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Sulfur Metabolism in *Escherichia coli* and Related Bacteria: Facts and Fiction

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Abstract

Living organisms are composed of macromolecules made of hydrogen, carbon, nitrogen, oxygen, phosphorus and sulfur. Much work has been devoted to the metabolism of the first five elements, but much remains to be understood about sulfur metabolism. We review here the situation in *Escherichia coli* and related bacteria, where more than one hundred genes involved in sulfur metabolism have already been discovered in this organism. Examination of the genome suggests that many more will be found, especially genes involved in regulation, scavenging of sulfur containing molecules and synthesis of coenzymes or prosthetic groups. Furthermore, the involvement of methionine as the universal start of proteins as well as that of its derivative S-adenosylmethionine in a vast variety of cell processes argue in favour of a major importance of sulfur metabolism in all organisms.

Introduction

Sulfur, an ubiquitous element of the Earth crust, is an essential component of life. However, its involvement in biological processes is limited to a series of highly specific compounds. This is probably due to the fact that it is a very reactive atom, and that many chemical reactions involving sulfur consume a large quantity of energy. It is therefore of prime importance to understand sulfur metabolism in model organisms. The various inorganic states of this atom have been well studied, and the corresponding knowledge is fundamental for understanding mineralogy and soil biology (for a review see Ehrlich, 1996). In contrast, the organic cycle of sulfur is less well known, and metabolism of sulfur, in spite of its central importance, has been relatively neglected. We review here general sulfur metabolism (anabolism, transport and catabolism and recycling), then emphasize the special situation of the metabolism of *S*-adenosylmethionine (AdoMet), ending with special situations where sulfur is involved, in particular synthesis of coenzyme and prosthetic groups as well as tRNA modifications. Finally, we point to the chemical kinship between sulfur and selenium, a building block of the 21st amino acid in proteins, selenocysteine.

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General Sulfur Metabolism

In the general metabolism of sulfur one must distinguish three well differentiated aspects: synthesis of the sulfur-containing amino acids (cysteine and methionine), together with that of the sulfur-containing coenzymes or prosthetic groups; catabolism and equilibration of the pool of sulfur containing molecules; and methionine recycling (a topic in itself, because of the role of methionine as the first residue of all proteins). Five databases for metabolism allow one to retrieve extant data relevant to sulfur metabolism: the Kyoto Encyclopedia of Genes and Genomes (KEGG:http://www.genome.ad.jp/kegg/kegg2.html), the WIT database (What is there?) of the Department of Energy at Argonne (http://wit.mcs.anl.gov/WIT2/), and the database of Pangea, EcoCyc (http://ecocyc.PangeaSystems.com:1555/server.html). Two specialized databases: Colibri (http://bioweb.pasteur.fr/GenoList/Colibri/) and SubtiList (http://bioweb.pasteur.fr/GenoList/SubtiList/) provide direct information about the genes and genomes of the model organisms *Escherichia coli* and *Bacillus subtilis*.

Anabolism

Sulfur Distribution

On average, the sulfur concentration at the surface of the Earth is estimated to be of ca 520 ppm. It varies in rocks between 270 and 2400 ppm. In fresh water, it is 3.7 ppm on average. In sea water, it reaches 905 ppm. In temperate regions, it varies between 100 and 1500 ppm in soil (Ehrlich, 1996). However, its concentration in plants is usually low. This element is present essentially in the form of the amino acids cysteine and methionine, their oxidation products, as well as molecules of reserve (or osmoprotectants, such as *S*-methylmethionine; Kocsis *et al.,* 1998) or various types of sulfonates (Friedrich, 1998; Cook *et al.,* 1999). It is also found in derivatives of secondary metabolism (in particular in garlic-related plants (Lim *et al.,* 1998), where these sulfur-containing metabolites play a very efficient antimicrobial role), and as sulfated carbohydrates or aminoglycosides (Guler *et al.,* 1996).

Oxido-Reduction and Assimilation of Sulfur

Sulfur metabolism, at least in the presence of oxygen, costs energy. As sulfate, it must first permeate the cell, where the intracellular electric potential is strongly negative (-70 mV), then change from a highly oxidized state to a reduced state. This requires a significant consumption of energy,

Table 1. Electron Transfers in the Absence of Photosynthesis.

Reducing agent	Redox couple[a]	$E^{0'}$ [mV][b]	$\Delta G^{0'}$ [kJ·mol^{-1}][c]	Organisms
Carbon monoxide	CO_2/CO e.g.*Pseudomonas*	-540	-261	'Carboxidobacteria'
Hydrogen	2 H^+/H_2	-410	-237	'Knallgas' bacteria
Sulfide	S_0/HS^-	-260	-207	*Thiobacillus, Beggiatoa, Wolinella succinogenes*
	HSO_3^-/HS^-	-110	-536	*Thiobacillus, Sulfolobus*
Sulfur	HSO_3^-/S_0	-45	-332	*Thiobacillus*
Sulfite	SO_4^{2-}/HSO_3^-	-520	-258	*Thiobacillus*
	APS/HSO_3^-	-60	-227	*Thiobacillus*
Ammonium	NO_2^-/NH_3	+340	-276	*Nitrosomonas*
Nitrite	NO_3^-/NO_2^-	+430	-75	*Nitrobacter*
Fe^{2+} (pH 2)	Fe^{3+}/Fe^{2+}	+770	-32	*Thiobacillus ferrooxidans, Sulfolobus*
Oxygen	O_2/H_2O	+816		Cyanobacteria

[a]The reaction procedes from the reduced state to the oxidized state, but convention asks that it is represented in the order: product/substrate.
[b]Redox reactions are reactions where a substrate is reduced (electron acceptor) and another one is oxidized (electron donor). ΔE is the difference of potential between the end and the start of the reaction. $\Delta E^{0'}$ is the redox potential difference (that can for example generate a protonmotive force) of a biochemical reaction in standard conditions: 298 K (25°C), pH 7.0 and where the concentration of each reagent is 1 mol/liter except for water (normal concentration 55.55 mol/liter) and gases (pressure of 101.3 kPa = 1 atm). The reaction occurs spontaneously only when the value of ΔE is negative, i.e. when the potential evolves towards more negative values. The redox potential is usually represented with the negative values above, and the spontaneous reactions procede from top to bottom.
[c]For each component of the system one attributes a quantity of free energy 'G', composed of an enthalpy 'H' (internal energy plus pressure multiplied by the volume) and of an entropy 'S' (measuring the degrees of freedom of the system, in terms of positions and energy levels available to its different components). In the cases where the absolute values are not large, the changes in G (ΔG) are decisive for the chemical reaction. The reaction occurs spontaneously only when the value of ΔG is negative. $\Delta G^{0'}$ is the ΔG of the biochemical reaction in standard conditions as defined above.

as well as the maintenance of a very low oxido-reduction potential, which seems difficult with the simultaneous presence of oxygen molecules (Table 1). Cell compartmentalization is therefore a prerequisite.

In *E. coli* the genes involved in these reduction steps are organized as several operons (Kredich, 1996). Operon *cysDNC* codes for subunit 2 of sulfate adenylyltransferase, subunit 1 of ATP sulfurylase (ATP:sulfate adenylyltransferase) and adenylylsulfate kinase. Genes *cysZ* and *cysK* may not be co-transcribed, although they are neighbours in the chromosome. CysZ is probably a membrane protein, but its function is unknown. CysK is *O*-acetylserine sulfhydrylase, phylogenetically related to tryptophan synthase (Lévy and Danchin, 1988). Operon *cysPUWAM* codes for a thiosulfate (and sulfate) periplasmic binding protein, an ABC-type membrane permease, and a minor *O*-acetylserine sulfhydrylase, specific for thiosulfate. Finally *cysE* codes for serine *O*-acetyl-transferase. It may lie in operon with genes *yibN*, *grxC*, *secB* and *gpsA* (coding respectively for: an unknown function, a glutaredoxin: glutathione-dependent redoxin, an element of the protein secretion machinery, and most probably a glycerol-phosphate hydrogenase). Gene *cysE* is the start point of cysteine metabolism, probably derived from an ancestral serine metabolism (Wächtershäuser, 1988; Danchin, 1989).

Sulfate is first transported into the cell, as we shall see, then it is assimilated in the form of adenosine phosphosulfate (APS), by an ATP sulfurylase (encoded by genes *cysD* and *cysN*). This reaction, which yields APS and pyrophosphate from ATP and sulfate, is strongly shifted towards ATP synthesis (and, therefore, not towards sulfate incorporation), because the $\Delta G^{0'}$ of hydrolysis of the APS phosphate-sulfate bond is considerably higher (-19 kcal/mol) than that of hydrolysis of the α,ß bond of ATP (-10,7 kcal/mol) (Liu *et al.,* 1998). It is therefore necessary that an important activity efficiently keeps the pyrophosphate concentration to a low level (through hydrolysis into inorganic phosphate) to pull the reaction towards the anabolic direction. This is assumed by one or several pyrophosphatases, whose essential role is to pull the pyrophosphate-producing macromolecular biosynthesis reactions towards the direction of anabolism (Figure 1). However, as witnessed by the high intracellular pyrophosphate concentration in E. *coli*, it seems that this reaction is far from equilibrium. It cannot therefore be sufficient to pull the reaction toward synthesis of APS (Liu *et al.,* 1998). This is why the synthesis of this latter molecule is also linked with hydrolysis of the ß,γ bond of GTP, which favors the reaction of sulfate incorporation (10^5-fold with respect to the reaction in absence of GTP). The coupling of the synthesis of APS with GTP hydrolysis displaces the equilibrium of the reaction towards sulfate assimilation ($\Delta G^{0'}$ = -6,8 kcal/mol) (Liu *et al.,* 1998). However, this is at the extremely high cost of incorporation of sulfur in the form of sulfate in these sulfur-containing molecules. This implies a strong selection pressure for the recovery of molecules which contain sulfur in reduced state.

APS is the substrate of a kinase (CysC), in a reaction that utilizes a second ATP molecule to phosphorylate the 3'OH position of APS, being transformed into 3'phosphoadenosine phosphosulfate (PAPS). One can think that the *raison d'être* of this reaction, which from ATP produces PAPS and ADP, is a supplementary means to pull the reaction toward the anabolic direction. In fact, not only does the 3' phosphate of PAPS have no specific function, but it must be metabolised into a by-product of the reaction, PAP (3'-5'ADP), with no known function in metabolism (Figure 1). In many bacteria (but we did not find these enzymes in *E. coli* or *B. subtilis*), the plants and the animals, PAPS is the necessary precursor for the sulfatation of various molecules (carbohydrates in particular) by sulfato-transferases (Kusche *et al.,* 1991; Suiko *et al.,* 1992; Varin and Ibrahim, 1992).

In the sulfur assimilation pathway, PAPS is reduced into SO_3^{2-}, yielding adenosine 3'-5' diphosphate (PAP) as a by-product. One should therefore investigate the fate of PAP after sulfate reduction. This is all the more important because this same molecule is produced in another reaction, beginning with coenzymeA, the transfer of the 4-phosphopantetheine group on the acyl carrier protein (ACP) of the complex synthesizing fatty acids. Finally synthesis

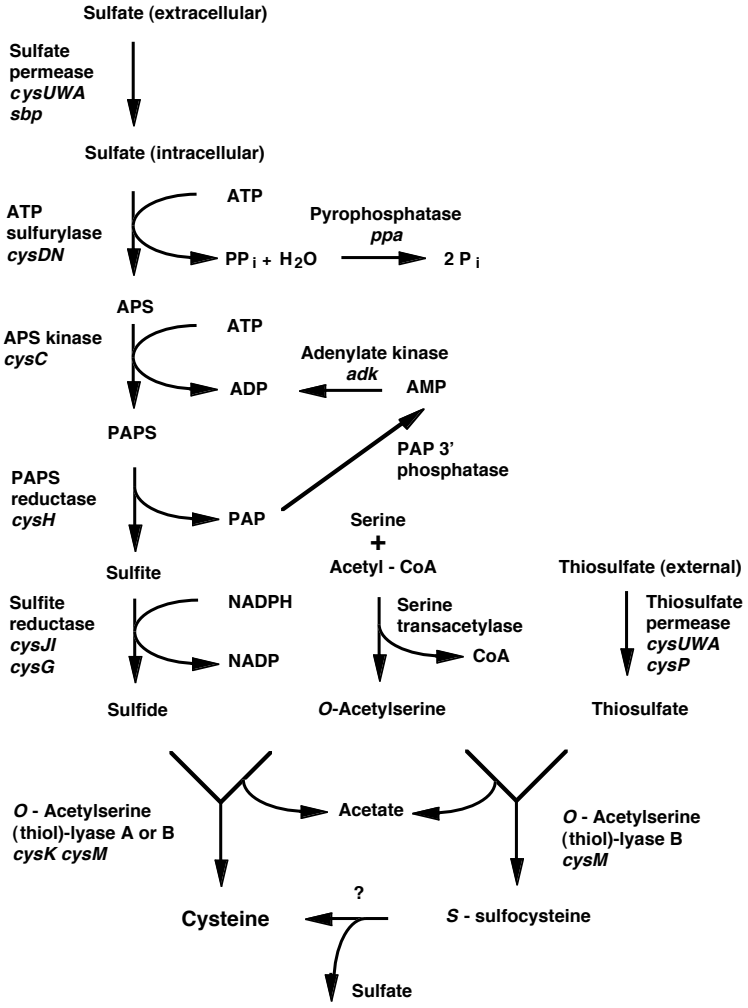

Figure 1. Sulfate assimilation pathway and cysteine biosynthesis in *Escherichia coli*.

of secondary metabolites such as peptide antibiotics, surfactin or polyketides also require the transfer of 4-phosphopantetheine from coenzymeA, producing PAP (Reuter *et al.,* 1999). There must exist therefore a 3'-5' diphosphoadenosine phosphatase, yielding 5'-AMP and phosphate. Protein CysQ (Swiss-Prot: P22255) may be the missing phosphatase, although we did not find explicit experimental data to support this. Indeed (York *et al.,* 1995), CysQ is similar to a plant adenosine diphosphate phosphatase (Gil-Mascarell *et al.,* 1999) involved in the reduction of sulfite to H_2S (Swiss-Prot: Q42546). This enzyme (HAL2=MET22 (Glaser *et al.,* 1993)) is probably also present in yeast, where its inactivation leads to methionine auxotrophy. The plant enzyme (encoded by the HAL2-like gene RHL (Peng and Verma, 1995)) is sensitive to the presence of sodium ions and its activity is associated with the resistance to osmotic stress. Because of its role one can wonder whether PAP phosphatase is not present in the cell in the neighborhood of adenylate kinase, which scavenges AMP. One should finally ask whether PAP does not play a regulatory role in a cell process controlling the entry of oxidized sulfur into the cell, eventually coupling this metabolism to that of lipids. Let us note in this respect that this molecule is very similar to cyclic AMP, a well-known regulator of gene expression (Ullmann and Danchin, 1983).

The enzyme involved in the reduction of sulfate, phosphoadenosine phosphosulfate reductase (EC 1.8.99.4) (thioredoxin-dependent PAPS reductase) (PADOPS reductase) is encoded by gene *cysH*.

5'-phosphoadenosine-3'-phosphosulfate + thioredoxin reduced <-> phosphoadenosine phosphate + thioredoxin oxidized + sulfite

The sulfite ion is reduced by NADPH-sulfite reductase (EC 1.8.1.2), an enzyme comprising two subunits, encoded by operon *cysJIH*. Subunit α (encoded by gene *cysJ*) involves FAD, whereas subunit ß (encoded by gene *cysI*) involves an iron-sulfur center and a siroheme prosthetic group (analogous to siroheme-dependent nitrite reductases). The sulfhydryl ion, HS^-, is quite reactive and toxic to the cell at high concentrations. The synthesis of siroheme is catalyzed by a SAM-dependent uroporphyrinogen III methylase (EC 2.1.1.107), encoded by gene *cysG* that forms an operon with genes *nirBDC* coding for the nitrite reductase and probably with genes *yhfLMNOPQR*. Siroheme is present in two enzymes, sulfite reductase and nitrite reductase. In addition, it is a precursor of the B12 coenzyme (which neither *E. coli* nor *B. subtilis* can entirely synthesize).

Many kinds of intermediary sulfur oxidation states exist: sulfite, thiosulfate, etc. Complex oxido-reduction systems permit reaching the ultimate reduction state, that of

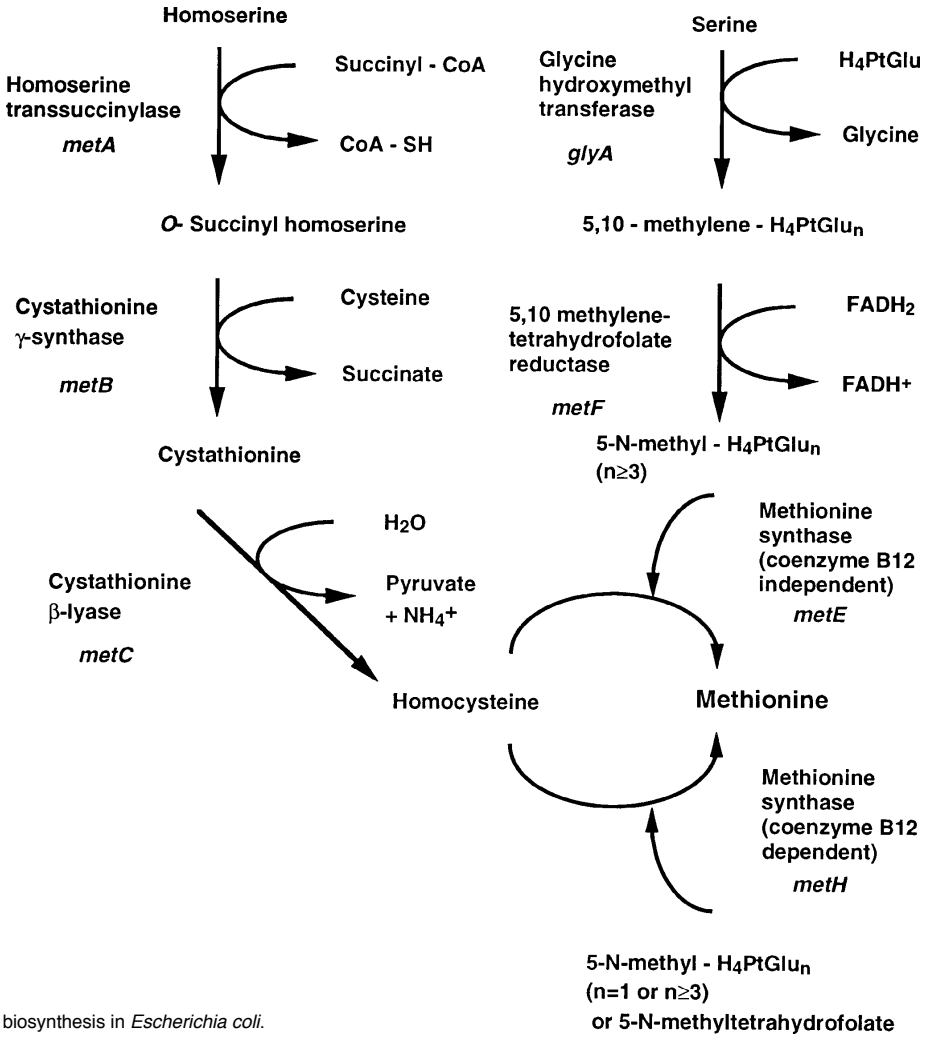

Figure 2. Methionine biosynthesis in *Escherichia coli*.

hydrogen sulfide, H_2S. These systems are often poorly known. However, one knows that, for example, thiosulfate can be an excellent sulfur source in *E. coli* (Sirko *et al.,* 1995). In the same way we do not know much about the use of organic sulfur sources.

Biosynthesis of Cysteine

The reaction catalyzed by CysE (serine transacetylase, EC 3.1.3.7) condenses an acetyl group from acetyl CoA on the hydroxyl group of serine, forming *O*-acetylserine. Sulfur, reduced as H_2S, reacts with *O*-acetylserine in *E. coli* to give cysteine. Genes *cysK* and *cysM* code for *O*-acetylserine (thiol)-lyase-A and -B, or *O*-acetylserine sulfhydrylase A and B (EC 4.2.99.8) (Lévy and Danchin, 1988; Kredich, 1996). Serine transacetylase and *O*-acetylserine sulfhydrylase A form an enzyme complex, cysteine synthase. In contrast, *O*-acetylserine sulfhydrylase B does not belong to an identified enzyme complex. Both *O*-acetylserine sulfhydrylases use sulfide as a nucleophile, but *O*-acetylserine sulfhydrylase B also possesses a characteristic feature, the ability to use thiosulfate in the place of H_2S, leading to the production of *S*-sulfocysteine. The conversion of *S*-sulfocysteine into cysteine has not been demonstrated in *E. coli*, and this casts doubt on the physiological importance of this activity. However, as we shall see later, there exist numerous sulfonatases that may act on this molecule, yielding cysteine and a sulfate ion, which would thus enter the normal pathway.

It is useful to understand the synthesis of *O*-acetylserine and its regulation, because intermediary metabolites are very often regulators of gene expression, as we shall see below for the role of N-acetylserine in *E. coli*. *N*-acetylserine (derived from O-acetylserine) is probably an inducer of the *cys* regulon. This molecule is formed by spontaneous cyclisation of *O*-acetylserine. The conversion of *O*-acetylserine into *N*-acetylserine occurs at the rate of 1% per minute at pH 7.6, and almost ten times faster at pH 8.6. The reverse reaction does not happen, which means that *O*-acetylserine but not *N*-acetylserine can serve as a sulfur acceptor. In contrast, *N*-acetylserine is approximatively 15-fold more efficient than *O*-acetylserine in its inducer action (Kredich, 1992).

Synthesis of Methionine

Methionine synthesis is linked to cysteine synthesis through metabolic pathways that differ according to the organisms. There often exist several different pathways in the same organism. In *E. coli*, the biosynthetic pathway is the

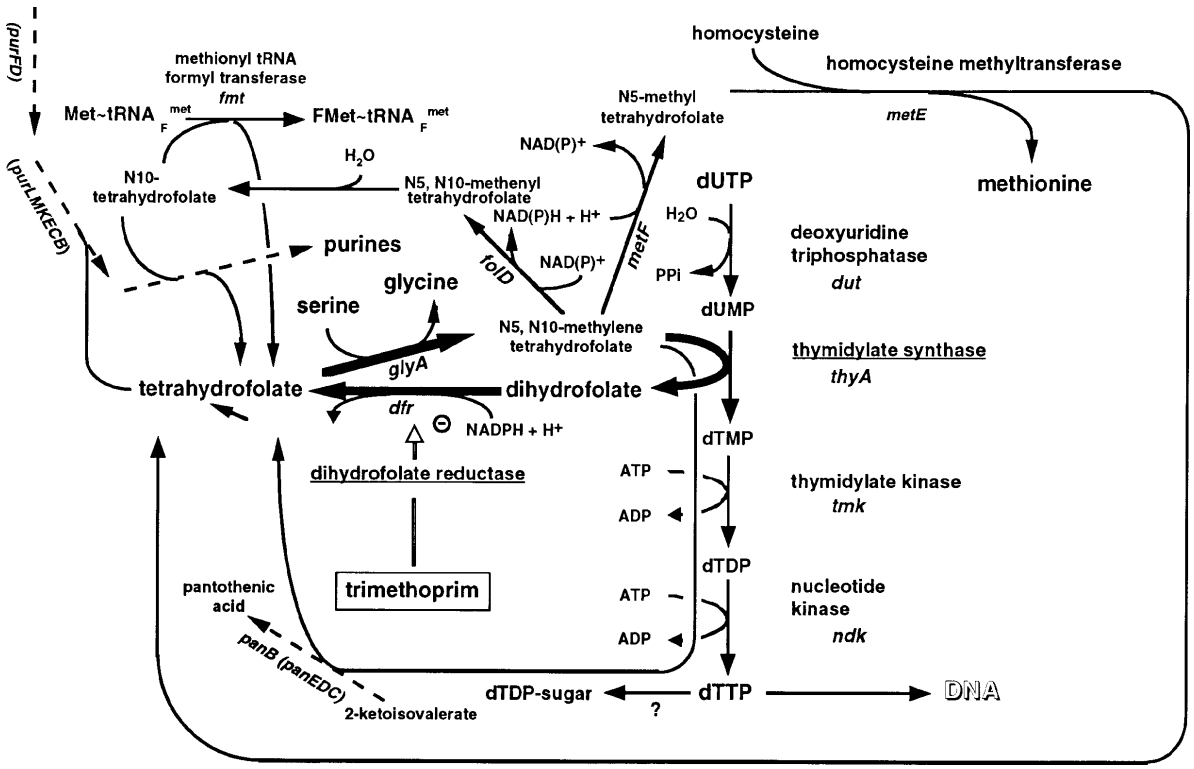

Figure 3. One-carbon residues cycle. Highlighted in the figure is the cycle leading to thymine synthesis, in which tetrahydrofolate is not simply recycled as a coenzyme, but used as a substrate (thick arrows).

following (Figure 2) (Greene, 1996). Biosynthesis of methionine originates from the homoserine pathway (which branches to lysine via diaminopimelate, an essential component of mureine, to threonine, and to isoleucine), starting from the synthesis of an activated derivative, O-succinylhomoserine. This activated homoserine condenses directly with cysteine, giving cystathionine. A *S*-lyase, belonging to a large family of enzymes that allow the cleavage of molecules of the X-CH_2-S-CH_2-Y, or X-CH_2-S-S-CH_2-Z type, from either side of the sulfur atom, liberates homocysteine and serine (which is cleaved into pyruvate and ammonium). Homocysteine is the precursor of methionine, whose methyl group comes from the one-carbon metabolism (Figure 3). In *E. coli*, two enzymes which catalyze this methylation exist. One of them, the product of the *metH* gene (EC 2.1.1.13), utilizes vitamin B12 as a cofactor, and the methyl group can be taken from 5-methyltetrahydrofolate or from its polyglutamyl derivative. The second one, the product of gene *metE* (EC 2.1.1.14), which is not coenzyme B12-dependent, catalyzes the methylation with 5-methyltetrahydropteroyltri-L-glutamate as the methyl group donor (Kung *et al.,* 1972). In both cases the original carbon derives from serine.

One can wonder why there exist two different genes for this metabolic step. In fact, one of them utilizes coenzyme B12 (which necessitates at least 26 steps for its synthesis, starting from uroporphyrinogen III; Michal, 1999) and consumes seven molecules of *S*-adenosylmethionine. *E. coli* does not synthesize coenzyme B12, but possesses a transport system (*btuBCDE* and *btuR*) highly specific and efficient for this coenzyme (Colibri: http://bioweb.pasteur.fr/GenoList/Colibri/). The reaction catalyzed by protein MetH with coenzyme B12 is more than one hundred-fold faster than that catalyzed by the B12-independent enzyme, MetE (EC 2.1.1.13) (Greene, 1996). It follows that the availability in methyl groups (we shall see below how important they are), via methionine, is provided much more easily in the presence than in the absence of coenzyme B12.

Methionine synthesis genes, *metA* (homoserine *O*-succinyltransferase, EC 2.3.1.46), *metB* (cystathionine γ-synthase EC 4.2.99.9), *metC* (cystathionine ß-lyase, EC 4.4.1.8), *metE* (5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, EC 2.1.1.14) and *metF* (5,10 methylenetetrahydrofolate reductase, EC 1.7.99.5) are more or less spread out in the chromosome. The *metB* operon includes another gene, *metL*, that codes for a bifunctional enzyme AKII-HDHII (aspartokinase II/homoserine hydrogenase II, EC 2.7.2.4 and EC 1.1.1.3), belonging to the part of the aspartate derived pathway which branches out to threonine, lysine and methionine synthesis (Saint-Girons *et al.,* 1988).

There exists finally a pathway for recycling of methyl groups, recently found, that utilizes *S*-methylmethionine as a methyl donor. This molecule, synthesized by plants, is scavenged by the product of gene *mmuM* (formerly *yagD*), with a strong similarity to *metH*, *S*-methylmethionine:homocysteine methyltransferase, which transfers methyl group directy onto homocysteine to give methionine (Thanbichler *et al.,* 1999; Table 2).

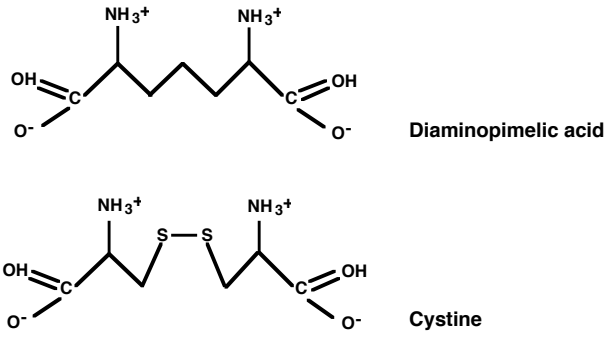

Figure 4. Comparison of cystine and diaminopimelate structure.

Transport of Sulfur-Containing Molecules and Sulfur Scavenging

Amino-Acid and Peptide Permeases

There certainly exist permeases for the sulfur-containing amino-acids: cysteine, its oxidation product cystine, homocysteine, homocystine, and methionine. They have however rarely been characterised without ambiguity. One may think that transport of methionine is carried out by branched-chain amino-acid permeases (*liv* in *E. coli*, *azlCD* and *braB brnQ* in *B. subtilis*), but one must wonder about the possible existence of specific permeases (Greene, 1996). Indeed, there exists a permease for *S*-methylmethionine, encoded by gene *mmuP* (formerly *yfkD*), in an operon with gene *mmuM* (Table 2).

Independently from their transport, the presence of reduced amino acids (cysteine and homocysteine) must pose a problem to the cell, since they are very reactive (reducers), and this must be taken into account when one explores the way in which they permeate the cell. In order to understand their possible effects, it is useful to remark that the distribution of proteins containing cysteine residues is not random in general. In the cytoplasm, one often finds proteins containing a metal, and in particular the diverse types of iron-sulfur centers (often with clustered cysteine residues), proteins containing isolated cysteine residues (often involved in the catalytic mechanism), and proteins containing cysteines burried in their hydrophobic regions. In contrast, the periplasm possesses a strongly oxidizing character (in the presence of oxygen), necessary for the formation of tertiary and quaternary structures of all kinds of proteins through disulfide bridges. Therefore proteins from this compartement are either poor in cysteines, or comprize cysteines involved in disulfide bridges. The intrusion of strongly reducing molecules may therefore have a deleterious role.

Probably, the problem of cysteine toxicity in its reduced form does not occur frequently, because it is not present in nature in significant amounts, but is perhaps present in environments devoid of oxygen. Cystine is likely to be more common, and it is probable that there exists a system permitting its transport, and its rapid metabolism (a ß-lyase for example). This seems more necessary because cystine is structurally similar to diaminopimelic acid (Figure 4), an essential component of many eubacteria cell walls, *E. coli*'s in particular. As in the case of every molecular mimic, it may take the corresponding place, and therefore interfere with the synthesis of mureine, leading to cell lysis (Richaud *et al.,* 1993).

There exist many proteases and peptidases in bacteria (Miller, 1996), and the external media (the gut in particular) such as those faced by *E. coli* often contain peptides. Several transport systems allow their salvage (Dpp, Tpp and Opp (Oliver, 1996)). A specially important case, despite the fact that it has hardly been studied, is that of peptides from the amino-terminal ends of proteins. They often carry a *N*-formyl-methionine group, specific for translation initiation. It has indeed been discovered that in the eucaryote hosts which live in the presence of commensal or pathogenic bacteria, there exist several recognition and transport systems for these peptides (Schiffmann *et al.,* 1975; Prossnitz *et al.,* 1999). This indicates that these molecules are produced and secreted in significant quantity. It seems likely that bacteria also possess transport systems allowing their salvage. Nobody knows, at this time, if the permeases Dpp, Tpp or Opp are adapted to this transport, or if there exist other similar permeases. In the same way, the pathways for the degradation of these peptides allowing methionine salvage, have not been studied. This would be of interest because these molecules can easily be visualized as participating in a new process allowing "quorum sensing".

Transport of Oxygenated Sulfur Sources

Sulfate and thiosulfate are transported into *E. coli* by proteins encoded by operon *cysPUWAM* and by an isolated gene, *spb*. These transport systems are composed of a single permease (encoded by genes *cysUWA*, the products of which are associated with the membrane) and two periplasmic binding proteins specific for these two anions: TSBP (thiosulfate-binding protein), encoded by gene *cysP,* and SPB (sulfate-binding protein), encoded by gene *spb* (Sirko *et al.,* 1995). Another protein, CysZ, is also necessary for transporting sulfate, but its exact function is not known. The two periplasmic proteins, TSBP and SBP, share their specificity for the same substrates. This accounts for the fact that a single mutant is always capable of transporting either anion (less efficiently, which slows down its growth as compared to the wild type only). A double mutant is incapable of transporting either anions.

Many molecules comprising sulfur in an oxidized state (sulfinates: -C-SO_2; sulfonates: -C-SO_3; sulfates -O-SO_3; thiosulfates -S-SO_3) may serve as sulfur sources (Cook *et al.,* 1999). There must exist several families of permeases specific for these different molecules. In *E. coli* only one has been identified and it transports taurine (van der Ploeg *et al.,* 1996). One expects that other ABC (ATP Binding Cassette) permeases transport these molecules. It is therefore probable that among the permeases of unknown function one will discover some that transport the sulfur-containing molecules of this family. As a working hypothesis it would be interesting to see whether there would be a permease in the operon containing gene *ycdM* (coding for a protein similar to a protein annotated in Swiss-Prot as "dibenzothiophene sulfurization enzyme A").

Once in the cell, under the action of appropriate enzymes, the molecules in question liberate their sulfur, in the form of sulfate or sulfite. These anions then enter the biosynthetic pathway that we have just described.

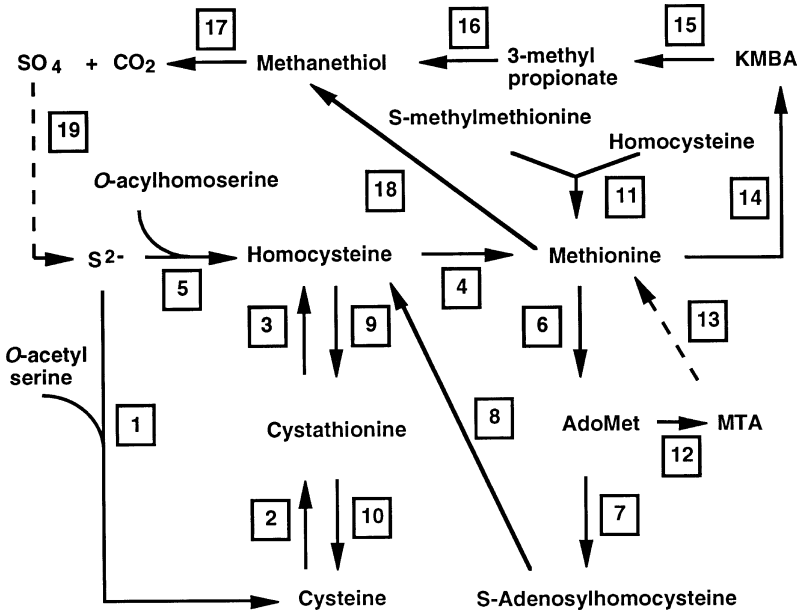

Figure 5. Methionine recycling. In the Figure are represented known pathways, presumed pathways, and unknown but likely pathways. There certainly exist other pathways that we were not able to imagine. 1 : *O*-acetylserine sulfhydrylase, 2 : cystathionine γ-synthase, 3 : cystathionine β-lyase, 4 : methionine synthase, 5 : *O*-acylhomoserine sulfhydrylase, 6 : SAM synthase, 7 : SAM methyltransferase, 8 : adenosylhomocysteine nucleosidase, 9 : cystathionine γ-lyase, 11 : S-methylmethionine:homocysteine methyltransferase, 12 : enzyme yielding MTA (e.g. spermidine synthase), 13 : pathway for MTA recycling (see Figure 9), 14 : methionine aminotransferase, 15 : KMBA decarboxylase, 16 : 3-methylpropionate lyase, 17 : methanethiol dioxygenase, 18 : methionine γ-lyase (this enzyme gives, in addition to methanethiol, ammonium and 2-ketobutyrate), 19 : sulfate assimilation pathway (see Figure 1).

Sulfonate Utilization and Scavenging

Organosulfonates are molecules that contain the SO_3^- group. It is possible to class them according to the nature of the bond they form with the sulfur atom. One thus finds *O*-sulfonates (often named sulfates, with an oxygen bond), *N*-sulfonates and *C*-sulfonates. *O*-sulfonates and *N*-sulfonates are degraded by hydrolases of the general classes EC 3.1.6.- or EC 3.10.1.- (action on the S-N bond), respectively. *C*-sulfonates, which are more stable, are not subjected to hydrolysis (Cook *et al.,* 1999). There exist three mechanisms for degradation of these compounds: (i) activation of carbon at the –C–SO_3^- bond and liberation of sulfite, a reaction catalyzed by thiamine, (ii) stabilization of the –C–SO_3^- bond by addition of an atom of oxygen to the carbon of this bond (directly by oxygenation), which liberates sulfite, and (iii) an unidentified mechanism of reduction. Taurine seems to be degraded, in the majority of cases, by the first mechanism (Cook *et al.,* 1999). In contrast, in *E. coli*, taurine (Eichhorn *et al.,* 1997) as well as most linear and aromatic sulfonates (alkyl-sulfonates and aryl-sulfonates) in most organisms, are degraded by the action of oxygenases (mono-oxygenases and dioxygenases). The general scheme for the reactions is as follows (it is sometimes repeated several times on the successive products of a same reaction):

$$NADH + O_2 + substrate + H^+ \rightarrow NAD^+ + product + HSO_3^-$$

In animals, cysteine oxidized as cysteic acid is decarboxylated into taurine and excreted. Taurine is transported by an ABC-permease (encoded by genes *tauABC* in *E. coli* (van der Ploeg *et al.,* 1996) and *ssuBAC* in *B. subtilis* (van der Ploeg *et al.,* 1998)). It is degraded by an α-ketoglutarate-dependent dioxygenase (*tauD*; (Eichhorn *et al.,* 1997)) into aminoacetaldehyde and sulfite. The fate of aminoacetaldehyde is not known, but there must exist a dehydrogenase to dispose of it. Sulfite subsequently enters the pathway of mineral sulfur assimilation.

In addition to this well characterized operon, there exist several operons in *E. coli* with features that are reminiscent of the systems for sulfur oxide scavenging. This is the case of likely monooxygenases (encoded by the gene *ycbN* in an operon with *ycbO* and *ycbM*), or of dioxygenases (YeaW and YeaX). Protein YcbN is a protein (monooxygenase) identifed in the study of the proteome as induced in sulfur starvation, which substantiates this hypothesis (Quadroni *et al.,* 1996 and Swiss-Prot P80645). However, its substrate is not known. In the case of dioxygenases one may wonder whether a dioxygenase identified as having 3-phenylpropionate as a substrate (YfhUVWXY) (Burlingame and Chapman, 1983; Burlingame *et al.,* 1986) may also act on sulfur-containing molecules. There exist other such enzymes as YeaW and YeaX, for which no substrate has been described. It seems interesting to perform a detailed analysis of what might be the natural substrates of reactions of this type in a biotope similar to that of *E. coli*. This would allow to explore much more efficiently the nature of many genes of unknown function in its genome. On the other hand, cysteine may have been oxidized to a lower state, as sulfinate, and it seems that some gene products related to the *nifS* gene may act as sulfinases (Mihara *et al.,* 1997).

Finally, it would be important to investigate the fate of *O*- or *N*-sulfonated carbohydrates, such as those present in many polysaccharides such as heparine, chondroïtine sulfate, and in plants, carragenanes or agar-agar. We have verified that *E. coli* cannot grow on agar-agar in the absence of sulfur source, but this does not tell us whether it can grow in presence of the corresponding monomers. Aryl-sulfonatases, most often studied for their action on xenobiotics, may well have a spectrum of action much wider than their action on aromatic substrates.

Methionine Recycling and Equilibriated Pools of Sulfur

In the metabolic pathways that have just been described, cystathionine plays a central role since, by its very structure, it allows going indiscriminately from cysteine to homocysteine and vice versa, as long as there exist ß-

lyase and γ-lyase, together with ß-synthase and γ-synthase activities (Figure 5). Therefore the organisms carrying these activities should grow equally well with cysteine or homocysteine as sulfur sources. Growth on methionine requires a methyl group transfer reaction. But as *S*-adenosylmethionine is involved in a large number of activities that produces homocysteine, this should be easily achieved. Furthermore, the reaction catalyzed by MmuM, shows that the transfer of a methyl group from a sulfonium group is not difficult to achieve (Thanbichler *et al.,* 1999). Finally, the reaction homologous to that leading from serine to cysteine must easily result from the evolution of the corresponding proteins and allow the use of homoserine as a substrate in the place of serine (*O*-acylhomoserine sulfhydrylase, or even homoserine phosphate sulfhydrylase; (Michal, 1999)). One can therefore easily appreciate the versatility in the utilization of sulfur sources in different organisms. Take the case of *Pseudomonas putida* for example: this organism can use not only the various standard sulfur molecules such as sulfur amino acids, but also all kinds of *C*-, *O*- or *N*-sulfonated molecules as sulfur sources (Vermeij and Kertesz, 1999). All depends on the selection pressure of the biotope in which bacteria strive to survive.

Considering its biotope, it is remarkable that *E. coli*, which can grow on cysteine, cannot grow on methionine (Kredich, 1996). This inability is certainly due to the lack of a pathway, the loss of which may result from the fact that the anaerobic medium in which *E. coli* generally lives is a sulfur-rich medium. When bacteria reach the stationary phase of growth, when they need not much neosyntheses, but are using S-adenosylmethionine for methylation and polyamine biosynthesis (see below), the problem that was posed to them may have been to eliminate the sulfur excess rather than its elaborate utilization. Recycling sulfur, which is essential during exponential growth, might have been a burden at this stage. In *E. coli*, there is no bypass from methionine (or homocysteine) to cysteine. *E. coli* metabolizes cystathionine only by the action of a ß-lyase (MetC), the γ-lyase being apparently absent from its genome. Interestingly, this lack of continuity in sulfur metabolism allows *E. coli* to use selenomethionine in the place of methionine, as was shown by Georges Cohen (Cohen and Cowie, 1957; Cowie and Cohen, 1957). We have indeed verified that *E. coli* was not able to grow with methionine as a sulfur source. It follows that *E. coli* is an organism of choice for testing by heterologous complementation the existence of sulfur equilibrating pathways in other microorganisms.

Methionine is the starting point of several cycles. The turnover rate of these cycles becomes particulary important when sulfur is limiting (in plants for example). The first of these cycles concerns the process of translation of messenger RNAs into proteins. All the proteins of the cell start with a methionine residue, which confers a particular importance to this amino acid. In Bacteria, translation begins in an original way. The initiator methionine is modified by a formyl group, and all neosynthesized proteins start with a *N*-formyl-methionine. A particular transfer RNA, $tRNA_F^{met}$ is first charged by methionine as its homologous $tRNA_M^{met}$, by the action of methionine tRNA synthetase, encoded by gene *metG* in *E. coli* (*metS* in *B. subtilis*). The remarkable feature comes now. This Met-$tRNA_F^{met}$ is formylated by a transformylase (identified both in *E. coli* and *B. subtilis*, *fmt*), that utilizes 10-formyltetrahydrofolate as donor of the formyl group (cf. (Danchin, 1973)). It is this FMet-$tRNA_F^{met}$ that, in the presence of translation initiation factor IF2 (encoded by gene *infB*) and GTP, will correctly position this charged tRNA at the translation initiation site of a messenger RNA (at codons AUG, UUG or GUG). In the presence of a transfer RNA corresponding to the subsequent codon, charged with its cognate amino acid and loaded on factor EF-Tu in the presence of GTP and then factor EF-G bound to GTP, the first peptidic link is formed. Let us note here a second oddity: the formation of this first peptidic link, except if it links FMet to an aromatic amino-acid, necessitates another factor, named EFP (encoded by gene *efp*) (Aoki *et al.,* 1997). The precise role of protein EFP, which is an essential protein, remains to be determined. Interestingly, we must remark that factor EFP is homologous to the eucaryotic factor eIF5-A (a family of highly conserved essential proteins), that carries a residue derived from spermidine, hypusine. The need for its presence suggests that it operates at a "fragile" step of translation, and that at this stage this process can be spontaneously interrupted. Also, the role of factor EFP in the liberation of short peptidyl-tRNA must be taken into account when one wishes to count the pool of methionine available in the cell. The synthesis of the protein then goes on normally.

One observes therefore that, as a function of the efficiency of this step (and more generally of every first steps of translation), one will obtain either a complete protein, carrying FMet at its extremity, or a transfer RNA carrying a peptide that will be hydrolyzed by peptidyl-tRNA hydrolase (EC 3.1.1.29), an essential protein encoded by gene *pth* (Heurgue-Hamard *et al.,* 1998). The need for the step of dissociation of peptidyl-tRNA from the ribosome is apparently necessary to ensure the accuracy of translation (Heurgue-Hamard *et al.,* 1998).

A general assessment of translation indicates that at least 10% of initiated translations are abortive (Heurgue-Hamard *et al.,* 1998). This shows that the effect of formation of the first peptidic link on the utilization of sulfur is far from negligible. As a consequence, during exponential growth when many proteins are synthesized, all starting with a formyl-methionine residue, there exists probably a recycling process permitting methionine salvage from peptidyl FMet-aa_n. An alternative to recycling is excretion of formylated peptides, but this leads to sulfur leakage, in the form of methionine. This possibility has not yet been explored. We have already indicated that FMet-containing peptides are recognized by receptors in eucaryotes (Prossnitz *et al.,* 1999), showing that this mechanism is most probably significant, at least under particular growth conditions. It may be involved in "quorum sensing", for instance.

A second series of reactions associated with translation plays a general role. The neosynthesized protein must be deformylated by a deformylase (specified by gene *def*, in an operon with *fmt*, in *E. coli* and *B. subtilis*). The sequence of these two steps - formylation and deformylation - must have an important role, since it is conserved throughout evolution in all eubacteria (and even in mitochondria and chloroplasts). But, oddly enough, it does not appear to be absolutely necessary since *E. coli* can grow without formylation, in particular when ribosomal protein S12 is altered to become streptomycin resistant (Danchin, 1973; Harvey, 1973; Petersen *et al.,* 1978; Mazel *et al.,* 1994).

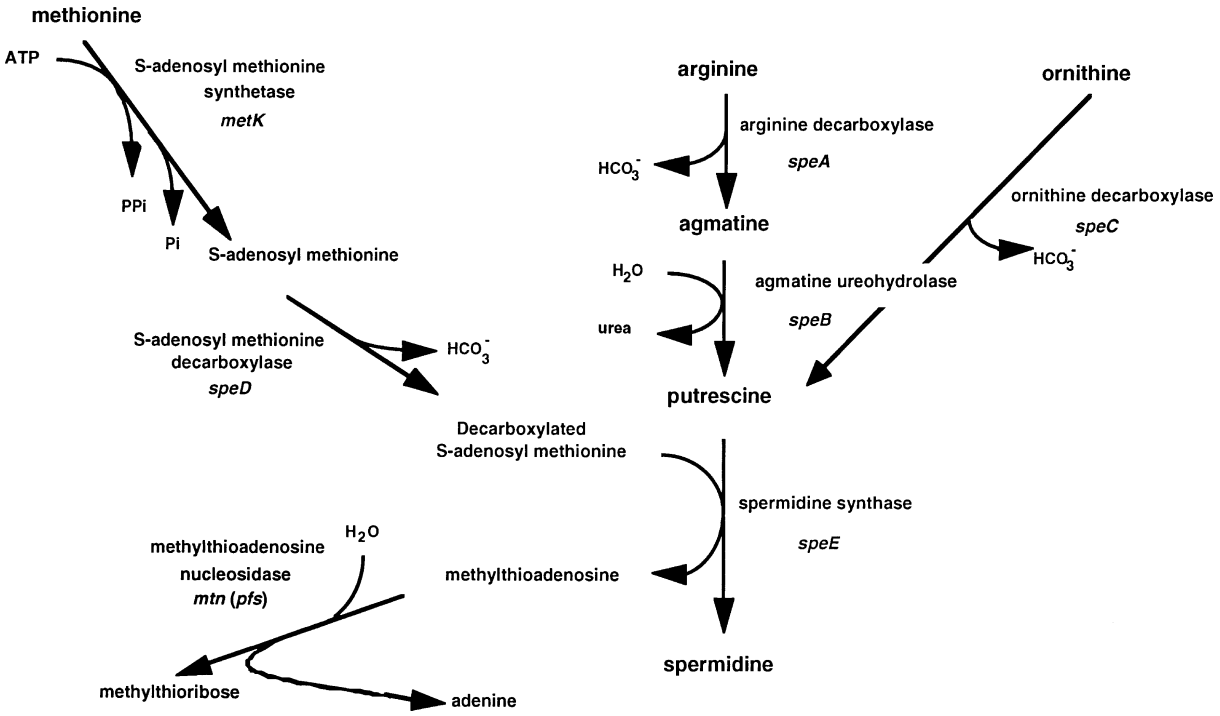

Figure 6. Polyamines biosynthesis in *Escherichia coli*.

Subsequently, methionine is cleaved by an original amino-peptidase.

The successive action of these two enzymes (deformylase and methionine aminopeptidase) recycles methionine. This liberates the amino-terminal extremity of the protein, which may thus be submitted to the degradative action of diverse aminopeptidases, allowing fine regulation of the concentration of the corresponding protein in the cell (see Varshavsky's "N-end rule" (Bachmair *et al.,* 1986)). It is essential to remark that the cleavage of the N-terminal methionine can only happen after deformylation (Solbiati *et al.,* 1999). The activity of deformylase is controlled by an essential ferrous ion, which makes it particularly sensitive to the presence of oxygen (Rajagopalan and Pei, 1998). Taken together, these observations show that these steps probably play a crucial role in the regulation of gene expression, in particular in the presence of oxygen.

Catabolism and Repair of Sulfur-Containing Amino Acids

Catabolism

The *E. coli* biotope is not likely to be limited in sulfur. Many observations substantiate this contention: cysteines are present in large quantity in its proteins. And, despite the need for a highly regulated cycle of methionine recycling during exponential growth, the *E. coli* metabolism does not seem to care for sulfur availability, at least during the stationary phase of growth. This is illustrated by the following example. Spermidine biosynthesis produces a sulfur-containing molecule - methylthioadenosine (MTA) (Figure 6 and see below). In a close relative of *E. coli, Klebsiella pneumoniae*, this molecule is transformed into methylthioribose-1-phosphate and recycled (see below). In *E. coli* however, it is excreted (Schroeder *et al.,* 1973), which implies an enormous loss of sulfur, because for each molecule of spermidine a molecule of methylthioribose (MTR) is produced and excreted (and we shall see that other reactions involving AdoMet produce also MTA)! This, however, may account for the fact that spermidine is not the major polyamine in *E. coli*, where putrescine appears to be present at a high concentration, in contrast to most other bacteria (Sekowska *et al.,* 1998; Cohen, 1998).

What do we know more generally about sulfur sources for *E. coli*? In its mammalian hosts, apart from quite varied sources (depending on the diet), which provide sulfur amino acids (at least when the host does not feed on purely vegetarian food) and sulfur containing vitamins, the major part derives probably from two pathways of cysteine degradation: cysteine-sulfinate (sulfinoalanine)-dependent, and cysteine-sulfinate independent, because methionine is recycled inside the cell (and its sulfur lost as MTR). Another sources is derived from the oxydation of cysteine into cysteic acid.

The cysteine-sulfinate-dependent pathway arises by oxidation of cysteine by cysteine dioxygenase (CDO, EC 1.13.11.20), which produces cysteine-sulfinate (sulfinoalanine). The latter can be catabolized in two different ways. Either it is transaminated by an aspartate aminotransferase (AAT, EC 2.6.1.1) with production of pyruvate and sulfate, or it is decarboxylated by cysteine sulfinate decarboxylase (sulfinoalanine decarboxylase, CSAD, EC 4.1.1.29) into hypotaurine. Hypotaurine is probably oxidized non-enzymatically into taurine (Bella and Stipanuk, 1996). Neither animals, nor plants (where it is rarer) can metabolize taurine. The excess of taurine produced by animals is excreted either directly in urine, or in bile in the form of taurocholate. This molecule is therefore abundant in *E. coli*'s diet. Cysteine, in the presence of

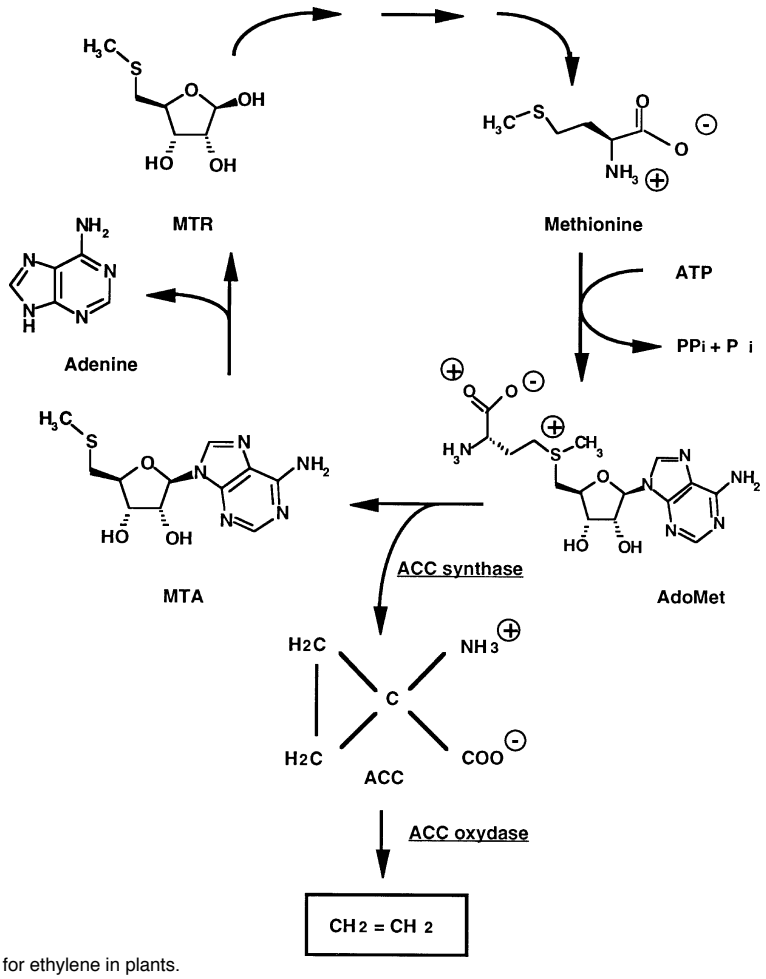

Figure 7. Biosynthesis pathway for ethylene in plants.

oxygen, can be oxidized spontaneously into cysteic acid (frequent oxidation in aging proteins). Cysteic acid is decarboxylated directly into taurine by sulfinoalanine decarboxylase (CSAD, also named sulfoalanine decarboxylase, an enzyme specific of two similar substrates, cysteine-sulfinate and cysteate; see Swiss-Prot: EC 4.1.1.29 and KEGG: MAP00430). Alternatively it is transformed in the presence of H_2S into cysteine and sulfite by a cysteine lyase (EC 4.4.1.10). However, most of these reactions are unknown (and, in fact, a cysteine sulfinate sulfinase has just been described; Mihara *et al.,* 1997), and these reactions (Swiss-Prot: http://www.expasy.ch/cgi-bin/nicezyme.pl?4.4.1.10) are just presented as hypotheses reflecting our ignorance, and as suggestions for further exploration.... As an illustration of this metabolism in bacteria (it does not appear to exist in *E. coli*, but it may exist in other enterobacteria), one finds in *B. subtilis* a gene (*yubC*) that looks like a cysteine dioxygenase, enzyme known to regulate the intracellular level of cysteine, of methionine and of glutathione in mammals (SubtiList: http://genomeweb.pasteur.fr/GenoList/SubtiList/) (Eppler and Dawson, 1998). This, together with the fact that *B. subtilis* possesses a very small number of cysteines in its proteins, may indicate a physiological role particular to this amino acid.

Metabolism and Repair of Oxidized Sulfur Amino Acids

Disulfide bridges are very important structural elements of many proteins. They are extremely rare in cytoplasmic proteins because of the reducing nature of the intracellular medium. In contrast, the periplasm possesses a strongly oxidizing character (in the presence of oxygen). An environment with this character is necessary to the formation of tertiary and quaternary structures of all kinds of proteins, via the formation of disulfide bridges. These covalent bonds are essential to the stability and activity of many extracellular proteins. This has been demonstrated in pathogenic bacteria in the case of some toxins, secreted cellulases and pectate lyases (Missiakas *et al.,* 1995). The corresponding oxidation mechanism (2 Cys -> Cys-S-S-Cys + 2 H^+ + 2 e^-), can be spontaneous in the presence of oxygen, but in *E. coli* the proteins of the family Dsb (DiSulfide Bond formation) that catalyze the formation of appropriate disulfide bridges, have an essential function. This family comprises proteins DsbA, DsbB, DsbC and DsbD (Missiakas *et al.,* 1995) and proteins DsbE and DsbF that have been recently discovered (Metheringham *et al.,* 1996). Among these proteins, DsbA and DsbC that are located in the periplasm have a strong oxidizing activity on cysteines. The membrane protein DsbB is essential for the recycling of the active site (active thiol) of DsbA, but the mechanism of this reaction is not yet known. Protein DsbD

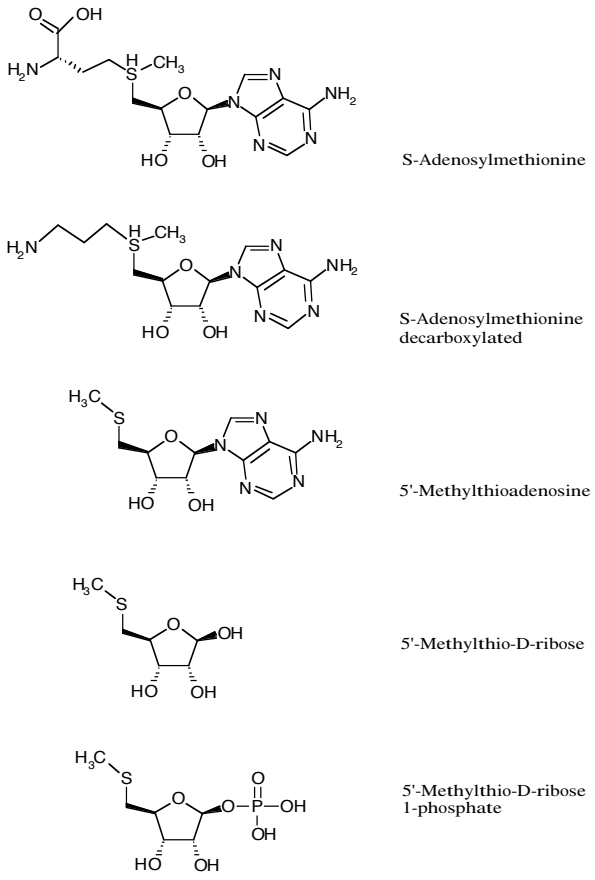

Figure 8. Structure of the *S*-adenosylmethionine derivatives.

counteracts the action of the two oxidizing forms (A and C) by reduction of disulfide bridges in an equilibrium between the thiols and the disulfide bonds in the periplasm. The role of Dsb proteins in the shaping of proteins is fundamental, and one can therefore wonder about the nature of their function: are they enzymes, or molecular chaperones? The case of protein DnaJ illustrates well the question. Identified as a chaperone, it possesses an enzymatic activity capable of reducing, oxidizing, or isomerizing disulfide bridges (Wang and Tsou, 1998).

Bader *et al.* reconstituted the oxidative folding system using purified DsbA and DsbB. They identified the sources of oxidative power for protein folding and showed how disulfide bond formation is linked to cellular metabolism: disulfide bond formation is directly coupled to the electron transport chain. DsbB uses quinones as electron acceptors, allowing various choices for electron transport to support disulfide bond formation. Electrons flow via cytochrome b_o oxidase to oxygen under aerobic conditions or via cytochrome b_d oxidase under partially anaerobic conditions. Under anaerobic conditions, menaquinone shuttles electrons to alternate final electron acceptors such as nitrate or fumarate (Bader *et al.,* 1999).

Intracellular proteins are mainly present in the thiol form, with a low representation of cysteines (1.6%), whereas extracellular proteins contain disulfide bridges and are rich in cysteines (4.1%) (Fahey *et al.,* 1977). This absence of disulfide bridges inside the cell, as we have seen, is linked to the strongly reducing character of the cytoplasm (production of NAD(P)H by the catabolic processes of respiration and glycolysis). In *E. coli*, many factors participate in the maintenance of the balance between thiols and disulfide bridges in the cytoplasm (disulfide bridges can be formed by the action of free radicals, active oxygen or cosmic rays, which lead to inactivation of the proteins sensitive to their action). In the cytoplasm the main system maintaining the correct ratio between thiols and disulfide bridges is constituted by a tripeptide containing cysteine, glutathione (see also below). Glutathione is present in *E. coli* at a high concentration (about 5 mM), and it is maintained almost entirely in the reduced form (redox potential of -230 mV). the ratio between reduced and oxidized glutathione is between 50:1 and 200:1 (Prinz *et al.,* 1997).

E. coli possesses at least four thiol-disulfide oxidoreductases that permit reduction of the disulfide bridges of cytoplasmic proteins: a thioredoxin (encoded by gene *trxA*) and three glutaredoxins (1, 2 and 3, encoded by genes *grxABC*). Thioredoxin is a reducing protein more efficient (redox potential of -270 mV) than glutaredoxins (redox potential of -233 mV to -198 mV). After reducing the disulfide bridges, the thiol-disulfide oxidoreductases are oxidized. A system based on the transfer of protons from NADPH to these proteins allows their reduction, a *sine qua non* step to keep them functional. Thioredoxins are reduced directly by a thioredoxin reductase (FAD enzyme, EC 1.6.4.5, encoded by gene *trxB*), whereas glutaredoxins are reduced by glutathione, that in its turn is reduced by glutathione reductase (FAD enzyme, EC 1.6.4.2, encoded by gene *gor*). In *E. coli*, these two systems: thioredoxin reductase and thioredoxin, and glutaredoxin reductase, glutathione and glutaredoxin, participate in the reduction of disulfide bridges of essential cytoplasmic enzymes that need this step of reduction in order to accomplish their catalytic function (for example ribonucleotide reductase, PAPS reductase or peptidyl methionine reductase).

One mechanism to regulate the activity of redox proteins in the cell is through reversible formation of disulfide bridges. This is often achieved with the help of glutathione, glutaredoxin, or thioredoxin. Thus, the activity of key redox regulatory proteins is responsive to the intracellular thiol-disulfide redox status. For example, OxyR, the prototypic redox-regulated transcription factor in *E. coli*, is activated through the formation of a disulfide bond using cysteine sulfenic acid as an intermediate, and is deactivated by enzymatic reduction of this disulfide bond with glutaredoxin 1 (Zheng *et al.,* 1998b; Aslund *et al.,* 1999). Likewise, the catalytic mechanism by which peroxiredoxins scavenge hydrogen peroxide or alkyl hydroperoxide also involves reduction and re-formation of disulfide bonds with a cysteine sulfenic acid as intermediate (reviewed in (Jin and Jeang, 1999)). Known peroxiredoxins in *E. coli* include AhpC and scavengase p20/Tpx (Chae *et al.,* 1994; Wan *et al.,* 1997; Zhou *et al.,* 1997).

The oxidation of methionine is a case of damage that can happen to proteins through action of endogenous or environmental oxidizing agents. Although in some cases oxidation does not have a large effect on the activity of proteins, in the majority of cases it abolishes their catalytic function. The cell has two ways to take into account proteins

containing oxidized methionine residues (Met(O)). Oxidized proteins are either degraded, or an enzyme can reduce the modified residues. This latter function is performed by peptidyl-methionine sulfoxide reductase MsrA (encoded by gene *msrA*) that reduces oxidized residues in the protein, restoring its function (Moskovitz *et al.,* 1995). This enzyme, apart from its capacity to repair oxidized residues in proteins, can also reduce methionine sulfoxide in its free amino-acid form. It permits therefore utilization of methionine sulfoxide as a source of sulfur in bacteria.

An interesting observation established recently a relationship between the capacity of repair of oxidized methionines and virulence in enterobacteria pathogenic for plants, *Erwinia chrysanthemi* (Hassouni *et al.,* 1999). Alteration of virulence (inability to cause systemic invasion) is associated with enhancement of sensitivity to oxidative stress and with the decrease of motility on a solid substrate. It involves the *msrA* gene, coding for peptidyl methionine sulfoxide reductase. Indeed, the mutants of this gene can no longer repair their proteins altered by the defensive action of plants (a process that utilizes active oxygen and free radicals). Moreover, motility, an essential factor of virulence, in particular motility on solid surface is affected in mutant *msrA*. Curiously, the *msrA* mutants remain motile in liquid medium. This shows that the affected mechanisms do not involve flagellae. It is possible that the target process is not the process of movement creation but, for example, sensing or adhesion. Combining these data, one can imagine that MsrA has the double function of a general repair system and a regulator of the production of extracellular appendages. The production of these appendages may be under the control of a regulator extremely sensitive to oxidative stress, normally repaired by MsrA. In any event, peptidyl methionine sulfoxide reductase is a particulary important protein since it seems to be present in all extant organisms: it belongs to the class of genes that Eugene Koonin named “the minimal gene set” (Mushegian and Koonin, 1996).

S-Adenosylmethionine and its Metabolism

Methionine is also an essential element in a large number of methylation processes - it is a universal methylating agent (and it was even proposed that it could act in the absence of enzymes, but this has not been found to be the case; Posnick and Samson, 1999) - via *S*-adenosylmethionine (SAM or AdoMet). Some of these methylations are essential to cell functioning, and SAM synthetase is therefore an essential enzyme. The exact role of these methylations is often poorly understood, but it is a type of chemical modification so frequent that it probably indicates a metabolic need linked to the availability in one-carbon residues (“one-carbon pool”). Sulfur in its sulfonium form is an ideal atom as an intermediate, being both a receptor and donor of alkyl groups. As a case in point, it appears now that another molecule contained in this group, *S*-methylmethionine, also plays an important role both in the storage and in the exchange of methyl groups (Thanbichler *et al.,* 1999).

Biosynthesis of *S*-Adenosylmethionine

AdoMet is synthesized from methionine and ATP by SAM synthase (EC 2.5.1.6), the product of gene *metK*. AdoMet is an essential molecule. This is due to the frequent involvement of AdoMet in the regulation of gene expression (cf. (Thomas *et al.,* 1991; Chen *et al.,* 1999)) and this accounts well for the ambiguous results of the genetics of *metK*. SAM synthase being essential, the *metK* gene product can never be totally inactive. If one obtains, among ethionine resistant mutants, mutants that make little AdoMet, they are nevertheless not entirely deprived of it (Saint-Girons *et al.,* 1988). One should not forget this fact, that has long made scientists think that there existed two AdoMet synthetases in *E. coli* (which is true in *S. cerevisiae*, but not in *E. coli*) (Thomas and Surdin-Kerjan, 1987; Greene, 1996).

AdoMet synthetase condenses methionine with ATP by hydrolyzing the latter into phosphate and pyrophosphate. In turn, pyrophosphate is cleaved by pyrophosphatase, pulling the reaction toward the biosynthesis direction:

$$ATP + methionine \rightarrow AdoMet + P_i + PP_i$$

This is therefore a very energy costly reaction since it consumes three energy-rich bonds, while creating only one, that of the sulfonium group. The sulfur atom of methionine is, in the molecule of AdoMet, positively charged. It is coordinated to three groups that it can theoretically donate in a more or less equivalent way: a methyl- group, an aminobutyryl- (or, more aptly, 3-amino-3-carboxylpropyl-) group and an adenosyl- group. It is generally the methyl-group which is donated, but the other reactions are possible (a variant is seen in the synthesis of spermidine, with transfer of the aminopropyl- group from dAdoMet). For example, some transfer RNA molecules ($tRNA^{Phe}$) can be modified with the 3-amino-3-carboxylpropyl- group (Nishimura *et al.,* 1974). A last type of transfer (transfer of the ribosyl- group) is the basis of another complicated modified base of tRNA, queuosine (Slany *et al.,* 1993).

Because of its reactivity this sulfonium group can be spontaneously hydrolyzed, and it is the masking from water molecules that allows its reactions with other acceptor substrates. In the presence of water it spontaneously yields methionine and adenosine, methylthioribose and homoserine, or methanol and adenosylhomocysteine. Therefore, a strict control of the pool of AdoMet in the cell must exist.

The main product of the transmethylation reaction is S-adenosylhomocysteine. In *E. coli*, this molecule seems to be recycled in the following way. A nucleosidase (EC 3.2.2.9), encoded by gene *pfs* (see below) hydrolyses this molecule into adenine and *S*-ribosylhomocysteine, which hydrolyses spontaneously into homocysteine and ribose (Della Ragione *et al.,* 1985). Homocysteine is converted into methionine, and ribose and adenine must be recycled (but we did not find a general assessment of these reactions). In plants and animals, there exists an adenosylhomocysteinase (EC 3.3.1.1) that produces adenosine and homocysteine directly. This seems more economical and produces adenosine, an important mediator of some cascades of regulation (Cohen, 1998). It seems clear that it would be useful to characterize more explicitly the outcome of these pathways in bacteria.

The Reactions of AdoMet Sulfonium Group

Methylations

Methylation reactions are very numerous. The best known, because they are better understood, are those which result in the methylation of DNA, in particular to protect DNA against restriction enzymes (in general the genes are in an operon coding for a DNA methylase, followed by the restriction enzyme: a case in point is the *hsdRM* operon in *E. coli* K12). They can also be involved in covalent modifications leading to processes of epigenetic heredity (as sexual imprint in vertebrates, or controlling the form of flowers (Cubas *et al.,* 1999)).

Methylation of Nucleic Acids

Most often, methylated sites are located at position N6 of adenine, or C5 of cytosine, but all kinds of reactions are possible: for example, positions 2' or 3' of ribose can also be methylated, as well as the amino group of adenine.

In *E. coli*, two major sites of methylation of DNA are observed: on the one hand the adenine present at site GATC, is methylated by Dam methylase, and on the other hand the second cytosine sites recognized by enzyme *Eco*RII CC(A/T)GG, by methylase Dcm (Urieli-Shoval *et al.,* 1983). GATC methylation sites are involved in at least three fundamental processes: (i) the replication proof-reading system ("long patch mismatch repair"), where protein MutH cleaves the unmethylated strand of sequence GATC, and hydrolyses the daughter strand when it interacts with a mismatch base pair recognized by the MutS MutL complex, followed by the repair of this strand by DNA polymerase I (Laengle-Rouault *et al.,* 1986); (ii) the fixation of protein DnaA, to permit correct initiation of replication of DNA at the Ori region (Campbell and Kleckner, 1990); (iii) the control, possibly epigenetic in part, of the transcription of genes involved in pili formation (Blyn *et al.,* 1990). Finally, an analysis of the genome sequence suggested a particular role of GATC sequences, perhaps hemimethylated, in the control of the transition of growth from anaerobiosis to growth in the presence of oxygen (Hénaut *et al.,* 1996). A recent work on the formation of *E. coli* biofilms controlled by the regulator OxyR which binds to regions containing several GATC substantiates experimentally this hypothesis (Hasman *et al.,* 1999). The case of DNA regions methylated by protein Dcm corresponds mostly to the correction of very local errors ("very short mismatch repair") (Lieb *et al.,* 1986).

RNA molecules are also often methylated: it is the case of many positions in transfer RNA (Bjork, 1996), in particular of the thymine residue in the TΨCG loop (by TrmA). Some positions in ribosomal RNA may be methylated permanently (Bjork, 1996), or in the presence of genes conferring resistance to some antibiotics (such as erythromycine (Thakker-Varia *et al.,* 1985)). The role of these methylations is poorly known, and inactivating the corresponding genes does not give a simple phenotype (apart from a variation in the accurracy of translation, (Bjork *et al.,* 1999)).

In the case of ribosomal RNA, eubacteria differ from eucaryotes and archaebacteria in that it is much less methylated, and probably methylated by methylases specific for each methylated region. In contrast, in the latter cases, it seems that a small set of methylases recognize structures made of ribosomal RNA associated with guide RNAs, that can methylate several regions of the rRNA.

Protein Methylation

Many proteins may also be methylated: ribosomal proteins (Chen *et al.,* 1977; Mardones *et al.,* 1980); chemotaxis control proteins (methylation of a glutamate residue in the chemotaxis receptors by methyltransferase CheR and demethylation by methylesterase CheB (Simms *et al.,* 1987)); *N*-methylation of the amino-terminal residue of some proteins (Stock *et al.,* 1987); or methylation of isoaspartate residues in aging proteins. In this latter case, methylation on the aspartate group creates an unstable methyl bond, that is corrected following spontaneous hydrolysis (Visick *et al.,* 1998).

The sites of these methylations are varied: it is often the ε-terminal group of lysines, but one also finds histidine (Chen and Bodley, 1988), aspartate, glutamate, arginine (Rawal *et al.,* 1995), and glycine (Ogawa *et al.,* 1998) residues, or the N-terminal extremity of proteins. In this latter case, the modifications concern methionine, phenylalanine, alanine or proline residues, which leads to the formation of monomethylmethionine, monomethylphenylalanine, monomethylalanine, trimethylalanine and dimethylproline (Stock *et al.,* 1987). Little is known concerning methylation of proteins in bacteria, and it is probable that the study of the proteome will lead to suprising discoveries in this domain.

Methylation of Metabolites

AdoMet is involved in the synthesis of a large number of metabolites, and in particular of essential coenzymes such as ubiquinone, menaquinone, siroheme and vitamin B12. One also finds detoxification systems (methyl-selenocysteine), antibiotics and all kinds of molecules involved in the cell's achitecture (terpene derivatives, sterols, lignin) or metabolites involved in the regulation of osmotic pressure (betaine, choline). In plants, one finds derivatives of methionine, such as *S*-methylmethionine (SMM) (James *et al.,* 1995) or *S*-methylcysteine (Chow *et al.,* 1972). The role of SMM is not clear but it is used by plants as an osmoprotectant. It has recently been implicated in the metabolism of selenium. Its methyl group can be transfered to homocysteine to give two molecules of methionine (role of storage for methyl groups). In plants, SMM can also be cleaved into dimethylmercaptan and homoserine or, finally, decarboxylated in the presence of pyridoxal-phosphate and metal ions. This decarboxylation product (dimethylsulfonium propylamine) is an element active in the anticancer molecule, bleomycine A_2 (Cohen, 1998). Some of these metabolites exist in diverse strains of *E. coli*, and it is likely that among the unknown genes of pathogenicity islands one finds genes involved in some of these methylations.

The Aminobutyryl Group and its Avatars

The second group which may be donated by AdoMet is the aminobutyryl group. It has been found recently that the aminobutyryl group is utilized by the cells in several reactions.

Quorum Sensing

Bacteria are often considered as isolated individuals, incapable of the organized behaviors observed in multicellular organisms. However, W. Hastings, who

studied marine bacteria forming luminescent colonies, discovered twenty years ago that their capacity to emit light was entirely determined by their relative number (Wilson and Hastings, 1998). These bacteria had to be concentrated enough to produce light. It appeared that they could measure their number to induce expression of the genes necessary for light production. This supposed, as in general assemblies, that a "quorum" was reached, hence the name of "quorum sensing" given to this phenomenon. "Quorum sensing" is a mechanism of communication between bacteria that leads them to display an organized collective behavior.

The following questions were asked: what are the molecules secreted by the bacteria, that permitted them to trigger this phenomenon, how these molecules were synthesized, and what did they control?

Among the many molecules that carry this information (autoinducers) one may distinguish at least two classes. Gram-negative bacteria produce N-acyl homoserine lactones (*N*-acyl HSL), small molecules composed of homoserine cyclised in lactone and an aliphatic chain. Other bacteria, such as *Staphylococcus aureus* or *B. subtilis*, produce peptidic autoinducers. In the case of Gram$^-$ bacteria, the paradigm for the study of quorum sensing is *Vibrio fisheri* or *V. harveyi*. In these bacteria, a protein belonging to the LuxI family catalyzes the formation of *N*-acyl HSL from a molecule of *S*-adenosylmethionine and acyl-ACP (acyl-Acyl Carrier Protein) (Val and Cronan, 1998; Parsek *et al.,* 1999). AdoMet is used in this reaction as a donor of the aminobutyryl group (it participates in the creation of the lactone cycle), which produces a molecule of methylthioadenosine (MTA). The aliphatic chain (in general short-chained) comes from the biosynthesis of fatty acids, and it is transported by ACP. Carrying an aliphatic chain of a certain length (but not too long) permits free diffusion of the molecule through the cell membrane, even in the absence of permease. This leads to a rapid equilibration of its concentration between the external medium and the inside of the cell.

When this concentration reaches a threshold value, the regulator effects of "quorum sensing" begin to control a large number of processes, such as: luminescence (*V. fisheri,* or *V. harveyi*), conjugative transfer of plasmids (*Agrobacterium tumefaciens*), collective movement of bacteria (swarming) (*Serratia liquefaciens*), synthesis of some reserve molecules (e.g. poly-3-hydroxybutyrate, *V. harveyi*), or finally virulence (Val and Cronan, 1998).

The Synthesis of Ethylene

The gas ethylene (CH_2=CH_2) is the first multifunctional gaseous hormone that has been discovered (in 1934, in plants), well before nitrogen monoxide, curiously now much more famous (Cohen, 1998). Ethylene participates, among other processes, in fruit ripening, plant aging and/or in the formation of roots or flowers. In plants, ethylene is derived from methionine, or more precisely of AdoMet. Ethylene is also synthesized in bacteria such as *E. coli*, *Pseudomonas syringae* or the fungus *Cryptococcus albidus*. The existence of microbes producing ethylene indicates a possible interference with the plant metabolism during infection of microbial origin.

In microorganisms, there are at least two known pathways for ethylene biosynthesis. A first one starts with methionine (as in plants) and the second one uses α-ketoglutarate as a precursor. In *Penicillium digitatum*, there exists yet another precursor, 2-keto-4-methylthiobutyrate (KMBA), produced by the transamination of methionine (or, as we shall see, the product of recycling of methylthioribose in *K. pneumoniae*). KMBA can be directly converted into ethylene. The main pathway using AdoMet as a precursor, is composed of two enzymes. The first one, ACC synthase (pyridoxal 5-phosphate enzyme, EC 4.4.1.14), converts AdoMet into 1-aminocyclopropane-1-carboxylic acid (ACC) yielding methylthioadenosine. Subsequently, ACC oxidase converts ACC into ethylene (Figure 7) (Cohen, 1998).

Cyanobacteria also produce ethylene, but its role is not well understood. For example, in *Fremyella displosiphon*, a photoreceptor protein which belongs to the family of two-component regulators is homologous to a gene governing the reponse to ethylene in *Arabidopsis thaliana* (Kehoe and Grossman, 1996). The authors speculate that this protein may be involved in the control of cellular processes regulated by light. It is possible that the receptors of ethylene and the phytochromes (red/infrared receptors) evolved from a common ancestor that was sensitive to the regulation by two stimuli: light and ethylene. We cannot exclude their possible role as mediators of differentiation in higher cyanobacteria, especially because there exists in these organisms an ethylene receptor binding protein homologous to that of *Arabidopsis thaliana* which seems to be functional (ethylene response sensor protein (ETR1) (Rodriguez *et al.,* 1999)).

Synthesis of Biotin

A step of biotin synthesis (*bioA*) uses AdoMet not as a source of methyl group, but as a source of amino groups, yielding S-adenosyl-4-methylthio-2-oxobutanoate as a by-product of the reaction. This molecule, which is similar to a precursor of ethylene synthesis, must therefore be recycled. This step has not yet been identified.

Modification of tRNA

Transfer RNAs are known to have a large number of modifications in their bases, some of which are frozen traces of ancestral functions (Danchin, 1990). The most recent works have identified 79 modifications in enterobacteria (Bjork *et al.,* 1999). One of these modifications results from transfer of the 3-amino-3-carboxypropyl group of *S*-adenosylmethionine (Nishimura *et al.,* 1974). This modification concerns phenylalanine tRNA and is on the uridine of the supplementary loop m^7G-X-C, where X represents 3-(3-amino-3-carboxypropyl)-uridine. As a product of the reaction, apart from the modified nucleoside, one obtains methylthioadenosine.

The Synthesis of Spermidine

The last point that we shall consider here is the transfer of the aminopropyl group of *S*-adenosylmethionine in the synthesis of polyamines. This reaction is of significant biological importance, despite the fact that, from a purely quantitative point of view, transmethylations are more significant. However, in the case of methylations, sulfur is immediaty recycled as homocysteine, which is not the case

in polyamine biosynthesis. In this case, AdoMet is first decarboxyled by *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) (Cohen, 1998). Subsequently, its aminopropyl group is transfered by spermidine synthase (aminopropyltransferase, EC 2.5.1.16) to putrescine resulting in a molecule of spermidine and a molecule of methylthioadenosine (see Figure 6). This reaction, which traps sulfur in a little studied molecule, is a universal reaction, present in all living organisms, with the exception of a few halophilic microorganisms that do not possess spermidine. In eucaryotes and some procaryotes, this aminopropyl group can be subsequently transfered onto spermidine to give spermine. This reaction is catalyzed by an enzyme very similar to spermidine synthases (spermine synthase, EC 2.5.1.22), with production of a molecule of methylthioadenosine, the fate of which will be considered below. Because of its availability, one may wonder about the possible existence of aminopropyl group transfers to other molecules: in principle one may expect that all the substrates of methylation, and in particular numerous proteins could be modified by this group. This may explain why analysis of bacterial proteome displays modified polypeptides in proportions much larger than expected.

Transfer of the Ribosyl Group Queuosine (Q)

[7-(((4,5-ci*S*-dihydroxy-2-cyclopenten-1-yl)amino)methyl)-7-deaza-guanosine], is a nucleoside that takes the place of guanosine at position 34 of some transfer RNAs (those of asparagine, aspartate, histidine and tyrosine) in Bacteria and in most Eucarya, but not in Archaea or yeast. Bacteria synthesize the base queuine de novo. In contrast, Eucarya must insert this base directly into the tRNA from a precursor they obtain from food. For a few years only, one knows that this nucleoside is a derivative of *S*-adenosylmethionine (Slany *et al.,* 1993). It is an extremely interesting case because AdoMet acts here as a donor of the ribosyl group. This is, as yet, the only known example of this reaction. Only two steps of the biosynthesis pathway of queuosine are well characterized. This pathway starts with GTP, which is converted by a unknown mechanism, requiring iron, into 7-(aminomethyl)7-deaza-guanine ($preQ_1$). $PreQ_1$ is then inserted at position 1 of the anticodon of some tRNAs in exchange for guanine by a tRNA-guanine transglycosylase (EC 2.4.2.29, encoded by gene *tgt*). The $preQ_1$ present in these tRNAs is converted into epoxyqueuosine (oQ) by attachement of the epoxycyclopentanediol group. This reaction is catalyzed by *S*-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-, encoded by gene *queA*). The cyclopentane group is the isomerized derivative of ribose coming from AdoMet. The outcome of adenine during this reaction is not known. In fact, because AdoMet labeled on ribose is not readily available, it is difficult to determine whether it is the ribosyl group alone or the adenosyl group that is used. The last step of reduction of epoxyqueuosine into queuosine is catalyzed by an unidentified enzyme using vitamin B12 as a cofactor (when present). Finally, queuosine or its derivatives is excreted at high levels in the medium. This may be used for quorum sensing and/or for interaction with the hosts.

It seems interesting that all the types of activated groups in AdoMet are used to provide modifications of transfer RNA. The methyl group is most often transferred, for example to form ribothymidine. The 3-amino-3-carboxypropyl group is used to give 3-(3-amino-3-carboxypropyl)-uridine and the ribosyl (or possibly adenosyl) group are involved in the formation of queuosine.

AdoMet also acts as a transient donor of the oxyadenosyl radical in the synthesis of L-b-lysine from L-lysine (reaction catalyzed by a lysine 2,3-aminomutase) in anaerobic conditions in *Clostridium subterminale* (Lieder *et al.,* 1998). In the same conditions, AdoMet plays a role in *E. coli* in the synthesis of deoxyribonucleotides, a reaction catalyzed by a ribonucleotide reductase (EC 1.17.4.2, encoded by genes *nrdD* and *nrdG*) (Ollagnier *et al.,* 1997). One sees therefore that the metabolism of this molecule is quite complicated.

Recycling of Methylthioadenosine

AdoMet can also be decarboxylated to dAdoMet. This molecule is the donor of the aminopropyl- group which is added to putrescine to form spermidine. During this reaction methylthioadenosine (MTA) is produced (Figure 8). Organic sulfur being often limiting, this molecule must be recycled in most cases. Several studies in bacteria related to *E. coli*, *K. pneumoniae*, suggest the metabolic pathway indicated in Figure 9.

In a first step in *E. coli*, MTA is hydrolyzed by a nucleosidase (EC 3.2.2.16, encoded by gene *pfs*), yielding methylthioribose (MTR) and adenine (Cornell *et al.,* 1996; Cornell and Riscoe, 1998). In *B. subtilis* the corresponding gene *yrrU*, is present in a complex operon *yrrTUyrhABC*, which appears to metabolize sulfur containing molecules (Sekowska and Danchin, 1999). In contrast, in animals and their parasites, MTA is phosphorolyzed in the presence of phosphate, giving methylthioribose-1-phosphate and adenine. In the case where sulfur is not limiting MTR is the final product and is excreted into the medium. This seems to be the case in *E. coli* (Schroeder *et al.,* 1973). In order to standardize nomencalture *pfs* and *yrrU* should perhaps be renamed *mtn*, for *m*ethyl*t*hioadenosine *n*ucleosidase (reserving *mtp* for the phosphorylase present in archaebacteria).

However, MTR is usually recycled (Figure 9). In the presence of oxygen, the pathway deciphered in *K. pneumoniae* begins with phosphorylation of MTR by a MTR kinase, which uses ATP as phosphate donor and produces MTR-1-phosphate (Wray and Abeles, 1995). MTR-1-P is transformed into methylthioribulose-1-phosphate (MTRu-1-P) by an aldose-ketose isomerase. MTRu-1-P is subsequently hydrated (and converted into a ketone) by a not yet identified hydratase, to give 2,3-diketo-5-methylthio-1-phosphopentane (DKM-1-P). This latter molecule is first converted into phosphoene-diol, then dephosphorylated to give 1,2-dihydroxy-3-keto-5-methylthiopentene (aci-reductone). These two steps are catalyzed by a bifunctional enzyme, enolase-phosphatase E-1. In *K. pneumoniae*, 1,2-dihydroxy-3-keto-5-methylthiopentene can be processed in two different ways, either by action of a dioxygenase E-2 (aci-reductone oxidase) into formate, 3-methylthiopropionate and CO (Wray and Abeles, 1993), or nonenzymatically into formate and a-keto-4-methylthiobutyrate (KMTB), the direct ketoacid precursor of methionine. Only the second pathway permits the direct way back to methionine thanks to the transamination of KMTB by an original tyrosine aminotransferase (TyrAT), that utilizes as an amino group

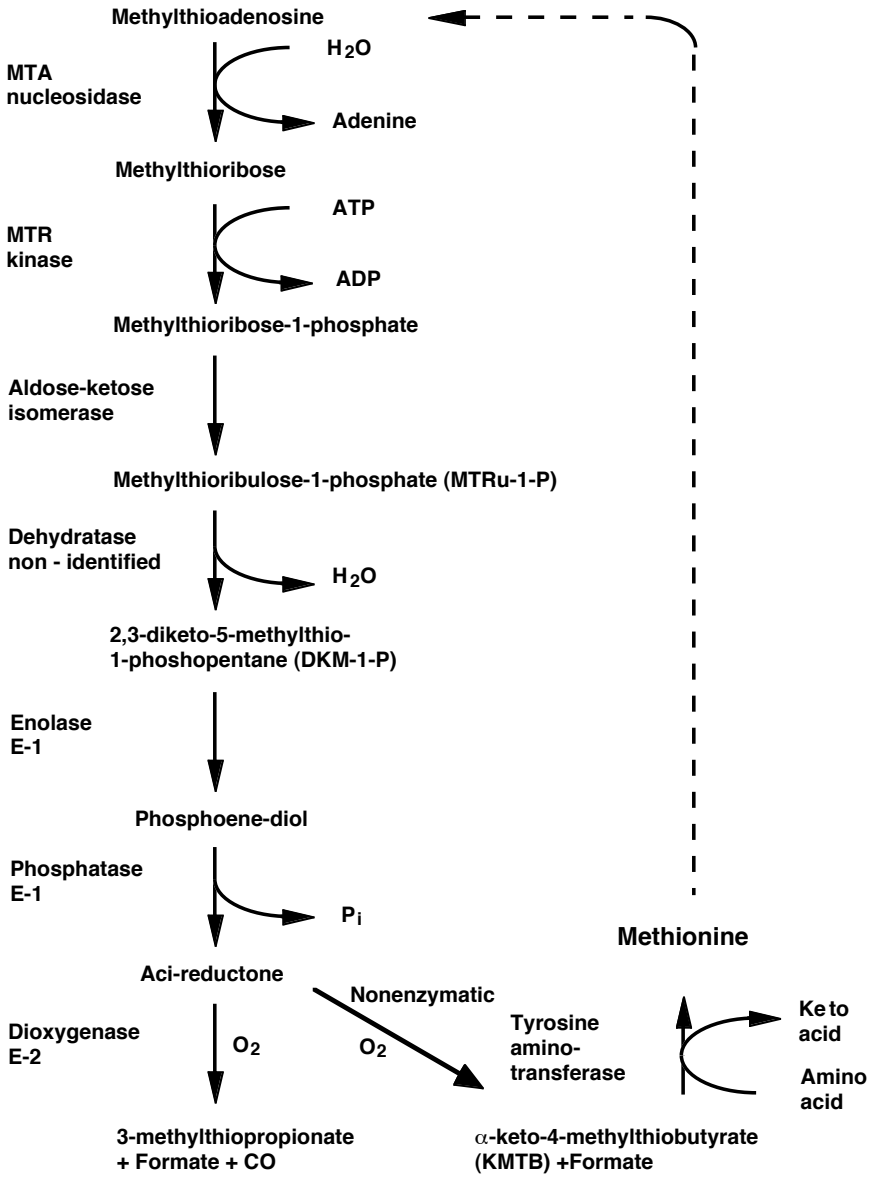

Figure 9. Methylthioribose recycling in *Klebsiella pneumoniae*.

donor glutamate or aromatic aminoacids (Heilbronn *et al.,* 1999) (Figure 9). It must be stressed that the carbon atoms 2, 3, 4 and 5 of ribose are found again in the methionine backbone, and that the methyl group carried by sulfur is not exchanged: this recycling process therefore does not require a source of one-carbon residues, but, in contrast, it produces one (in the form of formaldehyde or formate). This fact is quite remarkable (Figure 9). Indeed, this production of one-carbon residues in the form of a chain reaction is typically explosive. One expects therefore that methionine metabolism will be particularly stringently regulated in order to control these explosive reactions. In this context, the storage in the form of *S*-methylmethionine may be a way to modify rapidly the availabilty of methyl groups.

At this stage, it is very difficult to go further, with the results in the literature being fragmentary. One must remark here the special role played by dioxygenase E-2 (that gives an interesting idea of the general possible function of dioxygenases). Indeed, this enzyme directly consumes dioxygen, O_2, and may therefore be utilized to prevent the toxic effects of oxygen (more numerous than we can summarize here, see for example (Gardner *et al.,* 1998)). In such a case, the production of MTA (MTR) may be considered as a means (perhaps existing very early in evolution, before the evolving of respiration) to protect the cell against the toxic effects of oxygen. This may indirectly account for the antioxidizing properties of polyamines (Lovaas and Carlin, 1991; Pavlovic *et al.,* 1992).

Special Metabolism of Sulfur

The previous sections just broadly outlined the general metabolism of sulfur, which still exists a series of more or less unknown metabolic pathways where sulfur is involved in some way. Sulfur is an element present in molecules

such as glutathione. It is also a constituent of iron-sulfur centers or of essential coenzymes such as thiamine, biotin, molybdopterin, pantothenate and lipoic acid. It also belongs to the many modifications of the nucleotides that constitute transfer RNA, via reactions that are just beginning to be understood.

Glutathione

We begin with this cofactor because it is directy associated with cysteine metabolism. Its transfer onto spermidine probably leads to storage of the latter in stationary growth phase (Cohen, 1998). In *E. coli*, gene *gsp* is involved in this transfer. The corresponding protein, GSP, is a bifunctional protein that catalyzes the formation of glutathionyl-spermidine (glutathionyl-spermidine synthase, EC 6.3.1.8), but also its hydrolysis (glutathionyl-spermidine amidohydrolase, EC 3.5.1.78). The two catalytic activities lie in two distinct parts of the protein, the amidase function is located in the amino-terminal part, whereas the synthetase function is located in the carboxy-terminal part (Swiss-Prot: P43675). There exists also another protein, glutathione *S*-transferase or GST (EC 2.5.1.18, encoded by gene *gst*), that tranfers glutathione to several kinds of molecules.

Glutathione is an isotripeptide, found in cells of most organisms. It is derived from the condensation of cysteine on the γ-terminal COOH of glutamate. A glycine residue is subsequently added by formation of an usual peptidic bond. Glutathione is used to recycle the sulfide groups that can be spontaneously oxidized, following electron transfer due to catalysis or free radicals, or other oxidation phenomena. In the course of this process, two molecules of glutathione form a disulfide bridge that is thus reduced by glutathione oxidoreductase. In *E. coli*, the genes involved are the following:

- gene *gor* codes for glutathione oxidoreductase; it is in operon with an unknown gene *yhiR*.
- gene *gshA* codes for γ-glutamyl-cysteine synthetase; it lies in a likely operon *yqaBAgshAygaG* (YqaB and YqaA may be phosphatases, similar to the phosphatase of the HPr protein of *B. subtilis*).
- gene *gshB* codes for glutathione synthetase; it lies in a likely operon *gshByqgEyqgF* (YqgE and YqgF display some analogy with phosphotransferases).

These unexpected associations suggest an interesting track to explore: is the biosynthesis of glutathione controlled by protein phosphorylation?

The Iron-Sulfur Centers

Sulfur is also an element of iron-sulfur centers (minute analogues of iron pyrite) that are involved in a large number of oxido-reductions, and constitute the core of ferredoxins. Models for scenarios of the origin of life, mostly recent ones, rest on the hypothesis that the electron transfers between Fe^{2+} iron and Fe^{3+} iron and the numerous possible oxidation states of sulfur have played a role of prime importance in the first metabolic steps of life (Granick, 1957; Wächtershäuser, 1988; Danchin, 1990). It is therefore interesting to wonder how the iron-sulfur centers are formed in the cell.

One knows very little about the formation of elemental sulfur, apart from conditions where it acts in the electron transfers allowing anaerobic respiration (Ehrlich, 1996). It is very unlikely that it is sulfur in this oxidation state that is incorporated in the diverse types of centers $(Fe\text{-}S)_n$. A possible start point seems to be cystine, possibly by directly coupling a cysteine to a cysteine present in the protein that carries the iron-sulfur center, using a dehydrogenase. A ß-lyase, analogous to cystathionine ß-lyase may then provide the thiocysteine that serves as donor of sulfur atom. But it is the study of the protein NifS in *Azotobacter vinelandii* that brings most information on a possible mechanism. This protein is a cysteine sulfurylase that, from thiocysteine, liberates sulfur, yielding cysteine (Zheng *et al.,* 1998a). Several enzymes homologous to the product of gene *nifS* of *A. vinelandi* exist in E. *coli*. One of those seems to code for a cysteine sulfinate sulfinase (Mihara *et al.,* 1997). The existence of these reactions may provide tracks to assign a function to some genes labeled “y”.

Thiamine

Thiamine is an essential cofactor containing a sulfur heterocycle. Its biosynthesis needs two separated pathways. The first synthesizes the thiazole group (5-methyl-4-(-hydroxyethyl)thiazole), and the second a pyrimidine (4-amino-5-hydroxymethylpyrimidine pyrophosphate) (Figure 10). These two molecules are combined to yield thiamine pyrophosphate. The pyrimidine moiety is derived from 5-aminoimidazole ribotide. Thiazole is derived from tyrosine, cysteine, and 1-oxy-D-xylulose-5-phosphate. The enzymatic mechanisms involved in the formation of thiazole and pyrimidine are not completely understood. In particular, there is evidence for a synthesis pathway for pyrimidines, related to that of purines, but that does not stem from normal ammonium transfer to PrPP. This pathway involves several genes of unknown function such as *apbA*, *apbE* or *ybgF* (the homologous gene in *B. subtilis* is *yabC*). Their inactivation makes the mutants particularly sensitive to serine.

An operon of five genes (*thiCEFGH*) involved in the biosynthesis of thiamine has been cloned and characterized (Table 2 and Figure 10). Gene *thiC* complements mutants auxotrophic for 4-amino-5-hydroxymethylpyrimidine, genes *thiFGH* complement mutants auxotrophic for thiazole, and gene *thiE* codes for thiamine phosphate synthase. Another gene (*thiI*), necessary for the biosynthesis of thiazole in *Salmonella typhimurium* has recently been identified (Webb *et al.,* 1997). A gene homologous to *thiI* of *S. typhimurium* is located at 9.5 min in the chromosome of *E. coli* and complements a mutation *nuvC*. NuvC is necessary for the synthesis of the thiouridine present in tRNA and of the thiazole moiety of thiamine. This suggests that ThiI plays a role in the chemistry of sulfur transfer in the biosynthesis of thiazole.

A new observation recently completed this pathway. When the product of gene *thiG*, purified from a strain that expressed it, has been analyzed by mass spectrometry, it appeared that it was in fact made of two proteins. A subunit, now named ThiS, had a mass of 7311 Da. The second subunit, for which one keeps the name ThiG, had a mass of 26897 Da. In fact, although the mass anomaly of ThiG had been noted before, ThiS had escaped attention during the identification of coding sequences (CDSs) in the *E. coli* genome. This was caused in part by the short length of the

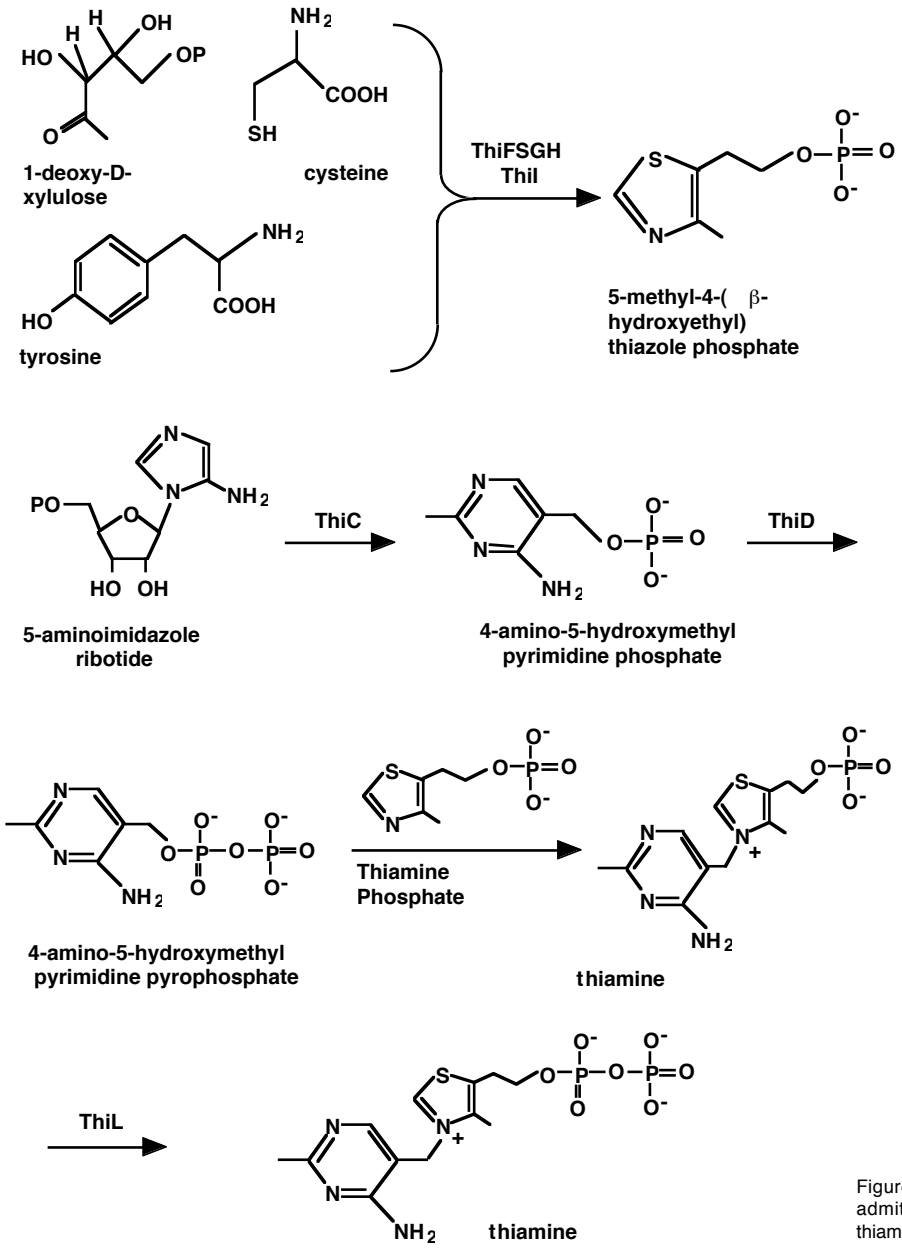

Figure 10. Pathway generally admitted for the synthesis of thiamine in *E. coli*.

corresponding CDS, and by the presence of sequence errors (quoted in Taylor *et al.,* 1998). To this difficulties we may add that ThiS is poorly colored with Coomassie blue and migrates with the front in the polyacrylamide gels. ThiS contains the -Gly-Gly sequence found at the carboxy-terminal end of ubiquitinylated human red cells. This similarity in sequence was more remarkable because ThiF is similar to an enzyme that activates ubiquitine. The similarity includes the presence of an ATP binding site. This suggested that ThiF may well catalyze the adenylation of ThiS, and that the adenylate derivative ThiS-CO-AMP may react with cysteine (or a sulfur donor derived from cysteine) to yield the thiocarboxylate derivative ThiS-COSH (Figure 11 B). Furthermore, the double role of ThiI in the biosynthesis of thiazole and 4-thiouridine indicated that ThiI may play a role in the reaction of sulfur transfer. This is what has been proposed by Taylor and his colleagues (Figure 11; Taylor *et al.,* 1998). Finally, the enzymatic mechanisms of sulfur transfer in the biosynthesis of thiamine, but also of molybdopterin, biotin, and lipoic acid have yet to be characterized.

Lipoic Acid

Thiamine is involved in the anabolic processes of decarboxylation (linked to dehydrogenation), where two cofactors operate successively, thiamine pyrophosphate and lipoic acid. Lipoate is synthesized by insertion of sulfur coming from cysteine into the hydrocarbon chain of octanoic acid (an intermediary in the biosynthesis of fatty acids) at position C-6 and C-8. This reaction is probably performed by a mechanism involving a radical (Michal,

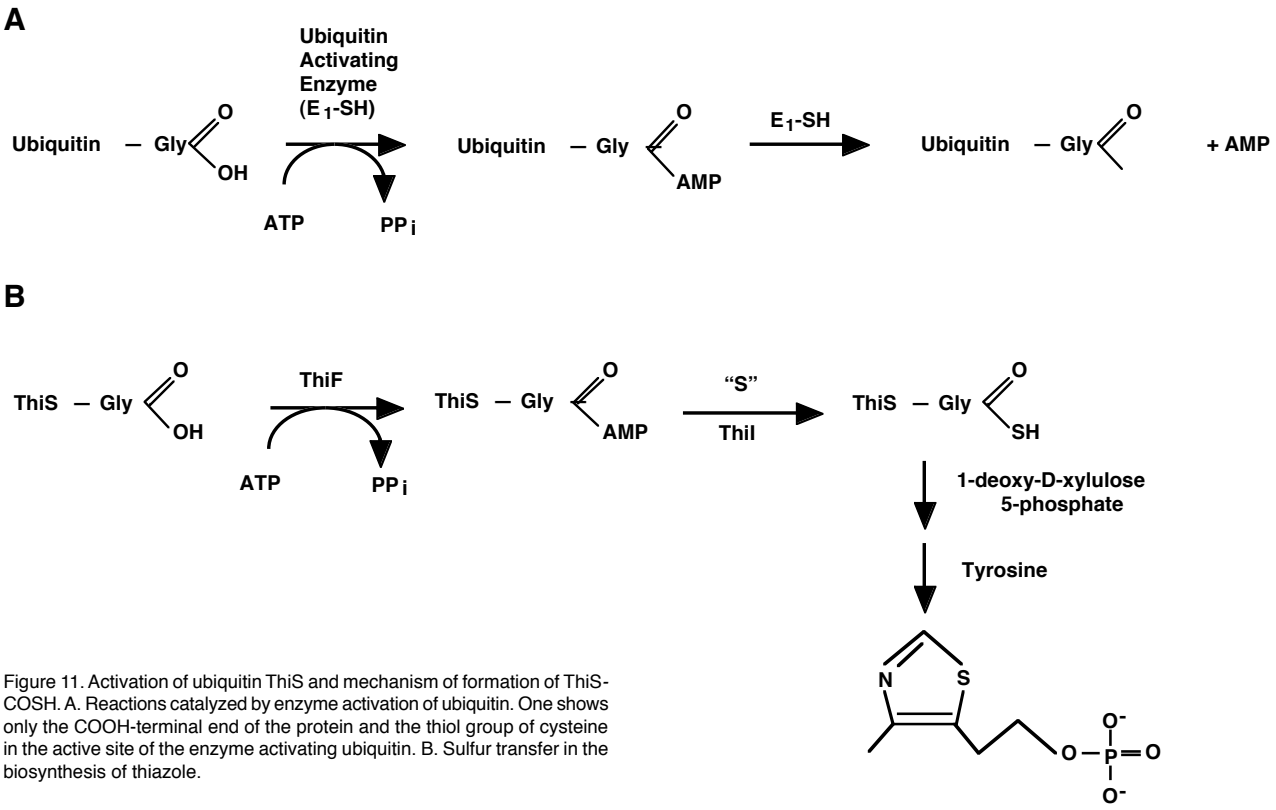

Figure 11. Activation of ubiquitin ThiS and mechanism of formation of ThiS-COSH. A. Reactions catalyzed by enzyme activation of ubiquitin. One shows only the COOH-terminal end of the protein and the thiol group of cysteine in the active site of the enzyme activating ubiquitin. B. Sulfur transfer in the biosynthesis of thiazole.

1999). The sources of sulfur used for the formation of biotin and lipoic acid are unknown, and the mechanism of insertion of sulfur into the protein has not yet been established. At the end of biosynthesis, a molecule of lipoate activated by ATP is linked to the ε-amino group of a lysine of the corresponding enzyme to yield the functional cofactor. Lipoic acid is a cofactor linked covalently to many dehydrogenases, such as pyruvate dehydrogenase (EC 3.3.1.) or α-ketoglutarate dehydrogenase (EC 3.8.1.). Most living organisms synthesize lipoic acid, which must be produced *in situ* to be functional, and seems to be difficult to recover from the environment. However, in some bacteria, lipoate is a growth factor (Michal, 1999).

Three genes of the biosynthesis pathway of lipoate have been identified: *lipA* (lipoate synthase, Swiss-Prot P25846), and *lplA* and *lipB* (lipoyl-protein ligases A and B, EC 6.-.-.-, Swiss-Prot P32099 and P30976, respectively). But it is clear that for understanding the whole pathway a lot of data are still missing. An operon comprising gene *ybeF* (YbeF is an activator of the LysR family, whose effector is unknown) and *lipA*, whose product may be involved in insertion of sulfur during lipoate biosynthesis is present in *E. coli*. There exist two proteins similar to LipA in *B. subtilis*, YutB (54% identity) and YqeV (23% identity). YqeV is remarkable because it is very conserved in all bacteria, but without known function. It is likely to have a role in the synthesis of lipoic acid (Table 2). LipB links lipoate to the ε-amino group of a lysine in a receptor protein, a reaction performed from a complex and not from free lipoate. In *E. coli* the context of the *lipB* gene is interesting. Several genes may form an operon: *dacA* (D-alanine carboxypeptidase), *ybeD* (similar to *yitC* of *B. subtilis*, similar to an oxo-acyl CoA carrier protein synthase) and *lipB*.

The Modifications of Transfer RNA

Among the 79 types of transfer RNA modifications that have been identified, at least 50 are present in *E. coli,* including three or four involving sulfur-containing molecules (Bjork, 1996).

In *E. coli* and related bacteria, the nucleotide in position 8 in transfer RNA is a modified uridine, 4-thiouridine. The principal role of this nucleotide is to protect cells against intense irradiation by near ultraviolet light (Mueller *et al.,* 1998). In the presence of sufficient irradiation at 365 nm, this nucleotide reacts with a neighbor cytosine producing a covalent bond. The tRNA molecule thus modified can no longer be charged by the corresponding amino acid, in most cases. This triggers the stringent response, whose signal is the ppGpp molecule, coupling translation to transcription. Macromolecular syntheses stop and bacteria are shifted to a state that permit them to better resist this situation. It is not known how the atom of sulfur is incorporated into the nucleotide. Although mutants controlling this process were isolated more than 25 years ago (*nuvA* and *nuvC;* Lipsett, 1978), the *thiI* gene involved in this reaction has only been characterized recently (Mueller *et al.,* 1998). Furthermore, this gene is involved in the synthesis of thiamine.

There exist several modifications at position 34 (first position of anticodon) in *E. coli*. In the case of tRNAs specific for glutamate, lysine and glutamine, the modified nucleoside is 5-methylaminomethyl-2-thiouridine (mnm^5s^2U34). The reaction of uridine 34 modification includes several steps, some of which are poorly characterized. The first step of thiolation at position two of uridine (s^2U34) is catalyzed by the product of gene *mnmA* (also known in the litterature as *trmU* or *asuE*). The product

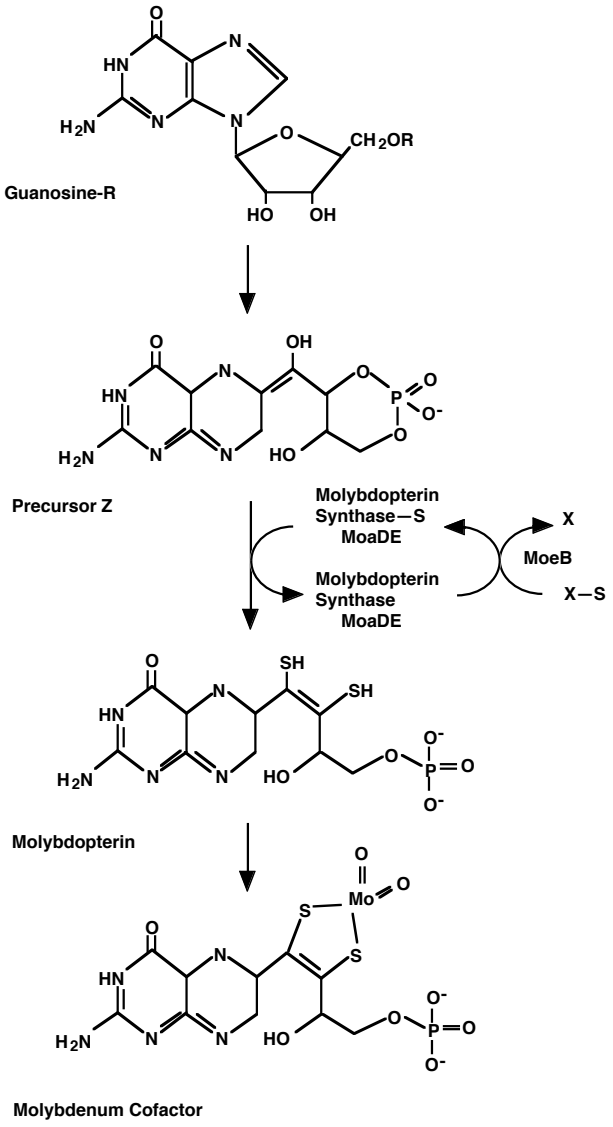

Figure 12. Structure of molecules and metabolic pathway for the synthesis of the molybdenum cofactor in *Escherichia coli*. At the beginning of the metabolic pathway, a derivative of guanosine is converted into precursor Z. Sulfur is subsequently added to precursor Z to form the thiolene active group.

of gene *mnmE* (*trmE*) catalyzes the first step of the modification at position five, but one does not know the steps that lead to the modification into 5-carbonylmethylaminomethyl-2-thiouridine ($cmnm^5s^2U34$). The two modifications at position two and five are synthesized independently of each other. The product of gene *mnmC* (*trmC*), which catalyzes the following step of the modification at position five, possesses two enzymatic activities that catalyze successively the reactions to liberate the acetate group of $cmnm^5s^2U34$ (which leads to nm^5s^2U34, 5-methylamino-2-thiouridine), and to methylate the nm^5s^2U34 in mnm^5s^2U34 (AdoMet-dependent reaction) (Hagervall *et al.,* 1998)).

The modification at position 34 participates probably in the discrimination between codons of the same family which, varying at their third position, code for different amino acids (for example lysine is encoded by two triplets AAA and AAG, which can lead to the confusion with asparagine codons AAU and AAC). However, this role is perhaps not direct, because mutants partially modified at position 34 of tRNA seem to be more accurate in translation (there is less confusion between lysine and asparagine) than the wild type, with a tRNA containing normally modified uridine. It has been proposed that this modification has a role in the interactions with the ribosome or with the translation elongation or termination factors, rather than in the direct codon discrimination (Hagervall *et al.,* 1998). In this case, the thiol group present in these modified bases stabilizes the structure of transfer RNA, which is centrally important to the recognition of the tRNAs by their corresponding synthetases and therefore their charging by the correct amino acids (Kruger and Sorensen, 1998).

At position 32 of some tRNAs of eubacteria, one finds another modification, 2-thiocytidine, but little is known about the genes involved in its synthesis and about the biological signification of this modification (Bjork, 1996).

Another modification (2-methylthio-N^6-isopentenyl adenosine (ms^2i^6A), adds a *S*-methyl group at position 2 of adenine at position 37 of transfer RNAs specific for codons beginning with U, with the exception of $tRNA^{Ser}$ I and V). It may result from the transfer of the methyl group of AdoMet, whereas the sulfur atom would come from cysteine. Two genes, *miaB* and *miaC*, are necessary for this reaction. Gene *miaA* catalyzes the first step of conversion of adenosine into 6-isopentenyl-adenosine (Esberg and Bjork, 1995). The position of these genes in the chromosome of *E. coli* was not clearly established until recently. The *miaB* gene has been shown to be identical to gene *yleA*. MiaB contains a cysteine cluster reminiscent of iron-binding sites. It is therefore assumed that it participates in the thiolation step of tRNA (Esberg *et al.,* 1999). In *S. typhimurium*, a fourth gene, *miaE*, oxidizes 2-methylthio-N^6-(isopentenyl)-adenosine into 2-methylthio-N^6-(4-hydroxyisopentenyl)-adenosine (ms^2io^6A) (Persson *et al.,* 1998).

Some modifications of tRNA are important for cellular metabolism (in *S. typhimurium*), or virulence (in *Shigella*) (Bjork *et al.,* 1999). Among others, these modifications concern position 37, such as the modification of adenosine just described (ms^2i^6A), or the modification of guanosine catalyzed by tRNA(m^1G37)methyltransferase, encoded by gene *trmD* (1-methyl-guanosine (m^1G37)), or another modification of guanosine at position 34 in queuosine.

The Molybdenum Coenzyme (Molybdopterin)

With the exception of nitrogenase, in all molybdoenzymes, the atom of molybdenum belongs to an organometallic structure named the molybdenum cofactor (MoCo). In this structure, molybdenum is linked to its organic ligand by a dithiolene group located in the 6-alkyl chain of molybdopterin (MPT). In *E. coli*, MoCo is present in the slightly different form of molybdopterin guanine dinucleotide (the conversion of MoCo in dinucleotide is placed just before incorporation of the cofactor in the protein). The biosynthesis of MPT starts with GTP as a precursor (Figure 12). There are several steps, that comprise the opening of the GTP cycle, leading to the direct precursor of MPT, precursor Z (Wuebbens and Rajagopalan, 1995). This latter

molecule is a 6-alkylpterine with a phosphate group at the extremity of the aliphatic chain. This phosphate group leads to the formation of a six atom heterocycle by using two carbons of the chain (C-2' and C-4') (Figure 12). Precursor Z contains none of the sulfur atoms present in MPT. The conversion of precursor Z into molybdopterin necessitates ring opening of the heterocycle and transfer of sulfur to create the dithiolene group needed for the chelation of molybdenum.

The transfer of sulfur in the biosynthesis of thiamine and molybdopterin follows the same course. A system of three enzymes (MoeB, MoaD, and MoaE) permitting the transfer of sulfur to precursor Z of molybdopterin has been reconstituted in *E. coli* (Pitterle *et al.,* 1993; Pitterle and Rajagopalan, 1993) (Figure 12) and in *Aspergillus nidulans* (Appleyard *et al.,* 1998).

The first enzyme, MoeB (also known as ChlN), molybdopterin synthase sulfurylase, is responsible for the activation of MoaD by adenylation, as well as the transfer of sulfur to activated MoaD (Appleyard *et al.,* 1998). MoaD (small subunit) with MoaE (large subunit) form molybdopterin synthase (known in the literature as "converting factor"). This enzyme possesses two catalytic activities: opening of the ring created by the phosphate group and sulfur transfer on precursor Z. Protein MoeB is very similar to ThiF; MoaD and ThiS comprise the same Gly-Gly dipeptide at their carboxyl-terminal end. One can therefore infer that sulfur is transfered in the same way by adenylation followed by formation of thiocarboxylate, as in the case of the biosynthesis of thiamine (Taylor *et al.,* 1998) (see Figure 11).

In *E. coli* the genes involved in the biosynthesis of MPT are situated in two operons: *moeA* (function unknown) *moeB*, and *moaABCDE*. *moaABC* have an unknown function (Table 2).

Biotin

Apart from the metabolism of energy, transamination and decarboxylation are the two most central steps of intermediary metabolism. In general, transamination is involved in anabolism, while decarboxylation is most often a first step of catabolism (cf. Danchin, 1990). A particular coenzyme, biotin, plays a central role in many decarboxylation reactions (but also carboxylations and transcarboxylations). Although generally essential, this coenzyme is rare as a free molecule in nature. For this reason, living organisms have developped many systems to capture and transport biotin. These systems are so powerful that they have been used as probes in many processes used in molecular biology. The biotin/(strept)avidin couple is often more efficient and more specific than the antigen/antibody couples. One can use biotin and streptavidine during the coupling of a reaction (molecular hybridization for example) with an enzymatic amplification system (Prescott and Fricker, 1999).

Biotin is synthesized by bacteria, yeast and plants essentially in the same way. However, this biosynthesis is not completely deciphered, and in particular one does not yet know how sulfur is incorporated into biotin. It begins probably by the condensation of three molecules of malonyl-CoA into pimeloyl-CoA with liberation of two molecules of carbon dioxide. Subsequently, the condensation of pimeloyl-CoA with alanine (pyridoxal-phosphate-dependent) leads to the formation of a molecule of 8-amino-7-oxopelargonate (KAPA) with the liberation of CO_2 and CoA-SH. KAPA is then converted into 7,8-diaminopelargonate (DAPA) by a transamination reaction. This reaction involves *S*-adenosylmethionine as an amino group donor. An ATP-dependent carboxylation leads to the closing of the imidazolidone ring and to the formation of dethiobiotin. The last step, that places sulfur in the molecule yields biotin by the closure of the thiophan ring (Michal, 1999) is still uncharacterized. It can neither use sulfur S_0, nor an iron-sulfur center. It probably uses sulfur coming from a protein with an iron-sulfur center, perhaps biotin synthase (Bui *et al.,* 1998), Because biotin (vitamin H) is an important food supplement, its production is of industrial interest.

In *E. coli*, we know that the synthesis of biotin is controlled by genes linked to locus *galETK* (lambda phage deletions often lead to auxotrophy for biotin), but all the steps are not yet identified. The genes involved in the synthesis of biotin are located in divergent operons. One has therefore possibly an operon of three genes: *bioA* (diaminopelargonic acid synthetase), *ybhB* (unknown gene that is similar to *yxkA* of *B. subtilis* (31% identity), itself similar to ... *bioA*) and *ybhC* (weakly similar to YdgB of *B. subtilis* (29% identity), without known function). The divergent operon is comprised of four genes: *bioB* (coding for biotin synthase), *bioF* (coding for 7-keto-8-aminopelargonic acid synthase), *bioC* (function unknown, the mutant of this gene is blocked in the formation of pimeloyl-CoA) and *bioD* (coding for dethiobiotin synthase). Gene *bioH* is located elsewhere in the chromosome, and its function is not known but the corresponding mutant is blocked in the formation of pimeloyl-CoA.

Pantothenic Acid and Coenzyme A

The presence of membranes in the form of a lipid bilayer is a universal feature of life. Lipids, and especially aliphatic long chain fatty acids, are ubiquitous constituents of membranes. To know their biosynthesis is therefore crucial in order to understand the metabolism of living organisms. This biosynthesis is based on the synthesis and degradation of thioester bonds, which have an ancient origin and probably existed before the origin of life. The mobile arm in these reactions catalyzed by ACP (Acyl Carrier Protein), is 4-phosphopantetheine. In some reactions this arm is linked to a nucleotide, and forms coenzymeA (CoA, or "acyl carrier group"). The biosynthetic mechanism of CoA synthesis is not entirely deciphered, although the main enzymatic steps have been identified (Figure 13).

The precursor of panthothenate biosynthesis is 2-oxoisovalerate, the valine transamination product. 2-oxoisovalerate is methylated by 5,10-methylene-THF, giving 2-hydropantoate, which is subsequently reduced to pantoate. An ATP-dependent condensation of the latter with ß-alanine (derived from aspartate α-decarboxylation, or from pyrimidine degradation) yields (*R*)-pantothenate. This latter product, which is largely excreted by E. *coli*, is source of this vitamin for mammals (Michal, 1999).

Coenzyme A is synthesized by all organisms from (*R*)-pantothenate that is first phosphorylated in (*R*)-4'-P-N-pantothenate. Its condensation with cysteine in presence of CTP produces pyrophosphate, CMP and (*R*)-4'-P-N-

pantothenoyl-cysteine. Following decarboxylation, one obtains 4'-P-pantetheine. Phosphopantetheine adenylyltransferase (PPAT), produces dephosphoCoA (Izard and Geerlof, 1999), that is subsequently phosphorylated by an unknown dephospho-CoA kinase to give the final product, CoA.

The 4-phosphopantetheine group of CoA is transfered on a serine residue of ACP (apo-ACP), the core protein of the fatty acids biosynthesis enzymes and also of peptide antibiotics. The same mechanism operates for the formation of an active protein EntF, necessary for the synthesis of siderophores (Gehring *et al.,* 1998). At this step, there is liberation of 3'5'adenosine diphosphate (PAP), the same molecule that is liberated during assimilation of sulfate, and that is hydrolysed in 5'AMP by the product of gene *cysQ*. It is therefore important to notice that PAP creates a possible link between sulfur assimilation, lipid biosynthesis, iron transport and antibiotic biosynthesis.

In *E. coli*, the few genes involved in the biosynthesis of pantothenate and of CoA are distributed in several operons dispersed in the chromosome (Table 2). They are indicated in Figure 13. We have operon *panBC* where *panB* codes for ketopantoate hydroxymethyltransferase, EC 2.1.2.11 and *panC* coding for pantothenate synthetase "panthoate activating enzyme", EC 6.3.2.1. Gene *panD* codes for aspartate 1-decarboxylase, EC 4.1.1.11 and *coaA* codes for pantothenate kinase, EC 2.7.1.33. The operon *kdtAB*, where *kdtB* has been wrongly annotated in Swiss-Prot, codes for phosphopantetheine adenyltransferase (Izard and Geerlof, 1999). Finally, operon *rnc era recO pdxJ acpS* contains *acpS* that codes for holo-ACP synthase (EC 2.7.8.7).

Some Elements of Regulation

A complex metabolism with so many interdependent steps requires an important coordination. Indeed, there exist, in *E. coli*, at least four general regulators (without counting specific regulators) that coordinate sulfur utilization both in the anabolic and in the catabolic direction. Three of these regulators, CysB, MetR and Cbl, are LysR-type activators (Greene, 1996; Kredich, 1996; van der Ploeg *et al.,* 1997). The regulators of this family (more than one hundred are known) are similar to each other in a region of about 280 residues, containing in its amino-terminal end a DNA binding sequence, the"helix-turn-helix" motif (Schell, 1993). To these regulators we can add protein MetJ, that has a totally different structure, with almost no equivalent among the proteins known to bind DNA (Greene, 1996).

CysB is a tetramer composed of identical subunits (M_r = 36 kDa), which controls expression of genes involved in the biosynthesis of cysteine in Gram-negative bacteria. This system includes many genes associated with sulfate transport, to its reduction into sulfide and to the formation of cysteine from serine and acetyl-CoA (Kredich, 1996). CysB is a transcriptional regulator that acts as an activator of transcription. It is also the repressor of its own synthesis (it is generally the case of LysR type regulators). The activity of CysB is modulated by a cofactor, *N*-acetylserine. As we have seen, *O*-acetylserine (the direct precursor of the synthesis of cysteine) is not stable. By internal cyclisation and transfer of the acetyl group, *O*-acetylserine leads to a more stable molecule, *N*-acetylserine. It is this molecule that plays the role of sensor of the available level of *O*-acetylserine in the cell, and regulates the cysteine biosynthesis pathway.

As in the case of many promotors functioning in the presence of an activator, the promotors of genes under the control of CysB possess a "-35" region that dif fers from the consensus sequence of sigma factor 70 (TTGACA). The activation promotors of this type requires the binding of the activator upstream from region "-35", which facilitates the formation of transcription initiation complexes (Kredich, 1992). It is difficult to predict the interaction sites of LysR type regulators. Their experimental identification (Schell, 1993) lead several authors to describe a consensus binding sequence for CysB to DNA TTANTNcNNtTNNNNNTNN and NNATNNNNAaNCNNTNNNT (Hryniewicz and Kredich, 1995). But because of its length and its small number of conserved residues a consensus of this type is probably without much significance. One must also remark that, curiously, the authors have never made a statistical study of the corresponding sequence. As we can see, the usual search for consensus sequences (generally disputable (Hénaut and Danchin, 1996)) seems therefore particulary ill suited to proteins of this type and to their operators.

The underlying molecular mechanisms of activation were not really understood until recently. It is often noted that the binding sites of these activator proteins apparently occur in strongly curved regions of DNA. Indeed, CysB is involved in the control of other responses where the supercoiling and the curvature of DNA have been noticed, such as the adaptation to acidic conditions (Rowbury, 1997). The analysis in electron microscopy of the structure of sigma 70 promotors, during transcription initiation, leads to a remarkable observation. It seems established that DNA encircles completely the molecule of RNA polymerase, forming a superhelix with a very small diameter (10 nm), which involves a strong DNA curvature at the promotor, placing the -70 region and the +24 region of promotor in close vicinity (Rivetti *et al.,* 1999). This makes therefore understandable that the stabilisation of curved structures could help transcription initiation. It must also be stressed that there exists a phenomenon of winding of the double helix, that can play an important role. This is illustrated by the promotors controlled in *B. subtilis* by factor Mta (Multidrug transporter activation), a member of the family of transcriptional regulators MerR, which have regions -10 and -35 spaced by 19 base pairs instead of 17 (Baranova *et al.,* 1999). One does not know yet, in the case of CysB, the relative contributions of the bending and torsion of the promotors that it regulates.

In the same way as we do not know their operators, it is difficult to identify the effector molecules for LysR type regulators. One has identified *in vitro* an inducer of CysB, but sulfide and thiosulfate reverse the effect of *N*-acetylserine on its binding with the promotors of regulon *cys*. They inhibit transcription initiation by exerting an anti-inducer role on CysB. The absence of physico-chemical kinship between these diverse molecules pose the question of identification of molecules that bind to CysB.

CysB protein has been crystallized, and its three-dimensional structure is known. The crystal has been obtained from a solution saturated in ammonium sulfate, then passed through several steps of sieving and purification in solutions containing no sulfate. However, in the crystal, a sulfate ion rests at the core of the protein.

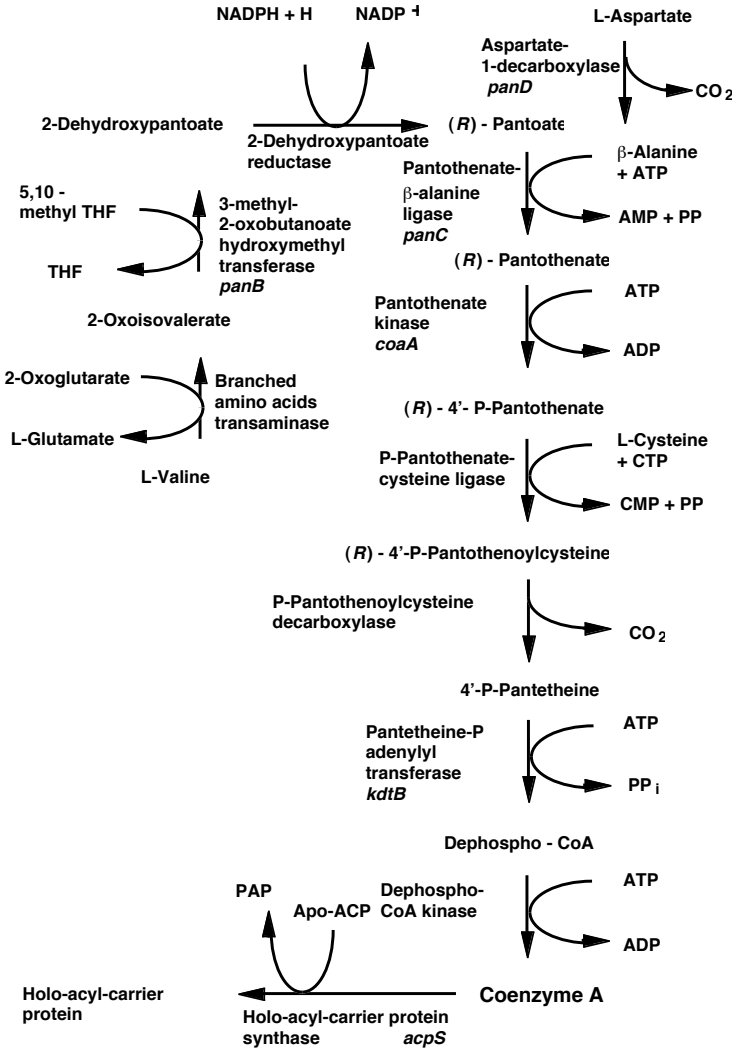

Figure 13. Coenzyme A and Acyl Carrier Protein biosynthesis pathway.

The authors of this work did not expect to find this ion there, although one could naturally expect its presence as regulator of this metabolic pathway (Tyrrell *et al.,* 1997). The sulfate ion is likely to be placed at the binding site for *N*-acetylserine, that seems be the true inducer of the pathway (Lynch *et al.,* 1994). One can therefore wonder whether sulfate does not play an antagonist role to *N*-acetylserine, for example to repress the pathway for sulfate transport. Another possibility is that the binding site of sulfate is the natural place of thiosulfate, that although structurally similar to sulfate, is an anti-inducer of regulon *cys* (Tyrrell *et al.,* 1997). Finally, this observation of the presence of sulfate in the inducer binding cavity is very interesting because of the very strong similarity of the three-dimensional structure of proteins of the LysR family (CysB in particular) with periplasmic proteins binding sulfate (Tyrrell *et al.,* 1997). It remains, however, to be proven that this is really a biologically significant phenomenon. This is the more so if one remarks that CysB contains a sequence (YVRLGLGVGVIASMAVD) which is remarkably similar to that of the consensus sequence of several AdoMet binding proteins [AMLIVF] [AMLIVF] [DE] [AMLIVF] G X G X G X [AMLIVF] X X X [AMLIVF] [AMLIVF] [DENQRKHST] (Wu *et al.,* 1992), suggesting that AdoMet could also modulate its activity.

The regulator Cbl is an activator of operon *tauABCD* and other genes belonging to the SSI class - "sulfate starvation-induced" - in cooperation with CysB (van der Ploeg *et al.,* 1997). One knows little about the operators recognized or on the effectors involved.

As for the pathway of cysteine biosynthesis, the pathway of methionine biosynthesis possesses also its regulators. MetR is an activator of expression of genes *glyA*, *metE* and *metH* and probably also of gene *metF* (Cowan *et al.,* 1993). The activation by MetR necessitates the presence of homocysteine as coactivator in the case of *glyA* and *metE* or as co-repressor (in the case of *metH*) (Plamann and Stauffer, 1989). The mechanisms of action of this activator have not yet been unravelled.

It remains finally a different regulator, MetJ, that controls the synthesis of methionine in reponse to AdoMet (for a review see (Greene, 1996)). MetJ belongs to the small class of transcriptional regulators that bind DNA by a motif "ribbon-helix-helix" (RHH) and not with "helix-turn-helix" as most regulators do in bacteria. This family contains, in addition to MetJ, the two repressors Arc and

Table 2. Correspondence Table for Sulfur Metabolism in *E.scherichia coli* and *Bacillus subtilis*

Operon *E. coli*	Possible Function and Comment	Similarities in *B. subtilis*	Possible Function and Comment
Biotin			
bioAybhBybhC			
bioA	diaminopelargonic acid synthetase	*bioA, yhxA, ycnG, yodT,* rocD, etc.	S-adenosylmethionine-8-amino-7-oxononanoate aminotransferase; operon *bioWAFDB ? bioI*
ybhB	unknown	*yxkA*	unknown
ybhC	≈ lipoprotein: cleavage site MNTFSVSRLALALAFGVTLTA ‡ C	*ydgB*	unknown
bioBFCD			
bioB	biotin synthetase	*bioB*	biotin synthetase
bioF	7-keto-8-aminopelargonic acid synthetase	*bioF*	7-ceto-8-aminopelargonic acid synthetase
bioC	unidentified upstream step	*ydaC, yodH, yxbB, yqeM*	≈ methyltransferase
bioD	dethiobiotin synthetase	*bioD, ytkP*	dethiobiotin synthetase
bioH	blocked before synthesis of pimeloyl CoA	*yisY, ytxM, yvfQ*	YisY: region YitAB; ≈ peroxidase; YtxM: ≈ enol-lactone hydrolase; YvfQ: ≈ enol-lactone hydrolase
murBbirA			
murB	UDP-N-acetylglucosaminyl-3-enol pyruvate reductase	*murB*	UDP-N-acetylglucosaminyl-3-enolpyruvate reductase; in a murein pyruvate reductase biosynthesis operon
birA	BirA acts both as repressor of the biotin operon and as an enzyme that synthesizes the corepressor; acetyl-CoA:CO_2- ligase. BirA activates biotin into biotinyl-5'-adenylate and transfers the biotin group to acceptor proteins.	*birA*	BirA acts both as repressor of the biotin operon and as an enzyme that synthesizes the corepressor; acetyl-CoA:CO_2- ligase. BirA activates biotin into biotinyl-5'-adenylate and transfers the biotin group to acceptor proteins; ≈ apparently heterogenous operon *ypjDdapBypjFGHccabirA*; this operon contains a murein biosynthesis gene (*dapB*: dihydrodipicolinate reductase), but different from that present in operon *EcmurBbirA*
Cysteine			
naccbl			
nac	LysR type regulator involved in the reponse to nitrogen	1. *gltC, yybE, yclA, yvbU, yofA, alsR, ywfK, ywbI, ywqM, yusT, ycgK, gltR, yoaU, ytlI, yrdO, yxjO, ykuM, citR, yraN* 2. *yybE, gltC, yclA, ywqM, yofA, yusT, ywfK, alsR*	Blast comparison with Nac either with the whole protein or with the protein truncated of its Helix-Turn-Helix region gives the following order of similar regulators: 1. with Helix-Turn-Helix region 2. swithout Helix-Turn-Helix region
cbl	member of the Cys regulon, related to CysB; ≈ LysR type regulator	1. *ywqM, gltC, gltR, yybE, yvbU, ywbI, yoaU, ywfK, yraN, yrdQ, yusT, alsR, yclA, citR, yxjO, ykuM, yofA, ycgK, ytlI* 2. *yybE, ywqM, ywfK, yvbU, gltR, gltC, yrdQ, yusT, yoaU, yraN, ywbI, alsR, ycgK, ykuM*	Blast comparison with Cbl either with the whole protein or with the protein truncated of its Helix-Turn-Helix region gives the following order of similar regulators: 1. with Helix-Turn-Helix region 2. without Helix-Turn-Helix region one remarks a significant difference between both lists
cysPUWAM	thiosulfate (sulfate) transport	*yvgK, yvgLM*	numerous ABC permeases, or parts of membrane sulfatases
cysP	periplamic thiosulfate binding protein	*yvgK*	transport ≈ sulfate, thiosulfate or molybdate; divergent from YvgLM, ≈ molybdate; downstream from YvgJ, ≈ sulfatase
cysU	ABC permease	*yvgM* , etc	ABC permease
cysW	ABC permease	*yvgM,* etc	ABC permease
cysA	ABC permease	*yurJ, msmX, opuCA, opuBA, yqiZ, opuAA, hisP, yckI, glnQ, yxeO, ssuB, yusC* etc	ABC permease: the similarity list is impressive; this is due to the conserved ATP binding site; yusC is a member of the "S-box" regulon
cysM	*O*-acetylsulfhydrylase M, probably thiosulfate specific; serine specific, similar to tryptophane synthase	*cysK, ytkP, yrhA*	*O*-acetyl homoserine could be the substrate of one among these activites, leading directly to homocysteine by sulfhydrylation
Upstream, same direction			
yfeT	=> RpiR ≈ repressor	*ybbH*	≈ repressor
cysB	pleiotropic regulator, crystallises with SO_4, but its normal regulator is *N*-acetylserine; there is a general difficulty to define the binding sites (bent DNA) and regulator molecules of LysR type activators	1. *gltC, yvbU, ywbI, ywfK, yybE, ywqM, alsR, yoaU, yclA, ytlI, ykuM, yusT, yrdQ, yraN, ycgK, yofA, yxjO* 2. *yvbU, gltC, yybE, ywfK, ywbI, ywqM, yusT, alsR, gltR, ytlI, yrdQ, yoaU, yofA, ycgK, yclA, ykuM, yraN, yxjO*	one finds here almost all LysR type regulators list 1 => whole protein list 2 => without Helix-Turn-Helix
cysCND	sulfate assimilation	*yitB !yitA yisZ*	two possible operons in *B. subtilis*
cysC	adenylyl sulfate kinase	*yisZ* *ylnC*	≈ adenylyl sulfate kinase; could be dephosphoCoA kinase ≈ adenylyl sulfate kinase; could be dephosphoCoA kinase
cysN	sulfate adenylyltransferase; this subunit catalyses hydrolysis of GTP coupled to APS biosynthesis	*tufA, lepA, ylaG, infB, fus*	similar to GTP binding proteins; coupling with GTP hydrolysis is probable in *B. subtilis* as in *E. coli*
cysD	sulfate adenylyltransferase	*cysH* *yitB*	≈ sulfate adenylyltransferase and ≈ in part to phosphoadenosine phosphosulfate reductase (= PAPS sulfotransferase); member of the "S-box" regulon homologous to *cysD* and to *cysH*; in an ≈ operon *yitBAyisZ*
yibNgrxC secB	probable operon apparently	*yacLMN gltXcysEcysS*	very complicated operon, *cysS* in part regulated by T-box; GltX
gpsA cysE	heterogeneous, divergent from *yibOPQ* ≈ phosphoglycerate mutase	*yazCyacOP*	aminoacylates both tRNA glt and gln; other unknown genes (ribose methyl transferases downstream?)
yibN	exists in *H. influenzae,* function unknown	*yqhL, yrkF, ybfQ, ytwF*	unknown, ≈ many other proteins, including a peptidyl-prolyl cis-trans isomerase and a protein for molybdopterin synthesis; *yrkF*: ≈ molybdopterin synthase sulfurylase: operon *yrkDEFGHIJ* very long Unknown upstream region and next to *bltR* (*bltR yrkByrkC*, ≈ operon), YrkH ≈ hydroxyacyl glutathione hydrolase; *ybfQ*: ≈ molybdopterin biosynthesis or prolyl isomerase, idem for *ytwF*, not far from and in the same orientation as operon *bioWAF*… sulfur metabolism island?
grxC	third glutaredoxin; glutathione reduction	*ytnI*, only representative in *B. subtilis* ≈ *yosR*	≈ (gluta)redoxin; upstream from gene *ytnJ*, ≈ sulfonatase; in ≈ operon *ytmIJKLMhisPytmO ytnIytnJribRhipOytnM* ≈ regulator *ytlI*; downstream (independent)? *ytoI* ≈ inosine monophosphate dehydrogenase
secB	chaperone required for secretion of a subclass of proteins, sensitive to glucose effects	no similarity	
gpsA	glycerol-3-phosphate dehydrogenase	*gpsA*	glycerol-3-phosphate dehydrogenase
cysE	serine acetyl transferase, at	1. *cysE, ykuQ, yvfD,*	1. ≈ serine acetyl transferase, operon regulated by attenuation (T-box)

	the end (80 bp gap) of a probable operon: *yibNgrxCsecBgpsAcysE*	*yyaI, yvoF* 2. *yvfD*	*gltX, cysE cysS yazZ yacO yacP* (YacO ≈ ribose methyl transferase) 2. ≈ probably capsule, not sulfur metabolism
nirBDCcysG	composite operon; siroheme-dependent	*cysH ylnABCDEF*	equivalent in part to operons *cysG* and *cysH*, assembled into a long operon
nirB	nitrite reductase (siroheme-dependent)	*nasB, nasD*	nitrite reductase operon (siroheme-dependent) *nasBCDEF*
nirD	nitrite reductase	*nasE*	nitrite reductase operon (siroheme-dependent) *nasBCDE*
nirC	≈ nitrite permease	*ywcJ, yrhG*	≈ nitrite permease; ≈ formate or nitrite permease
cysG	siroheme synthase	*ylnD, ylnF* *nasF*	≈ uroporphyrinogen III methyltransferase uroporphyrinogen III methyltransferase (siroheme nitrite reductase)
cysJIH		*yvgRQ*	
cysJ	sulfite reductase, alpha (siroheme- dependent)	1. *yvgR* 2. *ykuP, yetO, yrhJ, ykuN*	1. ≈ sulfite reductase, alpha 2. ≈ flavoproteins, undefined substrates
cysI	sulfite reductase, beta (siroheme- dependent)	*yvgQ*	≈ sulfite reductase, beta
cysH	adenylylsulfate reductase	*cysH, yitB*	adenylylsulfate reductase, cf *cysD*; member of the "S-box" regulon
		ylnA	≈ sulfate permease, similar to PitA and PitB in *E. coli*, tetraedral anion permeases
		! ylnB	≈ adenylyltransferase (archaebacteria, eucaryotes, cyanobacteria)
		ylnC	≈ adenylylsulfate kinase (archaebacteria, eucaryotes, cyanobacteria); could be dephosphoCoAkinase
		ylnD	≈ uroporphyrin-III C-methyltransferase
		! ylnE	
		ylnF	≈ siroheme synthase
cysZcysK	≈ operon?		
cysZ	membrane protein, transport?, iron-sulfur cluster formation?	*ycbT, yuiG*	*yuiG* is divergent from operon *yuiHI*, *yuiH* ≈ sulfite oxydase
cysK	*O*-acetylserine sulfhydrylase A; not clearly cotranscribed with *cysZ*	*cysK, ytkP, yrhA*	*O*-acetylserine sulfhydrylase, ≈*O*-acetylserine sulfhydrylase, ≈ *O*-acetylhomoserine sulfhydrylase
cysQ	PAP phosphatase, analogous to *HAL2 in Arabidopsis* and mammals and to MET22 in yeast, link between sulfur and fatty acids synthesis	*yktC*	≈ PAP phosphatase; in the neighborhood of *speA*
cysS	cysteine tRNA synthetase, isolated, divergent from *ppiB ybbF*	*=>gltXcysEcysS*	cysteine tRNA synthetase, member of the "T-box" regulon
Formylmethionine			
deffmtrsmB		*deffmtyloM*	
def	FMet-protein deformylase; the divergent operon is probably interesting	*def*	FMet-protein deformylase; downstream from primase and *yloI* ≈ pantothenate metabolism
		ykrB	
fmt	5-formyltetrahydrofolate MettRNAMetF transformylase	*fmt*	5-formyltetrahydrofolate MettRNAMetF transformylase
rsmB	16S RNA m5C967 methyltransferase	*yloM(yloNOPQRS)*	≈ protein Sun: RNA methylase; if the operon continues it contains a protein kinase and a protein phosphatase, then a ≈ ribulose-5-phosphate epimerase, and a ≈ thiamine pyrophosphokinase
Glutathione			
yqaBAgshAygaG			
yqaB	≈ phosphatase	*hprP, yvdM, yhcW*	P-Ser-HPr phosphatase
yqaA	≈ phosphatase	*ybfM, yddC*	≈ phosphatase
gshA	gamma-glutamyl-cysteine synthetase	no similarity	
ygaG	≈ quorum sensing LuxS	*ytjB*	≈ quorum sensing LuxS
ggt	gamma-glutamyl transpeptidase	*ggt, ywrD*	gamma-glutamyl transpeptidase; ≈ gamma-glutamyl transpeptidase
gshByqgE yqgF			
gshB		no similarity	
yqgE	unknown	*yrrK*	≈ phosphotransferase
yqgF	unknown	*yrrK*	≈ phosphotransferase
goryhiR			
gor	glutathione oxidoreductase	*pdhD, acoL, yqiV, ahpF, nasB, nasD*	≈ dehydrogenase, lipoate-dependent subunit E3 (transfer on lipoate)
yhiR	unknown	no similarity	
gsp	glutathionylspermidine synthetase/amidase	no similarity	
yghU divergent	member of the GST family	no similarity	
gst	glutathione S-transferase	no similarity	(very weak similarity with *ywhB*, *yflC*, *moeA*)
Lipoate			
ybeFlipA			
ybeF	≈ LysR type regulator		
lipA	lipoate synthesis; ≈ sulfur insertion	*yutB, yqeV, bioB*	lipoate synthesis; ≈ sulfur insertion
dacAybeDlipB			
dacA	D-alanine carboxypeptidase		
ybeD	exists in *H. influenzae*	*yitC*	unknown
lipB	lipoyl-protein ligase	no similarity	
smplplA			
smp	unknown	*yflR*	very weak similarity
lplA	creation of the amide bond between lipoate and the subunit that uses the co-enzyme	*yhfJ, yqhM*	YhfJ: ≈ lipoate-protein ligase; ≈ operon *yhflJK*; YqhM: ≈ lipoate-protein ligase, ≈ operon upstream from ≈ transcriptional regulator YqhN
Methionine			
metA	homoserine *O*-succinyltransferase	*metB*	≈ homoserine *O*-acetyltransferase
yjaB	divergent; ≈ acetyl transferase	*paiA, yyaT, ywnH, yjcF*	≈ acetyl transferase
metBLF			
metB	cystathionine gamma-synthase	*yrhB, yjcI, yjcJ*	several operons; ≈ cystathionine synthases or lyases; *yjcIJ* are members of the "S-box"regulon
metL	aspartokinase II-homoserine dehydrogenase II; bifunctional enzyme	*1. lysC, dapG* *2. hom, yclM*	1. aspartokinases II and I 2. homoserine dehydrogenases
metF	5,10-methylenetetrahydrofolate reductase; one-carbon	*yitJ*	≈ 5,10-methylenetetrahydrofolate reductase; member of the "S-box" regulon
metJ	divergent; regulator of *metBLF*	no similarity	
metC	cystathionine beta-lyase	*yjcJ, yrhB, yjcI*	several operons, cf *metB* in *E. coli*
metE	tetrahydropteroyltriglutamate methyltransferase	*1. metC* *2. yxjH, yxjG*	1. cobalamine-independent methionine synthase ; member of the "S-box" regulon 2. neighbors, members of the "S-box" regulon
metR	divergent, regulator	*1. gltR, ywqM, alsR, yybE, yofA, yvbU, yusT, gltC, yrdQ, ytlI, ywbI, ywfK, ycgK, yclA, ykuM, yxjO, yraN* *2. ywqM, gltR, alsR,*	1. LysR type regulators 2. idem, without the Helix-Turn-Helix region note that the order differs from that in the list of the CysB homologs in *E. coli*

		yybE, yrdQ, yusT, yofA, yvbU, ywfK	
metGyehHyehI			
metG	methionine tRNA synthetase	*metS*	methionine tRNA synthetase;
yehH	≈ metabolism of the molybdenum cofactor	*yjiB*	≈ monooxygenase
yehI	≈ metabolism of the molybdenum cofactor	no similarity	
mrp	divergent; ATP binding protein, link with methionine metabolism	*ybaL, minD, ylxH, ywqD, soj*	link with the cell architecture
ykfDyagD			
ykfD	S-methylmethionine permease	*ybgF, yveA, hutM, rocC*	≈ S-methylmethionine permease; amino acid permeases;
YveA:		*rocE* etc	≈ cation permease (sulfonium ?)
mmuM	=YagD methyl: group transfer to homocysteine from AdoMet or S-methylmethionine; selenium metabolism	*ybgG, yitJ*	≈ operon *ybgJHG*: ≈ transport and methyl group transfer from S-methyl methionine; *yitJ* member of the "S-box" regulon
metH	B12-dependent N5-methyltetrahydrofolate homocysteine transmethylase	1. *yitJ* 2. *ybgG*	1. ≈ 5,10-methylenetetrahydrofolate reductase; member of the "S-box" regulon 2. ≈ methionine methyltransferase; ≈ operon *ybgJHG*, containing a sodium co-transport methylmethionine permease downstream from ≈ two component system
***N*-terminal methionine**			
mapglnDdapDyaeIyaeH		*secYadkmap*	three genes in a very large ribosomal operon, highly conserved in Gram$^+$ organisms
map	methionine aminopeptidase	*map*	methionine aminopeptidase
glnD	uridyltranferase: modifies protein PII of glutamine synthetase by uridylation	*yflG* no similarity	≈ methionine aminopeptidase; next to *yflE* ≈ sulfatase
dapD	acetyltranferase of the CysE family, 4th step of diaminopimelate and lysine synthesis	*ykuQ, cysE*	≈ tetrahydropicolinate succinylase; neighborhood *EccysE*, *EccysJ*
yaeIH	≈ protein phosphatase (deuridylase?)	*ykuE, ykoQ, ypbG*	≈ phosphatases
divergent *rpsBtsfpyrHfrr*	divergent, containing, as suprisingly as in operon *map*, ribosomal protein S2, recycling factor EFTs, UMP kinase, ! and the ribosome recycling factor!	*rpsBtsfsmbAfrr*	SmbA is ≈ PyrH, this operon is extraordinarily conserved
Molybdopterin			
moeAB		*mobAmoeBAmobBmoaED*	
moeA	molybdopterin synthesis	*moeA*	molybdopterin synthesis
moeB	molybdopterin synthesis; ≈ adenylates MoaD	*moeB*	molybdopterin synthesis; ≈ adenylates MoaD
moaABCDE		*narQA, moaB, ydiGHIJ*	
moaA	molybdopterin synthesis	*narA, (ywiA)*	≈ molybdopterin synthesis
moaB	molybdopterin synthesis	*moaB*	≈ molybdopterin synthesis; region *ackA*, *moaB*, *argGH*
moaC	molybdopterin synthesis	*ydiG (yxiB, thiD)*	≈ molybdopterin synthesis; in an unknown operon
moaD	transfers sulfur to the cofactor precursor, small subunit. The protein is ≈ adenylated at the GG carboxy-terminal dipeptide, thiolated.	*moaD*	transfers sulfur to the cofactor precursor, small subunit. By analogy with *E. coli* one might postulate that the protein is ≈ adenylated at the GG carboxy-terminal dipeptide (conserved), thiolated and transfers sulfur
moaE	sulfur transfer, large subunit	*moaE*	sulfur transfer, large subunit
Iron-sulfur cluster			
csdAygdK			
csdA	cysteine sulfinate desulfinase	*yrvO, nifZ, nifS, yurW, ycbU*	≈ cysteine sulfinate desulfinase; ≈ lyase; ≈ operons *nifZytbJ*; *nifSyrxA*; *yrzCyrvO*; *yurYXWVU, ycbU*
ygdK	unknown; family UPF 0050, YnhA	no similarity	
yfhPnifSyfhNFhscBAfdx			
yfhJ			
yfhP	unknown; ≈ regulator	*yrzC, yhdE, ywgB*	unknown; YrzC ≈ regulator?; ≈ operon *yrzCyrvO*
nifS	= YfhO: cysteine sulfinate desulfinase	*yrvO, nifZ, nifS, yurW, ycbU*	≈ cysteine sulfinate desulfinase; ≈ lyase; cf *EccsdA*
yfhN	≈ NifU; sulfur recruitment	*yurV*	≈ NifU; sulfur recruitment; ≈ operon *yurYXWVU*
yfhF	≈ HesB	*yutM*	unknown, next to *paiAB*
hscB	family DnaJ; ≈ iron-sulfur cluster maturation; ≈ oxidation, reduction and isomerisation of disulfide bridges	*yqkE, ykzD*	very weak similarity
hscA	Hsp70 family protein, DnaK; ≈iron-sulfur cluster maturation	*dnaK, mreBH, mreB, mbl*	≈ Hsp70
fdx	ferredoxin	*yrhE, yjcS*	≈ ferredoxin
yfhJ	unknown	*yusH, spoIIAB, yorB*	≈ anti-sigmafactor , weak similarity
sufABCDSE	*ydiCynhEDCBA*	*yurYXWVU*	
sufA	*yfhF, yadR* ≈ synthesis of the iron-sulfur cluster	*yutM*	unknown, in operon with a probable diaminopimelate epimerase gene
sufB	*sufD*; ≈ ABC permease	*yurU, yurX*	≈ ABC permease
sufC	≈ sulfate permease	*yurY*	≈ ABC permease
sufD	*sufB*; ABC permease	*yurX, yurU, (nifZ)*	≈ ABC permease
sufS	≈ cysteine desulfurase	*yurW, yrvO, nifZ, nifS*	≈ cysteine desulfurase or cysteine sulfinate desulfinase
sufE	*ygdK*; unknown, in operon with *csdA*, coding for a cysteine sulfinate desulfinase	no similarity	
Pantothenate			
panBC		*panBCD*	
panB	ketopantoate hydroxymethyltransferase	*panB*	ketopantoate hydroxymethyltransferase
panC	pantothenate synthetase	*panC*	pantothenate synthetase
panD	aspartate 1-decarboxylase	*panD*	aspartate 1-decarboxylase
coaA	panthothenate kinase	*yqjS*	≈ pantothenate kinase; in ≈ operon *yqjST*
kdtAB			
kdtA	3-deoxy-D-manno-octulosonate-lipid A transferase	no similarity	
kdtB	identification error in SwissProt: phosphopantetheine adenylyltransferase (PPAT)	*ylbI*	≈ phosphopantetheine adenylyltransferase; in ≈ operon *ylbHI*; YlbH ≈ methylase
rncerarecOpdxJacpS			
rnc	RNase III; cleaves double stranded RNA	*rncS*	cleaves the 5' and 3' extremities of stable RNAs precursors; isolated
era	essential GTP and RNA binding protein; control of the cell cycle	*bex*	complements *era* from *E. coli*; in ≈ operon *yqfFGdgkAcddbexrecO*; YqfFG unknown; diacylglycerol kinase; cytidine deaminase (programmed frameshift)
recO	unknown, sensitivity to DNA damage	*recO*	sensitivity to DNA damage; the *erarecO* part of the operon is conserved
pdxJ	required with PdxA for the synthesis of pyridoxine 5 phosphate from 4-(phosphohydroxy)-L-threonine, NAD and 1-deoxy-D-xylulose-5-phosphate	no similarity	

acpS	holoACP synthase; binds the 4-phosphopantetheine arm to ACP and liberates PAP (cf CysQ)	*ydcB*	≈ holoACP synthase; in ≈ operon *ydcBC*; YdcC unknown
MTA recycling			
pfsyadTyadS		*yrrTUyrhABC*	
pfs	methylthioadenosine nucleosidase; S-adenosylhomocysteine nucleosidase; *E. coli* does not use MTA or MTR (secreted)	*yrrU*	methylthioadenosine nucleosidase; ≈ S-adenosylhomocysteine nucleosidase; *B. subtilis* uses MTA and MTR as sulfur sources.
yadT	≈ periplasmic protein	*yvrC, yfiY, yfmC, fhuD*	≈ iron binding protein; ≈ lipoprotein MKKRAGIWAALLLAAVMLAG #C
yadS	≈ membrane protein	*yvgT*	unknown; next to *yvgQR*: ≈ sulfite reductase
S-adenosylmethionine			
metK	S-adenosylmethionine synthetase	*metK*	S-adenosylmethionine synthetase; belongs to the "S-box" regulon
Sulfonatases			
tauABCD		*ssuA*	arylsulfonates binding lipoprotein; lipopeptide signal MKKGLIVLVAVIFLLAG #C
tauA	taurine periplasmic binding protein	*ssuB*	arylsulfonates ABC permease (ATP binding site)
tauB	taurine ABC permease (ATP binding site)	*ssuC*	arylsulfonates ABC permease (membrane protein)
tauC	taurine permease (membrane protein)	no similarity	
tauD	taurine dioxygenase		
aslA	≈ arylsulfonatase	no similarity	Unrelated aryl (alkyl)sulfonatases exist in *B. subtilis*
aslB	≈ arylsulfonatase or post-translation activity for the activation of arylsulfonatase (formation of formylglycine); activation of AslA	*yydG*	unknown
ydeNM			
ydeN	≈ hydrolase (sulfonatase)	*yqgS, yflE, yfnI, yvgJ*	≈ sulfonatase
ydeM	≈ activation of YdeN	*narA, ywiA*	≈ molybdopterin synthesis
yidKJ			
yidK	≈ permease (sodium symport)	*opuE, ycgO, ywcA, yodF, yhjB*	≈ permease
yidJ	*ydeN*, *aslA*, *yejM*; ≈ sulfonatase	*yflE, yvgJ*	≈ sulfonatase
yidF	*ydeM*, *aslB*; ≈ activation of YidJ	no similarity	
Thiamine			
apbAyajL			
apbA	2-dehydropantoate reductase; second pyrimidine synthesis pathway	*ylbQ*	≈ 2-dehydropantoate reductase; upstream from YllA: unknown
yajL	thiamine synthesis	*yoaZ*	very weak similarity
ompCapbEadaalkByojI			
ompC	porin; outer membrane		
apbE	second pyrimidinesynthesis pathway; ≈ conversion of aminoimidazole ribotide (AIR), into 4-amino-5-hydroxymethyl-2-methyl pyrimidine (HMP), thiamine precursor; lipoprotein	no similarity	
ada	dealkylase: stoichiometric transfer of the alkyl group from position O-6 of alkylated guanines in DNA to a cysteine of the enzyme; suicide reaction; also repairs O-4-methylthymine.	*adaA*	adaptive response to DNA alkylation; positive regulation of operon *adaAB*; AdaB: O6-methylguanidine-ADN methyltransferase
alkB	dealkylation of DNA	no similarity	
yojI	≈ ABC permease; ATP binding site	many homologs	ATP binding site
thiCEFSGH		*thiA*, *ywbIthiKC*, *tenAIgox ByjbSTUVW(yjbX)*	
thiC	synthesis of the hydroxymethylpyrimidine moiety of thiamine	*thiA*	synthesis of the hydroxymethylpyrimidine moiety of thiamine
thiE	thiamine-phosphate pyrophosphorylase	*thiC, tenI*	thiamine-phosphate pyrophosphorylase
thiF	synthesis of thiazole phosphate; similar to the enzyme responsible for ubiquitinylation of eucaryotic proteins ; ≈ adenylates ThiS	*yjbU, moeB*	≈ BsThiB; YjbU ≈ adenylates YjbS; ≈ in a very large operon *ywbIthiKC, tenAIgoxByjbSTUVW(yjbX)* containing a complex regulator (TenAI) and a glycine oxidase; MoeB ≈ adenylates MoaD
thiS	transfers sulfur to the thiazole protein precursor, small subunit. The protein is adenylated at the C-terminal GG, thiolated and transfers sulfur	*yjbS*	≈ transfers sulfur to the thiazole precursor, small subunit. The protein is adenylated at the C-terminal GG, thiolated and transfers sulfur
thiG	transfers sulfur to the thiazole precursor, large subunit	*yjbT, (thiC, yaaD)*	transfers sulfur to the thiazole precursor, large subunit
thiH	unknown	no similarity	
		nifZytbJ	
thiI	≈ transfers sulfur to thiazole via ThiS; = NuvC sulfur transfer to transfer RNA	*ytbJ*	In ≈ operon *nifZytbJ*; NifZ is an homolog of NifS, ≈ cysteine desulfurase, responsible for sulfur transfer to thiazole; the divergent *braB* gene ≈ branched chain amino acid permease, could transport cysteine, cystine or methionine
thiLpgpA		*ydiABCDE*	
thiL	thiamine monophosphate kinase	*ydiA*	≈ thiamine monophosphate kinase; YbiB: ≈ ATP binding site; YbiC YbiE: ≈ endopeptidases, YbiD: ≈ N-acetylase
pgpA	≈ phosphatidyl-glycerophosphate phosphatase	no similarity	
thiMDyegXW			
thiM	phosphorylation of 4-methyl-5-(beta-hydroxyethyl)thiazole (beta-hydroxyethyl)thiazole	*thiK*	phosphorylation of 4-methyl-5-(beta-hydroxeythyl)thiazole; downstream from a LysR type regulator (YwbI); divergent ≈ operon *ywbHGF* might code for the transport of a thiamine-like molecule
thiD	phosphomethylpyrimidine kinase; similar to PdxK	*yjbV, thiD*	YjbV ≈ phosphomethylpyrimidine kinase; *thiD*: annotated by comparison, ≈ pyridoxal kinase?
yegX	unknown	*yrzG*	very weak similarity
yegW	≈ transcription regulator	*yvoA, ymfC, yurK, ydhQ, ybgA, treR, yydK* etc.	≈ transcription regulator
pyrLBIyjgF		*yabHpurRyabJ, pyrPBCAAABDIIDFE*	
pyrB	aspartate transcarbamylase, catalytic subunit	*pyrB*	aspartate transcarbamylase, in a very long pyrimidine operon, upstream from *cysH*
pyrI	aspartate transcarbamylase, regulator subunit	no similarity	
yjgF	new pyrimidine pathway; involved in the synthesis of thiamine; serine effect	*yabJ*	involved in the control of purine synthesis (convergence point with pyrimidines); YabH: ≈ metabolite kinase
yabNtbpAthiPthiQ			
yabN	unknown		
tbpA	≈ thiamine binding protein	no similarity	
thiP	≈ thiamine pyrophosphate transport, thiamine and thiazole	no similarity	
thiQ	≈ thiamine pyrophosphate transport, thiamine and thiazole	ATP binding site	
tRNA thiolation		*thdFgidAgidByyA*	
trmE (mnmE)	tRNA modification; synthesis of 5-methyl-aminoethyl,	*thdF*	tRNA modification; synthesis of 5-methyl-aminoethyl, 2-thiouridine;

	2-thiouridine; ThdF: thiophene oxidation; ≈ GTP binding protein, member of Era family		≈ GTP binding protein, member of Era family; GidAB: cell division inhibitors in glucose (≈ oxidoreductase; new pyrimidine pathway?); YyaA: ≈ chromosome segregation
ymfCBtrmUycfCpurB			
ymfC	≈ pseudouridine synthase	*rluB, ytzF*	≈ pseudouridine synthase
ymfB	≈ bases phosphohydrolysis	*ytkD*	very weak similarity
trmU (mnmU)	synthesis of 2-thiouridine	*yrrA*	≈ synthesis of 2-thiouridine
ycfC	unknown	no similarity	
purB	adenylosuccinate lyase	*purB*	adenylosuccinate lyase
miaBybeZYXcutE			
miaB	methylthiolation of isopentenylated A37 derivatives	*ymcB, yqeV, (ytqA, yutB)*	YmcB and YqeV ≈ methylthiolation of isopentenylated A37 derivatives; YtqA, YutB ≈ synthesis of lipoic acid
ybeZ	≈ PhoH; phosphate starvation induced	*phoH, ylaK*	≈ PhoH; phosphate starvation induced
ybeY	UPF 0054 family	*yqfG*	≈ metalloenzyme (similar to *phoH*)
ybeX	UPF 0053 family	*yugS, yqhB, yrkA, yhdT*	≈ hydrolase; *yrkA* similar to *blt*
cutE	copper tolerance	no similarity	
Sulfate transport			
sbp	periplasmic sulfate binding protein, isolated gene	*yvgK, ytmJ*	≈ sulfate binding: the most likely is YvgK; YtmJ: sulfonate binding, lipoprotein MNKRKGLVLLLSVFALLGGG ‡ C
Sulfur or sulfate transfer			
sseA	≈ rhodanese; sensitivity to serine; ≈ thiosulfate sulfur transfer to cyanide	*yqhL*	very weak similarity to molybdenum cofactor synthesis gene
ydjXYZynjABCDE			
ydjX	≈ membrane protein	*ytxB, yqeD, yhjE, yngC, ypuB, yshB*	unknown; ≈ DNA metabolism (primosome)
ydjY	unknown	no similarity	
ydjZ	≈ membrane protein	*yhjE, yqeD, ytxB, ykoX, yngC, ybfM*	unknown; ≈ anion hydrolase
ynjA	unknown	*ydfG*	unknown
ynjB	unknown	no similarity	
ynjC	≈ membrane protein	*yvgM*	≈ molybdate or sulfate transport
ynjD	≈ anion permease, ATP binding site	*ygaL, opuBA, etc*	≈ anion permease, ATP binding site
ynjE	≈ thiosulfate sulfur transfer to cyanide	*ytwF*	some similarity to a part of YnjE; small protein, GG dipeptide near its C-terminal extremity; ≈ rhodanese; ≈ MoeB (enzyme for C-terminus adenylation); ≈ sulfurylase

Gene names are given following Demerec's nomenclature (three low case letters followed by a capital, in italics). Operons (or likely transcription units) are shown by their gene names with no space. When gene names are separated by a comma, they indicate some similarity; their order correspond to the list of BLAST scores. When a gene name is placed in parentheses it indicates a very weak similarity, that may be interesting in the particular context. In the *B. subtilis* gene list, a gene name preceded by an exclamation mark indicates that it is, inside an operon, a gene with no sequence equivalent in *E. coli*. The ≈ sign indicates a similarity considered as significant with the product having the activity or the structure named immediately after. Annotation are extracted from Colibri (http://bioweb.pasteur.fr/GenoList/Colibri/) and SubtiList (http://bioweb.pasteur.fr/GenoList/SubtiList/).

Mnt of bacteriophage P22 of *S. typhimurium* (He *et al.,* 1992). The regulators of the HU family of *B. stearothermophilus* also belong to this class (Vis *et al.,* 1994). Protein MetJ is a dimer composed of two identical subunits (M_r = 12 kDa), that regulate expression of all genes involved in the biosynthesis of methionine (with the exception of *metH*) and of gene *metK* coding for SAM-synthase (Greene, 1996). It also regulates its own expression (Saint-Girons *et al.,* 1984). MetJ binds upstream sequences of genes that have two to five repeats of the eight nucleotides 'AGACGTCT', the "methionine box". Each methionine box acts as a recognition site for a molecule of repressor. However, one needs at least two boxes in order to bind a molecule of repressor. The number of repeats (and their similarity with the consensus) determines the number of repressor molecules that will bind, and therefore the level of repression. MetJ is an aporepressor, that for its activity requires a co-repressor, AdoMet, whose affinity for the aporepressor is weak (K_d = 200 mM). The dissociation constant of the repressor-operator complex is of 1 nM in the presence of AdoMet and 10-fold less in its absence (Saint-Girons *et al.,* 1986).

As we can see, the regulation of sulfur utilization and the corresponding enzyme activities involve various and complicated mechanisms that are not always well understood. This regulation is linked to the methylation potential of the cell (it is the role of AdoMet) and possibly also to its oxido-reduction potential. The very nature of sulfur leads us to investigate the processes that permit the cell to manage its many oxidation states.

The situation in many organisms, and in particular in *E. coli*, is complicated because the metabolism of sulfur is associated with that of another atom, selenium, which has a particularly enigmatic role.

Selenium and Sulfur

Selenium is an element both indispensible and very toxic to living organisms. Its "normal" intracellular concentration is only ten-fold lower than the concentration for which selenium becomes toxic. This implies that the cell must manage the transport and the mechanisms of detoxification of this element. Selenium, which is very similar to sulfur, is the element immediately below sulfur in the same series of Mendeleieff table. It can therefore very easily take its place inside biological molecules. Selenium's atomic radius is however larger than that of sulfur, which means that the bonds involving selenium atoms are much longer and weaker. Replacement of sulfur by selenium therefore changes the form of molecules or the distances between the atoms. Moreover, the chemical properties of selenocysteine and cysteine are very different. As a consequence, at physiological pH, the selenol- group (-SeH) of selenocysteine is principally ionized, whereas the sulfhydryl group(-SH) of cysteine is principally protonated.

It follows that incorporation of selenocysteine in proteins has a very strong effect on their tertiary structure, and alters the catalytic activity of residues containing an atom of selenium. The chemical differences between selenomethionine and methionine are not that important,

but the sensitivity to oxygen of the former is however higher. The toxic effects of selenium are therefore due to the substitution of sulfur, leading mainly to the formation of selenocysteine.

Selenium is also more easily oxidized than sulfur. As sulfur, elementary selenium is not soluble. Its oxidized forms (selenate and selenite) are very soluble and constitute the source of selenium for the cell. Selenium oxides play in biological systems the role of a strong oxidizing centre. Selenite is an extremely toxic compound because it reacts with the sulfhydryl groups of glutathione (GSH) or of cysteine, producing molecules of the type RS-Se-SR or RSSR and Se^0. The reaction of selenite with thiols produces also H_2O_2 and O_2^-, participating to its toxicity and giving it a mutagenic role (Kramer and Ames, 1988).

Because of the significant chemical similarity between sulfur and selenium, many organisms cannot discriminate between these two elements. Selenium enters the pathway of sulfur assimilation and is non-specifically integrated in various molecules, that normally contain sulfur. The proportion of sulfur replacement by selenium depends on the intracellular concentration of these two elements, but also on the affinity of enzymes involved in these sulfur assimilation pathways, vis-a-vis substrates containing sulfur or selenium.

However, there exist systems permitting biological discrimination between sulfur and selenium. A first example is specific insertion of selenocysteine in some proteins, in the presence of codon UGA in the corresponding messenger RNA. Thus, selenocysteine plays the role of the twenty first amino acid of the genetic code. The synthesis of selenocysteine is separated from sulfur metabolism. It begins with a transfer RNA carrying an activated serine. In this case, selenium stems from monoselenophosphate, a molecule synthesized by selenophosphate synthase (product of gene *selD*) from selenide (HSe^-) and ATP (Lacourciere and Stadtman, 1998). Monoselenophosphate is also the selenium donor for the conversion of 2-thiouridine into 2-selenouridine in some tRNAs. The selenoproteins have an important role in the anaerobic metabolism of *E. coli* (for example the formate hydrogenase (Axley *et al.,* 1990)).

A second example of biological discrimination between sulfur and selenium exists in plants. It is useful to understand what happens in bacteria. It is manifested by greater tolerance to selenium, which is thus accumulated in a large number of organic molecules (Se-methylselenocysteine, γ-glutamyl-Se-methylselenocysteine or selenocystathionine). Most plants resistant to selenium belong to the genus *Astragalus* (*Fabaceae*). They are characterised by: (i) a strong accumulation of selenium in the form of Se-methylselenocysteine, (ii) enhancement of selenium tolerance, and (iii) a strong reduction of selenium incorporation into proteins (Neuhierl *et al.,* 1999). Until very recently, the mechanisms of resistance of plants to selenium, were not clearly established. One supposed that this resistance was linked to the presence of enzymes that, in recruiting storage metabolites containing sulfur, they transformed them into selenium derivatives that could no longer be incorporated into proteins. Indeed, a methyltransferase specific for selenocysteine (selenocysteine methyltransferase) that uses *S*-methylmethionine as donor of methyl group has been purified in *Astragalus bisulcatus* (species tolerant to selenium) (Neuhierl *et al.,* 1999). In *A. bisulcatus*, selenium is metabolized by the sulfur assimilation pathway, which leads to the formation of selenocysteine, as primary compound. Selenocysteine is subsequently methylated very efficiently by selenocysteine methyltransferase, which prevents incorporation of selenocysteine in these proteins or others molecules containing sulfur. It is probable that this compound is subsequently discarded and/or stored in vacuoles, but this remains to be seen.

In *E. coli*, there exists a homologue of selenocysteine methyltransferase, homocysteine methyltransferase encoded by gene *mmuA*. This enzyme presents only a slight preference for selenohomocysteine as compared to homocysteine, whereas the enzyme from *A. bisulcatus* is specific for selenohomocysteine or selenocysteine. It is very likely that the two enzymes are related, and that the enzyme involved in detoxification has evolved from an ancestral protein which did not discriminate between sulfur and selenium.

The specificity for *S*-methylmethionine (and probably also for the S (+) isomer of *S*-adenosylmethionine) is unusual for methyltransferases. However, the two methyltransferases described above use *S*-methylmethionine as donor of methyl group, this product being abundant in plants. Furthermore, both enzymes can use the non-physiological stereoisomer of AdoMet (a spontaneous product of the racemisation of groups coordinated to a sulfur atom). In addition to the role of these enzymes in the detoxification and catabolism of *S*-methylmethionine, they may thus have a function in the scavenging of these molecules (Neuhierl *et al.,* 1999).

Concluding Remarks

Sulfur is an essential component of cells. It has been associated with life from its very early steps, and it still plays a role of major importance. In *E. coli*, we have seen in this review that, among the ca 4,500 genes in the genome, more than one hundred genes are directly involved in some step of sulfur metabolism. Analysis of Table 2 suggests that many more genes are probably related to sulfur in a way or another. Curiously enough, not much work has been devoted not only to the identification of many metabolic steps involving sulfur, but also to the regulation of sulfur availability, disposal or to the metabolic steps controlled by sulfur containing molecules. We hope that the present work will be an incentive for further exploration of this enigmatic domain of gene functions, especially in the context of genetics of genomes.

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