to prove the hypothetical involvement of Str in cyanide detoxification in plants. Thus far, the evidence for a role of plant Str in cyanide detoxification is rather low: Str activity was detected in the same order of magnitude in cyanogenic and non-cyanogenic plants (Chew 1973) The results were confirmed in the way that no correlation between cyanogenesis and rhodanese activity was found (Kakes and Hakvoort 1992). An involvement of AtStr1 in cyanide detoxification as in animals appears rather unlikely since the expression level of *AtStr1* and Str activity in total protein extracts remained unchanged in cyanide treated plants (Meyer et al. 2003).

A function in providing reduced sulfur for Fe–S cluster was investigated with contradictory results. Pagani et al. (1984) demonstrated the transfer of reduced sulfur into the Fe–S cluster of ferredoxin in spinach. Whereas the preliminary analysis of an *atStr1* T-DNA insertion mutant showed that the mutation had no effect on the activity levels of Fe–S cluster containing proteins, suggesting that AtStr1 is not directly involved in Fe–S cluster assembly (Nakamura et al. 2000). However, Fe–S cluster are very sensitive to oxidative stress. Therefore, decreasing oxidative stress by regulation of redox homeostasis as thioredoxin peroxidase could also protect Fe–S cluster stability.

In radish and tobacco dark-inducible, senescence-associated homologs of AtStr15 encoded by *Rsdin1* and *Ntdin*, respectively, were identified that accumulated upon prolonged darkness, ethylene, cytokinin, and heat stress (Azumi and Watanabe 1991; Shimada et al. 1998; Yang et al. 2003). Due to similarity to this proteins most group VI AtStr have been annotated in the databases as senescence-associated proteins. The proteins share similarity to the C-terminal domain of AtStr1, to the GlpE protein in *E. coli* (Ray et al. 2000) as well as to other distinct stress-related proteins, such as sulfide dehydrogenase from *Wolinella succinogenes* (Klimmek et al. 1999), phage-shock protein PspE from *E. coli* (Adams et al. 2002), and to the hsp67B2 from *Drosophila* (Shimada et al. 1998).

In agreement with the database annotations the expression of *AtStr15*, *AtStr16*, and *AtStr18* as well as Str activity in total protein extracts have been shown to increase with progressing age (Bartels et al. 2007b). In contrast, the expression of *AtStr14* decreased in 5-week-old *Arabidopsis* plants and also at low sulfate supply, indicating a distinct role in sulfur metabolism. While the 3-MP Str activity in total protein extracts increased continuously with progressing age of the plants, the TS Str activity was strongly induced from week 5 to the end of the experiment after 6 weeks. The expression patterns of *AtStr16* and *AtStr18* as well as that of the senescence-associated *SAG13*, used as a marker for senescence in a previous work (Meyer et al. 2003), paralleled with the pattern of TS Str activity (Bartels et al. 2007b). AtStr16 (At5g66040) and AtStr18 (At5g66170) have been identified as TS Str proteins (Bauer and Papenbrock 2002) and are likely to play a role in senescence.

The most interesting and by far best characterized of the six proteins in group VI is AtStr15 (AtSEN1). The senescence-associated *AtStr15* gene is induced commonly during senescence caused by several senescence inducing factors including age, darkness, and phytohormones as well as by inoculation with diverse pathogens (Oh et al. 1996; Chung et al. 1997; Weaver et al. 1998; Schenk et al. 2005). *AtStr15* expression was clearly induced at nutritional stress conditions (low sulfate or phosphate, added TS) and in darkness, and increased with age (Bartels et al. 2007b). In contrast to the results obtained by Bartels et al. (2007b), the expression of *AtStr15* was slightly reduced in leaves and roots at low phosphate in a recent microarray analysis (Misson et al. 2005). Despite all these information the biological function of the *AtStr15* gene product could not be determined yet. In tobacco, a homolog of AtStr15, Ntdin1, was recently shown to be involved in molybdenum cofactor biosynthesis (Yang et al. 2003). According to results from Bartels (2006) a role of AtStr15 in molybdenum cofactor biosynthesis could be excluded. This was confirmed by recent results on the function of AtStr15/AtSEN1 (Schenk et al. 2005). Taking together the information obtained on AtStr15/AtSEN1 thus far, the protein may have a function in general stress response of the plant caused by various biotic and abiotic triggers. AtStr13

(At5g55130) in group V was shown to act as molybdopterin synthase sulfurylase (Mendel 2007). The AtStr12/SIR1 protein is composed of a rhodanese-like domain and an ubiquitin-activating enzyme E1-like domain. SIR1 is a regulator of many auxin-inducible genes. The *sir1* mutant was resistant to sirtinol, a small molecule activating many auxin-inducible genes, and displays auxin-related developmental phenotypes. An involvement of the protein in the propagation of auxin-signals in *Arabidopsis* was suggested (Zhao et al. 2003).

Another putative activity of a rhodanese-containing *Arabidopsis* protein was found by chance. Arsenate reductase (AR) activity was determined in an arsenate-hyperaccumulating fern. The reaction mechanism was very similar to the previously reported activity of Acr2p from yeast, using glutathione as the electron donor. A T-DNA knockout mutant of *Arabidopsis* with disruption in the putative Acr2 gene had no AR activity (Duan et al. 2005). According to the nomenclature by Bartels et al. (2007b) the orthologous protein in *Arabidopsis* corresponds to AtStr5 (At5g03455), a nuclear protein with a Cys in the active center. So far the AR activity could not be confirmed for the recombinant *Arabidopsis* protein (Papenbrock, unpublished results). Interestingly, the same protein was shown to act as Cdc25 dual-specificity tyrosine-phosphatase (Landrieu et al. 2004). Therefore, further experiments are needed to clarify the cellular function of AtStr5.

In a proteome-wide characterization of seed aging, it was shown that a loss in seed vigor is associated with a decreased level of 3-MP Str, highlighting further the importance of sulfur metabolism and homeostasis in seeds. As yet unknown important role(s) of Str in seed physiology and quality is predicted (Rajjou et al. 2008).

Str might be involved in defence mechanisms against pathogens

It is known since antiquity that sulfur has protective effects against pests and diseases. Sulfur supply thus influences plant resistance. In a recent study the increased disease susceptibility of sulfur deficient *Brassica napus* plants toward distinct fungal and bacterial pathogens was shown to be caused at least partially by a reduction of sulfur-dependent phytoanticipins (Dubuis et al. 2005). Elemental sulfur, glutathione, sulfite, H_2S, and TS can be products of specific Str reactions. Besides other sulfur containing compounds like Cys-rich antifungal proteins, glucosinolates and phytoalexins, these substances might play important roles in plant disease resistance (Cooper et al. 1996; Williams et al. 2002; Cooper and Williams 2004; Williams and Cooper 2004; Rausch and Wachter 2005). There are hints that Str proteins might also play a role in pathogen defence reactions of the plant. In a suppression subtractive hybridization of non-infected and with *Verticillium dahlia* infected tomato plants a tomato Str homolog to AtStr1 could be isolated (Jonathan Howarth, Rothamsted, UK, personal communication). In a differential display analysis aiming to isolate genes related to resistance toward the powdery mildew fungus *Erysiphe graminis* in wheat (*Triticum aestivum* L.) a Str gene displaying similarity to AtStr1 was identified that might be involved in pathogen resistance against *E. graminis* in wheat (Niu et al. 2002). A rhodanese-like protein displaying similarity to AtStr17 was identified in phloem exudates of *Curcubita maxima* Duch. by two-dimensional gel electrophoresis and subsequent mass spectrometry. The protein is suggested to be involved in stress and defence responses of the plant by acting as phytohormone and/or in signaling (Walz et al. 2004). Since Str proteins have been proposed to detoxify reactive oxygen species in mammals (Nandi et al. 2000) one could speculate about a possible protective function of the AtStr proteins during pathogen defence by protecting healthy tissue from oxidative damage, again by regulation of redox homeostasis as thioredoxin peroxidase.

Are Str a relict in evolution?

Some authors consider rhodanese as a biochemical relic of a time when the earth's atmosphere contained large amounts of hydrogen cyanide (Pagani et al. 1987). But this is a rather pessimistic view of such an ubiquitous enzyme (Hama et al. 1994). It is thought, on the basis of the amino acid sequence evolution around the catalytic Cys that the C-terminal catalytically active domain of prokaryotic TS Str is an ancestral protein of the rhodanese family and the duplicated molecule is an 3-MP Str precursor (Nagahara 2007). A change of 3-MP Str occurred in the oxidizing atmosphere of the earth after the appearance of cyanobacteria. In this process a leucine residue was replaced with a Cys residue to regulate cellular redox homeostasis in the new oxidizing earth atmosphere. Because plants evolved under reducing conditions, there is a leucine residue at the redox-sensing switch. In a phylogenetic evolution life forms acquired the redox-sensing switch in 3-MP Str in adaptation to the oxidizing atmosphere in the earth (Nagahara 2007).

Conclusions

Str belong to a very exciting protein family and we are just at the beginning to elucidate their functions in the organism. It was shown already that Str are involved in regulation of metabolism including protein biosynthesis, co-factor biosynthesis, protection against biotic and abiotic stress, regulation of redox homeostasis, seed development

among others. Probably from the current systematic analysis of *Arabidopsis* mutants we will be able to learn about many more functions in plants and maybe also in other organisms.

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References

Abeles FB, Morgan PW, Saltveit ME (1992) Ethylene biosynthesis in plant biology. Academic Press, San Diego

Adams H, Teertstra W, Koster M, Tommassen J (2002) PspE (phage-shock protein E) of *Escherichia coli* is a rhodanese. FEBS Lett 518:173–176

Alphey MS, Williams RA, Mottram JC, Coombs GH, Hunter WN (2003) The crystal structure of *Leishmania major* 3-mercapto-pyruvate sulfurtransferase. A three-domain architecture with a serine protease-like triad at the active site. J Biol Chem 278:48219–48227

Azumi Y, Watanabe A (1991) Evidence for a senescence associated gene induced by darkness. Plant Physiol 95:577–583

Balmer Y, Vensel WH, Tanaka CK, Hurkman WJ, Gelhaye E, Rouhier N, Jacquot JP, Manieri W, Schürmann P, Droux M, Buchanan BB (2004) Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. Proc Natl Acad Sci USA 101:2642–2647

Bartels A (2006) Functional characterisation of sulfurtransferase proteins in higher plants. PhD thesis, Leibniz Universität Hannover

Bartels A, Forlani F, Pagani S, Papenbrock J (2007a) Conformational studies on *Arabidopsis* sulfurtransferase AtStr1 analysed by spectroscopic methods. Biol Chem 388:53–59

Bartels A, Mock HP, Papenbrock J (2007b) Differential expression of *Arabidopsis* sulfurtransferases under various growth conditions. Plant Physiol Biochem 45:178–187

Bauer M, Papenbrock J (2002) Identification and characterization of single-domain thiosulfate sulfurtransferases from *Arabidopsis thaliana*. FEBS Lett 532:427–431

Bauer M, Dietrich C, Nowak K, Sierralta WD, Papenbrock J (2004) Intracellular localization of sulfurtransferases from *Arabidopsis thaliana*. Plant Physiol 135:916–926

Blumenthal S, Hendrickson H, Abrol Y, Conn E (1968) Cyanide metabolism in higher plants: III. The biosynthesis of β-cyano-alanine. J Biol Chem 243:5302–5307

Bonomi F, Pagani S, Cerletti P, Cannella C (1977) Rhodanese-mediated sulfur transfer to succinate dehydrogenase. Eur J Biochem 72:17–24

Bordo D, Bork P (2002) The rhodanese/Cdc25 phosphatase superfamily. Sequence-structure-function relations. EMBO Rep 3:741–746

Bordo D, Deriu D, Colnaghi R, Carpen A, Pagani S, Bolognesi M (2000) The crystal structure of a sulfurtransferase from *Azotobacter vinelandii* highlights the evolutionary relationship between the rhodanese and phosphatase enzyme families. J Mol Biol 298:691–704

Bracha-Drori K, Shichrur K, Katz A, Oliva M, Angelovici R, Yalovsky S, Ohad N (2004) Detection of protein–protein interactions in plants using bimolecular fluorescence complementation. Plant J 40:419–427

Burow M, Kessler D, Papenbrock J (2002) Enzymatic activity of the *Arabidopsis* sulfurtransferase resides in the C-terminal domain but is boosted by the N-terminal domain and the linker peptide in the full-length enzyme. Biol Chem 383:1363–1372

Cannella C, Berni R, Ricci G (1984) Determination of rhodanese activity by tetrazolium reduction. Anal Biochem 142:159–162

Chew NY (1973) Rhodanese in higher plants. Phytochemistry 12:2365–2367

Chung B-C, Lee SY, Oh SA, Rhew TH, Nam HG, Lee C-H (1997) The promoter activity of *sen1*, a senescence-associated gene of *Arabidopsis*, is repressed by sugars. J Plant Physiol 151:339–345

Colnaghi R, Cassinelli G, Drummond M, Forlani F, Pagani S (2001) Properties of the *Escherichia coli* rhodanese-like protein SseA: contribution of the active-site residue Ser240 to sulfur donor recognition. FEBS Lett 500:153–156

Cooper RM, Williams JS (2004) Elemental sulphur as an induced antifungal substance in plant defence. J Exp Bot 55:1947–1953

Cooper RM, Resende ML, Flood J, Rowan MG, Beale MH, Potter U (1996) Detection and cellular localization of elemental sulphur in disease-resistant genotypes of *Theobroma cacao*. Nature 379:159–162

Cornilescu G, Vinarov DV, Tyler EM, Merkley JL, Cornilescu CC (2006) Solution structure of a single-domain from *Arabidopsis thaliana*. Protein Sci 15:2836–2841

Donadio S, Shafiee A, Hutchinson R (1990) Disruption of a rhodanese like gene results in cysteine auxotrophy in *Saccharopolyspora erythraea*. J Bacteriol 172:350–360

Duan GL, Zhu YG, Tong YP, Cai C, Kneer R (2005) Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator. Plant Physiol 138:461–469

Dubuis P-H, Marazzi C, Staedler E, Mauch F (2005) Sulphur deficiency causes a reduction in antimicrobial potential and leads to increased disease susceptibility of oilseed rape. J Phytopathol 153:27–36

Gemeinhardt K (1938) The thiocyanate content of plants. Ber Deut Botan Ges 56:275

Glatz Z, Bouchal P, Janiczek O, Mandl M, Ceskova P (1999) Determination of rhodanese enzyme activity by capillary zone electrophoresis. J Chromat A 838:139–148

Gliubich F, Gazerro M, Canotti G, Delbono S, Bombieri G, Berni R (1996) Active site structural features for chemically modified forms of rhodanese. J Biol Chem 271:21054–21061

Hama H, Kayahara T, Ogawa W, Tsuda M, Tsuchiya T (1994) Enhancement of serine-sensitivity by a gene encoding rhodanese-like protein in *Escherichia coli*. J Biochem 115:1135–1140

Hatzfeld Y, Saito K (2000) Evidence for the existence of rhodanese (thiosulfate:cyanide sulfurtransferase) in plants: preliminary characterization of two rhodanese cDNAs from *Arabidopsis thaliana*. FEBS Lett 470:147–150

Heazlewood JL, Tonti-Filippini JS, Gout AM, Day DA, Whelan J, Millar AH (2004) Experimental analysis of the *Arabidopsis* mitochondrial proteome highlights signaling and regulatory components, provides assessment of targeting prediction programs, and indicates plant-specific mitochondrial proteins. Plant Cell 16:241–256

Hildebrandt TM, Grieshaber MK (2008) Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. FEBS J 275:3352–3361

Huang J, Niknahad H, Khan S, O'Brien PJ (1998) Hepatocyte-catalysed detoxification of cyanide by L-and D-cysteine. Biochem Pharmacol 55:1983–1990

Jarabak R, Westley J (1978) Steady-state kinetics of 3-mercaptopyruvate sulfurtransferase from bovine kidney Arch. Biochem Biophys 185:458–465

Kakes P, Hakvoort H (1992) Is there rhodanese activity in plants? Phytochemistry 31:1501–1505

Kende H (1993) Ethylene biosynthesis. Ann Rev Plant Physiol Plant Mol Biol 44:283–307

Klimmek O, Stein T, Pisa R, Simon J, Kroger A (1999) The single cysteine residue of the Sud protein is required for its function as a polysulfide-sulfur transferase in *Wolinella succinogenes*. Eur J Biochem 263:79–84

Laloi C, Rayapuram N, Chartier Y, Grienenberger JM, Bonnard G, Meyer Y (2001) Identification and characterization of a mitochondrial thioredoxin system in plants. Proc Natl Acad Sci USA 98:14144–14149

Landrieu I, da Costa M, De Veylder L, Dewitte F, Vandepoele K, Hassan S, Wieruszeski JM, Corellou F, Faure JD, Van Montagu M, Inzé D, Lippens G (2004) A small CDC25 dual-specificity tyrosine-phosphatase isoform in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 101:13380–13385

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

Leimkuehler S, Rajagopalan KV (2001) A sulfurtransferase is required in the transfer of cysteine sulfur in the in vitro synthesis of molybdopterin from precursor Z in *Escherichia coli*. J Biol Chem 276:22024–22031

Louie M, Kondor N, DeWitt JG (2003) Gene expression in cadmium-tolerant *Datura innoxia*: detection and characterization of cDNAs induced in response to Cd^{2+}. Plant Mol Biol 52:81–89

Luo GX, Horowitz PM (1994) The sulfurtransferase activity and structure of rhodanese are affected by site-directed replacement of Arg-186 or Lys-249. J Biol Chem 269:8220–8225

Manning K (1988) Detoxification of cyanide by plants and hormone action. Ciba Found Symp 140:92–110

Marchand C, Le Marechal P, Meyer Y, Decottignies P (2006) Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. Proteomics 6:6528–6537

Matthies A, Rajagopalan KV, Mendel RR, Leimkuehler S (2004) Evidence for the physiological role of a rhodanese-like protein for the biosynthesis of the molybdenum cofactor in humans. Proc Natl Acad Sci USA 101:5946–5951

Melino S, Cicero DO, Orsale M, Forlani F, Pagani S, Paci M (2003) *Azotobacter vinelandii* rhodanese: selenium loading and ion interaction studies. Eur J Biochem 270:4208–4215

Mendel RR (2007) Biology of the molybdenum cofactor. J Exp Bot 58:2289–2296

Meyer T, Burow M, Bauer M, Papenbrock J (2003) *Arabidopsis* sulfurtransferases: investigation of their function during senescence and in cyanide detoxification. Planta 217:1–10

Meyer Y, Reichheld JP, Vignols F (2005) Thioredoxins in *Arabidopsis* and other plants. Photosynth Res 86:419–433

Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Ortet P, Creff A, Somerville S, Rolland N, Doumas P, Nacry P, Herrerra-Estrella L, Nussaume L, Thibaud MC (2005) A genome-wide transcriptional analysis using *Arabidopsis thaliana* affymetrix gene chips determined plant responses to phosphate deprivation. Proc Natl Acad Sci USA 102:11934–11939

Nagahara N (2007) Molecular evolution of thioredoxin-dependent redox-sensing switch in mercaptopyruvate sulfurtransferase. Res Adv Biol Chem 1:19–26

Nagahara N, Katayama A (2005) Post-translational regulation of mercaptopyruvate sulfurtransferase via a low redox potential cysteine-sulfenate in the maintenance of redox homeostasis. J Biol Chem 280:34569–34776

Nagahara N, Nishino T (1996) Role of amino acid residues in the active site of rat liver mercaptopyruvate sulfurtransferase. cDNA cloning, overexpression, and site-directed mutagenesis. J Biol Chem 271:27395–27401

Nagahara N, Okazaki T, Nishino T (1995) Cytosolic mercaptopyruvate sulfurtransferase is evolutionary related to mitochondrial rhodanese. J Biol Chem 270:16230–16235

Nagahara N, Ito T, Kitamura H, Nishino T (1998) Tissue and subcellular distribution of mercaptopyruvate sulfurtransferase in the rat: confocal laser fluorescence and immunoelectron microscopic studies combined with biochemical analysis. Histochem Cell Biol 110:243–250

Nagahara N, Ito T, Minami M (1999) Mercaptopyruvate sulfurtransferase as a defense against cyanide toxication: Molecular properties and mode of detoxification. Histol Histopathol 14:1277–1286

Nagahara N, Yoshii T, Abe Y, Matsumura T (2007) Thioredoxin-dependent enzymatic activation of mercaptopyruvate sulfurtransferase. An intersubunit disulfide bond serves as a redox switch for activation. J Biol Chem 282:1561–1569

Nakamura T, Yamaguchi Y, Sano H (2000) Plant mercaptopyruvate sulfurtransferases: molecular cloning, subcellular localization and enzymatic activities. Eur J Biochem 267:5621–5630

Nandi DL, Horowitz PM, Westley J (2000) Rhodanese as a thioredoxin oxidase. Int J Biochem Cell Biol 32:465–473

Niu JS, Yu L, Ma Z-Q, Chen P-D, Liu D-J (2002) Molecular cloning, characterization and mapping of a rhodanese like gene in wheat. Acta Genet Sin 29:266–272

Ogasawara Y, Lacourciere G, Stadtman T (2001) Formation of a selenium-substituted rhodanese by reaction with selenite and glutathione: possible role of a protein perselenide in a selenium delivery system. Proc Natl Acad Sci USA 98:9494–9498

Oh SA, Lee SY, Chung IK, Lee CH, Nam HG (1996) A senescence-associated gene of *Arabidopsis thaliana* is distinctively regulated during natural and artificially induced leaf senescence. Plant Mol Biol 30:739–754

Okolie PN, Obasi BN (1993) Diurnal variation of cyanogenic glucosides, thiocyanate and rhodanese in cassava. Phytochemistry 33:775–778

Okubara PA, Berry PM (1999) A mRNA (Accession No. AF109156) from symbiotic root nodules of Datisca glomerata with homology to thiosulfate sulfurtransferase genes. PGR 99–027. Plant Physiol 119:1147

Pagani S, Bonomi F, Cerletti P (1984) Enzymic synthesis of the iron-sulfur cluster of spinach ferredoxin. Eur J Biochem 142:361–366

Pagani S, Eldridge M, Eady RR (1987) Nitrogenase of *Klebsiella pneumoniae*: rhodanese-catalyzed restoration of activity of inactive 2Fe species of the Fe protein. Biochem J 244:485–488

Palenchar PM, Buck CJ, Cheng H, Larson TJ, Mueller EG (2000) Evidence that thiI, an enzyme shared between thiamine and 4-thiouridine biosynthesis, may be a sulfurtransferase that proceeds through a persulfide intermediate. J Biol Chem 275:8283–8286

Pantoja-Uceda D, López-Méndez B, Koshiba S, Inoue M, Rigawa T, Terada T, Shirouzu M, Tanaka A, Seki M, Shinozaki K, Yokoyama S, Güntert P (2005) Solution structure of the rhodanese homology domain At4g01050 (175–295) from *Arabidopsis thaliana*. Protein Sci 14:224–230

Papenbrock J, Grimm B (2001) Regulatory network of tetrapyrrole biosynthesis—studies of intracellular signalling involved in metabolic and developmental control of plastids. Planta 213:667–681

Papenbrock J, Schmidt A (2000a) Characterization of a sulfurtransferase from *Arabidopsis thaliana*. Eur J Biochem 267:145–154

Papenbrock J, Schmidt A (2000b) Characterization of two sulfurtransferase isozymes from *Arabidopsis thaliana*. Eur J Biochem 267:5571–5579

Papenbrock J, Bartels A, Hartmann F, Hartmann J, Triulzi T (2009) Reduced sulfur in the plant cell–enzymatic formation and functional roles. In: Sirko A et al (eds) Sulfur metabolism in higher plants. Backhuys Publishers, Leiden, pp 217–219

Peltier JB, Ytterberg AJ, Sun Q, van Wijk KJ (2004) New functions of the thylakoid membrane proteome of *Arabidopsis thaliana* revealed by a simple, fast, and versatile fractionation strategy. J Biol Chem 279:49367–49383

Piotrowski M, Schönfelder S, Weiler EW (2001) The *Arabidopsis thaliana* isogene NIT4 and its orthologs in tobacco encode *β*-cyano-L-alanine hydratase/nitrilase. J Biol Chem 276:2616–2621

Ploegman JH, Drent G, Kalk KH, Hol WGJ, Heinrikson RL, Keim P, Weng L, Russell J (1978) The covalent and tertiary structure of bovine liver rhodanese. Nature 273:124–129

Prieto JL, Perez-Castineira JR, Vega JM (1997) Thiosulfate reductase from *Chlamydomonas*. J Plant Physiol 151:385–389

Rajjou L, Lovigny Y, Groot SP, Belghazi M, Job C, Job D (2008) Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. Plant Physiol 148:620–641

Rausch T, Wachter A (2005) Sulfur metabolism: a versatile platform for launching defence operations. Trends Plant Sci 10:503–509

Ray WK, Zeng G, Potters MB, Mansuri AM, Larson TJ (2000) Characterization of a 12-kilodalton rhodanese encoded by *glpE* of *Escherichia coli* and its interaction with thioredoxin. J Bacteriol 182:2277–2284

Russell J, Weng L, Keim PS, Heinrikson RL (1978) The covalent structure of bovine liver rhodanese. J Biol Chem 253:8102–8108

Sabelli R, Iorio E, De Martino A, Podo F, Ricci A, Viticchiè G, Rotilio G, Paci M, Melino S (2008) Rhodanese-thioredoxin system and allyl sulfur compounds. FEBS J 275:3884–3899

Schenk PM, Kazan K, Rusu AG, Manners JM, Maclean DJ (2005) The *SEN1* gene of *Arabidopsis* is regulated by signals that link plant defence responses and senescence. Plant Physiol Biochem 43:997–1005

Schievelbein H, Baumeister R, Vogel R (1969) Comparative investigations on the activity of thiosulphate-sulphur transferase. Naturwissenschaften 56:416–417

Schmidt A (1984) Occurrence of mercaptopyruvate sulfotransferase activity in photosynthetic organisms. Z Naturforsch 39c:916–921

Schmidt A, Erdle I, Gamon B (1984) Isolation and characterization of thiosulfate reductases from the green alga *Chlorella fusca*. Planta 162:243–249

Shimada Y, Wu GJ, Watanabe A (1998) A protein encoded by din1, a dark-inducible and senescence-associated gene of radish, can be imported by isolated chloroplasts and has sequence similarity to sulphide dehydrogenase and other small stress proteins. Plant Cell Physiol 39:139–143

Sörbo BH (1955) Rhodanese. Methods Enzymol 2:334–337

Spallarossa A, Donahue JL, Larson TJ, Bolognesi M, Bordo D (2001) *Escherichia coli* GlpE is a prototype sulfurtransferase for the single-domain rhodanese homology superfamily. Structure 9:1117–1125

Spallarossa A, Forlani F, Carpen A, Armirotti A, Pagani S, Bolognesi M, Bordo D (2004) The "rhodanese" fold and catalytic mechanism of 3-mercaptopyruvate sulfurtransferases: crystal structure of SseA from *Escherichia coli*. J Mol Biol 335:583–593

Toohey JI (1989) Sulphane sulphur in biological systems: a possible regulatory role. Biochem J 264:625–632

Vennesland B, Castric PA, Conn EE, Solomonson LP, Volini M, Westley J (1982) Cyanide metabolism. Fed Proc 41:2639–2648

Walter M, Chaban C, Schutze K, Batistic O, Weckermann K, Nake C, Blazevic D, Grefen C, Schumacher K, Oecking C, Harter K, Kudla J (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. Plant J 40:428–438

Waltner M, Weiner H (1995) Conversion of a nonprocessed mitochondrial precursor protein into one that is processed by the mitochondrial processing peptidase. J Biol Chem 270:26311–26317

Walz C, Giavalisco P, Schad M, Juenger M, Klose J, Kehr J (2004) Proteomics of curcurbit phloem exudate reveals a network of defence proteins. Phytochemistry 65:1795–1804

Weaver LM, Gan S, Quirino B, Amasino RM (1998) A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. Plant Mol Biol 37:455–469

Westley J (1973) Rhodanese. Adv Enzymol 39:327–368

Westley J (1981) Thiosulfate:cyanide sulfurtransferase (rhodanese). Methods Enzymol 77:285–291

Westley J, Heyse D (1971) Mechanisms of sulfur transfer catalysis sulfhydryl-catalyzed transfer of thiosulfonate sulfur. J Biol Chem 246:1468–1474

White RH (1982) Metabolism of L-[sulfane-^{34}S]thiocystine by *Escherichia coli*. Biochemistry 21:4271–4275

Williams JS, Cooper RM (2004) The oldest fungicide and newest phytoalexin–a reappraisal of the fungitoxicity of elemental sulphur. Plant Pathol 53:263–279

Williams JS, Hall SA, Hawkesford MJ, Beale MH, Cooper RM (2002) Elemental sulfur and thiol accumulation in tomato and defense against a fungal vascular pathogen. Plant Physiol 128:150–159

Williams RA, Kelly SM, Mottram JC, Coombs GH (2003) 3-Mercaptopyruvate sulfurtransferase of *Leishmania* contains an unusual C-terminal extension and is involved in thioredoxin and antioxidant metabolism. J Biol Chem 278:1480–1486

Wood JL (1987) Sulfane sulfur. Methods Enzymol 143:25–29

Wróbel M, Lewandowska I, Bronowicka-Adamska P, Paszewski A (2009) The level of sulfane sulfur in the fungus *Aspergillus nidulans* wild type and mutant strains. Amino Acids 37:565–571

Yang SH, Berberich T, Miyazaki A, Sano H, Kusano T (2003) *Ntdin*, a tobacco senescence-associated gene, is involved in molybdenum cofactor biosynthesis. Plant Cell Physiol 44:1037–1044

Zhao Y, Dai X, Blackwell HE, Schreiber SL, Chory J (2003) SIR1, an upstream component in auxin signaling identified by chemical genetics. Science 301:1107–1110