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Bacterial transport of sulfate, molybdate, and related oxyanions

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Abstract Sulfur is an essential element for microorganisms and it can be obtained from varied compounds, sulfate being the preferred source. The first step for sulfate assimilation, sulfate uptake, has been studied in several bacterial species. This article reviews the properties of different bacterial (and archaeal) transporters for sulfate, molybdate, and related oxyanions. Sulfate uptake is carried out by sulfate permeases that belong to the SulT (CysPTWA), SulP, CysP/(PiT), and CysZ families. The oxyanions molybdate, tungstate, selenate and chromate are structurally related to sulfate. Molybdate is transported mainly by the high-affinity ModABC system and tungstate by the TupABC and WtpABC systems. CysPTWA, ModABC, TupABC, and WtpABC are homologous ATP-binding cassette (ABC)-type transporters with similar organization and properties. Uptake of selenate and chromate oxyanions occurs mainly through sulfate permeases.

Keywords Sulfate permeases · Molybdate transport · Tungsten transport · Selenate transport · Chromate transport

Introduction

Sulfur (S) is an essential element that is widely required by living organisms because it plays several important roles in cells. Sulfur is a component of the amino acids cysteine and methionine as well as of cellular cofactors including biotin, coenzyme A, S-adenosylmethionine, thiamine, glutathione, lipoic acid, and iron-sulfur clusters (Scott et al. 2007). Sulfate (SO_4^{2-}) is the preferred sulfur source for the majority of organisms and is the second most abundant soluble oxyanion, after phosphate, in the bacterial cell (Silver and Walderhaug 1992). Sulfur in sulfate is in the +VI oxidation state, thus sulfate belongs to the group VI of oxyanions, which includes other structurally similar members such as molybdate, tungstate, selenate, and chromate (Markovich 2001). Another pathway of sulfur assimilation involves thiosulfate ($S_2O_3^{2-}$). Sulfate and thiosulfate are taken up by membrane transporters called sulfate permeases (Fig. 1). Once inside the cell, sulfate is first reduced to sulfite and then to sulfide, which is

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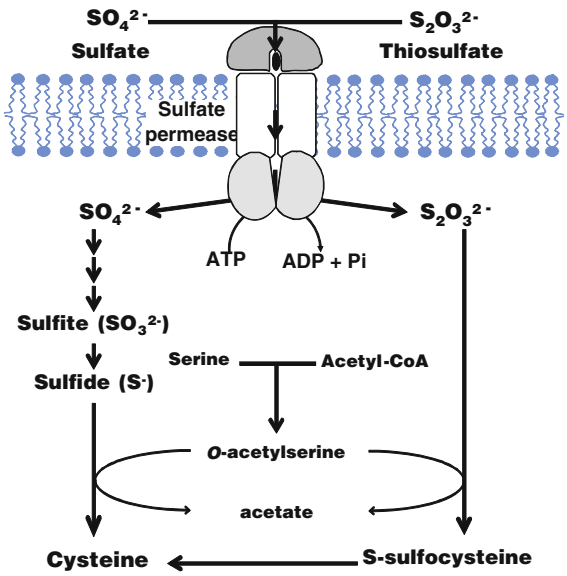


Fig. 1 Sulfate and thiosulfate transport and assimilation in *Salmonella typhimurium* and *Escherichia coli*. Sulfate is first taken up by the sulfate permease and then is reduced to sulfite and sulfide, which reacts with *O*-acetylserine to produce cysteine. *O*-acetylserine is formed by the reaction of serine with acetyl-CoA. Thiosulfate is taken up by the sulfate permease and then reacts directly with *O*-acetylserine to produce S-sulfocysteine, which is subsequently transformed into cysteine

utilized to synthesize cysteine; thiosulfate reacts with O-acetylserine to give S-sulfocysteine, which is then reduced to cysteine (Sekowska et al. 2000; Guédon and Martin-Verstraete 2007) (Fig. 1). Bacterial sulfate permeases belong to several protein families such as ATP-binding cassette (ABC)-type and SulP, PiT or CysZ transporters. Due to their similar structural characteristics, related oxyanions can be transported inside the cell by the same type of carriers.

The molybdate (MoO_4^{2-}) and tungstate (WO_4^{2-}) oxyanions are the main sources of essential metals molybdenum (Mo) and tungsten (W) in bacterial cells (Hille 2002). Molybdenum is an essential trace element for the majority of living organisms. Molybdenum-containing enzymes are ubiquitous in nature and play important roles in the global cycles of nitrogen, carbon, and sulfur (Kisker et al. 1997). Tungsten has been also reported as an essential trace element for some bacteria and archaea (Johnson et al. 1996). Molybdate and tungstate are taken up by high-affinity ABC-type transporters (Schwarz et al. 2007).

Selenium is an essential element for microorganisms and its common biological forms are selenocysteine (the so-called twenty-first amino acid) and selenomethionine (Stolz et al. 2006). Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are the predominant inorganic forms of selenium in aerobic environments. High concentrations of selenium oxyanions are highly toxic and mutagenic for bacteria and mammals. Selenate is taken up by the sulfate permeases and by an alternative system that also transports selenite (Lindblow-Kull et al. 1985; Turner et al. 1998).

In contrast to essential sulfur, molybdenum, tungsten, and selenium, chromium is a controversial element (Stearns 2000). Chromium (III) is considered an essential nutrient required by humans and animals (Vincent 2004). However, chromium essentiality is challenged because no specific enzyme or cofactor containing chromium has been identified (Stearns 2000; Vincent 2010). Chromium is considered as nonessential for microorganisms. Chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) are Cr(VI) derivatives that are highly toxic for the majority of cells. Chromate is transported inside bacterial cells mainly by sulfate transporters (reviewed in Ramírez-Díaz et al. 2008). Due to the toxicity of chromate, some bacteria possess the CHR transport system, which extrudes chromate out of the cell (Cervantes et al. 2001).

Because sulfate, molybdate, tungstate, and selenate are the principal sources of the essential elements sulfur, molybdenum, tungsten, and selenium, respectively, it is important to understand how these oxyanions are transported inside the cells. This review is focused on bacterial sulfate transporters Sbp/CysPTWA, SulP, CysP and CysZ, some of which have not been considered in detail in other reviews (Kertesz 2001). In addition, a comparative description of the bacterial transporters for molybdate, tungstate, selenate, and chromate is presented. When available, information on oxyanion transport by archaeal species is also described.

Sulfate transport

Sulfate is transported inside bacterial cells by carriers belonging to the SulT family, to the SulP family (Kertesz 2001), by the CysP transporter, that belongs to the phosphate inorganic transporter (PiT) family (Mansilla and de Mendoza 2000), and by the CysZ

Table 1 Bacterial transporters of sulfate

Transporter	Family	TC number[a]	Organisms	References
Sulfate-thiosulfate permease (CysPTWA)	Sulfate/tungstate uptake transporter SulT	3.A.1.6	*Salmonella typhimurium*	Ohta et al. (1971)
			Escherichia coli	Sirko et al. (1990)
			Mycobacterium tuberculosis	Wooff et al. (2002)
			Synechococcus elongatus	Laudenbach and Grossman (1991)
			Burkholderia cenocepacia	Farmer and Thomas (2004)
SulP	Sulfate permease SulP	2.A.53	*Acidithiobacillus ferrooxidans*	Valdés et al. (2003)
			Mycobacterium tuberculosis	Zolotarev et al. (2008)
CysP/(PiT)	Inorganic phosphate transporter PiT	2.A.20	*Bacillus subtilis*	Mansilla and de Mendoza (2000)
CysZ	Putative sulfate transporter	9.B.7	*Escherichia coli*	Parra et al. (1983)
CysZ	Putative 4-toluene sulfonate uptake permease (TSUP)	9.A.29	*Corynebacterium glutamicum*	Rückert et al. (2005)

[a] According to the Transport Classification Database (TCDB)

putative sulfate transporters (Rückert et al. 2005) (Table 1) (Fig. 2). Sulfate can also be transported by the ModABC molybdate transport system (Table 2). These transporters are described in the following sections.

Sulfate permeases of the SulT family

The sulfate-thiosulfate permease belongs to the sulfate/tungstate uptake transporter (SulT) family (TC# 3.A.1.6) of the ABC superfamily of transporters. The SulT family is constituted by sulfate and tungstate porters, according to the Transporter classification database (TCDB) (Saier 2000; Kertesz 2001; Saier et al. 2006), a system that classifies transporters according to mode of action and phylogeny.

Sulfate and thiosulfate transport is an energy-dependent process carried out by the same transporter (Fig. 1), which is inhibited by sulfite, molybdate, selenite, selenate, and chromate (Furlong 1987; Silver and Walderhaug 1992). The SulT sulfate-thiosulfate permeases from *Salmonella typhimurium* and *Escherichia coli* are constituted by: (i) one of two periplasmic proteins, Sbp, the sulfate-binding protein (Pflugrath and Quiocho 1985), or CysP, the thiosulfate-binding protein (Hryniewicz et al. 1990); (ii) membrane proteins CysT and CysW; and (iii) the

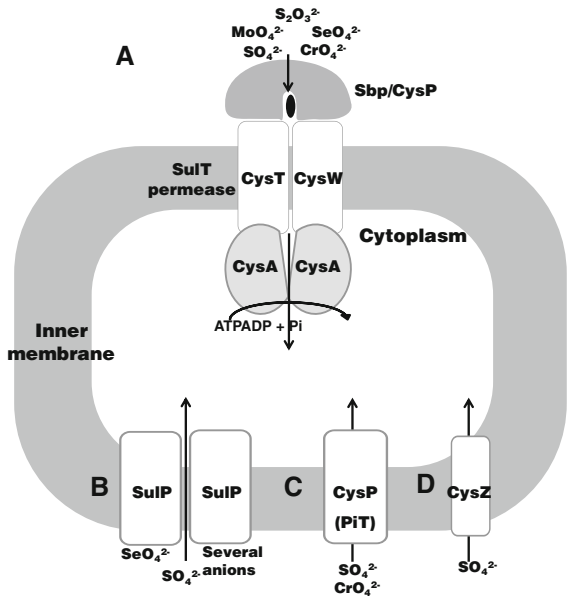


Fig. 2 Bacterial sulfate transporters. **a** Sulfate-thiosulfate SulT permease constituted of the sulfate- or thiosulfate-binding proteins Sbp or CysP, respectively, the membrane proteins CysT and CysW, and the CysA ATPase. **b** SulP transporter. **c** CysP/(PiT) transporter. **d** CysZ transporter. The oxyanions transported by each system are indicated. Sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$), molybdate (MoO_4^{2-}), selenate (SeO_4^{2-}), chromate (CrO_4^{2-}). Details for each system are provided in the text

Table 2 Bacterial transporters of molybdate and tungstate

Transporter	Family	TC number[a]	Substrates	Organisms	References
ModABC	Molybdate uptake transporters MolT	3.A.1.8	Molybdate Tungstate Sulfate	*Escherichia coli*	Maupin-Furlow et al. (1995)
Sulfate/thiosulfate permease (CysPTWA)	Sulfate/tungstate uptake transporter SulT	3.A.1.6	Sulfate Chromate Molybdate Selenate	*Escherichia coli*	Rosentel et al. (1995) Turner et al. (1998)
Nonspecific anion transport system	N.R.[b]	N.R.	Molybdate Sulfate Selenate Selenite	*Escherichia coli*	Rosentel et al. (1995)
TupABC	Sulfate/tungstate uptake transporter SulT	3.A.1.6	Tungstate	*Eubacterium acidaminophilum*	Makdessi et al. (2001)
WtpABC	Sulfate/tungstate uptake transporter SulT	3.A.1.6	Tungstate Molybdate	*Pyrococcus furiosus*	Bevers et al. (2006)

[a] According to the Transport Classification Database (TCDB)

[b] *N.R.* no reported in the TCDB (gene not identified)

ATP-binding protein, CysA (Fig. 2a). SulT subunits are encoded by the *cysPTWA* operon and by the *sbp* gene, located either in another chromosomal region or instead of *cysP* in the same operon (Table 1). Also as part of this operon, can be found the *cysM* gene that encodes *O*-acetylserine sulfhydrylase B, which is not involved in transport but participates in the central anabolic pathway of bacteria to produce cysteine (reviewed in Silver and Walderhaug 1992).

Periplasmic sulfate (Sbp)- and thiosulfate (CysP)-binding proteins

The Sbp protein of *S. typhimurium* (accession number P02906) was the first periplasmic substrate-binding protein identified and crystallized. Sbp shares structural similarities to numerous periplasmic binding proteins of Gram-negative bacteria (Pflugrath and Quiocho 1988; Tam and Saier 1993). Sbp is an ellipsoid that consists of two similar globular domains with the ligand-binding site located deep in a cleft between the domains, in which sulfate is held tightly in place by seven hydrogen bonds in a completely desolvated state (Pflugrath and Quiocho 1985, 1988). There are no positively-charged residues nor cations or water molecules within van der Waals distance to the sulfate dianion, and stabilization of the isolated charges of sulfate in the Sbp-sulfate complex involves local dipoles (He and Quiocho 1993). The Sbp protein exhibits high specificity for sulfate (*Km* of 0.1 μM) and for other tetrahedral fully ionized oxyanions, and can discriminate for the structurally similar oxyanion phosphate (Jacobson and Quiocho 1988; Quiocho 1996). Sbp of *E. coli* displays 94% identity, the same size and antigenicity to the *S. typhimurium* protein, and probably binds sulfate by a similar mechanism (Jacobson et al. 1991).

CysP, the thiosulfate-binding protein, shows 44% sequence similarity with Sbp from both *E. coli* and *S. typhimurium*; they belong to two different but related protein families (COG4150 and COG1613, respectively). Interestingly, the amino acid residues essential for sulfate binding are conserved in Sbp and CysP proteins (Hryniewicz et al. 1990). Sbp and CysP have partially overlapping activities, and it has been suggested that both proteins interact with membrane proteins CysT and CysW of the SulT permease, which explains the failure to isolate transport-defective mutants affected in either *sbp* or *cysP* (Hryniewicz et al. 1990). The presence of two alternative substrate-

binding proteins in the SulT permease whose function overlaps may be an adaptive advantage for bacterial cells under certain environmental conditions.

CysT and CysW membrane proteins

Membrane proteins CysT (also called CysU, accession number P16701) and CysW (accession number P0AEB0) constitute the transport channel of the SulT permease (Sirko et al. 1990) (Fig. 2a). CysT and CysW belong to two different but related protein families (COG0555 and COG4208, respectively). Both proteins display six putative transmembrane segments (TMSs) (Sirko et al. 1990). In *E. coli* and several other bacteria, mutations in the *cysTWA* gene cluster confer a requirement for cysteine or for a sulfur source different from sulfate, but in the β-proteobacterium *Burkholderia cenocepacia*, transposon insertions in the *cysW* gene do not result in cysteine auxotrophy (Hryniewicz et al. 1990; Farmer and Thomas 2004). *cysW* mutants of *B. cenocepacia* have similar growth properties as the wild-type strain in the presence of $\geq$0.5 mM of sulfate, even when sulfate uptake by the mutants was 50-fold less efficient. Because the genome of *B. cenocepacia* apparently does not possess additional copies of *cysW* genes, it was proposed that CysT may partially substitute for CysW in sulfate uptake by this bacterium (Farmer and Thomas 2004).

CysA, the ATPase subunit

CysA (COG1118), the ATPase subunit of the SulT transporter, couples the hydrolysis of ATP to the translocation of sulfate (Sirko et al. 1990) (Fig. 2a). The crystal structure of CysA from the Gram-positive thermoacidophilic bacterium *Alicyclobacillus acidocaldarius* has been solved to a resolution of 2.0 Å (Scheffel et al. 2005); this is the only CysA protein that has been structurally analyzed to date. CysA displays two different dimer forms in the asymmetric unit: the well-defined CysA-1 dimer, and the CysA-2 form, which is fairly flexible. In the CysA functional dimer, each monomer is composed of a regulatory domain, which is present only in some ABC-ATPases, and the nucleotide-binding domain (NBD), which has a fold similar to that of other ABC-ATPases. The regulatory domain has also a similar fold in all the ABC-ATPases in which it is present, and it has been suggested that this might function as a signal-transduction module (Scheffel et al. 2005). The NBD of CysA is subdivided into a catalytic subdomain and a helical subdomain. NBDs of the ABC-ATPases possess three highly conserved motifs, the 'Walker' A motif, with consensus sequence GXXGXGKS/T, in which X can be any residue, the 'Walker' B motif, with sequence hhhhD, in which h stands for hydrophobic amino acids, and the ABC signature motif LSGGQ (also called C-loop), which is unique to the ABC transporter superfamily. NBDs also possess several motifs with only one highly-conserved residue, such as the Q-, Pro-, D- and H-loops (Oswald et al. 2006). All of these motifs are well conserved in CysA proteins.

Distribution of the cysPTWA operon

The *cysPTWA* operon is widely distributed among bacteria and displays two different alternative forms, depending on the gene encoding the substrate-binding protein, the *sbp*-containing operons and the *cysP*-containing operons (Fig. 3). The *sbp*-containing operon is unique in some members of α-, β-, and γ-proteobacteria, and appears to be the only operon in all δ-, ε-proteobacteria, actinobacteria, firmicutes and cyanobacteria. A few members of α-, β-, and γ-proteobacteria, such as *Silicibacter* sp. and *Herminiimonas arsenicoxydans*, possess only the *cysP*-containing operon. However, the two alternative operons are present in several members of α-, β-, and γ-proteobacteria, such as *Agrobacterium tumefaciens*, *Janthinobacterium* sp., *and Pseudomonas mendocina* (Fig. 3).

The *sbp*- and the *cysP*-containing operons show distinct gene arrangements among the different taxonomic groups. Both operons have a minimum of four genes: *sbp*, *cysT*, *cysW* and *cysA*. Exceptions are the α-proteobacterium *Magnetospirillum magneticum* and *H. arsenicoxydans*, which show only three genes: *cysP*, *cysT* and *cysW*. In various bacteria, such as *E. coli*, the *sbp* gene is located in another region of the chromosome. The *cysPTWA* operons of some bacteria also contain genes involved in sulfur metabolism (Fig. 3).

The prevalence of the *sbp*-containing operon is probably due to the preference of sulfate vs. thiosulfate as a sulfur source. The presence of two operon

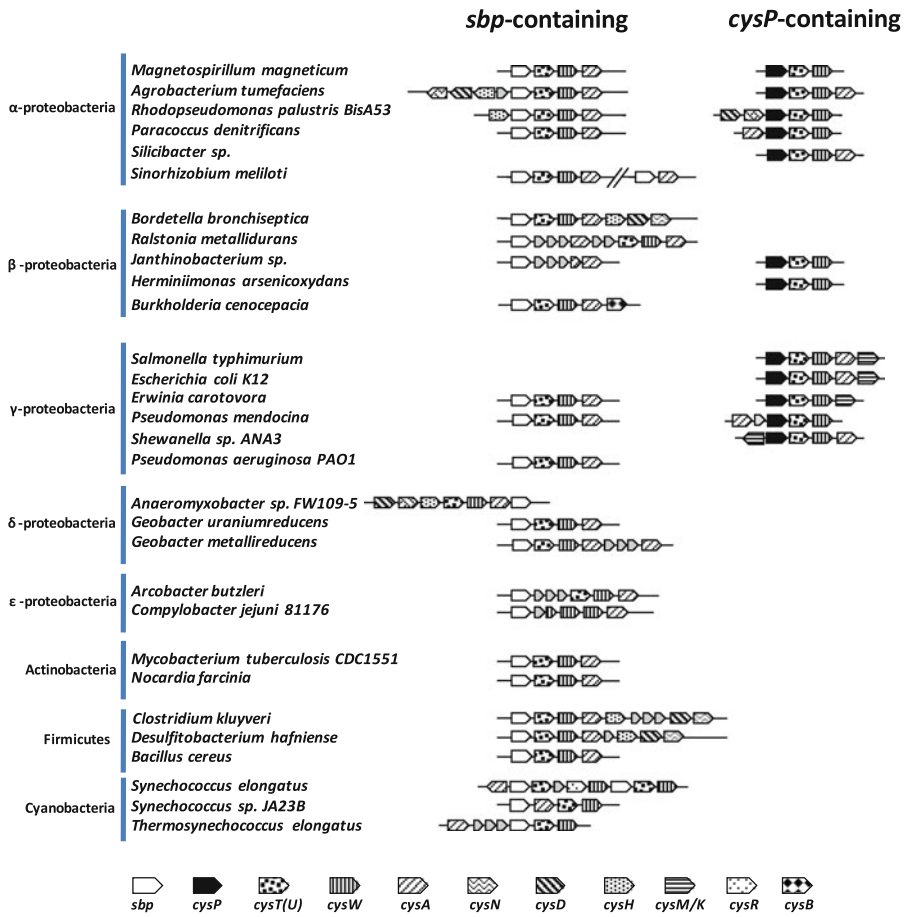


Fig. 3 Distibution and organization of the *cysPTWA* operon and *cys*-related genes. The clades of the different analyzed species are shown to the *left*. The name of the representative species and the distribution of the *sbp*- and *cysP*-containing operons for each are indicated. *Open arrows* represent the genes and direction of transcription. Genes with the same shading encode proteins with similar functions. The code name for each gene is provided in the bottom. Distribution of the different operons was constructed with the STRING database (von Mering et al. 2007) using the sequences of *sbp* and *cysP* genes

forms in several bacterial taxa indicates the importance of the redundancy of the uptake systems, and a possible advantage for these organisms. In *E. coli* EC2541, a mutant affected in a gene encoding a transcriptional factor that regulates expression of genes required for sulfonate-sulfur utilization, the level of the Sbp protein is greatly diminished; however, this mutant can grow on sulfate probably because CysP acts as a functional backup for Sbp (van der Ploeg et al. 1997; Han and Lee 2006).

Sulfate permeases with similar characteristics to the SulT system have been described in Actinobacteria. Such is the case for *Mycobacterium tuberculosis*, in which the sulfate permease components are encoded by a cluster formed by the *subI* and *cysTWA* genes. *subI* encodes a sulfate-binding protein that displays 36% identity (54% similarity) to Sbp. The *Km* for sulfate uptake of the *M. tuberculosis* permease is 36 μM (Wooff et al. 2002), a value identical to the *Km* of the *S. typhimurium* SulT permease. In *B. cenocepacia*, the sulfate transporter is encoded by the *sbp cysTWA* gene cluster (Farmer and Thomas 2004), which forms part of an operon that is probably expressed from a promoter located upstream of the *sbp* gene (Iwanicka-Nowicka et al. 2007).

A sulfate-thiosulfate SulT permease has been also described in the Cyanobacterium *Synechococcus elongatus* PCC 7942 (Green and Grossman 1988; Laudenbach and Grossman 1991). Sulfate transport in *S. elongatus* is light- and energy-dependent, and, as occurs in *S. typhimurium*, is inhibited by thiosulfate, selenate and chromate. The genes that encode *S. elongatus* sulfate permease (*cysA-sbpA-cysT-orf81-cysR-cysW*) have a different arrangement from those of *E. coli* and *S. typhimurium* operons (Laudenbach and Grossman 1991) (Fig. 3). Mutations on *cysT* and *cysW* decrease the V_{max} for sulfate transport ca. 25 times compared to the wild-type strain. Neither the *cysR* gene

nor the *orf81* products are required for growth on sulfate or thiosulfate, but mutant strains affected in these genes are unable to grow on thiocyanate as a sulfur source.

Regulation of the cysPTWA operon

Sulfate and thiosulfate uptake varies according to the availability of cysteine or other sulfur sources. Sulfate transport is repressed when cells are grown in the presence of cysteine and is derepressed in the absence of sulfate. Gene products required for transport of sulfate and thiosulfate are regulated as part of the cysteine regulon (Hryniewicz and Kredich 1991), which includes the genes involved in the biosynthesis of cysteine from sulfate (Kredich 1992). Maximal expression of the cysteine regulon requires both sulfur limitation and the presence of the inducers *O*-acetylserine, which is a precursor in the biosynthesis of cysteine (Fig. 1), or *N*-acetylserine, which is derived nonenzymatically from *O*-acetylserine by an intramolecular *O*-to-*N*-acetyl shift. Repression of the cysteine regulon by growth on cysteine, or other readily utilizable sulfur sources, is due to the inhibition of serine acetyltransferase by cysteine, that results in a decrease in the synthesis of the inducer *O*-acetylserine (Kredich 1992).

In *E. coli,* excess of sulfate in the growth medium reduces the expression of *cys* genes by 40–50% of fully derepressed values, whereas in the presence of cysteine gene expression is completely repressed (van der Ploeg et al. 1996). A similar regulation occurs in *Pseudomonas aeruginosa*, in which the *sbp* and *cysTWA* genes are overexpressed when cells are grown in the absence of sulfate (Hummerjohann et al. 1998; Tralau et al. 2007).

CysB is the global regulator for sulfur assimilation from inorganic sulfate (Kredich 1992; Kertesz 2001; Guédon and Martin-Verstraete 2007). CysB is a homotetramer of 36-kDa subunits, which possesses several functional domains for DNA binding, inducer response, and oligomerization (Lochowska et al. 2001). CysB belongs to the LysR family of regulators (Kredich 1992), and acts with a dual function capable of either activating or repressing transcription from different target promoters (Lochowska et al. 2001). Inducers *O*-acetylserine and *N*-acetylserine, which are essential for maximal expression, stimulate binding of CysB to promoters of the *cysJIH* operon (that encodes the enzymes involved in the reduction of sulfate and sulfite from the route of cysteine biosynthesis), and of the *cysK*, *cysP*, and *cysB* genes (Monroe et al. 1990; Hryniewicz and Kredich 1991; Ostrowski and Kredich 1991). In *S. typhimurium*, several different types of CysB-binding sites (CBS) have been identified, which are categorized by function and by their responses to the inducer (Hryniewicz and Kredich 1995). The *cysB* gene is negatively autoregulated by the binding of CysB to positions −10 to +36 relative to the major *cysB* transcription start site (Ostrowski and Kredich 1991). In this case, the inducer *N*-acetylserine inhibits binding of CysB to the *cysB* promoter and partially reverses inhibition of transcription initiation caused by CysB; this means that CysB has the ability to respond to acetylserine with either an increase or a decrease in affinity for different DNA sequences (Ostrowski and Kredich 1991). In contrast, in *B. cenocepacia* and *Pseudomonas putida* the function of CysB appears to be independent of the presence of *O*-acetylserine, indicating that CysB action may vary with respect to the recognition of an inducer (Iwanicka-Nowicka et al. 2007; Kouzuma et al. 2008).

The function of CysB is not restricted to regulation of the assimilatory sulfate reduction pathway, it also controls sulfur assimilation at a global level. When the preferred inorganic sulfur compounds are not available, bacteria produce a set of proteins known as sulfate-starvation-induced (SSI) proteins, which include metabolic enzymes, regulatory proteins, and transport systems, for scavenging sulfur from organosulfur compounds (Kertesz et al. 1993; van der Ploeg et al. 1996; Hummerjohann et al. 1998; Scott et al. 2007).

Sulfate permeases of the SulP family

The sulfate permease SulP superfamily (TC# 2.A.53) is a large and ubiquitous protein family with hundreds of sequenced members derived from the three life domains (Table 1); however, only a few proteins belonging to this family have been functionally characterized (Saier 2000; Kertesz 2001; Saier et al. 2006). The SulP proteins contain from 430–900 aa (Saier 2000), but the bacterial members are of 434–573 residues, with some exceptions. In contrast to the SulT-type sulfate permeases, which transport sulfate and related oxyanions, SulP family members are inorganic anion uptake carriers or anion:anion

exchange transporters (Felce and Saier 2004) (Fig. 2b). Some proteins of the SulP family may be fused to different catalytic or ligand-binding domains or may be associated with enzymes. The fused domain can determine substrate specificity for many of these proteins. If the carrier is fused to enzymes related to sulfur metabolism, for example, this suggests that it may function in sulfate uptake (Felce and Saier 2004).

The genome of the γ-proteobacterium *Acidithiobacillus ferrooxidans* contains the *sulP* gene that encodes a SulP-type sulfate permease, which exhibits 11 predicted TMSs (Valdés et al. 2003). The s*ulP* gene is separated by 10 base pairs from the upstream gene *cab1* encoding a carbonic anhydrase. The juxtaposition of *cab1* and *sulP* in *A. ferrooxidans* suggests that SulP functions as a SO_4^{2-}:HCO_3^- antiporter (Valdés et al. 2003).

SLC26 anion exchangers are a protein family that is a part of the large SulP superfamily; they transport a wide number of monovalent and divalent anions such as sulfate, chloride, iodide, formate, oxalate, the hydroxyl ion, and bicarbonate (Mount and Romero 2004). SLC26 proteins are predicted to have 10–14 TMSs and a C-terminal extension, which appears to play a role in the function/regulation of transport activity (Shibagaki and Grossman 2006). The SLC26 proteins appear to be assembled as homodimers with the amino and carboxyl termini at the intracellular membrane side (Detro-Dassen et al. 2008), which suggests that all SulP family members are dimers (Fig. 2b).

The genome of *M. tuberculosis* encodes the SulP-type protein Rv1739c, which is related to SLC26 (Zolotarev et al. 2008). Expression of Rv1739c in *E. coli* increased the uptake of sulfate but not of chloride, formate, or oxalate; sulfate uptake was inhibited by sulfite, thiosulfate, and selenate. In *Mycobacterium bovis* BCG with the *cysA* gene deleted, expression of Rv1739c did not complement sulfate auxotrophy, suggesting that Rv1739c requires the *cysA* gene product for sulfate transport (Zolotarev et al. 2008).

Several proteins annotated as probable sulfate transporters of the SulP family have not been functionally characterized and may be carriers of other ions. For example, the BicA protein of the Cyanobacterium *Synechococcus* sp. PCC 700 is a HCO_3^- ion transporter that was initially reported as a sulfate transporter (Price et al. 2004).

The CysP sulfate permease from gram-positive bacteria

Contrary to the extensive knowledge available for sulfate transport in Gram-negative bacteria, mainly through the studies in *E. coli* and *S. typhimurium*, there is little detailed information reported in Gram-positive bacteria. One of the sulfate transporters analyzed is the *Bacillus subtilis* sulfate permease CysP, encoded by the *cysP* gene (previously denominated *ylnA*) (Mansilla and de Mendoza 2000) (Fig. 2c). Although they share the same name, this permease is unrelated to the CysP thiosulfate-binding protein from *E. coli* and *S. typhimurium* (described in "Periplasmic sulfate (Sbp)- and thiosulfate (CysP)-binding proteins" section). Expression of the *cysP* gene from *B. subtilis* in *E. coli* mutants affected in *cysA97*, *cysT* or *cysP-sbp* genes restored the ability of the strains to grow in minimal medium with sulfate as sole sulfur source; moreover, an *E. coli* mutant strain carrying a plasmid with the *B. subtilis cysP* gene accumulated more sulfate, as compared to the *E. coli* strain without the *cysP* gene, indicating that *B. subtilis* CysP is a sulfate transporter (Mansilla and de Mendoza 2000).

The *B. subtilis* CysP permease is predicted as a membrane protein with 11 TMSs and two homologous domains that might have arisen by means of a tandem internal gene duplication event (Mansilla and de Mendoza 2000; Salaun et al. 2001).

Relation of CysP with PiT phosphate transporter family

CysP has no sequence homology with SulT- or SulP-type sulfate permeases, but shows similarity with phosphate permeases of the inorganic phosphate transporter (PiT) family (TC# 2.A.20) (Table 1). CysP possesses the Pho4 domain (COG0306) present in all PiT family members, confirming its relationship with this family (Mansilla and de Mendoza 2000). However, it is not known whether, in addition to functioning as a sulfate permease, CysP is also able to transport inorganic phosphate. To obtain additional insights on this latter possibility, a phylogenetic tree was constructed with 786 protein sequences that belong to the PiT family (including the CysP permease). Genes encoding PiT family transporters are widespread throughout the three life domains,

suggesting an ancient origin for this protein group. Archaeal and bacterial proteins are 300-550 aa in length, whereas the larger eukaryotic proteins range from 500–1,100 aa in length because they have an additional intracellular domain.

Three major clusters are observed in the phylogenetic tree for the PiT family (Fig. 4a). Cluster I, on the right side of the tree, is the most numerous group (374 sequences) and is mainly represented by a mixture of archaeal and bacterial clades. The inorganic phosphate transporters reported in *E. coli*, PitA and PitB, are included in this cluster (Harris et al. 2001). Cluster II (bottom left side of the tree) is nearly as numerous as cluster I (340 sequences) and comprises members of the three life domains. This group includes the following characterized eukaryotic phosphate transporters: mPit-2 from mouse (Bai et al. 2000), Pht2;1 from potato (Rausch et al. 2004); Pht2;1 from Arabidopsis (Daram et al. 1999), and Pit-1 and Pit-3 from human (Ravera et al. 2007). Cluster III (top left side of the tree) comprises 72 proteins (41 from archaea and 31 from bacteria). The sulfate permease CysP from *B. subtilis* is included within this group, but no additional characterized sequences belong to the cluster.

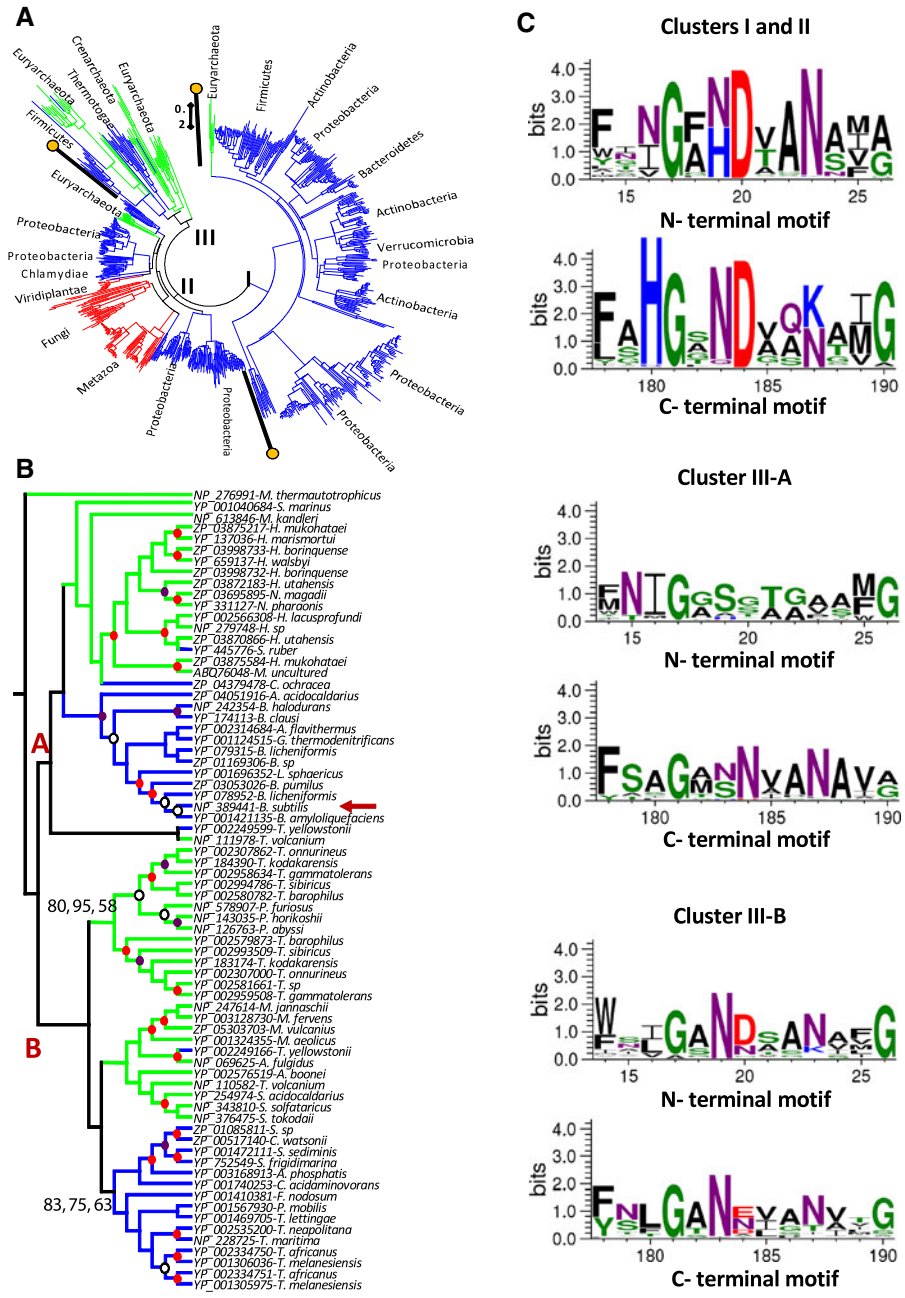
Figure 4b depicts a detailed view of cluster III. The group comprises two branches: subgroup III-A is formed mainly by members of the bacterial clade Firmicutes (including *B. subtilis* CysP) and the archaeal clade Euryarchaeota, whereas subgroup III-B is made up of members of the bacterial clades Thermotogae, Cyanobacteria and Proteobacteria, and the archaeal clades Euryarchaeota and Crenarchaeota. CysP from *B. subtilis* belongs to an operon that comprises four genes encoding enzymes involved in sulfur metabolism (Dam et al. 2007). This genetic arrangement is also present in *Bacillus licheniformis*; several other species included in subgroup III-A possess orthologous *cysP* genes associated with sulfur metabolism genes, which suggests that CysP from *B. subtilis* is not the only PiT family member involved in sulfate transport.

Bøttger and Pedersen (2005) identified inside PiT family sequences two related motifs containing highly conserved aspartate residues critical for phosphate transport. Indeed, replacement of these aspartates with asparagines severely impaired transport function, but did not perturb overall protein architecture. However, further analysis showed that aspartate residues critical for phosphate transport are not strictly conserved in all PiT family members (Bøttger and Pedersen 2005). For example, CysP from *B. subtilis* possesses the two conserved motifs, but the aspartate residues are replaced by glycine and asparagine. This finding agrees with the fact that CysP is a sulfate permease and suggests that it is not able to transport inorganic phosphate. It is noteworthy that the two motifs are conserved without exception in all 786 sequences shown in Fig. 4a as members of the PiT family.

In order to compare these motifs among PiT family members protein sequences, we performed a sequence logos analysis (Fig. 4c). The motif logos of clusters I and II (that comprise 714 sequences, including all the experimentally characterized phosphate transport proteins), demonstrate that both critical aspartate residues are strictly conserved, with no exception. This suggests that all members of clusters I and II are phosphate transporters. In contrast, logos analysis of cluster III-A (comprising 30 sequences, including CysP from *B. subtilis*) shows that no acidic residues are conserved in both N and C motifs, suggesting that the members of this cluster are not phosphate transporters and probably they function as sulfate permeases. Logos analysis of cluster III-B (41 sequences with no characterized protein) shows that the acidic residues are present, but not strictly conserved; therefore their transport function is uncertain. Additional experimental data are needed to prove the above mentioned hypotheses. Interestingly, indels analysis of cluster III-A sequences revealed that archaeal sequences possess a specific insertion (41–71 aa) located between the last two TMSs, which is absent in the rest of PiT family members.

The CysZ sulfate permeases

The genome of *E. coli* possesses a gene that encodes the CysZ protein (Table 1), reported as a sulfate transporter in the TCDB database (TC# 9.B.7) (Fig. 2d). CysZ is a membrane protein with five putative TMSs and belongs to the DUF540 or CysZ protein family (COG2981). Members of this protein family exist only in bacteria. In *E. coli*, *S. typhimurium,* and other Proteobacteria, the *cysZ* gene is located upstream of the *cysK* gene, which encodes *O*-acetylserine sulfhydrylase A, an enzyme involved in the biosynthesis of cysteine. *cysZ* mutants of *E. coli* are deficient in sulfate transport (Britton et al. 1983;



Parra et al. 1983). Thus, CysZ from *E. coli* is described as a sulfate permease, although *S. typhimurium cysZ* mutants can grow normally in 1 mM sulfate (Byrne et al. 1988). No additional CysZ homologous proteins have been experimentally tested.

The genome of the Gram-positive soil bacterium *Corynebacterium glutamicum* lacks sulfate permeases similar to SulT, SulP, or CysP; instead, *C. glutamicum* has a CysZ protein, which is proposed as the principal sulfate permease in this microorganism (Rückert et al. 2005). This protein is not homologous to CysZ from *E. coli,* sharing only 13% identity. CysZ from *C. glutamicum* belongs to the putative 4-Toluene sulfonate uptake permease (TSUP) family, also known as the DUF81 or COG0730 protein family (TC# 9.A.29) (Table 1). These proteins are mainly

◀ **Fig. 4** Phylogenetic analysis and sequence logos of the CysP/(PiT) protein superfamily. **a** Phylogenetic tree constructed with the Minimum evolution (ME) method using an alignment of 786 protein sequences from the CysP/(PiT) protein superfamily. Sequences are distributed in three main clusters (*see text*). Similar topologies were obtained using the Neighbor-joining (NJ) and Maximum parsimony (MP) methods. Bacteria, Archaea, and Eukarya domains are indicated with *blue*, *green* and *red* lines, respectively. Scale bar represents 0.2 amino acid substitutions per site. Trees were calculated with MEGA 4.0 (Tamura et al. 2007) using the TJJ amino acid substitution model. **b** Detailed phylogenetic tree of cluster III (CysP/(PiT) family) that comprises two branches: subgroups IIIA and IIIB. A *red arrow* signals the location of *Bacillus subtilis* CysP(PiT). *Circles* indicate nodes with bootstrap values of >70% (*white*), 80% (*purple*) or 90% (*red*) in 1,000 random replicates in all methods employed. Bootstrap values for critical nodes are explicitly shown utilizing NJ/MP/ME methods. Entries include sequence access number and species name. **C**, Sequence logos of conserved N- and C-terminal motifs of PiT family members. The sequences of PiT family were sorted into clusters according to the results of phylogenetic analysis. Numbering is according to the sequence of CysP from *B. subtilis* (accession number: NP_389441); residues 14–26 and 178–190 correspond to the N- and C-motifs, respectively. Sequence logos were constructed using WebLogo (http://weblogo.threeplusone.com/). Each logo consists of stacks of amino acid letters, one stack for each position in the sequence. The overall height of the stack indicates the sequence conservation at that position, while the height of letters within the stack indicates the relative frequency of each amino acid at that position (Crooks et al. 2004). A ProfileGrid (Roca et al. 2008) is provided as supplementary material (Figs. 1s, 2s, 3s). (Color figure online)

present in bacteria and archaea, only a few are found in eukaryotes, and have been described as 4-toluene sulfonate carriers (Mampel et al. 2004), sulfite exporters (Weinitschke et al. 2007), as well as sulfate permeases (Rückert et al. 2005). CysZ from *C. glutamicum*, predicted to possess six TMSs, is encoded by the *cysZ* gene of the *cysIXHDNYZ* operon. Enzymes encoded by *cysIHDN* genes catalyze similar reactions to those of the known orthologous ones in *E. coli* (Rückert et al. 2005). In contrast, *cysXYZ* genes were described for the first time. Strains of *C. glutamicum* with deletions in each of the *cysIHDNYZ* genes were unable to grow in a medium containing sulfate or sulfite as sulfur sources, showing that they are involved in the transport or reduction of inorganic sulfur compounds (Rückert et al. 2005). CysZ is a high-affinity sulfate transporter because a *C. glutamicum* mutant strain affected in *cysZ* was unable to grow with <5 mM sulfate as sole sulfur source. Growth of the mutant was restored by increasing sulfate, and at >30 mM sulfate growth was similar to that of the wild-type strain, suggesting the presence of a low-affinity sulfate transporter that functions at high sulfate concentrations. CysX and CysY proteins are probably involved in electron transfer and in the biosynthesis of the siroheme cofactor of sulfite reductase, respectively; they probably participate in the sulfite into sulfide reduction (Rückert et al. 2005).

Molybdate and tungstate transport

Molybdenum is a transition trace metal that is required by nearly all living organisms, including microorganisms, plants, and animals. The few species that do not require molybdenum use tungsten. Molybdenum-containing enzymes, or molybdoenzymes, are found in all aerobic organisms, whereas tungsten-containing enzymes are mainly found in anaerobic prokaryotes, such as hyperthermophilic archaea, which are obligately tungsten-dependent; tungstoenzymes have been also identified in aerobic proteobacteria (Hille 2002; Schwarz et al. 2007). Molybdenum and tungsten are incorporated into pterin molecules, thus generating the molybdenum cofactor (Moco or molybdopterin) and the tungsten cofactor (Wco or tungstopterin), respectively, which are required for assembly and function of molybdoenzymes (except for the Fe-Mo cofactor in nitrogenase) and tungstoenzymes, respectively (Hille 2002; Schwarz et al. 2007).

Molybdenum and tungsten are transported in the form of the oxyanions molybdate (MoO_4^{2-}) and tungstate (WO_4^{2-}), respectively, which are taken up through the membrane by high-affinity ABC-type transporters. These transporters allow bacteria to scavenge the less abundant molybdate or tungstate ions in the presence of sulfate, whose concentration in sea water is ca. 10^5 times higher than that of molybdate (Schwarz et al. 2007). Due to the similarity between molybdate and tungstate oxyanions these may be transported by the same carrier (Grunden and Shanmugam 1997; Bevers et al. 2006). Some bacteria produce small cytoplasmic molybdate/tungstate Mop proteins (ca. 7 kDa), which are reported to be involved in oxyanion storage or homeostasis (Wagner et al. 2000; Makdessi et al. 2004).

In *E. coli*, molybdate may be taken up through three transport systems (reviewed in Self et al. 2001; Pau 2004) (Table 2) (Figs. 2, 5): (1) the high-affinity ModABC system (Maupin-Furlow et al. 1995); (2)

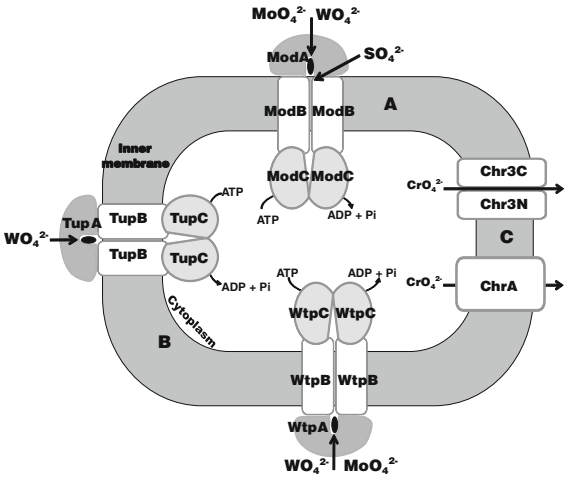


Fig. 5 Bacterial oxyanion transporters. **a** Molybdate transporters. The ModABC transporter is formed by ModA, the molybdate-binding protein, ModB, the membrane protein, and ModC, the ATPase. **b** Tungstate transporters. TupABC and WtpABC systems are constituted by TupA and WtpA, the substrate-binding proteins, TupB and WtpB, the membrane proteins, and TupC and WtpC, the ATPases. **c** Chromate efflux systems. Bidomain ChrA and paired Chr3N-Chr3C transporters are shown. The oxyanions transported for each system are indicated. Molybdate (MoO_4^{2-}), tungstate (WO_4^{2-}), sulfate (SO_4^{2-}), chromate (CrO_4^{2-}). Details for each system are provided in the text

the CysPTWA (SulT) sulfate-thiosulfate permease, with lower affinity (Rosentel et al. 1995), and (3) a non-specific low-efficiency anion transport system that requires high molybdate concentrations, and which also transports sulfate, selenate, and selenite; this transporter, however, has not been further characterized (Rosentel et al. 1995). Recently, a novel permease that takes up molybdate at micromolar concentrations was identified in the α-proteobacterium *Rhodobacter capsulatus* (Gisin et al. 2010). This permease, denominated PerO, seems to import also sulfate, tungstate, and vanadate, suggesting that functions as a general oxyanion transporter. PerO belongs to the ArsB/NhaD ion transporter family, which has mostly uncharacterized members from all three life domains (Gisin et al. 2010).

The ModABC molybdate transporter

The high-affinity ModABC molybdate system, which also transports tungstate and sulfate (Grunden and Shanmugam 1997; Self et al. 2001) (Table 2) (Fig. 5a), belongs to the Molybdate uptake transporter (MolT) family (TC# 3.A.1.8). In *E. coli*, the ModABC transporter is encoded by the *modABC* operon. The *modA* gene encodes the periplasmic molybdate-binding protein ModA (COG0725), which specifically binds molybdate or tungstate with a *Kd* of ca. 20 nM (Imperial et al. 1998). A lower affinity is shown by ModA from the phytopathogen bacterium *Xanthomonas axonopodis* pv. *citri* (*Kd* of 0.29 μM for molybdate and 0.58 μM for tungstate) (Balan et al. 2006). The crystal structure of ModA proteins from the bacteria *E. coli*, *Azotobacter vinelandii* and *X. axonopodis* (Hu et al. 1997; Lawson et al. 1998; Balan et al. 2008), and the ModA/WtpA molybdate- or tungstate-binding proteins (see "The TupABC and WtpABC tungstate transporters" section for tungstate transport) from the archaea *Archaeoglobus fulgidus*, *Methanosarcina acetivorans*, *Methanocaldococcus jannaschii*, *Pyrococcus horikoshii* and *Pyrococcus furiosus* have been solved to high resolution (Hollenstein et al. 2009). The conformation of ModA is ellipsoidal and binding of molybdate is through seven hydrogen bonds, similar to the sulfate-binding Sbp protein. Bacterial and archaeal ModA proteins have a highly similar fold, the main structural differences of archaeal proteins include an additional *β*-sheet located on the protein surface and, most notably, an oxyanion-binding site with octahedral coordination for molybdenum and tungsten atoms (Hollenstein et al. 2009); in contrast, bacterial ModA proteins possess tetrahedral coordination (Hu et al. 1997; Lawson et al. 1998; Balan et al. 2008). These differences suggest distinct oxyanion-binding modes of ModA/WtpA proteins from archaea and bacteria.

The *modB* gene encodes the integral membrane protein ModB, which builds the membrane channel of the ModABC transporter (Fig. 5a). Crystallization of ModB proteins from *A. fulgidus* and *M. acetivorans* has shown that ModB possesses six TMSs (Hollenstein et al. 2007; Gerber et al. 2008). ModB functions as a homodimer to form the channel for molybdate transport (Fig. 5a) (Grunden and Shanmugam 1997). ModB proteins have several conserved motifs also conserved in CysT, CysW, and in the phosphate permeases PstA and PstC (Self et al. 2001).

ModC, encoded by the *modC* gene, is the ATPase subunit of the ModABC complex that energizes molybdate transport. The crystal structures of ModC proteins from *A. fulgidus* and *M. acetivorans* showed that these have similar folds and that they contain the

highly-conserved motifs of ABC ATPases (Hollenstein et al. 2007; Gerber et al. 2008). The two ModC subunits exhibit a "head-to-tail" arrangement, with the conserved phosphate-binding loop (P-loops) juxtaposed to the ABC signature motifs (LSGGQ) of the opposite subunit. Even when ModC proteins exhibit a similar fold, only ModC from *M. acetivorans* possesses a regulatory domain; this is a region of about 120 aa appended to the C-terminus, which is also present in the CysA protein (Gerber et al. 2008).

Structure of the ModABC complex

Crystal structures of putative ModABC transporters of *A. fulgidus*, with ModA bound to molybdate or tungstate (Hollenstein et al. 2007) (Fig. 6), and of *M. acetivorans*, were recently solved (Gerber et al. 2008). The *A. fulgidus* ModABC transporter is made up of a single ModA protein with the molybdate

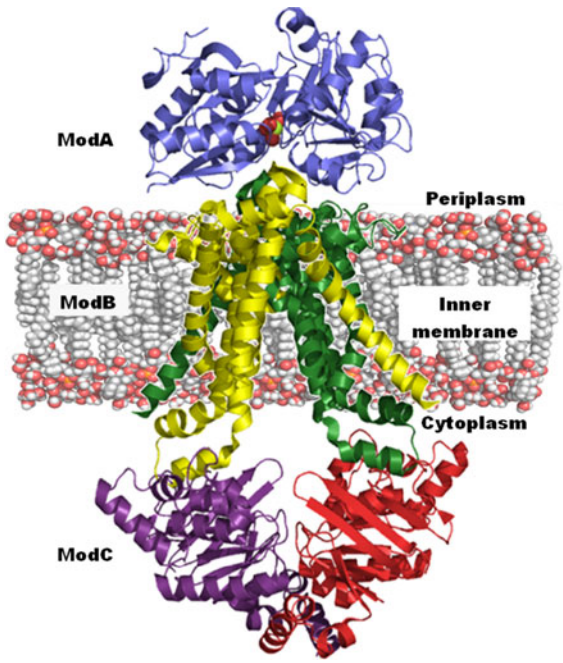


Fig. 6 Structure of the ModABC transporter from *Archaeoglobus fulgidus*. Front view of the $ModAB_2C_2$ complex. The complex in ribbon representation displays the ModA binding protein colored blue, the ModB subunits colored *yellow* and *green*, and the ModC subunits colored *red* and *magenta*. The oxyanion bound to the center of ModA is shown as *green* (tungsten) and *red* (oxygen) spheres. The lipid bilayer is also shown. The figure was constructed with VMD and PyMol V 0.99, using data from Hollenstein et al. (2007). (Color figure online)

oxyanion bound to the external side of a $ModB_2C_2$ complex. Molybdate or tungstate oxyanions are bound in a cleft between two lobes in ModA; both lobes interact with ModB and there are several charged residues localized on the interface. ModA aligns the substrate-binding cleft with the entrance to the translocation pathway on ModB, which is constituted of two identical subunits that create a large cavity, the translocation pathway. The ModB–ModC interface transmits critical conformational changes, thus coupling ATP binding and hydrolysis to transport. Based on the structure of the $ModAB_2C_2$ complex, a stoichiometry of two ATP per imported molybdate has been proposed (Hollenstein et al. 2007).

Distribution of the modABC operon

Characterized *modABC* operons encoding molybdate transport proteins include those of *R. capsulatus* (Wang et al. 1993), *A. vinelandii* (Mouncey et al. 1995), *Staphylococcus carnosus* (Neubauer et al. 1999), *Klebsiella pneumoniae* (Grunden and Shanmugam 1997), *Anabaena variabilis* (Zahalak et al. 2004), *Bradyrhizobium japonicum* (Delgado et al. 2006), *X. axonopodis* pv. *citri* (Balan et al. 2006), and *Campylobacter jejuni* (Smart et al. 2009; Taveirne et al. 2009). A comparative genomic analysis to examine the occurrence of molybdenum utilization showed the presence of molybdate transporter *modABC* genes in 294 genomes of bacteria, which accounts for 90% of molybdenum-utilizing bacteria identified, and 14 genomes of archaea, which represent 39% of molybdenum-utilizing archaea identified (Zhang and Gladyshev 2008).

Regulation of the modABC operon

In *E. coli*, transcription of the *modABC* operon is negatively controlled by the molybdate-responsive ModE protein (Mouncey et al. 1995; Walkenhorst et al. 1995; Grunden et al. 1996). The active form of ModE, which binds to the *modA* operator, is the ModE-molybdate complex (ModE-Mo) (Anderson et al. 1997). The apparent *Kd* for interaction between the *modA* operator and ModE-Mo is 0.3 nM, and this value increases >25 times in the absence of molybdate (Self et al. 2001). *E. coli* ModE functions as a homodimer and binds two molecules of molybdate

with high affinity ($Kd = 0.8$ μM); ModE may also bind tungstate (Anderson et al. 1997). Molybdate or tungstate binds directly to ModE without the requirement of an additional component; binding of the oxyanion induces a conformational change in the protein (Anderson et al. 1997; Gourley et al. 2001; Schüttelkopf et al. 2003). Each ModE monomer consists of two domains: the N-terminal domain, which possesses the HTH motif for DNA binding and is primarily responsible for ModE dimerization, and the C-terminal domain, which is the putative molybdate-binding component (Hall et al. 1999). ModE discriminates among oxyanions based on size and charge, binding molybdate or tungstate but not other tetrahedral oxyanions such as phosphate, sulfate, or vanadate (Gourley et al. 2001).

A molybdenum cofactor (Moco)-sensing riboswitch candidate has been recently identified in several bacteria (Regulski et al. 2008). The highly-conserved RNA motif (Moco RNA) is located upstream of genes encoding the molybdate transporter, molybdenum cofactor (Moco) biosynthesis enzymes, and other enzymes that utilize Moco as a coenzyme. Moco RNA selectively senses Moco and controls the expression of adjacent genes in response to changing levels of the coenzyme. The location of Moco RNA upstream of the *modABC* operon suggests an additional level of regulation of the transporter. A structural variant of Moco RNA has been identified in bacteria that use a tungsten cofactor (Tuco), suggesting that these RNA types allow the selective recognition of either Moco or Tuco (Regulski et al. 2008).

The TupABC and WtpABC tungstate transporters

The tungstate oxyanion (WO_4^{2-}) is taken up by bacterial cells through three ABC-type transporters (Table 2) (Fig. 5): (1) TupABC, which is highly specific for tungstate (Makdessi et al. 2001); (2) WtpABC, which transports both tungstate and molybdate (Bevers et al. 2006), and (3) the already described molybdate ModABC transporter (Grunden and Shanmugam 1997).

TupABC, the first tungstate-specific transporter described, is encoded by the *tupABC* operon identified in the Gram-positive anaerobic bacterium *Eubacterium acidaminophilum* (Makdessi et al. 2001; Andreesen and Makdessi 2008). TupA, a tungstate-binding protein, is highly specific for tungstate and does not bind molybdate, sulfate, chromate, selenate, phosphate, or chlorate. TupB is an inner membrane protein that possesses five TMSs and has low similarity (<20%) to the ModB protein. TupC is the ATPase subunit of the complex (Fig. 5b). TupABC homologs have been also characterized from the Gram-negative microaerophile *C. jejuni* (Smart et al. 2009; Taveirne et al. 2009). Other homologs to this transport system have been localized in 85 genomes of bacteria and in 12 genomes of archaea (Zhang and Gladyshev 2008).

The WtpABC transporter, identified in the archaeon *P. furiosus*, is able to take up both tungstate and molybdate (Table 2) (Fig. 5b). WtpA binds tungstate with higher affinity (Kd = 0.017 nM) than TupA (Kd = 500 nM) and ModA (Kd = 20 nM) (Bevers et al. 2006). Affinity for molybdate (Kd = 20 nM) is similar to that of ModA (Kd = 11 nM). WtpA has a low level of identity/similarity with ModA (18/30%) and TupA (16/31%), indicating that this is a new class of tungstate- and molybdate-binding proteins. Sequence similarities of WtpB with ModB and TupB are 53 and 50%, respectively, and those of WtpC with ModC and TupC are 51 and 56%, respectively (Bevers et al. 2006). The WtpABC system is present in some archaea that do not express homologs of TupA or ModA, such as *P. furiosus* and *Methanococcus jannaschii* (Bevers et al. 2006). Some archaea and bacteria have homologous genes for more than one type of tungstate transporter systems, such as *M. acetivorans*, which possesses ModA and WtpA transporters, and the sulfate-reducing bacterium *Desulfovibrio vulgaris*, which has TupA and ModA homologs (Bevers et al. 2006). Homologs of WtpABC transport system have been identified in 10 genomes of bacteria and in 23 genomes of archaea (Zhang and Gladyshev 2008), emphasizing that TupA and ModA occur predominantly in bacteria whereas WtpA appears to be an archaeal tungstate transporter.

Selenate transport

Selenium is an analog of sulfur and may substitute for sulfur in certain thiols. Although selenium is an essential element for microorganisms, it is toxic at elevated concentrations. The common organic forms of selenium are the selenocysteine and selenomethionine amino acid analogs (Stolz et al. 2006). In

E. coli, a single carrier has been described that can transport selenate (SeO_4^{2-}), selenite (SeO_3^{2-}) and sulfate. Sulfate is the preferred substrate, with an affinity ($Km = 2.1$ μM) ca. eight times higher than that for selenate ($Km = 17.1$ μM) and ca. 50 times higher for selenite ($Km = 102$ μM). All three oxyanions compete with each other for their transport (Lindblow-Kull et al. 1985).

Transport of selenate also occurs through the SulT sulfate permease system (Turner et al. 1998) (Fig. 2a). An *E. coli* mutant strain affected in the *cysA* ATPase gene showed impaired selenate reduction due to its inability to take up selenate (Bébien et al. 2002). Selenate uptake into *E. coli* cells also requires the YbaT protein, a putative transporter of amino acids or other metabolites, and the outer-membrane porin NmpC, because mutations of the corresponding genes inhibited selenate reduction with no change in growth rate (Bébien et al. 2002).

Selenate transport through a sulfate permease has been also reported in the *β*-proteobacterium *Cupriavidus metallidurans* CH34 (previously *Alcaligenes eutrophus* or *Ralstonia metallidurans*) (Avoscan et al. 2009). Cells grown under sulfate-limited conditions (0.3 mM sulfate) accumulated up to six times more selenate than cells grown in sulfate-rich (3 mM) medium, probably due to induction of sulfate permeases (Avoscan et al. 2009). These findings indicate that *C. metallidurans* takes up selenate utilizing a sulfate permease. Sulfate transport by the previously mentioned SulP-type Rv1739c permease of *M. tuberculosis*, expressed in *E. coli*, was inhibited by selenate and selenite (Zolotarev et al. 2008), which suggests that both selenium oxyanions can also be taken up through this permease (Fig. 2b).

Chromate transport

Chromium, a non-essential metal for microorganisms and plants, exists in nature as two main chemical species: the trivalent form, Cr(III), which is relatively innocuous because it is water-insoluble at physiological pH, and the hexavalent form, Cr(VI), considered a more toxic species. Inside the cell, Cr(VI) is readily reduced to Cr(III) by the action of various enzymatic and non-enzymatic activities; Cr(III) generated may then exert diverse toxic effects in the cytoplasm (reviewed in Ramírez-Díaz et al. 2008).

Chromate uptake

Chromate, an analog of sulfate, is transported into the cells through sulfate transport systems (Fig. 2), and chromate has been identified as a competitive inhibitor of sulfate transport (reviewed in Cervantes et al. 2001). Genes encoding sulfate transporters and enzymes involved in sulfur metabolism are strongly induced when bacteria are under chromate exposure, which mimics intracellular sulfur limitation (Brown et al. 2006; Henne et al. 2009). The previously described sulfate permease CysP from *B. subtilis* also transports chromate inside the cell (Fig. 2c). *E. coli* cysteine-auxotroph mutants with altered *cysT*, *cysP*, *sbp* or *cysA97* genes are chromate-resistant, probably due to their inability to transport the toxic ion inside the cell. Expression of *B. subtilis* CysP in these chromate-resistant strains conferred sensitivity to chromate, confirming that this oxyanion can be transported inside *B. subtilis* cells by the CysP permease (Mansilla and de Mendoza 2000).

Chromate efflux

The most studied chromate transport system is the ChrA protein, which is encoded by the *P. aeruginosa* pUM505 plasmid (Cervantes et al. 1990). *P. aeruginosa* ChrA is a 416-aa protein that displays a topology of 13 TMSs (Jímenez-Mejía et al. 2006). The ChrA transporter functions as a chemiosmotic pump that extrudes chromate from the cytoplasm using the proton-motive force (Alvarez et al. 1999; Pimentel et al. 2002) (Fig. 5c). Efflux of chromate by *P. aeruginosa* cells shows typical saturation kinetics with an apparent *Km* of 82 μM chromate and a V_{max} of 0.13 nmol chromate min^{-1} mg $protein^{-1}$. Sulfate and molybdate inhibit chromate efflux in a concentration-dependent fashion, which suggests that the ChrA protein also binds these oxyanions (Pimentel et al. 2002).

In *C. metallidurans*, two ChrA proteins have been identified: $ChrA_1$ encoded by the pMOL28 plasmid, and $ChrA_2$ encoded by a chromosomal gene; these proteins confer chromate resistance by a similar efflux mechanism to that of the *P. aeruginosa* transporter (Juhnke et al. 2002). A ChrA homolog has been also identified in the facultative anaerobic Gram-negative bacteria *Shewanella* sp. ANA-3; in contrast with ChrA proteins from *P. aeruginosa and*

C. metallidurans, which are functional only in their respective hosts (Cervantes et al. 1990; Nies et al. 1990), ChrA from *Shewanella* conferred chromate resistance when expressed in both *E. coli* and *P. aeruginosa* (Aguilar-Barajas et al. 2008). ChrA transporters with a similar function in chromate efflux were recently identified in a chromosomally located transposon of the α-proteobacterium *Ochrobactrum tritici* strain 5bvl1 (Branco et al. 2008) and in a plasmid from the Gram-positive *Arthrobacter* sp. strain FB24 (Henne et al. 2009); both strains were isolated from chromate-polluted environments. The pANL plasmid from *S. elongatus* PCC 7942 encodes a CHR homolog (named SrpC); interestingly, this homolog was located in an island rich in sulfur-related genes regulated by sulfate (Chen et al. 2008). *Synechococcus* SrpC confers chromate resistance on *E. coli*, probably by an efflux mechanism (Aguilar-Barajas, unpublished results).

ChrA proteins belong to the CHR superfamily of chromate ion transporters, which currently comprises hundreds of homologs from all three life domains (Nies et al. 1998; Nies 2003; Díaz-Pérez et al. 2007). The CHR superfamily is constituted of members of two sizes: short-chain proteins (the SCHR family) of ca. 200 aa with only one domain and that are commonly encoded by adjacent gene pairs, and long-chain proteins (the LCHR family) of ca. 400 aa with homologous N- and C-terminal halves. LCHR proteins are proposed as having derived from gene duplication/fusion events of ancestral SCHR-encoding genes (Díaz-Pérez et al. 2007). The ChrA proteins characterized from *P. aeruginosa, C. metallidurans, Shewanella*, *O. tritici*, *Arthrobacter*, and *Synechococcus* described above all belong to the LCHR family.

The function of the SCHR proteins ChrN (denominated as the amino domain) and ChrC (the carboxyl domain) from *B. subtilis* was recently demonstrated (Díaz-Magaña et al. 2009). These short-chain proteins are encoded by adjacent paired genes, *chr3N–chr3C*, which are transcribed as part of a bicistronic mRNA probably forming an operon. Expression of the *Bacillus* paired *chr* genes in *E. coli* conferred chromate resistance, whereas expression of single *chr3N* or *chr3C* genes did not confer this, indicating that both genes are required for function. Also, expression of *B. subtilis chr3N–chr3C* genes caused diminished chromate uptake by *E. coli* cells. It was proposed that SCHR proteins form oppositely-oriented heterodimers in the membrane that efflux chromate ions from cytoplasm by a similar mechanism as that of long-chain LCHR proteins (Díaz-Magaña et al. 2009) (Fig. 5c).

The genomes of some bacteria, such as *C. metallidurans* and several *Burkholderia* species, encode multiple SCHR and LCHR homologs. Dramatic examples of the possession of redundant genes encoding CHR homologs are *Burkholderia vietnamiensis* TVV75 (five LCHRs and two pairs of SCHRs) and *Burkholderia xenovorans* LB400 (four LCHRs and two pairs of SCHRs) (Díaz-Pérez et al. 2007). The six CHR genes from *B. xenovorans* LB400 have been shown to confer chromate resistance when expressed in *E. coli*, although each homolog does this under different growth conditions (M. Luna-Luna, unpublished results).

Conclusions

Sulfate is used preferentially as a sulfur source by bacterial cells, and probably for this reason, bacterial sulfate permeases constitute a diverse set of membrane transporters grouped into several different protein families. Nevertheless, the number of sulfate permeases biochemically tested remains low, including transporters of the SulT, SulP, CysP/(PiT), and two CysZ families. Sulfate-thiosulfate SulT permeases of the ABC transporter superfamily are the most widely distributed and characterized sulfate transporters. Interestingly, the CysP sulfate permease from *B. subtilis* belongs to the PiT family of phosphate transporters, and phylogenetic and logos analysis strongly suggest that other PiT members in Gram-positive bacteria may also transport sulfate. Permeases for molybdate and tungstate belong mainly to the ABC-type transporter family, which, unlike sulfate permeases, are more specific for their substrates. In contrast, there are no specific uptake systems for selenate and chromate; instead, these are taken up by transporters of structurally related oxyanions, such as sulfate or molybdate. Because chromate ions are highly toxic for bacteria, they can be extruded from the cytoplasm through the widely distributed efflux systems of the CHR superfamily.

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References

Aguilar-Barajas E, Paluscio E, Cervantes C, Rensing C (2008) Expression of chromate resistance genes from *Shewanella* sp. strain ANA-3 in *Escherichia coli*. FEMS Microbiol Lett 285:97–100

Alvarez AH, Moreno-Sánchez R, Cervantes C (1999) Chromate efflux by means of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*. J Bacteriol 181: 7398–7400

Anderson LA, Palmer T, Price NC, Bornemann S, Boxer DH, Pau RN (1997) Characterisation of the molybdenum-responsive ModE regulatory protein and its binding to the promoter region of the *modABCD* (molybdenum transport) operon of *Escherichia coli*. Eur J Biochem 246: 119–126

Andreesen JR, Makdessi K (2008) Tungsten, the surprisingly positively acting heavy metal element for prokaryotes. Ann NY Acad Sci 1125:215–219

Avoscan L, Carrière M, Proux O, Sarret G, Degrouard J, Covès J, Gouget B (2009) Enhanced selenate accumulation in *Cupriavidus metallidurans* CH34 does not trigger a detoxification pathway. Appl Environ Microbiol 75:2250–2252

Bai L, Collins JF, Ghishan FK (2000) Cloning and characterization of a type III Na-dependent phosphate cotransporter from mouse intestine. Am J Physiol Cell Physiol 279: C1135–C1143

Balan A, Santacruz CP, Moutran A, Ferreira RC, Medrano FJ, Pérez CA, Ramos CH, Ferreira LC (2006) The molybdate-binding protein (ModA) of the plant pathogen *Xanthomonas axonopodis* pv *citri*. Protein Expr Purif 50:215–222

Balan A, Santacruz-Pérez C, Moutran A, Ferreira LC, Neshich G, Goncalves Barbosa JA (2008) Crystallographic structure and substrate-binding interactions of the molybdate-binding protein of the phytopathogen *Xanthomonas axonopodis* pv *citri*. Biochim Biophys Acta 1784:393–399

Bébien M, Kirsch J, Méjean V, Verméglio A (2002) Involvement of a putative molybdenum enzyme in the reduction of selenate by *Escherichia coli*. Microbiology 148:3865–3872

Bevers LE, Hagedoorn PL, Krijger GC, Hagen WR (2006) Tungsten transport protein A (WtpA) in *Pyrococcus furiosus*: the first member of a new class of tungstate and molybdate transporters. J Bacteriol 188:6498–6505

Bøttger P, Pedersen L (2005) Evolutionary and experimental analyses of inorganic phosphate transporter PiT family reveals two related signature sequences harboring highly conserved aspartic acids critical for sodium-dependent phosphate transport function of human PiT2. FEBS J 272:3060–3074

Branco R, Chung AP, Johnston T, Gurel V, Morais P, Zhitkovich A (2008) The chromate-inducible *chrBACF* operon from the transposable element Tn*OtChr* confers resistance to chromium(VI) and superoxide. J Bacteriol 190:6996–7003

Britton P, Boronat A, Hartley DA, Jones-Mortimer MC, Kornberg HL, Parra F (1983) Phosphotransferase-mediated regulation of carbohydrate utilization in *Escherichia coli* K12: location of the *gsr* (*tgs*) and *iex* (*crr*) genes by specialized transduction. J Gen Microbiol 129:349–356

Brown SD, Thompson MR, Verberkmoes NC, Chourey K, Shah M, Zhou J, Hettich RL, Thompson DK (2006) Molecular dynamics of the *Shewanella oneidensis* response to chromate stress. Mol Cell Proteomics 5:1054–1071

Byrne CR, Monroe RS, Ward KA, Kredich NM (1988) DNA Sequences of the *cysK* regions of *Salmonella typhimurium* and *Escherichia coli* and linkage of the *cysK* regions to *ptsH*. J Bacteriol 170:3150–3157

Cervantes C, Ohtake H, Chu L, Misra TK, Silver S (1990) Cloning, nucleotide sequence, and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. J Bacteriol 172:287–291

Cervantes C, Campos-García J, Devars S, Gutiérrez-Corona F, Loza-Tavera H, Torres-Guzmán JC, Moreno-Sánchez R (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 25:335–347

Chen Y, Holtman CK, Magnuson RD, Youderian PA, Golden SS (2008) The complete sequence and functional analysis of pANL, the large plasmid of the unicellular cyanobacterium *Synechococcus elongatus* PCC 7942. Plasmid 59: 176–192

Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14: 1188–1190

Dam P, Olman V, Harris K, Su Z, Xu Y (2007) Operon prediction using both genome-specific and general genomic information. Nucleic Acids Res 35:288–298

Daram P, Brunner S, Rausch C, Steiner C, Amrhein N, Bucher M (1999) Pht2;1 encodes a low-affinity phosphate transporter from Arabidopsis. Plant Cell 11:2153–2166

Delgado MJ, Tresierra-Ayala A, Talbi C, Bedmar EJ (2006) Functional characterization of the *Bradyrhizobium japonicum modA* and *modB* genes involved in molybdenum transport. Microbiology 152:199–207

Detro-Dassen S, Schänzler M, Lauks H, Martin I, zu Berstenhorst SM, Nothmann D, Torres-Salazar D, Hidalgo P, Schmalzing G, Fahlke C (2008) Conserved dimeric subunit stoichiometry of SLC26 multifunctional anion exchangers. J Biol Chem 283:4177–4188

Díaz-Magaña A, Aguilar-Barajas E, Moreno-Sánchez R, Ramírez-Díaz MI, Riveros-Rosas H, Vargas E, Cervantes C (2009) Short-chain chromate ion transporter proteins from *Bacillus subtilis* confer chromate resistance in *Escherichia coli*. J Bacteriol 191:5441–5445

Díaz-Pérez C, Cervantes C, Campos-García J, Julian-Sánchez A, Riveros-Rosas H (2007) Phylogenetic analysis of the chromate ion transporter (CHR) superfamily. FEBS J 274: 6215–6227

Farmer KL, Thomas MS (2004) Isolation and characterization of *Burkholderia cenocepacia* mutants deficient in pyochelin production: pyochelin biosynthesis is sensitive to sulfur availability. J Bacteriol 186:270–277

Felce J, Saier MH Jr (2004) Carbonic anhydrases fused to anion transporters of the SulP family: evidence for a novel type of bicarbonate transporter. J Mol Microbiol Biotechnol 8:169–176

Furlong CL (1987) Osmotic-shock-sensitive transport systems. In: Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, DC, pp 768–796

Gerber S, Comellas-Bigler M, Goetz BA, Locher KP (2008) Structural basis of trans-inhibition in a molybdate/tungstate ABC transporter. Science 321:246–250

Gisin J, Muller A, Pfander Y, Leimkuhler S, Narberhaus F, Masepohl B (2010) A *Rhodobacter capsulatus* member of a universal permease family imports molybdate and other oxyanions. J Bacteriol 192:5943–5952

Gourley DG, Schuttelkopf AW, Anderson LA, Price NC, Boxer DH, Hunter WN (2001) Oxyanion binding alters conformation and quaternary structure of the c-terminal domain of the transcriptional regulator *modE* Implications for molybdate-dependent regulation, signaling, storage, and transport. J Biol Chem 276:20641–20647

Green LS, Grossman AR (1988) Changes in sulfate transport characteristics and protein composition of *Anacystis nidulans* R2 during sulfur deprivation. J Bacteriol 170:583–587

Grunden AM, Shanmugam KT (1997) Molybdate transport and regulation in bacteria. Arch Microbiol 168:345–354

Grunden AM, Ray RM, Rosentel JK, Healy FG, Shanmugam KT (1996) Repression of the *Escherichia coli modABCD* (molybdate transport) operon by ModE. J Bacteriol 178:735–744

Guédon E, Martin-Verstraete I (2007) Cysteine metabolism and its regulation in bacteria. In: Wendisch VF (ed) Amino acids biosynthesis pathways, regulation and metabolic engineering, vol 5. Springer-Verlag, Berlin, Heidelberg, pp 195–218

Hall DR, Gourley DG, Leonard GA, Duke EM, Anderson LA, Boxer DH, Hunter WN (1999) The high-resolution crystal structure of the molybdate-dependent transcriptional regulator (ModE) from *Escherichia coli*: a novel combination of domain folds. EMBO J 18:1435–1446

Han MJ, Lee SY (2006) The *Escherichia coli* proteome: past, present, and future prospects. Microbiol Mol Biol Rev 70:362–439

Harris RM, Webb DC, Howitt SM, Cox GB (2001) Characterization of PitA and PitB from *Escherichia coli*. J Bacteriol 183:5008–5014

He JJ, Quiocho FA (1993) Dominant role of local dipoles in stabilizing uncompensated charges on a sulfate sequestered in a periplasmic active transport protein. Protein Sci 2:1643–1647

Henne KL, Nakatsu CH, Thompson DK, Konopka AE (2009) High-level chromate resistance in *Arthrobacter* sp strain FB24 requires previously uncharacterized accessory genes. BMC Microbiol 9:199

Hille R (2002) Molybdenum and tungsten in biology. Trends Biochem Sci 27:360–367

Hollenstein K, Frei DC, Locher KP (2007) Structure of an ABC transporter in complex with its binding protein. Nature 446:213–216

Hollenstein K, Comellas-Bigler M, Bevers LE, Feiters MC, Meyer-Klaucke W, Hagedoorn PL, Locher KP (2009) Distorted octahedral coordination of tungstate in a subfamily of specific binding proteins. J Biol Inorg Chem 14:663–672

Hryniewicz MM, Kredich NM (1991) The *cysP* promoter of *Salmonella typhimurium*: characterization of two binding sites for CysB protein, studies of in vivo transcription initiation, and demonstration of the anti-inducer effects of thiosulfate. J Bacteriol 173:5876–5886

Hryniewicz MM, Kredich NM (1995) Hydroxyl radical footprints and half-site arrangements of binding sites for the CysB transcriptional activator of *Salmonella typhimurium*. J Bacteriol 177:2343–2353

Hryniewicz M, Sirko A, Palucha A, Bock A, Hulanicka D (1990) Sulfate and thiosulfate transport in *Escherichia coli* K-12: identification of a gene encoding a novel protein involved in thiosulfate binding. J Bacteriol 172:3358–3366

Hu Y, Rech S, Gunsalus RP, Rees DC (1997) Crystal structure of the molybdate binding protein ModA. Nat Struct Biol 4:703–707

Hummerjohann J, Kuttel E, Quadroni M, Ragaller J, Leisinger T, Kertesz MA (1998) Regulation of the sulfate starvation response in *Pseudomonas aeruginosa*: role of cysteine biosynthetic intermediates. Microbiology 144:1375–1386

Imperial J, Hadi M, Amy NK (1998) Molybdate binding by ModA, the periplasmic component of the *Escherichia coli mod* molybdate transport system. Biochim Biophys Acta 1370:337–346

Iwanicka-Nowicka R, Zielak A, Cook AM, Thomas MS, Hryniewicz MM (2007) Regulation of sulfur assimilation pathways in *Burkholderia cenocepacia*: identification of transcription factors CysB and SsuR and their role in control of target genes. J Bacteriol 189:1675–1688

Jacobson BL, Quiocho FA (1988) Sulfate-binding protein dislikes protonated oxyacids. A molecular explanation. J Mol Biol 204:783–787

Jacobson BL, He JJ, Vermersch PS, Lemon DD, Quiocho FA (1991) Engineered interdomain disulfide in the periplasmic receptor for sulfate transport reduces flexibility. Site-directed mutagenesis and ligand-binding studies. J Biol Chem 266:5220–5225

Jímenez-Mejía R, Campos-Garcia J, Cervantes C (2006) Membrane topology of the chromate transporter ChrA of *Pseudomonas aeruginosa*. FEMS Microbiol Lett 262:178–184

Johnson MK, Rees DC, Adams MW (1996) Tungstoenzymes. Chem Rev 96:2817–2840

Juhnke S, Peitzsch N, Hübener N, Große C, Nies DH (2002) New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 179:15–25

Kertesz MA (2001) Bacterial transporters for sulfate and organosulfur compounds. Res Microbiol 152:279–290

Kertesz MA, Leisinger T, Cook AM (1993) Proteins induced by sulfate limitation in *Escherichia coli*, *Pseudomonas*

putida, or *Staphylococcus aureus*. J Bacteriol 175:1187–1190

Kisker C, Schindelin H, Rees DC (1997) Molybdenum-cofactor-containing enzymes: structure and mechanism. Annu Rev Biochem 66:233–267

Kouzuma A, Endoh T, Omori T, Nojiri H, Yamane H, Habe H (2008) Transcription factors CysB and SfnR constitute the hierarchical regulatory system for the sulfate starvation response in *Pseudomonas putida*. J Bacteriol 190: 4521–4531

Kredich NM (1992) The molecular basis for positive regulation of *cys* promoters in *Salmonella typhimurium* and *Escherichia coli*. Mol Microbiol 6:2747–2753

Laudenbach DE, Grossman AR (1991) Characterization and mutagenesis of sulfur-regulated genes in a cyanobacterium: evidence for function in sulfate transport. J Bacteriol 173:2739–2750

Lawson DM, Williams CE, Mitchenall LA, Pau RN (1998) Ligand size is a major determinant of specificity in periplasmic oxyanion-binding proteins: the 12 A resolution crystal structure of *Azotobacter vinelandii* ModA. Structure 6:1529–1539

Lindblow-Kull C, Kull FJ, Shrift A (1985) Single transporter for sulfate, selenate, and selenite in *Escherichia coli* K-12. J Bacteriol 163:1267–1269

Lochowska A, Iwanicka-Nowicka R, Plochocka D, Hryniewicz MM (2001) Functional dissection of the LysR-type CysB transcriptional regulator Regions important for DNA binding, inducer response, oligomerization, and positive control. J Biol Chem 276:2098–2107

Makdessi K, Andreesen JR, Pich A (2001) Tungstate uptake by a highly specific ABC transporter in *Eubacterium acidaminophilum*. J Biol Chem 276:24557–24564

Makdessi K, Fritsche K, Pich A, Andreesen JR (2004) Identification and characterization of the cytoplasmic tungstate/molybdate-binding protein (Mop) from *Eubacterium acidaminophilum*. Arch Microbiol 181:45–51

Mampel J, Maier E, Tralau T, Ruff J, Benz R, Cook AM (2004) A novel outer-membrane anion channel (porin) as part of a putatively two-component transport system for 4-toluenesulphonate in *Comamonas testosteroni* T-2. Biochem J 383:91–99

Mansilla MC, de Mendoza D (2000) The *Bacillus subtilis cysP* gene encodes a novel sulphate permease related to the inorganic phosphate transporter (Pit) family. Microbiology 146:815–821

Markovich D (2001) Physiological roles and regulation of mammalian sulfate transporters. Physiol Rev 81:1499–1533

Maupin-Furlow JA, Rosentel JK, Lee JH, Deppenmeier U, Gunsalus RP, Shanmugam KT (1995) Genetic analysis of the *modABCD* (molybdate transport) operon of *Escherichia coli*. J Bacteriol 177:4851–4856

Monroe RS, Ostrowski J, Hryniewicz MM, Kredich NM (1990) In vitro interactions of CysB protein with the *cysK* and *cysJIH* promoter regions of *Salmonella typhimurium*. J Bacteriol 172:6919–6929

Mouncey NJ, Mitchenall LA, Pau RN (1995) Mutational analysis of genes of the *mod* locus involved in molybdenum transport, homeostasis, and processing in *Azotobacter vinelandii*. J Bacteriol 177:5294–5302

Mount DB, Romero MF (2004) The SLC26 gene family of multifunctional anion exchangers. Pflugers Arch 447: 710–721

Neubauer H, Pantel I, Lindgren PE, Götz F (1999) Characterization of the molybdate transport system ModABC of *Staphylococcus carnosus*. Arch Microbiol 172:109–115

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27:313–339

Nies A, Nies DH, Silver S (1990) Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. J Biol Chem 265:5648–5653

Nies DH, Koch S, Wachi S, Peitzsch N, Saier MH Jr (1998) CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate antiporters. J Bacteriol 180:5799–5802

Ohta N, Galsworthy PR, Pardee AB (1971) Genetics of sulfate transport by *Salmonella typhimurium*. J Bacteriol 105:1053–1062

Ostrowski J, Kredich NM (1991) Negative autoregulation of *cysB* in *Salmonella typhimurium*: in vitro interactions of CysB protein with the *cysB* promoter. J Bacteriol 173: 2212–2218

Oswald C, Holland IB, Schmitt L (2006) The motor domains of ABC-transporters. What can structures tell us? Naunyn Schmiedebergs Arch Pharmacol 372:385–399

Parra F, Britton P, Castle C, Jones-Mortimer MC, Kornberg HL (1983) Two separate genes involved in sulphate transport in *Escherichia coli* K12. J Gen Microbiol 129: 357–358

Pau RN (2004) Molybdenum uptake and homeostasis. In: Klipp W, Masepohl B, Gallon JR, Newton WE (eds) Genetics and regulation of nitrogen fixation in free-living bacteria. Kluwer Academic Publishers, Netherlands, pp 225–256

Pflugrath JW, Quiocho FA (1985) Sulphate sequestered in the sulphate-binding protein of *Salmonella typhimurium* is bound solely by hydrogen bond. Nature 314:257–260

Pflugrath JW, Quiocho FA (1988) The 2 Å resolution structure of the sulfate-binding protein involved in active transport in *Salmonella typhimurium*. J Mol Biol 200:163–180

Pimentel BE, Moreno-Sánchez R, Cervantes C (2002) Efflux of chromate by *Pseudomonas aeruginosa* cells expressing the ChrA protein. FEMS Microbiol Lett 212:249–254

Price GD, Woodger FJ, Badger MR, Howitt SM, Tucker L (2004) Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. Proc Natl Acad Sci USA 101:18228–18233

Quiocho FA (1996) Atomic basis of the exquisite specificity of phosphate and sulfate transport receptors. Kidney Int 49:943–946

Ramírez-Díaz MI, Díaz-Pérez C, Vargas E, Riveros-Rosas H, Campos-García J, Cervantes C (2008) Mechanisms of bacterial resistance to chromium compounds. Biometals 21:321–332

Rausch C, Zimmermann P, Amrhein N, Bucher M (2004) Expression analysis suggests novel roles for the plastidic phosphate transporter Pht2;1 in auto- and heterotrophic tissues in potato and Arabidopsis. Plant J 39:13–28

Ravera S, Virkki LV, Murer H, Forster IC (2007) Deciphering PiT transport kinetics and substrate specificity using

electrophysiology and flux measurements. Am J Physiol Cell Physiol 293:C606–C620

Regulski EE, Moy RH, Weinberg Z, Barrick JE, Yao Z, Ruzzo WL, Breaker RR (2008) A widespread riboswitch candidate that controls bacterial genes involved in molybdenum cofactor and tungsten cofactor metabolism. Mol Microbiol 68:918–932

Roca AI, Almada AE, Abajian AC (2008) ProfileGrids as a new visual representation of large multiple sequence alignments: a case study of the RecA protein family. BMC Bioinformatics 9:554

Rosentel JK, Healy F, Maupin-Furlow JA, Lee JH, Shanmugam KT (1995) Molybdate and regulation of *mod* (molybdate transport), *fdhF*, and *hyc* (formate hydrogenlyase) operons in *Escherichia coli*. J Bacteriol 177:4857–4864

Rückert C, Koch DJ, Rey DA, Albersmeier A, Mormann S, Puhler A, Kalinowski J (2005) Functional genomics and expression analysis of the *Corynebacterium glutamicum fpr2-cysIXHDNYZ* gene cluster involved in assimilatory sulphate reduction. BMC Genomics 6:121

Saier MH Jr (2000) A functional-phylogenetic classification system for transmembrane solute transporters. Microbiol Mol Biol Rev 64:354–411

Saier MH Jr, Tran CV, Barabote RD (2006) TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucleic Acids Res 34:D181–D186

Salaun C, Rodrigues P, Heard JM (2001) Transmembrane topology of PiT-2, a phosphate transporter-retrovirus receptor. J Virol 75:5584–5592

Scheffel F, Demmer U, Warkentin E, Hülsmann A, Schneider E, Ermler U (2005) Structure of the ATPase subunit CysA of the putative sulfate ATP-binding cassette (ABC) transporter from *Alicyclobacillus acidocaldarius*. FEBS Lett 579:2953–2958

Schüttelkopf AW, Boxer DH, Hunter WN (2003) Crystal structure of activated ModE reveals conformational changes involving both oxyanion and DNA-binding domains. J Mol Biol 326:761–767

Schwarz G, Hagedoorn P, Fischer K (2007) Molybdate and tungstate: uptake, homeostasis, cofactors, and enzymes. In: Nies DH, Silver S (eds) Molecular microbiology of heavy metals, vol 6. Springer-Verlag, Berlin, pp 421–451

Scott C, Hilton ME, Coppin CW, Russell RJ, Oakeshott JG, Sutherland TD (2007) A global response to sulfur starvation in *Pseudomonas putida* and its relationship to the expression of low-sulfur-content proteins. FEMS Microbiol Lett 267:184–193

Sekowska A, Kung HF, Danchin A (2000) Sulfur metabolism in *Escherichia coli* and related bacteria: facts and fiction. J Mol Microbiol Biotechnol 2:145–177

Self WT, Grunden AM, Hasona A, Shanmugam KT (2001) Molybdate transport. Res Microbiol 152:311–321

Shibagaki N, Grossman AR (2006) The role of the STAS domain in the function and biogenesis of a sulfate transporter as probed by random mutagenesis. J Biol Chem 281:22964–22973

Silver S, Walderhaug M (1992) Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol Rev 56:195–228

Sirko A, Hryniewicz M, Hulanicka D, Böck A (1990) Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the *cysTWAM* gene cluster. J Bacteriol 172:3351–3357

Smart JP, Cliff MJ, Kelly DJ (2009) A role for tungsten in the biology of *Campylobacter jejuni*: tungstate stimulates formate dehydrogenase activity and is transported via an ultra-high affinity ABC system distinct from the molybdate transporter. Mol Microbiol 74:742–757

Stearns DM (2000) Is chromium a trace essential metal? Biofactors 11:149–162

Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60:107–130

Tam R, Saier MH Jr (1993) Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol Rev 57:320–346

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 40. Mol Biol Evol 24:1596–1599

Taveirne ME, Sikes ML, Olson JW (2009) Molybdenum and tungsten in *Campylobacter jejuni*: their physiological role and identification of separate transporters regulated by a single ModE-like protein. Mol Microbiol 74:758–771

Tralau T, Vuilleumier S, Thibault C, Campbell BJ, Hart CA, Kertesz MA (2007) Transcriptomic analysis of the sulfate starvation response of *Pseudomonas aeruginosa*. J Bacteriol 189:6743–6750

Turner RJ, Weiner JH, Taylor DE (1998) Selenium metabolism in *Escherichia coli*. Biometals 11:223–227

Valdés J, Veloso F, Jedlicki E, Holmes D (2003) Metabolic reconstruction of sulfur assimilation in the extremophile *Acidithiobacillus ferrooxidans* based on genome analysis. BMC Genomics 4:51

van der Ploeg JR, Weiss MA, Saller E, Nashimoto H, Saito N, Kertesz MA, Leisinger T (1996) Identification of sulfate starvation-regulated genes in *Escherichia coli*: a gene cluster involved in the utilization of taurine as a sulfur source. J Bacteriol 178:5438–5446

van der Ploeg JR, Iwanicka-Nowicka R, Kertesz MA, Leisinger T, Hryniewicz MM (1997) Involvement of CysB and Cbl regulatory proteins in expression of the *tauABCD* operon and other sulfate starvation-inducible genes in *Escherichia coli*. J Bacteriol 179:7671–7678

Vincent JB (2004) Recent developments in the biochemistry of chromium(III). Biol Trace Elem Res 99:1–16

Vincent JB (2010) Chromium: celebrating 50 years as an essential element? Dalton Trans 39:3787–3794

von Mering C, Jensen LJ, Kuhn M, Chaffron S, Doerks T, Kruger B, Snel B, Bork P (2007) STRING 7—recent developments in the integration and prediction of protein interactions. Nucleic Acids Res 35:D358–D362

Wagner UG, Stupperich E, Kratky C (2000) Structure of the molybdate/tungstate binding protein Mop from *Sporomusa ovata*. Structure 8:1127–1136

Walkenhorst HM, Hemschemeier SK, Eichenlaub R (1995) Molecular analysis of the molybdate uptake operon, *modABCD*, of *Escherichia coli* and *modR*, a regulatory gene. Microbiol Res 150:347–361

Wang G, Angermuller S, Klipp W (1993) Characterization of *Rhodobacter capsulatus* genes encoding a molybdenum

transport system and putative molybdenum-pterin-binding proteins. J Bacteriol 175:3031–3042

Weinitschke S, Denger K, Cook AM, Smits TH (2007) The DUF81 protein TauE in *Cupriavidus necator* H16, a sulfite exporter in the metabolism of C2 sulfonates. Microbiology 153:3055–3060

Wooff E, Michell SL, Gordon SV, Chambers MA, Bardarov S, Jacobs WR Jr, Hewinson RG, Wheeler PR (2002) Functional genomics reveals the sole sulphate transporter of the *Mycobacterium tuberculosis* complex and its relevance to the acquisition of sulphur in vivo. Mol Microbiol 43:653–663

Zahalak M, Pratte B, Werth KJ, Thiel T (2004) Molybdate transport and its effect on nitrogen utilization in the cyanobacterium *Anabaena variabilis* ATCC 29413. Mol Microbiol 51:539–549

Zhang Y, Gladyshev VN (2008) Molybdoproteomes and evolution of molybdenum utilization. J Mol Biol 379:881–899

Zolotarev AS, Unnikrishnan M, Shmukler BE, Clark JS, Vandorpe DH, Grigorieff N, Rubin EJ, Alper SL (2008) Increased sulfate uptake by *E. coli* overexpressing the SLC26-related SulP protein Rv1739c from *Mycobacterium tuberculosis*. Comp Biochem Physiol A Mol Integr Physiol 149:255–266