Review

Sulfate permeases — phylogenetic diversity of sulfate transport

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Sulfate uptake, the first step of sulfate assimilation in all organisms, is a highly endoergic, ATP requiring process. It is under tight control at the transcriptional level and is additionally modulated by posttranslational modifications, which are not yet fully characterized. Sulfate anion is taken up into the cell by specific transporters, named sulfate permeases, located in the cell membrane. Bacterial sulfate permeases differ significantly from the eukaryotic transporters in their evolutionary origins, structure and subunit composition. This review focuses on the diversity and regulation of sulfate permeases in various groups of organisms.

INTRODUCTION

Sulfate is a widespread inorganic anion in the Earth's crust. Bacteria, fungi, and plants can utilize sulfate for the synthesis of sulfur-containing organic compounds. In the initial step of assimilation, sulfate is taken up into the cell by an energy-dependent process carried out by specific proteins known as sulfate permeases. In the endoergic assimilatory pathway, inorganic sulfur is reduced and incorporated into a carbon backbone forming cysteine, which is a substrate in methionine synthesis. This first step of sulfate uptake through hydrophobic membranes is a pivotal point in the regulation of sulfate assimilation. In the presence of a favorable organic sulfur source in the environment, the cell immediately switches off the ATP-dependent sulfate assimilation pathway beginning with sulfate permease. Sulfate permeases, due to their low substrate specificity, may also transport other, mostly highly toxic, tetra-oxyanions like selenate, tellurate, chromate, tungstate and molybdate. Most of them are lethal for the cell.

The initial three steps of sulfate assimilation are similar in all organisms: inorganic sulfate is transported into the cell by a sulfate permease and subsequently activated by ATP forming APS (adenosine-5-phosphosulfate), which is further phosphorylated by a second ATP molecule yielding a high-energy sulfate donor PAPS (3'-phosphoadenosine-5-phosphosulfate) known as "active sulfate" (Fig. 1).

The latter reaction is the last step of sulfate assimilation in mammals. Sulfur autotrophs, like bacteria and fungi, can subsequently reduce PAPS to sulfide (S^{2-}), which is then incorporated into the carbon backbone of serine or homoserine to form the first sulfur-containing amino acid — cysteine/homocysteine. In plants, sulfide is produced directly by APS reduction.

SULFATE UPTAKE SYSTEMS

Sulfate permeases are diverse proteins localized mostly in the cytoplasmic membrane. Following the Transporter Classification (TC) system, all

$$SO_4^- \xrightarrow{1} SO_4^- \xrightarrow{2} APS \xrightarrow{3} PAPS \xrightarrow{4} SO_3^- \xrightarrow{5} S^- \rightarrowtail cys \rightarrowtail met$$

Figure 1. Sulfate assimilation pathway.
1 – sulfate permease, 2 – ATP sulfurylase, 3 – APS kinase, 4 – PAPS reductase, 5 – sulfite reductase.

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Abbreviations: ABC, ATP-binding cassette; APS, adenosine-5-phosphosulfate; STAS, sulfate transporter and anti-sigma antagonist.

Table 1. Classification of transport proteins according to the TC system (including subclasses of known sulfate transporters) with examples mentioned in the text.

Classes/Subclasses			Transport proteins	Examples
1.			Channels/Pores	
2.			Electrochemical Potential-driven Transporters	
	2.A.		Porters (uniporters, symporters, antiporters)	
		2.A.1.	**The Major Facilitator Superfamily (MFS)**	AstA *Aspergillus nidulans*
		2.A.20.	**The Inorganic Phosphate Transporter (PiT) Family**	CysP *Bacillus subtilis*
		2.A.29.	**The Mitochondrial Carrier (MC) Family**	DIC *Homo sapiens*
		2.A.47.	**The Divalent Anion:Na⁺ Symporter (DASS) Family**	NaS-1 *Mus musculus*
		2.A.53.	**The Sulfate Permease (SulP) Family**	SB *Aspergillus nidulans*
3.			Primary Active Transporters	
	3.A.		P-P-bond-hydrolysis-driven transporters	
		3.A.1.	**The ATP-binding Cassette (ABC) Superfamily**	CysP *Escherichia coli*
4.			Group Translocators	
5.			Transport Electron Carriers	
8.			Accessory Factors Involved in Transport	
9.			Incompletely Characterized Transport Systems	
	9.A.		Recognized transporters of unknown biochemical mechanism	
		9.A.29.	**The Putative 4-Toluene Sulfonate Uptake Permease (TSUP) Family**	CysZ *Corynebacterium glutamicum*

known membrane transporters are classified into nine classes (Table 1). The TC system is analogous to the Enzymes Classification (EC) system for functional classification of enzymes but, in addition, it incorporates phylogenetic information. So far, most of known sulfate permeases belong to three subclasses of two transporter classes (Table 1): eukaryotic sulfate transporters belong to the second class, forming two main subclasses (TC 2.A.47. and TC 2.A.53.; names in bold), while prokaryotes possess only one well characterized family, belonging to the third class (TC 3.A.1.).

PROKARYOTIC TRANSPORTERS

The SulT group (TC 3.A.1.6.) is the main transporter family responsible for sulfate and thiosulfate uptake in prokaryotes (Saier, 1999). It belongs to a superfamily of proteins having an ATP-binding cassette (ABC) and acting in a complex with four other proteins (Kertesz, 2001).

In Gram-negative bacteria, *Escherichia coli* and *Salmonella typhimurium*, the sulfate-thiosulfate permease is a complex of five types of subunits encoded by: *sbp*, *cysP*, *cysT* (currently named *cysU*), *cysW* and *cysA* genes (Kredich, 1996). Sulfate assimilation is initiated by periplasmic sulfate (Sbp) and thiosulfate binding (CysP) proteins (Hryniewicz *et al.*, 1990; Sirko *et al.*, 1990) (Fig. 2).

The channel for sulfate transport is composed of two transmembrane proteins: CysT(U) and CysW. Homodimer-forming CysA comprises catalytic and helical domains: a nucleotide-binding (ATP-bind-

ing) and a regulatory domain (beta sandwich) which changes conformation upon binding of ATP (Scheffel *et al.*, 2005). Interestingly, CysA was also found in a high-throughput screen to bind directly to cysteine synthase CysK, the last enzyme of sulfate assimilation pathway (Arifuzzaman *et al.*, 2006).

This five-component system (Sbp, CysA, CysP, CysT, CysW) seems to be similar to that found in cyanobacteria, for instance in *Synechococcus* sp. (Laudenbach & Grossman, 1991). Genes encoding these components as well as some other genes involved in sulfur metabolism are tightly regulated at the transcriptional level. This complex response engaging two transcription factors, CysB
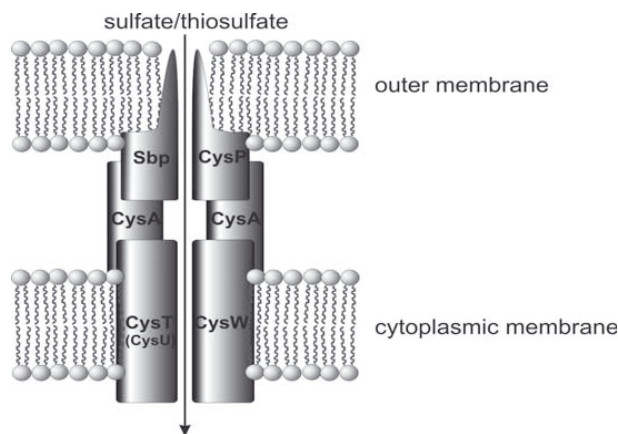

Figure 2. Outline of gram-negative bacterial sulfate/thiosulfate transporters.
The transporter is composed of ABC-type subunits which form a channel. Because of a lack of structural studies of the entire complex, the diagram is based on terse literature descriptions.

and Cbl, has been well characterized in *E. coli* and *S. typhimurium*. CysB plays the main role in activation of genes encoding the sulfate uptake complex upon sulfur starvation (Iwanicka-Nowicka & Hryniewicz, 1995; Kredich, 1996). Cbl acts preferentially in the regulation of genes responsible for the uptake of organosulfur compounds. Both proteins belong to the LysR family of transcriptional regulators (LTTRs) and their activity is dependent on ligands such as APS, thiosulfate or *O*-acetylserine (Kredich, 1992; 1996).

Unlike Gram-negative bacteria, Gram-positive ones are poorly characterized with respect to sulfate transport: it has been described only in two bacterial species. One of them, *Bacillus subtilis*, has an unusual sulfate uptake system which consists of transmembrane protein CysP. Homologs of this transporter represent a broad family of phosphate permeases, described in *Neurospora crassa* (PHO4) and *E. coli* (PitA) (Mansilla & Mendoza, 2000). It should be noted that despite identical gene symbols, *cysP* from *B. subtilis* is not a homolog of the *E. coli cysP*. The second species, *Corynebacterium glutamicum*, carries the *fpr2-cysIXHDNYZ* gene cluster involved in assimilatory sulfate reduction, in which *cysZ* was identified as encoding the main sulfate permease with an atypical structure of six transmembrane helices (Rückert *et al.*, 2005). In addition, *C. glutamicum* has an as yet uncharacterized second low affinity sulfate transporter.

Sequencing of many bacterial genomes has revealed that some species also possess genes encoding proteins of the SulP family but they have not been shown to be active components of sulfate transport, although it was reported that overexpression of the Rv1739c transporter (which is a SulP member) from the Gram-positive *Mycobacterium tuberculosis* is able to increase sulfate transport in *E. coli* (Zolotarev *et al.*, 2008). However, this transporter did not complement the *E. coli cysA* mutants, impaired in the main ABC sulfate uptake system. Thus, Rv1739c may be a CysTWA-dependent sulfate transporter, however, there is no evidence that the SulP-encoding genes are regulated by sulfur sources.

Members of the SulP family, carrying additional non-transporter domains, have been described in some prokaryotes. One SulP subfamily includes transporters fused to homologs of carbonic anhydrase, suggesting that these chimeric proteins function in bicarbonate or carbonate transport. In another subfamily, a SulP protein is joined to the rhodanese catalytic domain (thiosulfate:cyanide sulfotransferase), indicating that this carrier may also be involved in sulfur metabolism (Felce & Saier, 2004). Some SulP proteins possess putative $Na^+:H^+$ antiporter or $Na^+:$bicarbonate symporter domains. It has been shown that one of distant SulP homologs identified in oceanic cyanobacteria is a bicarbonate:Na^+ symporter (Felce & Saier, 2004; Price *et al.*, 2004).

EUKARYOTIC TRANSPORTERS

While the role of the SulP transporters in prokaryotes is not clear, most eukaryotic members of this family have actually been shown to be involved in sulfate uptake (Sandal & Marcker, 1994; Smith *et al.*, 1995). These proteins are inorganic anion transporters or anion:anion exchangers. Many of them have been well characterized functionally. They differ in their affinities to substrates. Some may function as sulfate:H^+ or sulfate:bicarbonate symporters, but generally anion:anion antiport has been reported for several SulP homologs in vertebrates. For instance, the mouse homolog, SLC26A6, can transport sulfate, formate, oxalate, chloride and bicarbonate, exchanging any one of these anions for another one (Jiang *et al.*, 2002). These proteins have a common topology of twelve transmembrane helices within the sulfate transport domain (Lohi *et al.*, 2002) (Fig. 3A). Positively charged arginine residues present in extracellular loops play an important role in sulfate recruitment (Smith *et al.*, 1995). Biochemical studies suggested that the SulP sulfate permeases may be regulated at the posttranslational level by phosphorylation (Chernova *et al.*, 2003; Rouached *et al.*, 2005). Recently, it has been found that most of eukaryotic SulP homologs possess a conserved regulatory domain in their C-terminal part. The structure of this region is similar to that of the bacterial anti-sigma factor antagonist from *B. subtilis* which is responsible for binding of nucleotides (Aravind & Koonin, 2000). In eukaryotes, this domain, designated STAS (**s**ulfate **t**ransporter and **a**nti-**s**igma antagonist; Fig. 3A), plays an important role in sulfate transport regulation, as shown in *Arabidopsis thaliana* (Rouached *et al.*, 2005). Mutations affecting phosphorylation of the STAS domain lead to a complete loss of sulfate transport capacity. Detailed structures of SulP transporters remain unknown, although their 3D topology might be similar to that of pres-
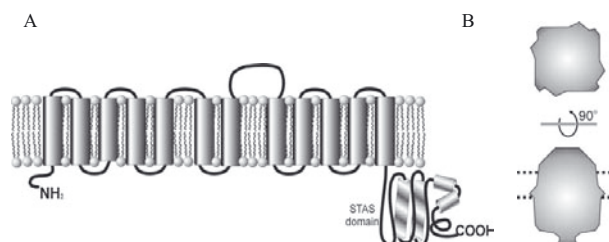

Figure 3A. Predicted topology of a sulfate permease from the SulP family. B. Three-dimensional projection of prestin tetramer, a member of SLC26 family (Mio *et al.*, 2008).

tin, a mammalian SLC26A5 member, which acts as a Cl^-/HCO_3^--binding molecular motor in outer hair cells of Corti's organ. Interestingly, nonmammalian vertebrate orthologs of prestin have been also described as active Cl^-/divalent (sulfate/oxalate) anion exchangers (Schaechinger & Oliver, 2007). Immersed in the lipid bilayer, prestin forms a bullet-shaped tetramer whose cytoplasmic bulk is formed by the STAS domains located in the C-terminal region (Fig. 3B; Mio *et al.*, 2008).

SULFATE TRANSPORT IN ANIMALS

Animals cannot reduce sulfate or synthesize sulfur-containing amino acids *de novo*, but sulfate, as an anion or ester, is essential for their existence. It is one of the most important anions in cells, being the fourth most abundant in the human plasma (300 μM) where it participates in maintaining ionic homeostasis. In animal liver, sulfate acts in a variety of activation and detoxification processes of many endogenous (including glycosaminoglycans, cerebrosides, steroids and catecholamines) and exogenous (drugs and other xenobiotics) compounds, which are conjugated into organic sulfate esters and excreted (Markovich, 2001). Therefore, proper distribution of sulfate across the hydrophobic lipid layer represents an important metabolic challenge. Active translocation of sulfate through the intestinal lumen, and between different tissues has been well characterized in mammals: human, mouse and rat. Human sulfate permeases have been studied in detail for health protection reasons in connection with severe illnesses. Mutations in the *DTD* gene encoding a sulfate transporter are responsible for diastrophic dysplasia, a severe human genetic disease. Defects in this gene lead to insufficient sulfation of proteoglycans in the cartilage matrix, which is manifested in skeletal abnormalities in the early stages of fetal development (Hastbacka *et al.*, 1994).

Two families of solute carrier transporters (SLC) which participate in sulfate transport have been described. Members of the main SulP family (SLC26 family in mammals) differ with regard to their substrates. Some of them, of low substrate specificity, can transport sulfate as well as bicarbonate or chloride. There is evidence that various isoforms of these permeases are expressed in different tissues or developmental stages. They are products of alternative splicing of the same pre-mRNA and show different substrate specificity (Lohi *et al.*, 2003).

Members of the second family of mammalian transporters, divalent anion:Na^+ symporters (DASS; also called the SLC13 family, Table 1. TC 2.A.47.), transport polyvalent anions (dicarboxylic acids, phosphate and sulfate) with sodium cations. SLC13 proteins can be divided into the NaS sulfate-preferring subfamily (these include the renal sulfate transporter NaS-1) and the NaC subfamily, the members of which transport mainly Krebs cycle intermediates (Markovich & Murer, 2004). In contrast to the tetrameric structure of prestin (SLC26A5), DASS members probably act as dimers, as shown in the case of the NaS-1 transporter (Regeer *et al.*, 2007).

In spite of the relatively good biochemical characterization of sulfate transporters in animals, little is known about the molecular mechanisms of the regulation of the encoding genes. It has been demonstrated that the human gene encoding the proximal tubular BBM Na^+-sulfate cotransporter NaS-1 (SLC13) is strongly regulated *in vivo* under various dietary/hormonal conditions (Markovich *et al.*, 1998; Sagawa *et al.*, 1998; Lee *et al.*, 2000). In addition, the mouse NaSi-1 (SLC13A1) gene was found to be regulated at the transcriptional level by vitamin D (Beck & Markovich, 2000), but also by corticoids, growth hormone and prostaglandins (Markovich, 2001). The regulation by lipids (also by the steroid/secosteroid-type ones) was found to involve farnesoid X receptor alpha (FXR) (Hubbert *et al.*, 2007), which is consistent with the finding that this receptor is activated by bile acids and polyunsaturated fatty acids (Zhao *et al.*, 2004; Gineste *et al.*, 2008).

Another mouse gene, encoding Sat1 transporter belonging to the SLC26A1 class, is strongly regulated at the transcriptional level by thyroid hormones. Numerous binding sites of triiodothyronine-responsive elements (T3RE) were found in the gene's promoter and it was demonstrated their functionality (Markovich, 2001; Lee *et al.*, 2003).

Other sulfate transporters are known which do not belong to the two families mentioned above. For instance, human dicarboxylate carrier (DIC; SLC25A10 family; TC 2.A.29.2.), located in the inner mitochondrial membrane, can transport malonate, malate and succinate in exchange for phosphate, sulfate, sulfite or thiosulfate (Crompton *et al.*, 1974a; 1974b; Fiermonte *et al.*, 1999). It was shown that the DIC-encoding gene is highly expressed in mammalian liver and kidney where the protein plays an important role in gluconeogenesis, in urea synthesis and in sulfur metabolism (Fiermonte *et al.*, 1999).

SULFATE PERMEASES IN PLANTS

Plants occupy a crucial position in the nutrient chain of terrestrial animals, serving, among other functions, as the main source of sulfur-containing amino acids. As higher eukaryotes, they have developed a wide range of tissue-specific sulfate transporters (Smith *et al.*, 1997). Most of them are encoded by a gene family closely related to that coding for
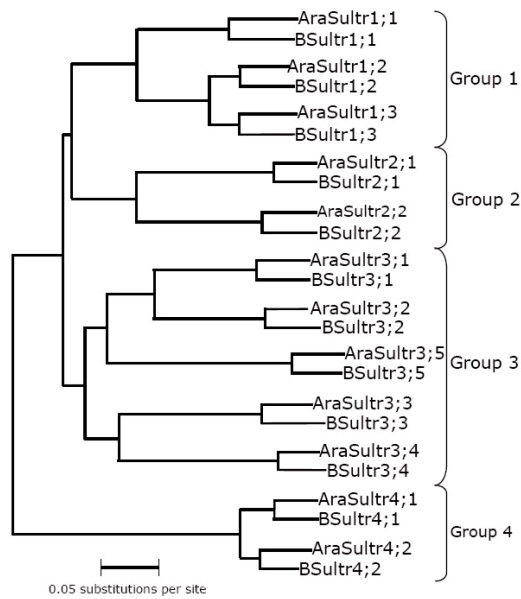
Figure 4. Phylogenetic neighbour-joining tree of sulP genes from *A. thaliana* (AraSultr) and cabbage (BSultr) (Buchner *et al.*, 2004).

animal and fungal sulfate:H^+ cotransporters of the SulP family. Plant genes encoding sulfate transporters were cloned and characterized for the first time from a tropical legume *Stylosanthes hamata* by complementation of a yeast sulfate permease-deficient mutant (Smith *et al.*, 1995). Basing on transcriptomic data from *Arabidopsis thaliana* and cabbage (*Brassica oleracea*), plant sulP members have been subdivided into five groups, depending on their properties, localization and substrate affinity (Hawkesford, 2003; Buchner *et al.*, 2004). Four of them are presented in Fig. 4. Studies on *A. thaliana*, *S. hamata*, barley, maize and cabbage have revealed the occurrence of twelve types of sulfate transporters from these four groups, and characterized them (Hawkesford, 2000). All of them are induced transcriptionally by sulfur availability.

Individual transporters have specific functions since they differ in the affinity for the transported ions; three types with a high affinity ($K_m \approx 9\ \mu M$) — *SULTR1;1, SULTR2;1* and *SULTR2;2* are expressed preferentially in roots (Leustek & Saito, 1999; Takahashi *et al.*, 2000; Yoshimoto *et al.*, 2002), whereas low-affinity forms (with a $K_m \approx 100\ \mu M$) are present in all tissues, especially in leaves (Leustek & Saito, 1999).

SULTR1;3, SULTR2;1, SULTR2;2, and SULTR3;5 are involved in long-distance sulfate transport, among them SULTR1;3 delivers sulfate through phloem (Takahashi *et al.*, 2000; Yoshimoto *et al.*, 2003; Kataoka *et al.*, 2004a). Of these transporters, only three have been shown to be involved in intracellular sulfate transport: SULTR4;1 in leaf chloroplasts (Takahashi *et al.*, 1999), and SULTR4;1 and

SULTR4;2 through the tonoplast (Hawkesford, 2000; Kataoka *et al.*, 2004b).

The fifth group of transporters, subdivided in two subgroups, have been identified in *Arabidopsis*, rice, and cabbage (Hawkesford, 2003; 2008). Their common feature is the absence of the STAS domain (Hawkesford, 2003). Members of this group vary in substrate specificity, localization and regulation. For instance, the *Arabidopsis* AtSULTR5;2 due to its affinity for molybdate anion has been renamed MOT1 (molybdenum transporter 1) (Tomatsu *et al.*, 2007; Baxter *et al.*, 2008). In *Brassica napus*, the SULTR5;1 transporter, which is localized in the roots, stem, leaves and intracellulary in the tonoplast, can transport sulfate. Interestingly, expression of the *Sultr5;1* gene is not increased upon sulfur starvation (Parmar *et al.*, 2007).

DIC transporters, previously mentioned as animal mitochondrial proteins, have also been described in *A. thaliana* (Palmieri *et al.*, 2008). Three of them transport malate, succinate, phosphate, oxaloacetate, sulfate and thiosulfate at high rates, the three latter anions more efficiently than do animal DICs. Transcripts of *DIC1* and *DIC2* are present in all plant tissues and their level is only slightly affected under some stress conditions.

Some plants and green algae possess unique types of sulfate transporters, mostly of prokaryotic origin, which are exceptions among eukaryotic sulfate permeases. A new type of sulfate permease was identified in chloroplasts of the green alga *Chlamydomonas reinhardtii* (Chen *et al.*, 2003). It is a SulP protein belonging to the ABC transporter superfamily (TC 3.A.1.6.), which contains seven transmembrane helices and two large hydrophilic loops. The gene encoding this permease has probably migrated from the chloroplast to the nuclear genome during evolution of *C. reinhardtii*. This gene has not been retained in vascular plants, e.g., *A. thaliana*, but it occurs in the chloroplast genome of some lower plants, like the liverwort *Marchantia polymorpha*.

The transcriptional regulation of sulfate transporter-encoding genes has been first characterized in the tropical legume *Stylosanthes hamata* (Smith *et al.*, 1995). The *Shst1* and *Shst2* genes both code for high affinity root sulfate permeases. Their transcription is strongly enhanced right at the onset of sulfur starvation, while expression of a third gene — *Shst3*, which occurs in leaves, is significantly delayed.

Some *cis-* and *trans-*acting regulatory elements engaged in transcriptional control of sulfur uptake genes have been identified in *A. thaliana* (for a detailed review see: Lewandowska & Sirko, 2008). Sulfur-responsive elements (SURE) consisting of GAGAC or GTCTC sequences have been found in the promoter of the *AtSultr1;1* gene (Maruyama-Nakashita *et al.*, 2005). In spite of the similarity of

the SURE sequence to the auxin response factor (ARF) binding sites, only the former functions under sulfate deficiency. The positively-acting transcription factor SLIM1 (**s**ulfur **lim**itation 1) specifically regulates transcription of genes from the sulfate assimilation pathway. This factor, primarily found as an activator of AtSultr1;2, belongs to the EIL (**e**thylene-**i**nsensitive3-**l**ike) transcription factor family (Maruyama-Nakashita *et al.*, 2006). SLIM1 also regulates genes encoding other sulfate transporters from the SULTR1, SULTR3 and SULTR4 groups.

Recently, it has been shown that SLIM1 regulation can be modified by microRNA-395 (miR395) (Kawashima *et al.*, 2009). Six *Arabidopsis* miR395 loci from chromosome I are induced differently in plant tissues and regulate expression of some *Sultr* genes. For instance, in leaves *Sultr2;1* mRNA is silenced by miR395, while in roots *Sultr2;1* is up-regulated upon sulfur limitation despite miR395 induction. These results reveal that in higher eukaryotes, e.g. plants, the transcriptional regulation of sulfate assimilation engaging the positive-acting transcription factor SLIM1 also in the silencing of some Sultr transcripts is much more complex than in microorganisms.

Some other proteins, such as a transporter-like protein involved in polar auxin delivery in *Arabidopsis*, encoded by the *BIG* gene (Kasajima *et al.*, 2007), may slow down sulfate uptake. Mutations in this gene lead to pleiotropic effects, including a slightly increased *Sultr2;2* expression.

The STAS domain mentioned earlier has been reported as an element of post-translational regulation of sulfate transporters (Aravind & Koonin, 2000; Chernova *et al.*, 2003; Rouached *et al.*, 2005; Yoshimoto *et al.*, 2007). Besides undergoing phosphorylation, this domain forms a complex with a cytosolic isoform of cysteine synthase (CS) occuring in *A. thaliana* root cortex cells[1]. It is worth noting that *O*-acetylserine, a substrate for CS, is a low molecular weight effector which positively regulates sulfate assimilation in plants (Hawkesford & Smith, 1997).

Little is known about molecular players in the signaling pathway activated during sulfur starvation in plants. So far only an SNRK2-type kinase responsible for a specific response in the signaling cascade of sulfur starvation in *C. reihardtii* has been identified (Davies *et al.*, 1999; Moseley *et al.*, 2009).

SULFATE PERMEASES IN FUNGI

Investigations on sulfate transport in fungi have so far been limited to a few species: the yeast *Saccharomyces cerevisiae* (Breton & Surdin-Kerjan, 1977; Cherest *et al.*, 1997), and the molds *Neurospora crassa* (Ketter & Marzluf, 1988; Marzluf, 1997) and *Penicillium chrysogenum* (van de Kamp *et al.*, 1999; 2000). Two sulfate permeases belonging to the SulP family have been found in all these species. One sulfate permease gene (*sB*) was also reported in *Aspergillus nidulans* (Arst, 1968; Piłsyk *et al.*, 2007). Mutations which abolish sulfate uptake lead to resistance to toxic analogs of sulfate, like selenate, chromate, tungstate and molybdate. The permease-encoding genes were found to be strongly regulated at the transcriptional level (Li *et al.*, 1996; Cherest *et al.*, 1997; van de Kamp *et al.*, 1999; Paszewski *et al.*, 2000; Natorff *et al.*, 2003).

In the yeast, transcripts of two permease genes, *SUL1* and *SUL2*, appeared 20 min after the onset of derepression conditions and quickly rose in time (Cherest *et al.*, 1997). The sequences of Sul1p and Sul2p indicate that they are integral membrane proteins with twelve transmembrane helices and share a high degree of similarity with each other. Kinetic studies show that both proteins belong to the high affinity sulfate transport class.

An atypical sulfate transporter of low specifity OAC (oxaloacetate carrier; PMT, TC 2.A.29.15.1.) has been identified in yeast mitochondria (Palmieri *et al.*, 1999). It belongs to DICs-type transporters, described earlier in plants and animals, and can transport oxaloacetate, malonate, thiosulfate or sulfate, but the latter does not seem to be its main substrate.

The *N. crassa* sulfate transporters are encoded by the *cys-13* (permease I) and *cys-14* (permease II) genes (Ketter & Marzluf, 1988; Marzluf, 1997). *cys-14* was the first cloned and well characterized eukaryotic gene encoding a sulfate transporter. In addition, *cys-14* was used as the main reporter gene in elucidation of the regulation of the sulfate assimilation pathway on the molecular level in *N. crassa*. Kinetic studies have revealed that CYS14 is the main high affinity sulfate permease, which is primarily expressed in mycelia, while CYS13 functions in conidia. Both genes are regulated at the transcriptional level by sulfur sources, like sulfate or methionine, and their transcripts appear to be highly expressed under sulfur starvation.

P. chrysogenum also has two genes — *sutA* and *sutB* — encoding sulfate permeases (van de Kamp *et al.*, 1999; 2000). The *sutB*-encoded protein is the major sulfate permease in this fungus, as demonstrated by gene disruption. The function of the *sutA* gene remains unclear. Expression of both *sutA* and *sutB* in *P. chrysogenum* is induced under sulfur starvation, although *sutA* is expressed at a much lower level than *sutB*.

Trichoderma reesei lacks an ortholog of *N. crassa* sulfate permease CYS14, while expression of the *cys-*

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[1]Shibagaki N, Grossman AR (2007) Functions of the STAS domain in the *Arabidopsis* sulfate transporter, Sultr1;2. *Botany and Plant Biology Joint Congress*. p. Abst. 1462 poster P11018.

13-orthologous gene is undetectable (Gremel *et al.*, 2008). In the genome of *T. reesei* five other sequences have been found showing similarity to genes encoding sulfate permeases in fungi, but nothing is known about their real function and regulation.

Screening of the *Aspergillus* sp. genome sequence databases showed that *Aspergilli* possess only one gene encoding a protein readily identified as a sulfate permease. In *A. nidulans* it is the *sB* gene located on chromosome VI (Arst, 1968; Piłsyk *et al.*, 2007). The *sB* gene is under transcriptional regulation dependent on sulfur sources, similarly to other fungi. Strains defective in the *sB* gene do not grow on sulfate as the sole sulfur source, but grow well on choline sulfate, which is taken up by a different permease. These strains are also resistant to toxic analogs of sulfate, selenate and chromate, in the presence of non-repressing methionine concentrations (Arst, 1968). It was found that *sB* mutations are complemented by the *P. chrysogenum sutB* gene (van de Kamp *et al.*, 1999).

An unusual sulfate transporter has also been identified in the nonreferential Japanese strain of *A. nidulans*. It is encoded by the *astA* gene (**a**lternative **s**ulfate **t**ransporter; Major Facilitator Superfamily TC 2.A.1.14.) (Piłsyk *et al.*, 2007). AstA orthologs are present only in a few fungal species, like *Fusarium* sp., *Nectria haematococca*, *Chaetomium globosum*, *Podospora anserina* and *Neosartorya fischeri*, all of which are plant pathogens/saprophytes. On the phylogenetic tree AstA orthologs form a separate branch of the Dal5 (allantoate permease) family, defining a new subfamily of fungal transporters. The presence of the *astA* gene exclusively in this group of filamentous fungi suggests that the gene plays a physiological role under some, yet undetermined, specific conditions, possibly related to interactions with plants.

Sulfate uptake is subject to regulation common for all steps of sulfate assimilation. The regulatory system involved seems to be similar in all fungi that have been studied in this respect. Sulfate assimilation, including sulfate uptake, is strongly repressed in *S. cerevisiae*, *N. crassa* and *A. nidulans* when they are grown in methionine-supplemented medium, but experiments with the use of mutants impaired in conversion of methionine to cysteine indicated that in fact the latter is the regulatory effector (Paszewski & Grabski, 1974; Paszewski & Ono, 1992; Hansen & Johannesen, 2000). This regulation occurs at the transcriptional level and is dependent on the sulfur metabolite repression (SMR) system (Marzluf, 1997; Paszewski *et al.*, 2000; Natorff *et al.*, 2003). This system consists of a positive-acting bZIP transcription factor needed for transcription of several sulfur-related genes (Paietta, 1992; Thomas & Surdin-Kerjan, 1997; Natorff *et al.*, 2003) and a negative-acting ubiquitin-ligase complex SCF that inacti-

vates the transcription factor under repressive conditions, i.e., excess of cysteine. This complex was first described in *S. cerevisiae* (Kaiser *et al.*, 2000; Rouillon *et al.*, 2000). In this organism it has a unique organization as it involves two bZIP transcription factors, the main Met4p and an auxillary one, Met28p, and an additional helix-loop-helix-type centromere-binding protein, Cbf1p. These three proteins are responsible for the activation of genes encoding enzymes from the sulfate assimilatory pathway. In filamentous fungi, like *N. crassa* and *A. nidulans*, this system is based on one bZIP transcription factor.

A separate, but interesting topic is the transport of molybdate since it is taken up by proteins related to sulfate transporters. These proteins have been characterized in bacteria and plants (Tejada-Jiménez *et al.*, 2007; Tomatsu *et al.*, 2007; Baxter *et al.*, 2008; Fitzpatrick *et al.*, 2008). In prokaryotes molybdate and tungstate are taken up by proteins belonging, like the sulfate channels, to the ABC family (TC 3.A.1.6/8.), while in eukaryotes, especially in plants, by members of the SulP family (TC 2.A.53.1.3) (Tomatsu *et al.*, 2007; Baxter *et al.*, 2008). These proteins are also low affinity sulfate transporters (Fitzpatrick *et al.*, 2008). They are localized in mitochondria and usually do not have a STAS domain. So far, there are no data describing molybdate transport in fungi, although genome analyses have shown the presence of ATP-binding proteins with a bacterial-type molybdate transporter domain modC. This suggests that fungi may utilize a transport system adopted from prokaryotes. In addition some molybdate may enter the cell through sulfate transporters.

CONCLUSIONS

Sulfate is taken into the cell by sulfate permeases, which comprise a variety of transporters represented by three main families: bacterial SulT (ATP-binding cassette), eukaryotic SulP/SLC26, and mammalian SLC13. In prototrophic organisms the sulfate assimilation pathway is highly endoergic so studies of sulfate transport are also important with respect to utilization of cellular energy. The pathway is repressed when a favourable sulfur source, like cysteine or methionine, is available. The expression of genes encoding sulfate transporters is strongly regulated at the transcriptional level as well as post-translationally.

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