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MINI-REVIEW

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Sulfonate-sulfur metabolism and its regulation in *Escherichia coli*

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Abstract In the absence of sulfate and cysteine, *Escherichia coli* can use aliphatic sulfonates as a source of sulfur for growth. Starvation for sulfate leads to the expression of the *tauABCD* and *ssuEADCB* genes. Each of these gene clusters encodes an ABC-type transport system required for uptake of aliphatic sulfonates and a desulfonation enzyme. The TauD protein is an α-ketoglutarate-dependent dioxygenase that preferentially liberates sulfite from taurine (2-aminoethanesulfonic acid). SsuD is a monooxygenase that catalyzes the oxygenolytic desulfonation of a range of aliphatic sulfonates other than taurine. Its cosubstrate is $FMNH_2$, which is provided by SsuE, an NAD(P)H-dependent FMN reductase. In contrast to many other bacteria, *E. coli* is unable to grow with arylsulfonates or with sulfate esters as sulfur source. The *tau* and *ssu* systems thus provide all genes for the utilization of known organosulfur sources by this organism, except the as yet unidentified gene(s) that enable some *E. coli* strains to grow with methanesulfonate or cysteate as a sulfur source. Expression of the *tau* and *ssu* genes requires the LysR-type transcriptional regulatory proteins CysB and Cbl. Synthesis of Cbl itself is under control of the CysB protein, and the CysB protein may therefore be regarded as the master regulator for sulfur assimilation in *E. coli*, while the Cbl protein functions as an accessory element specific for utilization of sulfur from organosulfur sources.

Keywords Sulfonate · Sulfate starvation · Cysteine biosynthesis · *Escherichia coli* · Taurine · Oxygenase · ABC transporter · Dioxygenase · CysB · Cbl

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Introduction

Sulfur, an essential element for bacterial growth, may be assimilated from a range of sources (Fig. 1). Cysteine is the preferred source of sulfur for *Escherichia coli* and many other bacterial species. Its presence in the medium results in repression of the enzymes involved in sulfur assimilation from sulfate (Fig. 1). Likewise, sulfide and thiosulfate repress the uptake, activation and reduction of sulfate (Kredich 1996). Cysteine is thus favored over sulfate and reduced inorganic sulfur compounds are favored over oxidized ones.

When cysteine and inorganic sulfur are both absent from the growth medium, *E. coli* can utilize aliphatic sulfonates such as taurine (2-aminoethanesulfonate) as source of sulfur. This was first observed in 1955 (Roberts et al. 1955), but it took more than 40 years until the enzymes responsible for the liberation of sulfite from taurine and other aliphatic sulfonates were purified and characterized (Eichhorn et al. 1997). Sulfonates have the general formula $R–C\text{-}SO_3^-$ and they are very stable compounds. In recent years it has become apparent that many bacterial species can utilize organosulfur compounds such as sulfonates and sulfate esters as source of sulfur, aerobically as well as anaerobically (for reviews, see Seitz and Leadbetter 1995; Cook et al. 1998; Kertesz 2000). Here, we review the biochemistry and regulation of sulfur assimilation from aliphatic sulfonates by *E. coli*.

The genes and enzymes involved in sulfonate-sulfur utilization were searched for by two independent approaches, both based on the assumption that their expression would be repressed by sulfate and cysteine. Two-dimensional gel electrophoresis (Quadroni et al. 1996) and transposon mutagenesis with λp*lac*Mu9 (Van der Ploeg et al. 1996) were used to identify proteins and genes whose expression was absent or decreased during growth with sulfate or cysteine as compared to growth with other sulfur sources. Some of the eight sulfate-starvation-induced (Ssi) proteins observed in the proteome study were identified as proteins involved in the uptake of cystine (FliY, cystine binding protein) or

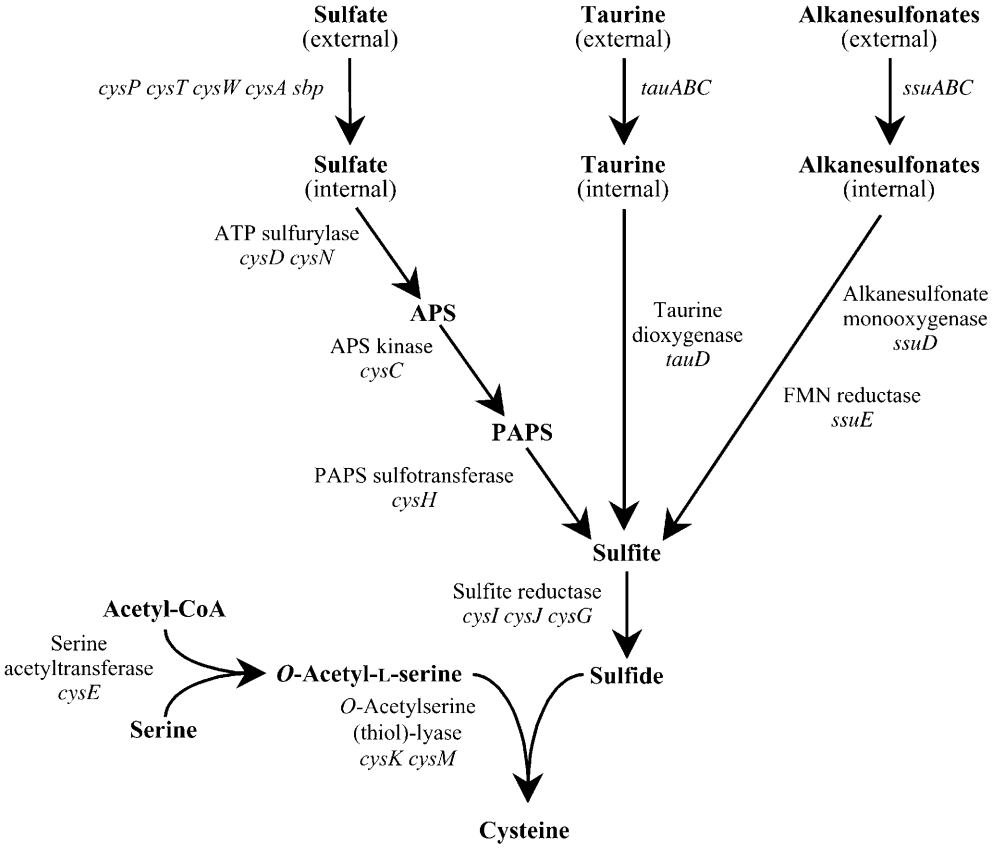

Fig. 1 Cysteine biosynthesis from sulfate and alkanesulfonates in *Escherichia coli*. *APS* Adenosine 5′-phosphosulfate, *PAPS* 3′-phosphoadenosine 5′-phosphosulfate

in the biosynthesis of cysteine from sulfate, notably CysK (cysteine synthase) and the sulfate binding protein (Sbp). The function of the remaining Ssi proteins was unknown at that time, but transposon mutagenesis with λp*lac*Mu9 led to the identification of the *tauABCD* gene cluster (Fig. 2), which encoded two further Ssi proteins (Van der Ploeg et al. 1996). A second system, the *ssuEADCB* gene cluster (Fig. 2), was subsequently identified by hybridization analysis using probes derived from the remaining unknown Ssi proteins (Van der Ploeg et al. 1999).

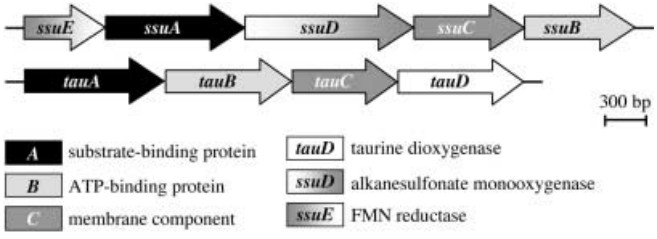

Fig. 2 Organization of the *E. coli ssuEADCB* and *tauABCD* gene clusters and functions of the encoded proteins. TauABC and SsuABC are two distinct ABC-type transporters. TauA and SsuA are periplasmic binding proteins, TauB and SsuB are ATP-hydrolyzing enzymes, TauC and SsuC are the integral membrane components of the corresponding transporter. TauD is an α-ketoglutarate-dependent taurine dioxygenase, SsuDE is a two-component alkanesulfonate monooxygenase. The Swiss-Prot accession numbers for the *tau* and *ssu* encoded proteins are: Q47537 (TauA), Q47538 (TauB), Q47539 (TauC), P37610 (TauD), P80644 (SsuE), P75853 (SsuA), P80645 (SsuD), P75851 (SsuC), P38053 (SsuB)

The function of the proteins in both clusters could be inferred from the growth properties of mutants and from the similarity of their sequences to proteins of known function. Both gene clusters encode an ABC transport system (*tauABC* and *ssuABC*) and an oxygenase system (*tauD* and *ssuDE*; Fig. 2). Mutants with transposon insertions in the genes of the *tauABCD* cluster were unable to use taurine as sulfur source, whereas deletion of the *ssu* gene cluster led to the inability to use aliphatic sulfonates other than taurine.

TauD, an α-ketoglutarate-dependent taurine dioxygenase

The sequence of the *tauD* gene product shows 28% identity to that of TfdA, an α-ketoglutarate-dependent 2,4-dichlorophenoxyacetate dioxygenase from *Ralstonia eutropha* (Fukumori and Hausinger 1993). Together with the phenotype of a *tauD* mutant, this suggested that TauD is an α-ketoglutarate (α-KG)-dependent dioxygenase using taurine as substrate. Indeed, the purified enzyme, a dimer of 81 kDa, converted taurine to sulfite and aminoacetaldehyde, most likely via the unstable intermediate 1-hydroxyaminoethanesulfonate (Eichhorn et al. 1997). This conversion was absolutely dependent on the presence of iron (II), molecular oxygen and α-KG. The overall reaction led to the formation of equimolar amounts of sulfite and succinate, indicating that taurine desulfonation and α-KG de-

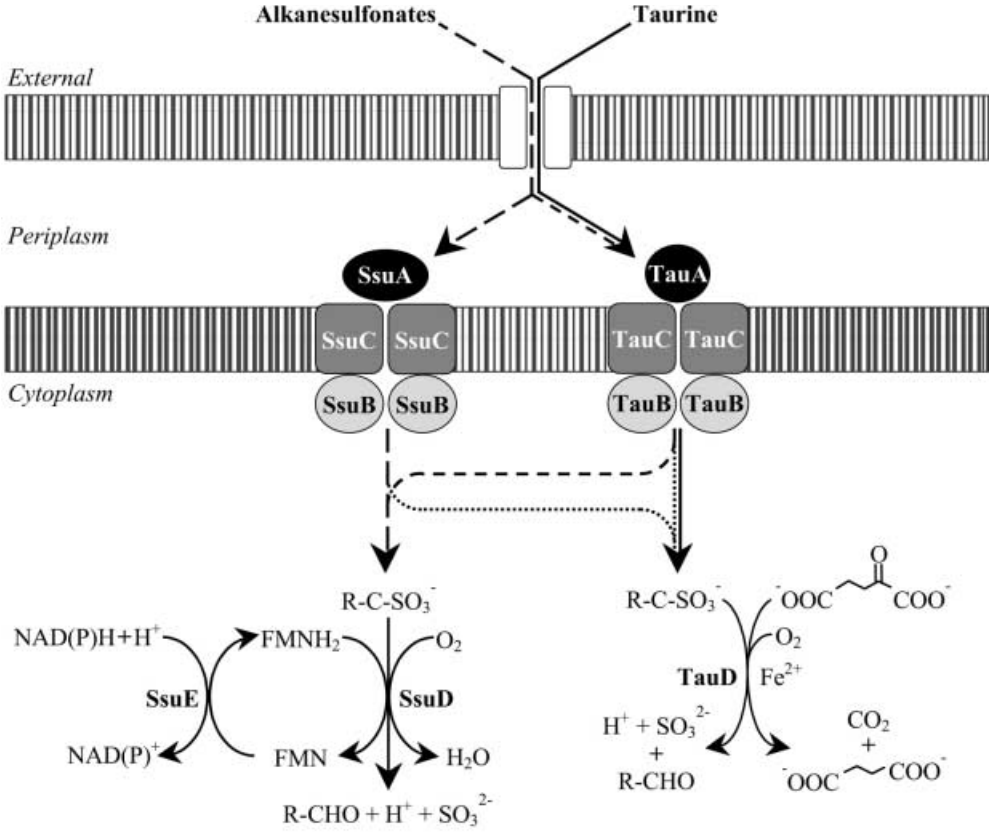

Fig. 3 Uptake and desulfonation of taurine and alkanesulfonates in *E. coli*. Taurine is exclusively transported into the cell and desulfonated by TauABCD (*solid lines*). All other alkanesulfonates are substrates of the SsuEADCB system (*large dashed lines*). Some of these can enter the cell via the TauABC transporter but are not desulfonated by TauD (*small dashed lines*). Some alkanesulfonates other than taurine can be desulfonated by TauD (*dotted lines*). It is not known how taurine and other alkanesulfonates pass through the outer membrane

carboxylation are strictly coupled (Fig. 3). Other sulfonates, for example butanesulfonate, pentanesulfonate and MOPS (3-(*N*-morpholino) propanesulfonic acid), served also as substrates for TauD, although with lower affinity than taurine (Eichhorn et al. 1997).

α-KG-dependent dioxygenases form a family of proteins that has been divided in three different groups, based on their sequence similarity (Hogan et al. 2000). The members of this family have a very diverse substrate range and catalyze different two-electron oxidation reactions, which include hydroxylation, desaturation and oxidative ring closure. They all contain a 2-His-1-carboxylate (Asp/Glu) motif which is proposed to bind iron. The TauD protein belongs to group II of the family (Hogan et al. 2000), which also includes TfdA.

Stopped flow kinetic analysis with TauD indicated that α-KG binds first in a six-coordinate Fe(II) complex (Ryle et al. 1999). The second substrate, taurine, enters fast and gives rise to a five-coordinated Fe(II) complex. Subsequently, O_2 is bound in a fast step. Succinate and CO_2 are then cleaved off and a Fe(IV)=O intermediate is formed, from which oxygen is inserted into taurine. In the last step, sulfite and aminoacetaldehyde leave the active center. Spectroscopic studies (Hegg et al. 1999) and site-directed mutagenesis (Hogan et al. 2000) confirmed this mechanism for TfdA. The residues proposed to be involved in iron binding, His-113, Asp-115 and His-262, are conserved among the group II α-KG-dioxygenases. In addition, an arginine/lysine residue is conserved in all representatives of this group (Hogan et al. 2000). This residue might be involved in binding of the carboxylate moiety of α-KG as has been found for the group I enzymes (Roach et al. 1995).

Recently, two other members of the Tfda/TauD family (group II) have been characterized, the YSD protein from *Saccharomyces cerevisiae* (Hogan et al. 1999) and the AtsK protein from *Pseudomonas putida* (Kahnert and Kertesz 2000). YSD is a sulfonate dioxygenase, with a somewhat broader substrate range than TauD (Hogan et al. 1999). AtsK acts on aliphatic sulfate esters, but does not use taurine as substrate (Kahnert and Kertesz 2000). The close relationship in sequence between TauD, AtsK, YSD and TfdA could indicate that the latter protein is also involved in sulfur assimilation from sulfonates or sulfate esters, or that it has only recently evolved to deal with 2,4-dichlorophenoxyacetic acid. This hypothesis is supported by the finding that the *tfdA* gene is widespread among soil isolates in the bacterial kingdom, also among isolates from pristine environments (Hogan et al. 1997).

SsuD, an $FMNH_2$-dependent sulfonate monooxygenase

The principal substrate for the TauD protein is taurine. Other sulfonates that can be utilized by *E. coli* as sulfur source are desulfonated by SsuD, a flavin-dependent monooxygenase that requires reduced FMN, which is delivered by SsuE (Eichhorn et al. 1999; Fig. 3). As is the case for TauD-catalyzed desulfonation, catalysis by SsuD probably results in an unstable 1-hydroxysulfonate that spontaneously decomposes to the corresponding aldehyde and sulfite.

Using pentanesulfonate as substrate, pentanal and sulfite were detected as products (Eichhorn et al. 1999).

SsuE, a homodimeric protein of 58.4 kDa, catalyzes the reduction of flavins by NADPH or NADH (Eichhorn et al. 1999). The preferred flavin substrate is FMN, but it can also reduce FAD or riboflavin.

The substrate range of SsuD, a homotetrameric enzyme of 181 kDa, includes linear aliphatic sulfonates ranging from ethanesulfonate to tetradecanesulfonate (Eichhorn et al. 1999). Among the best substrates are 1,3-dioxo-2-isoindolineethanesulfonate and 2-(4-pyridyl)ethanesulfonate. The significance of this is not clear, but these substrates may be related in structure to naturally occurring sulfonates. Among the sulfonates not desulfonated by pure SsuD are aromatic sulfonates, taurine, cysteate and methanesulfonate (Eichhorn et al. 1999). Whereas *E. coli* K-12 strains cannot use cysteate or methanesulfonate as source of sulfur, other *E. coli* strains can. This indicates that desulfonation of these compounds requires either an oxygenase with different substrate specificity or an additional protein that extends the substrate range of SsuD or TauD to cysteate and methanesulfonate (E. Eichhorn, unpublished results). *Pseudomonas aeruginosa*, which can also grow with methanesulfonate, contains two related $FMNH_2$-dependent monooxygenases, MsuD and SsuD. SsuD from *P. aeruginosa* is 77% identical to SsuD from *E. coli*, but its substrate range is unknown. However, the principal substrate for MsuD, which is 66% identical to *E. coli* SsuD, is methanesulfonate and activity decreases with increasing chain length of the substrate (Kertesz et al. 1999).

The SsuD protein is a member of the family of monooxygenases that require reduced flavins for activity. In these enzymes, the flavins do not function as a prosthetic group, but serve as cosubstrates. In many cases, the flavin reductase is encoded in the same locus as the oxygenase, although the oxygenase does not appear to be strictly dependent on the cognate reductase for reduced FMN. It has been shown, for example, that the FMN reductase NmoB from *Chelatobacter heintzii* can be replaced by NADP(H):FMN reductase from *Photobacterium fischeri* to deliver reduced FMN to NmoA, an oxygenase involved in degradation of nitrilotriacetate (Xu et al. 1997). Given this absence of specificity, it appears unlikely that the reductase and the oxygenase interact physically, although it would make sense in order to prevent $FMNH_2$ from being reoxidized by reactions occurring uncoupled from SsuD catalysis.

Although there is very little sequence similarity (Eichhorn et al. 1999), the SsuD protein may be similar in structure to the β-subunit of luciferase from *Vibrio harveyi* (Fisher et al. 1996) and to F_{420}-dependent N^5,N^{10}-methylenetetrahydro-methanopterin reductase (Shima et al. 2000), whose structures have been solved. Crystals of SsuD have been obtained (E. Eichhorn, unpublished results) and determination of the three-dimensional structure is in progress.

SsuE shows sequence similarity to some flavoproteins of archaea and bacteria (Eichhorn et al. 1999). These proteins contain a conserved motif which is likely to function as an FMN binding site. Some of these flavoproteins in addition contain a 4-cysteine motif as part of an iron-sulfur cluster which is used to transfer electrons to FMN. In SsuE, this iron-sulfur cluster is absent, which could indicate that electrons are directly transferred from NADPH or NADH to FMN.

The sulfonate transport systems

The *tauD* and *ssuDE* genes are accompanied by the *tauABC* and *ssuABC* genes, respectively, that encode ABC transport systems. TauA and SsuA are thought to function as periplasmic sulfonate binding proteins, whereas TauB and SsuB, as well as TauC and SsuC could act as ATP binding proteins and membrane components of the transporter, respectively. Biochemical studies are lacking, but phenotypic analysis of mutants containing in-frame deletions in the individual components of the putative transport genes indicated that the substrates transported by the two systems were largely reflected by the substrate ranges of the oxygenases TauD and SsuD (Eichhorn et al. 2000). Thus, taurine, the best substrate for the TauD protein, was only transported by the TauABC system, since deletions in *tauA* or in *tauBC* resulted in lack of growth with this sulfur source. Likewise, longer-chain aliphatic sulfonates were exclusively transported by the SsuABC system. Some substrates, for example 2-hydroxyethanesulfonate, butanesulfonate and 3-aminopropanesulfonate, were translocated by both systems and in many cases, but not in all, these sulfonates were substrates for both desulfonating enzymes (Eichhorn et al. 2000).

Another major conclusion of this work was that the components of the TauABC and SsuABC transport systems are not interchangeable (Eichhorn et al. 2000). Although the substrates transported are very similar, the periplasmic binding proteins of the two systems do not share a single membrane component and ATPase, as is the case for the *E. coli* sulfate binding protein Sbp and the thiosulfate binding protein CysP. These proteins possess an overlapping substrate range and share the membrane components CysT and CysW and the ATP binding protein CysA. But Sbp and CysP are 45.8% identical at the protein sequence level, whereas TauA and SsuA share only 22.7% identity. Another example is that of the arylsulfonate binding protein AsfC from *P. putida*, which probably shares the membrane component AtsB and the ATPase AtsC with the sulfate ester binding protein AtsR (Vermeij et al. 1999). AtsR and AsfC are 48% identical in amino acid sequence.

Bacterial ABC-type uptake permeases have been divided into 20 different groups on the basis of sequence similarity between the periplasmic binding proteins (http://www.biology.ucsd.edu/~ipaulsen/transport). The SsuABC and TauABC transporters belong to a separate group of ABC transporters that is distinct, for example, from the group of sulfate transporters. To this group of sulfonate transporters belong probably also the AtsR proteins from *P. aeruginosa* and *P. putida* and the AsfC protein from *P. putida*, which are thought to function as sulfate ester and arylsulfonate binding proteins, respectively (Kertesz 2000).

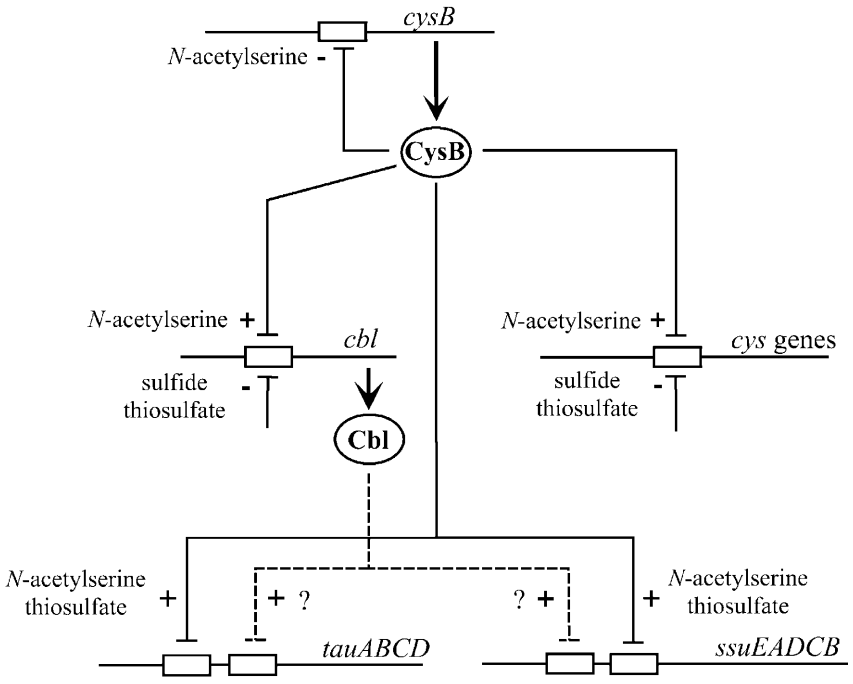

Fig. 4 Model for the regulation of sulfur assimilation by CysB and Cbl in *E. coli*. The interactions between the CysB protein, effectors and the promoter regions of the *cys* genes and of *cysB* itself have been well-characterized (Kredich 1996). The model for regulation of the *ssu* and *tau* genes is supported by results from gene fusion analysis, gel mobility-shift assays and footprinting experiments (Van der Ploeg et al. 1997, 1999), but awaits further clarification. In particular, the identities of the coinducer and the anti-inducer of Cbl are unknown. The binding sites for CysB and Cbl on the *tau* and *ssu* promoter do not share obvious similarity

Although the systems that transport sulfonates over the cytoplasmic membrane in *E. coli* have been characterized at the genetic level, it is not known how, and by which proteins, transport of sulfonates through the outer membrane is mediated. We assume that non-specific porins are involved in this process.

Regulation of organosulfur utilization, an extension of the *cys* regulon

It has been known for some time that the CysB protein, a LysR-type transcriptional regulator, positively regulates the expression of the genes encoding the assimilatory sulfate reduction pathway (Kredich 1996). However, recent findings have shown that the function of CysB is not restricted to regulation of the assimilatory sulfate reduction pathway, but that it may be at the top of a regulatory system that controls the regulation of sulfur assimilation at a global level (Fig. 4).

Full expression of the *cys* genes requires CysB and the coinducer *N*-acetylserine, which is non-enzymatically derived from *O*-acetylserine (Kredich 1996). Synthesis of *O*-acetylserine is catalyzed by serine acetyltransferase (Fig. 1). The activity of this enzyme is regulated through feedback inhibition by cysteine. Therefore, the concentration of intracellular cysteine indirectly controls expression of the *cys* genes. On the other hand, sulfide and thiosulfate compete for binding with *N*-acetylserine. Sulfate itself has no direct effect on expression, but its conversion to sulfide and cysteine results in about two-fold reduction of the expression levels of the *cys* genes.

In contrast, the *ssu* and *tau* genes are fully repressed when *E. coli* is grown in the presence of sulfate. A second regulatory protein, Cbl, is required for expression of the *tau* and *ssu* genes. Expression of *cbl* itself is under control of CysB (Iwanicka-Nowicka and Hryniewicz 1995), which thereby indirectly regulates expression of the *ssu* and *tau* operons. Promoter-*lacZ* fusions and DNA binding experiments have shown that Cbl binds just upstream of the –35 region of the *ssu* promoter (Van der Ploeg et al. 1999). Removal of this binding site eliminates expression, which indicates that Cbl acts as an archetypal LysR-type transcriptional activator. However, the situation is probably more complex, since the *ssu* promoter region contains also binding sites for the CysB protein and for the integration host factor (Van der Ploeg et al. 1999). Removal of these binding sites did not have a strong effect on expression from the *ssu* promoter, so their significance is not clear at the moment. This contrasts with the situation in the promoter region of the *tau* operon, where it was shown that both Cbl and CysB bind to DNA are needed in concert for expression (Van der Ploeg et al. 1997)

The three-dimensional structure of the C-terminal part of CysB (residues 88–324) from *Klebsiella pneumoniae*, which comprises the binding site for *N*-acetylserine, has been determined (Tyrrell et al. 1997). The protein contains two α/β domains which enclose a cavity that is proposed to serve as binding site for acetylserine. The Cbl protein is 45% identical in sequence to CysB, and many of the residues that shape the cavity are conserved. This suggests that the structure of the coinducer recognized by Cbl could be very similar to acetylserine, the coinducer for CysB. However, in contrast to binding of CysB to the *cys* promoter regions, binding of Cbl to the *tau* and *ssu* promoter regions is not influenced by *O*-acetylserine (Van der Ploeg et al. 1997, 1999). Moreover, transcription experiments in vitro have also shown that *O*-acetylserine has no effect on

the formation of transcript (M. Hryniewicz, Warsaw, personal communication).

Since the *tau* and *ssu* genes are repressed by sulfate, it would appear that sulfate functions as anti-inducer. However, in mutants that are unable to activate sulfate to adenosine phosphosulfate (APS), expression of *tau* and *ssu* is not repressed by sulfate, whereas these genes are still repressed by sulfate in a mutant that is unable to reduce sulfite to sulfide (J. R. van der Ploeg, unpublished results). Therefore, we suggest that an intermediate of the cysteine biosynthetic pathway between sulfate and cysteine functions as anti-inducer.

Certain mutants of CysB cause constitutive expression of the *cys* genes, probably because of a conformational change of the protein that leads to activation of expression even in the absence of *N*-acetylserine (Colyer and Kredich 1994). Several mutants exhibiting the constitutive phenotype carry mutations in residue Thr-149 (Colyer and Kredich 1994), which is part of the cavity proposed to bind *N*-acetylserine. In the Cbl protein, residue 149 is a serine, which when changed to methionine causes constitutive expression of the *tau* and *ssu* operons (J. R. van der Ploeg, unpublished results).

Two-dimensional gel electrophoresis of extracts from a *cbl* mutant revealed that, apart from the TauA, TauD, SsuD and SsuE proteins, the Ssi proteins CysK and FliY were also absent and Sbp was strongly reduced (Van der Ploeg et al. 1997). This could indicate that expression of *cysK*, *fliY* and *sbp* is also controlled by Cbl, although this has not yet been confirmed by transcriptional analysis. An indication for the dependence of expression of *cysK* and *sbp* on Cbl is the reduced growth rate of a *cbl* mutant with sulfate (Iwanicka-Nowicka and Hryniewicz 1995).

The number of target genes regulated by Cbl may be even larger than found until now, at least in *K. aerogenes*. In this organism, unlike in *E. coli*, a transsulfurylation pathway for recycling of methionine into cysteine appears to be retained (Seiflein and Lawrence 2001). It was suggested that the regulation of this pathway involves the CysB protein either directly or indirectly, since a *cysB* mutant used methionine only after a very long lag-phase. Moreover, one of the enzymes in this pathway, γ-cystathionine lyase, is repressed by cysteine. Since it was not only repressed by cysteine, but also by sulfate, it was hypothesized that a putative, as yet unknown regulatory protein, MtcR, was involved, with APS as molecule that senses the concentration of sulfate (Seiflein and Lawrence 2001). The question arises as to whether the putative MtcR from *K. aerogenes* and Cbl from *E. coli* could be structurally and functionally similar proteins. A *cbl* gene is present in *Klebsiella* (Iwanicka-Nowicka and Hryniewicz 1995) and its gene product is 86% identical in sequence to the *E. coli* Cbl protein.

Apart from methionine and aliphatic sulfonates, *K. aerogenes* can utilize arylsulfate esters as source of organic sulfur, since it contains an arylsulfatase, encoded by the *atsA* gene. Like the enzymes involved in utilization of the other organic sulfur sources, expression of *atsA* is repressed by sulfate and by cysteine (Adachi et al. 1975). This could indicate that Cbl is also a regulator of arylsulfate utilization.

In *E. coli*, the *cbl* gene is positioned downstream of the *nac* gene, which encodes a LysR-type transcriptional activator that positively regulates expression of operons that encode utilization of poor nitrogen sources, and negatively regulates expression of operons that encode utilization of preferred nitrogen sources like ammonium (Bender 1991). Transcriptional analysis of *cbl* using DNA arrays has shown that *cbl* is cotranscribed and coregulated with the *nac* gene (Zimmer et al. 2000). This could mean that, apart from its function in regulation of organosulfur utilization, *cbl* has an accessory function in regulating nitrogen assimilation.

Occurrence of the *tau* and *ssu* genes in bacteria

Escherichia coli spends most of its lifetime in the gut, a mostly anoxic environment with enough sulfate available. It may therefore seem surprising that *E. coli* and some other enterics (Uria-Nickelsen et al. 1993) are able to grow with sulfonates as sources of sulfur. These compounds are mainly found in soil environments (Autry 1990), where sulfate may be limiting and where *E. coli* may spend some of its existence.

A search in fully or partially sequenced bacterial genomes revealed that both *tauD* and *ssuD* genes, defined as ORFs whose translation products have more than 50% identity to TauD and SsuD from *E. coli*, respectively, are present in *Yersinia pestis, K. aerogenes, P. aeruginosa* and *P. putida*, whereas *Bacillus subtilis* contains only the *ssu* genes (Eichhorn 2000). Since this survey, many more bacterial genome sequence projects have been initiated, and *ssuD* appears also to be present in *Ralstonia metalliduran*s, *Burkholderia cepacia*, *Sphingomonas aromaticivorans*, *Pseudomonas fluorescens*, *Yersinia pseudotuberculosis, Rhodopseudomonas palustris, Nostoc punctiforme* and *Bacillus anthracis*, whereas the *tauD* gene is also found in *Yersinia pseudotuberculosis.*

Among the enteric bacteria, *Salmonella typhimurium* does not grow with sulfonates as sulfur source. Although the genome sequence of *S. typhimurium* has not yet been completed, the *ssu* and *tau* genes as well as the *cbl* gene appear to be absent in this bacterium.

Concluding remarks and unresolved questions

The ability of *E. coli* to utilize taurine and other aliphatic sulfonates as sulfur sources under conditions of sulfate starvation is due to the presence of two systems, each composed of an ABC-type transporter and an oxygenolytic enzyme that liberates sulfite from the substrate. The oxygenase systems do not possess heme or iron-sulfur centers, which is in line with the observation that proteins specifically expressed under conditions of sulfate starvation (Ssi proteins) have an extremely low cysteine content. The TauD protein depends on ferrous iron as a cofactor

and electrons are provided by α-KG. The SsuD protein uses reduced FMN as a source of electrons.

The two transport systems and the two oxygenases have a complementary substrate range, although there is some overlap. This partial redundancy could be of physiological relevance, but it is quite possible that we do not know the natural substrates, in particular for the *ssu* system. The principal substrate for the *tau* system is most likely taurine, since it is a naturally occurring compound. Surprisingly, *E. coli* K12 strains cannot utilize methanesulfonate or cysteate, which are sulfonates that also occur in natural environments.

CysB is a global regulator for sulfur assimilation from inorganic sulfate and from organic sulfur sources. The CysB protein regulates the expression of the *cys* genes and that of *cbl*, which in turn is required for expression of the *ssu* and *tau* genes. Although this appears to be a simple regulatory cascade, many questions remain. It is, for example, not known why the CysB protein binds to the promoter regions of *tau* and *ssu*. Whereas CysB might function as repressor (Van der Ploeg et al. 1999) for the *ssu* system, it is required for expression of the *tau* genes (Van der Ploeg et al. 1997). This indicates that there are differences in the regulation of the two systems. Another important unresolved question concerns the mechanism by which the presence of sulfate is sensed and how the signal indicating sulfate sufficiency causes repression of the *tau* and *ssu* genes. Since the TauD and SsuD proteins require molecular oxygen for activity, it would make sense if the genes encoding these proteins were turned off when oxygen is absent from the medium. However, until now, this issue has not been investigated.

Whereas the role of Cbl in regulation of sulfonate-sulfur utilization is clear, it is unknown whether Cbl also functions in regulation of expression of genes required for the utilization of other, as yet unknown, organic sulfur sources by *E. coli* and other enteric bacteria.

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