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Minireview

Sulfur metabolism in archaea reveals novel processes

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Summary

Studies on sulfur metabolism in archaea have revealed many novel enzymes and pathways and have advanced our understanding on metabolic processes, not only of the archaea, but of biology in general. A variety of dissimilatory sulfur metabolisms, i.e. reactions used for energy conservation, are found in archaea from both the *Crenarchaeota* and *Euryarchaeota* phyla. Although not yet fully characterized, major processes include aerobic elemental sulfur (S^0) oxidation, anaerobic S^0 reduction, anaerobic sulfate/sulfite reduction and anaerobic respiration of organic sulfur. Assimilatory sulfur metabolism, i.e. reactions used for biosynthesis of sulfur-containing compounds, also possesses some novel features. Cysteine biosynthesis in some archaea uses a unique tRNA-dependent pathway. Fe-S cluster biogenesis in many archaea differs from that in bacteria and eukaryotes and requires unidentified components. The eukaryotic ubiquitin system is conserved in archaea and involved in both protein degradation and biosynthesis of sulfur-containing cofactors. Lastly, specific pathways are utilized for the biosynthesis of coenzyme M and coenzyme B, the sulfur-containing cofactors required for methanogenesis.

Introduction

Organisms of the *Archaea* domain possess many unique and interesting characteristics that clearly distinguish them from bacterial prokaryotes. These characteristics include novel membrane lipids based upon phytanyl ether lipids, information processing systems (DNA replication, transcription and translation) similar to those found in eukaryotes, and unique metabolic pathways, such as methanogenesis and anaerobic methane oxidation (Cavicchioli, 2011). Initial studies on archaea identified three physiological groups: methane-producing archaea (methanogens), extremely halophilic archaea (haloarchaea) and sulfur-dependent thermophilic archaea. However, with the growing knowledge of their physiological and phylogenetic diversities, archaea are currently classified based upon their small subunit ribosomal RNA sequences into several major phyla, including *Crenarchaeota*, *Euryarchaeota*, *Thaumarchaeota*, '*Aigarchaeota*', *Korarchaeota* and *Nanoarchaeota* (Brochier-Armanet *et al*., 2011).

Many archaea utilize sulfur compounds (sulfate, sulfite, thiosulfate, elemental sulfur, sulfide, polysulfide, metal sulfides and organic sulfur) as electron donors or acceptors for energy production (Kletzin, 2007). Some of these processes are shared with bacteria (e.g. dissimilatory sulfate, sulfite and elemental sulfur reduction), while others are only found in archaea so far (e.g. respiratory reduction of the heterodisulfide of coenzyme M and coenzyme B in methanogens). Sulfur compounds are also assimilated for the biosynthesis of sulfur-containing amino acids (Cys and Met), cofactors (e.g. Fe-S clusters, *S*-adenosylmethionine, coenzyme A, biotin, molybdopterin, thiamine, lipoic acid, coenzyme M and coenzyme B) and nucleosides (e.g. 2-thiouridine and 4-thiouridine). Similar to dissimilatory sulfur metabolism, some of the assimilatory processes are common to those found in bacteria and eukaryotes (e.g. the ubiquitin-like system for sulfur transfers), while others are unique to archaea (e.g. the tRNA-dependent cysteine biosynthesis). Overall, studying archaeal sulfur metabolism is important for not only understanding the physiology of archaea but also providing insights into sulfur metabolisms in general and the global sulfur cycle.

Dissimilatory sulfur metabolism

Aerobic sulfur oxidation

Aerobic sulfur oxidation is common in the order *Sulfolobales* of the *Crenarchaeota*. Members of the *Sulfolobales* are exclusively thermoacidophiles with optimal growth

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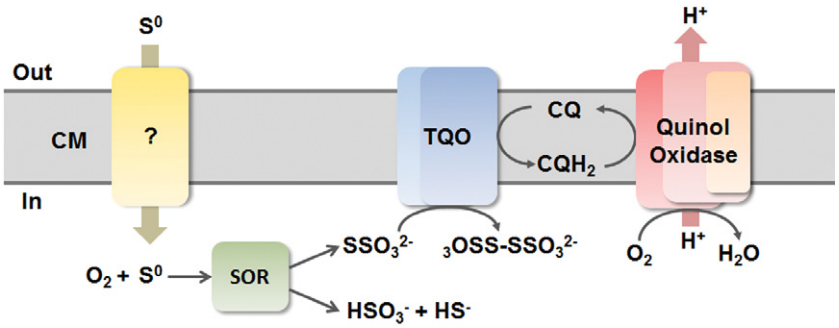

Fig. 1. Model of the coupling of S^0-oxidation to the aerobic respiratory chain with thiosulfate (SSO_3^{2-}) as an intermediate in *Acidianus ambivalens*. S^0 is disproportionated by SOR to form sulfite (HSO_3^-) and sulfide (HS^-) in the presence of O_2, and SSO_3^{2-} is derived from a non-enzymatic condensation of HSO_3^- with S^0. SSO_3^{2-} is oxidized by TQO to form tetrathionate ($S_4O_6^{2-}$), and the electrons are transported through the caldariella quinone (CQ) pool to the terminal quinol oxidase for the reduction of O_2 to H_2O. The terminal quinol oxidase may pump protons for energy conservation. CM, cytoplasmic membrane.

temperatures of 60–90°C and optimal pH of 2–4.5 (Huber and Stetter, 2001; Huber and Prangishvili, 2006). They frequently thrive in continental solfataric fields and have been isolated from such habitats worldwide (Huber and Prangishvili, 2006). They exhibit a broad metabolic diversity and often derive energy by aerobic S^0 oxidation or anaerobic S^0 reduction. The aerobic S^0-oxidation pathway has been studied with *Acidianus ambivalens* as the model organism (Kletzin *et al*., 2004; Kletzin, 2007; 2008).

A proposed scheme of S^0-oxidation in *A. ambivalens* is represented in Fig. 1. The initial step of S^0-oxidation involves a cytoplasmic sulfur oxygenase reductase (SOR), which catalyses the O_2-dependent disproportionation of S^0 to produce sulfite and sulfide ($4\ S^0 + O_2 + 4\ H_2O \rightarrow 2\ HSO_3^- + 2\ H_2S + 2H^+$), and then thiosulfate is likely produced from a nonenzymatic reaction ($S^0 + HSO_3^- \rightarrow S_2O_3^{2-} + H^+$) (Kletzin, 1989). The SOR is a homo-oligomer composed of 24 monomers, which form a hollow sphere enclosing a positively charged nanocompartment (Urich *et al*., 2006; Li *et al*., 2008). The catalytic pocket of each subunit contains three conserved cysteinyl residues (Cys31, Cys101 and Cys104 in *A. ambivalens* SOR) and a low-potential mononuclear non-haem iron centre (Urich *et al*., 2004; 2005; Chen *et al*., 2005). Cys31 exhibits a persulfide modification (R-S-SH), and the persulfide may covalently bind linear sulfur species to form polysulfides that serve as the actual substrate (Urich *et al*., 2006). The iron centre is likely the site for both sulfur oxidation and reduction, and other cofactors or electron carriers are not required for activity (Urich *et al*., 2006). Since SOR in *A. ambivalens* is not coupled to the electron transport chain or substrate-level phosphorylation, its significance in energy conservation is unclear. The SOR homologues have been identified in several other organisms including the crenarchaeotes *Acidianus brierleyi*, *Acidianus tengchongensis* and *Sulfolobus tokodaii*, the euryarchaeotes *Ferroplasma acidarmanus* and *Picrophilus torridus*, and the hyperthermophilic bacterium *Aquifex aeolicus*. The *A. brierleyi* (Emmel *et al*., 1986), *A. tengchongensis* (Chen *et al*., 2005) and *S. tokodaii* (Liu, 2008) SORs have been confirmed to be active, but the activities and physiological functions of other SORs need further investigation.

The products of the initial S^0-oxidation step (sulfide, sulfite and thiosulfate) are presumably further oxidized to sulfate for energy conservation (Zimmermann *et al*., 1999; Kletzin, 2008), although only the thiosulfate oxidation pathway in *A. ambivalens* has been characterized at molecular levels. Two membrane-bound enzyme complexes are involved in thiosulfate oxidation: the thiosulfate : quinone oxidoreductase (TQO), which oxidizes thiosulfate to form tetrathionate with caldariella quinone as the electron acceptor (Müller *et al*., 2004), and the terminal aa_3-type quinol oxidase, which shuttles electrons from the caldariella quinone pool to O_2 (Purschke *et al*., 1997). The first complex, TQO, consists of two subunits (DoxA and DoxD), which are encoded in a bicistronic operon in the *A. ambivalens* genome (Purschke *et al*., 1997). Homologues of *doxA* and *doxD* are also present in the genomes of the crenarchaeotes *Sulfolobus solfataricus*, *S. tokodaii*, *Metallosphaera sedula*, *Acidilobus saccharovorans*, *Vulcanisaeta distributa* and *Vulcanisaeta moutnovskia*, the euryarchaeote *P. torridus*, and the bacteria *Acidithiobacillus ferrooxidans* and *Bacteroides* spp. The second complex, the terminal quinol oxidase, belongs to the haem-copper oxygen reductase superfamily and consists of two large subunits (DoxB and DoxC) and one small subunit (DoxE), which are encoded in a single operon in the *A. ambivalens* genome (Purschke *et al*., 1997). Although this oxidase has been demonstrated to function as a proton pump (Gomes *et al*., 2001), it lacks many key residues that delineate the proton-pumping channel in canonical oxidases (Purschke *et al*., 1997). Instead, this enzyme exhibits a redox-linked reversible conformational change in the

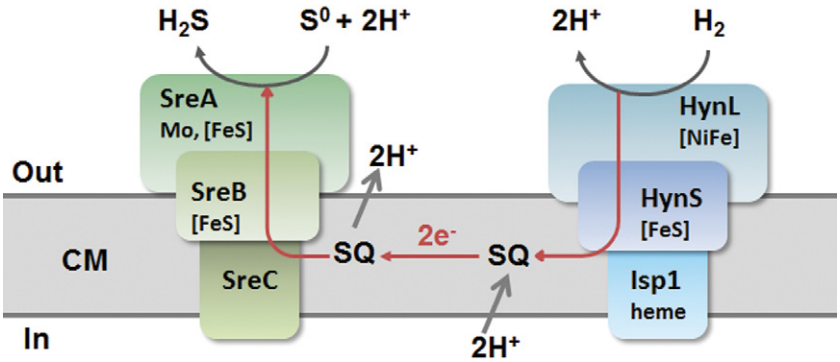

Fig. 2. Model of the coupling of S^0 reduction to the anaerobic electron transport chain in *Acidianus ambivalens*. Two membrane-bound enzyme complexes are required for this process: a sulfur/polysulfide reductase (Sre) (with three major structural subunits: SreA, SreB, and SreC) and a NiFe hydrogenase (Hyn) (with three major structural subunits: HynL, HynS, and Isp1). Subunits of these two enzyme complexes with unknown functions are omitted in this scheme. The electron flow, which is likely mediated by sulfolobus quinone (SQ), is represented by red arrows. A proton gradient is possibly generated by transfer of protons from the inside to the outside of the membrane with SQ as a carrier (Laska *et al.*, 2003). CM, cytoplasmic membrane.

haem a_3-Cu_B centre, and this transition may contribute to proton translocation by a unique mechanism (Das *et al.*, 1999; 2004).

Anaerobic sulfur reduction

Anaerobic reduction of S^0 is a widespread ability in archaea (Blank, 2009). In the *Crenarchaeota*, S^0 reduction is found in the orders *Thermoproteales*, *Sulfolobales* and *Desulfurococcales*; in the *Euryarchaeota*, S^0 reduction is found in the orders *Thermococcales* and *Thermoplasmatales* and many methanogens. At least four mechanisms of S^0 reduction are known in archaea: (i) respiration of S^0 with H_2 as the electron donor (e.g. some crenarchaeotes within the genera *Acidianus*, *Pyrodictium*, *Thermoproteus*, *Pyrobaculum*, and *Ignicoccus* and some euryarchaeotes within the genus *Thermoplasma*), (ii) respiration of S^0 with organic compounds as electron donors (e.g. some crenarchaeotes within the genera *Desulfurococcus*, *Thermodiscus*, *Stetteria*, *Thermoproteus*, *Pyrobaculum*, *Thermocladium*, *Caldivirga* and *Thermofilum*), (iii) fermentation of organic compounds with S^0 as the electron acceptor (e.g. some crenarchaeotes within the genera *Desulfurococcus*, *Thermodiscus*, *Staphylothermus*, and *Hyperthermus* and some euryarchaeotes within the genera *Thermococcus*, *Pyrococcus* and *Palaeococcus*), and lastly (iv) assimilatory reduction of S^0 to H_2S in methanogens, which is not a means of energy conservation. Extensive studies on S^0 reduction have been performed with *A. ambivalens*, *Pyrodictium abyssi*, *Pyrodictium brockii* and *Pyrococcus furiosus* as model organisms.

The mechanism of S^0 respiration with H_2 in some members of the *Crenarchaeota* (e.g. *A. ambivalens* and *Pyrodictium* spp.) is similar to that in some bacteria, such as *Wolinella succinogenes*. This process involves two membrane-bound, multi-subunit enzymes: sulfur or polysulfide reductase (SR/PSR) and NiFe hydrogenase (Hedderich *et al.*, 1998; Kletzin *et al.*, 2004). These two enzymes together reduce S^0 to H_2S with H_2 as the electron donor (Fig. 2). Electron transfer is most likely mediated by sulfolobus quinone in *A. ambivalens* (Laska *et al.*, 2003), by quinone and cytochrome *c* in *P. brockii* (Pihl *et al.*, 1992), and by cytochromes *b* and *c* in *P. abyssi* (Dirmeier *et al.*, 1998). The genes encoding the *A. ambivalens* SR and NiFe hydrogenase have been identified (Fig. 2) (Laska *et al.*, 2003). The first enzyme complex, SR, is encoded by an operon with five open reading frames (ORFs) and consists of the SR large subunit (SreA), the SR FeS subunit (SreB), the membrane anchor protein (SreC), and two proteins with unknown functions (SreD and SreE). SreA and SreB share sequence similarities with the DMSO reductase family and potentially contain Fe-S clusters and molybdopterin (Laska *et al.*, 2003). The second enzyme complex, the NiFe hydrogenase, is encoded by an operon with 12 ORFs and consists of the hydrogenase large subunit (HynL), the hydrogenase small subunit (HynS), the membrane anchor protein (Isp1), some of the maturation proteins (HypCDE and HoxM), and several proteins with unknown functions (Isp2, HynYZ and HypYZ) (Laska *et al.*, 2003). HynL and HynS share sequence similarity with the Group I [NiFe] hydrogenase (membrane-bound, H_2-uptake hydrogenase) (Vignais *et al.*, 2001) and potentially contains [NiFe] and Fe-S clusters (Laska *et al.*, 2003). The *A. ambivalens* SR and hydrogenase genes are more similar in their sequences to bacterial than archaeal homologues, suggesting that these gene clusters were obtained by lateral gene transfer from bacteria (Laska *et al.*, 2003; Blank, 2009).

The mechanism of the fermentation-based S^0 reduction has been studied with *P. furiosus* as the model organism

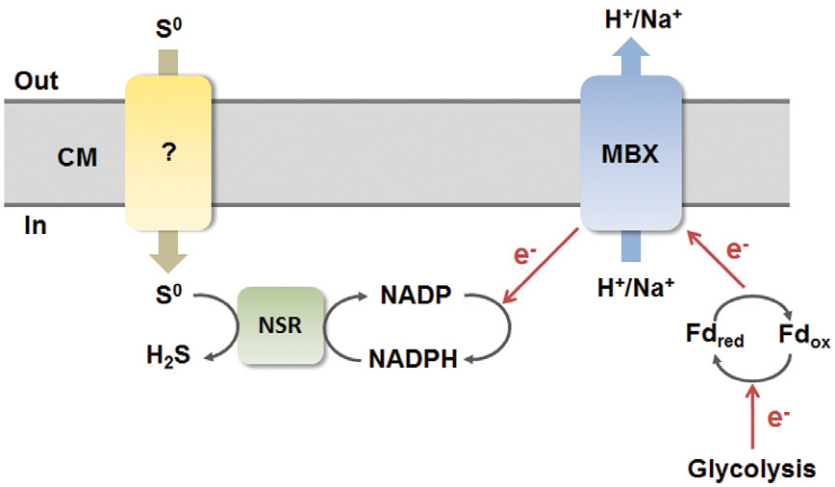

Fig. 3. Model of the coupling of S^0 reduction to glycolysis in *Pyrococcus furiosus*. S^0 is reduced to H_2S mainly by NSR with NADPH as the electron donor. The membrane-bound, multi-subunit MBX is proposed to reduce NADP with Fd_{red}, which is regenerated by the glycolysis pathway. MBX is homologous to the ferredoxin-oxidizing, proton-pumping NiFe hydrogenase, so MBX is proposed to conserve energy by pumping H^+ or Na^+ (Bridger *et al.*, 2011). The electron flow is represented by red arrows. CM, cytoplasmic membrane; Fd, ferredoxin.

(Fig. 3). This organism gains energy by fermentation of peptides or carbohydrates. S^0 is required for growth with peptides but has little effect on growth with maltose (Adams *et al.*, 2001). Two enzymes play key roles in the metabolism of S^0: a cytoplasmic coenzyme A (CoA)-dependent NAD(P)H sulfur oxidoreductase (NSR) and a membrane-bound oxidoreductase complex (MBX). The first enzyme, NSR, is a homodimeric flavoprotein and reduces S^0 to H_2S with NADPH as the electron donor (Schut *et al.*, 2007). Although this enzyme exhibits a high S^0 reduction activity, it is not essential for growth with S^0 (Bridger *et al.*, 2011). Presumably, other multifunctional enzymes, including sulfide dehydrogenases (SuDH I and SuDH II), cytoplasmic hydrogenases (SHI and SHII), and pyruvate oxidoreductase, can compensate for the absence of NSR for S^0 reduction (Bridger *et al.*, 2011). The second enzyme, MBX, is encoded by an operon with 13 ORFs and plays an essential role in mediating electron flow to S^0 (Bridger *et al.*, 2011). MBX shares high sequence similarity with the H_2-evolving, energy-conserving, membrane-bound NiFe-hydrogenase (Sapra *et al.*, 2003) and is proposed to conserve energy by pumping H^+ or Na^+ (Bridger *et al.*, 2011). Although an *in vitro* activity has not yet been established, MBX likely connects glycolysis and NADPH-dependent S^0-reduction via ferredoxin (Bridger *et al.*, 2011). This NSR-MBX S^0-reduction system has only been found in the *Thermococcales* so far.

Anaerobic sulfate and sulfite reduction

Dissimilatory sulfate, sulfite and thiosulfate reduction are present in the genus *Archaeoglobus* of the *Euryarchaeota* (Hartzell and Reed, 2006) and the genera *Pyrobaculum*, *Thermocladium* and *Caldivirga* of the *Crenarchaeota* (Huber *et al.*, 2006). These organisms are hyperthermophiles and able to gain energy by reducing sulfate, thiosulfate, or sulfite to H_2S with organic substrates and/or H_2 as electron donors. *Archaeoglobus fulgidus*, which has been studied as the model organism, contains the complete bacterial pathway for dissimilatory sulfate reduction (Dahl *et al.*, 1994); however, the connection of this process to energy conservation is still unclear.

Three cytoplasmic enzymes are involved in dissimilatory sulfate reduction in *Archaeoglobus*: ATP sulfurylase (or ATP : sulfate adenylytransferases), adenosine 5'-phosphosulfate (APS) reductase and dissimilatory sulfite reductase (dSiR). The first enzyme, ATP sulfurylase, is a homo-oligomer and catalyses the activation of sulfate with ATP to generate APS and pyrophosphate (Sperling *et al.*, 2001). The second enzyme, APS reductase, is a $\alpha_2\beta_2$-heterotetrameric iron-sulfur flavoenzyme and catalyses the reversible reduction of APS to AMP and sulfite (Dahl and Trüper, 2001). The two electrons required for APS reduction are likely transferred from an unknown electron donor to FAD via two [4Fe-4S] clusters (Fritz *et al.*, 2002a,b; Schiffer *et al.*, 2006). The third enzyme, dSiR (encoded by *dsrA* and *dsrB*), is a $\alpha_2\beta_2$-heterotetrameric sirohaem-[4Fe-4S]-containing enzyme and catalyses the reduction of sulfite to sulfide (Oliveira *et al.*, 2008; Schiffer *et al.*, 2008; Parey *et al.*, 2010). This enzyme is conserved in sulfate- and sulfite-reducing prokaryotes, but its reaction mechanism is poorly understood. Several unsolved questions of the final step include whether thiosulfate ($S_2O_3^{2-}$) and trithionate ($S_3O_6^{2-}$) are necessary intermediates, what is the physiological electron donor, and how sulfite reduction is coupled to energy conservation. Recent crystal structural work of dSiR from *Desulfovibrio vulgaris* (Oliveira *et al.*, 2008) and *A. fulgidus* (Schiffer *et al.*, 2008; Parey *et al.*, 2010) has indicated that dSiR reduces sulfite via a series of two-electron transfers. Furthermore, the *D. vulgaris* dSiR has been proposed to reduce sulfite with unknown electron donors to form a S^0-intermediate, which is then transferred to a

cysteinyl residue on DsrC, a protein tightly associated with dSiR, to form a persulfide (Oliveira *et al*., 2008). This persulfide is then reduced by another cysteinyl residue of DsrC to release H_2S and form a disulfide (Oliveira *et al*., 2008). The disulfide is then reduced by the membrane quinone pool and, thus, connected to energy conservation (Oliveira *et al*., 2008). However, the dSiR purified from *A. fulgidus* does not include DsrC (Schiffer *et al*., 2008; Parey *et al*., 2010), and therefore the reaction mechanism of archaeal dSiR awaits further investigation.

Anaerobic dimethylsulfoxide (DMSO) reduction

Anaerobic DMSO respiration has been found in some haloarchaea. Haloarchaea generally grow heterotrophically under aerobic conditions, although many of them can also grow anaerobically and conserve energy via photophosphorylation, fermentation of arginine, and anaerobic respiration with electron acceptors such as nitrate, DMSO, trimethylamine-*N*-oxide (TMAO), and fumarate (Hartmann *et al*., 1980; Oren and Trüper, 1990; Oren, 1991; 2006). The molecular basis of DMSO reduction has been characterized in *Halobacterium* sp. strain NRC-1 (Müller and DasSarma, 2005).

DMSO and TMAO respiration in *Halobacterium* sp. strain NRC-1 requires a six-gene operon *dmsREABCD*, which encodes a heterotrimeric DMSO/TMAO reductase (DmsABC), a putative activator (DmsR), and two putative molecular chaperones necessary for DMSO reductase maturation (DmsD and DmsE) (Müller and DasSarma, 2005). The molecular components of the haloarchaeal DMSO/TMAO reductase is similar to that in *Escherichia coli*, which consists a molybdopterin-containing catalytic subunit (DmsA), a Fe-S cluster-containing electron transfer subunit (DmsB) and a membrane anchor subunit (DmsC) (Gennis and Stewart, 1996). The electrons are likely transferred to the DMSO/TMAO reductase via menaquinone, and this process presumably results in the generation of a proton motive force (Müller and DasSarma, 2005). Homologues of the DMSO/TMAO reductase are present in several other haloarchaea including *Haloarcula marismortui*, *Halobacterium salinarum*, *Haloferax volcanii* and *Halomicrobium mukohataei*.

Biosynthesis of sulfur compounds

Cysteine

The synthesis of L-cysteine is a major process by which inorganic sulfur is incorporated into organic compounds. At least two novel cysteine biosynthesis pathways are present in archaea (Fig. 4). Both pathways start from *O*-phosphoserine (Sep), which is synthesized from D-3-phosphoglycerate and is also an intermediate in the pathways for serine and cystathionine biosynthesis (Helgadóttir *et al*., 2007). Since Sep is much more thermostable than *O*-acetylserine, which is a common intermediate for cysteine biosynthesis in plants and many bacteria, the utilization of Sep for cysteine biosynthesis may represent an adaptation of certain archaea to high growth temperatures. The first pathway was identified in the hyperthermophilic crenarchaeon *Aeropyrum pernix*. In this pathway Sep is directly sulfhydrylated with sulfide to form cysteine (Mino and Ishikawa, 2003a,b). This reaction is catalysed by *O*-phosphoserine sulfhydrylase, which is homologous to *O*-acetylserine sulfhydrylase and cystathionine β-synthase in bacteria (Oda *et al*., 2005). The second pathway was identified in methanogenic archaea. In this pathway Sep is first aminoacylated to $tRNA^{Cys}$, and then Sep-$tRNA^{Cys}$ is converted to Cys-$tRNA^{Cys}$ with an unknown sulfur donor (Sauerwald *et al*., 2005; Hauenstein and Perona, 2008; Liu *et al*., 2012a). These two steps are catalysed by *O*-phosphoseryl-tRNA synthetase (SepRS) and Sep-tRNA : Cys-tRNA synthase (SepCysS) respectively (Sauerwald *et al*., 2005). Many *Methanococcales* and thermophilic *Methanobacteriales* species contain only the tRNA-dependent pathway for *de novo* cysteine biosynthesis. However, *Methanosarcinales* species also possess serine acetyltransferase and *O*-acetylserine sulfhydrylase, the bacterial enzymes for cysteine biosynthesis (Fig. 4) in addition to the tRNA-dependent pathway (Borup and Ferry, 2000a,b; Kitabatake *et al*., 2000); therefore, cysteine biosynthesis pathways may be redundant in some methanogens.

Fe-S clusters

The process of Fe-S cluster assembly in archaea remains a key issue for future research. Three multiprotein systems are known for Fe-S cluster biogenesis in bacteria and eukaryotes: the nitrogen fixation (NIF), the iron-sulfur cluster (ISC) and the mobilization of sulfur (SUF) systems (reviewed in Johnson *et al*., 2005; Lill, 2009; Py and Barras, 2010). All three systems involve a cysteine desulfurase that transfers sulfur from free cysteine to Fe-S clusters through generation of a persulfide enzyme adduct. A scaffold protein also provides the chemical and structural environment that facilitates the assembly and delivery of Fe-S clusters. The cysteine desulfurase of the SUF system (SufS) in haloarchaea is active for generating a persulfide intermediate using free cysteine as the sulfur donor (Zafrilla *et al*., 2010), which suggests that some archaea utilize the bacteria route for sulfur incorporation into Fe-S clusters. However, many methanogens as well as many non-methanogenic archaea from solfataric hydrothermal systems lack homologues of cysteine desulfurase in their genomes. Moreover, in *Methanococcus maripaludis*, the sulfur in Fe-S clusters does not originate

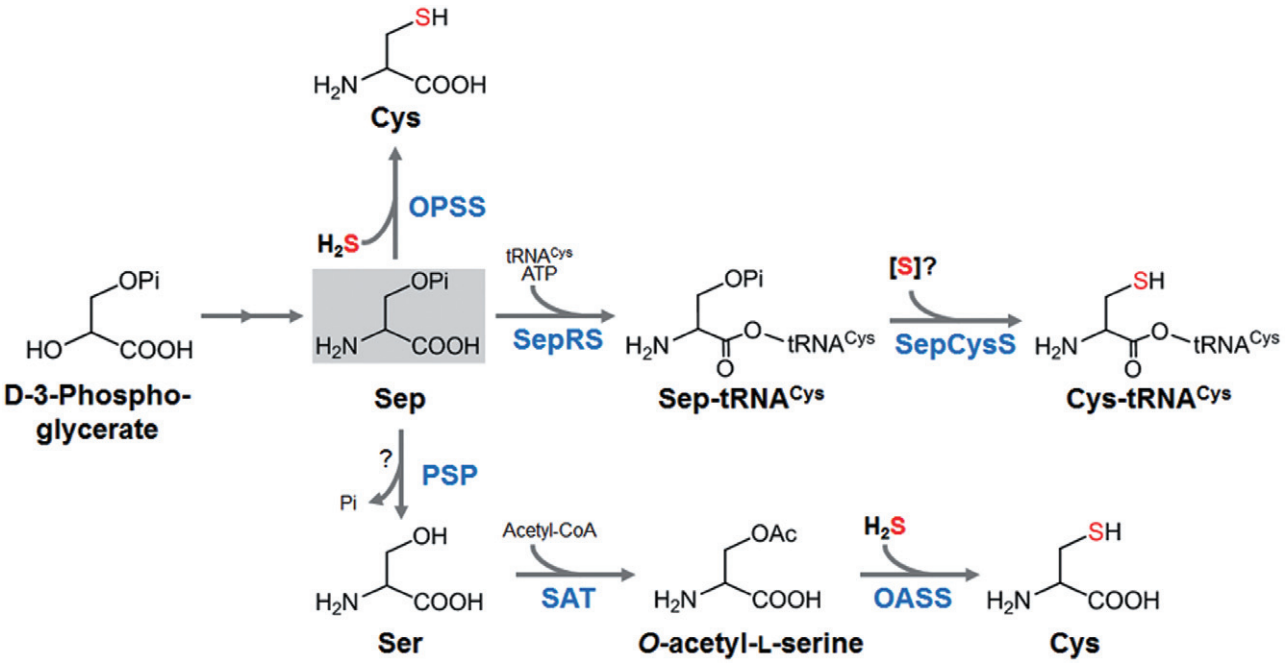

Fig. 4. Cysteine biosynthesis pathways in archaea. D-3-phosphoglycerate is converted to *O*-phospho-L-serine (Sep) via an oxidation and a transamination step (Helgadóttir *et al*., 2007). In the hyperthermophilic crenarchaeon *Aeropyrum pernix*, Sep is sulfhydrylated with sulfide by *O*-phosphoserine sulfhydrylase (OPSS) to form cysteine (Mino and Ishikawa, 2003a,b; Oda *et al*., 2005). In methanogenic archaea and *Archaeoglobus* spp., $tRNA^{Cys}$ is aminoacylated with Sep by *O*-phosphoseryl-tRNA synthetase (SepRS), and then Sep-$tRNA^{Cys}$ is converted with an unknown sulfur donor to form Cys-$tRNA^{Cys}$ by Sep-tRNA : Cys-tRNA synthase (SepCysS) (Sauerwald *et al*., 2005; Hauenstein and Perona, 2008; Liu *et al*., 2012b). In some *Methanosarcinales* species, the bacterial cysteine biosynthesis pathway is also present in addition to the tRNA-dependent SepRS/SepCysS pathway. In this pathway, Sep is likely dephosphorylated by *O*-phosphoserine phosphatase (PSP) to form Ser, activated with acetyl-CoA by serine acetyltransferase (SAT) to form *O*-acetyl-serine, and sulfhydrylated with sulfide by *O*-acetylserine sulfhydrylase (OASS) to form Cys (Borup and Ferry, 2000a,b; Kitabatake *et al*., 2000).

from cysteine, which suggests that these methanogenic archaea use an unknown mechanism for sulfur incorporation (Liu *et al*., 2010; 2012b).

Only SufB, SufC and ApbC/Nbp35 of the known Fe-S cluster assembly proteins are conserved across archaea (Boyd *et al*., 2009; Iwasaki, 2010; Liu *et al*., 2010), suggesting that many of the proteins required for Fe-S cluster biogenesis in archaea have yet to be identified (Fig. 5). While the SUF system in *E. coli* operates under oxidative stress and iron starvation conditions (Outten *et al*., 2004; Yeo *et al*., 2006; Lee *et al*., 2008), it is the only Fe-S cluster assembly system in certain bacteria, such as *Thermatoga maritima*, *Mycobacterium tuberculosis* and cyanobacteria (Takahashi and Tokumoto, 2002; Huet *et al*., 2005). Thus, SUF is also capable of serving as the major assembly system. The SufBCD complex has sequence hallmarks of the ATP binding cassette (ABC)-type transporters and may function as the assembling centre for Fe-S clusters, with SufB as a scaffold and SufC as an ATPase (Eccleston *et al*., 2006; Layer *et al*., 2007). In most archaea, *sufB* and *sufC* are arranged as neighbouring genes. SufB and SufC in *M. maripaludis* are likely to be essential (F. Sarmiento, unpubl. data), which is consistent with a major role in Fe-S cluster biogenesis. In

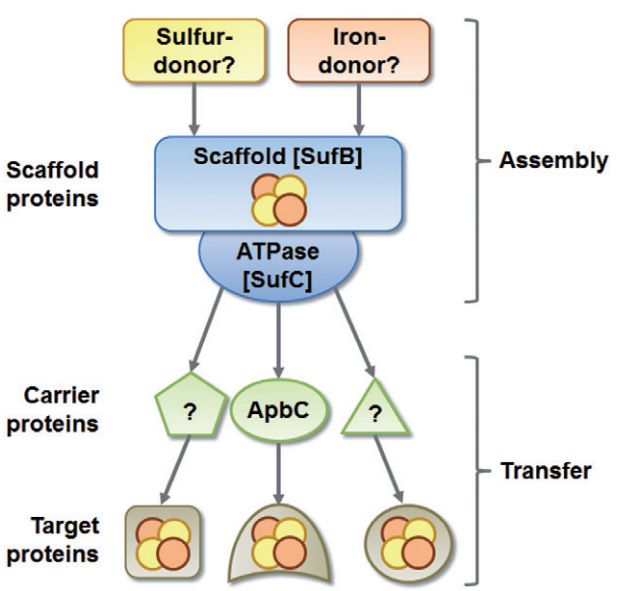

Fig. 5. Model of Fe-S cluster biogenesis in archaea. Fe-S cluster biogenesis involves two stages: assembly and transfer. In the assembly stage, a scaffold protein (SufB) receives sulfur and iron from unknown donors and builds Fe-S clusters. In the transfer stage, an ATPase (SufC) facilitates the release of Fe-S clusters from the scaffold, and then the Fe-S clusters are transported to target apoproteins via different carrier proteins (e.g. ApbC).

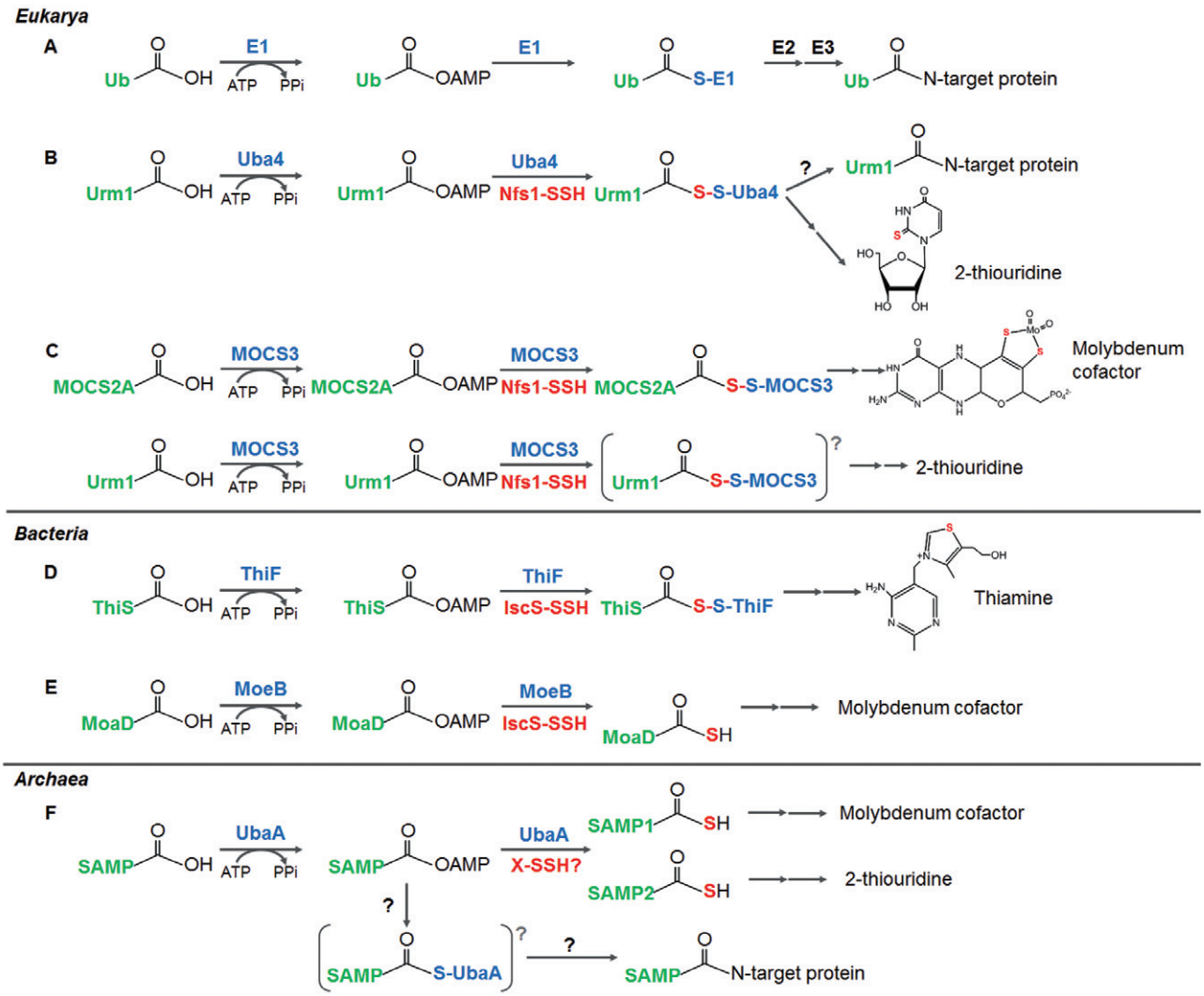

Fig. 6. The common mechanism of sulfur transfer and protein conjugation via ubiquitin-related systems: (A) the eukaryotic ubiquitin pathway for protein modification; (B) the yeast Urm1 pathway for protein modification and tRNA thiolation; (C) the human pathway for molybdenum cofactor biosynthesis and tRNA thiolation; (D) the bacterial pathway for thiamine biosynthesis; (E) the bacterial pathway for molybdenum cofactor biosynthesis; (F) the archaeal SAMP pathway for molybdenum cofactor biosynthesis, tRNA thiolation, and protein modification. All pathways require E1-like proteins (in blue) to activate the ubiquitin-like (Ubl) proteins (in green) by adenylation of their carboxyl-terminal glycine. For sulfur transfer processes, the Ubl proteins receive sulfur from a protein-bound cysteinyl persulfide (in red) and form either a covalent complex with the E1-like protein or a carboxyl-terminal thiocarboxylate. The Ubl protein then donates sulfur for downstream processes.

bacteria and eukaryotes, ApbC/Nbp35 acts as a Fe-S cluster carrier protein that transfers clusters from a scaffold protein to a target apoprotein (Roy *et al.*, 2003; Hausmann *et al.*, 2005; Netz *et al.*, 2007; Boyd *et al.*, 2008). While the ApbC homologue from *M. maripaludis* can reconstitute Fe-S clusters with Fe^{3+}, dithiothreitol, and S^{2-} *in vitro* (Boyd *et al.*, 2009), the physiological function of this protein in archaea remains to be clarified.

Molybdenum cofactor and 2-thiouridine

Sulfur incorporation into molybdenum cofactor (MoCo) and 2-thiouridine (s^2U) in haloarchaea utilize small archaeal modifier proteins (SAMPs), which are also involved in protein conjugation that targets proteins for degradation (Fig. 6) (Humbard *et al.*, 2010; Maupin-Furlow, 2011; Miranda *et al.*, 2011). SAMPs have a β-grasp fold structure and a Gly-Gly motif at the carboxyl-terminus, which are similar to ubiquitin (Ub) and ubiquitin-like (Ubl) proteins (Humbard *et al.*, 2010; Ranjan *et al.*, 2010; Jeong *et al.*, 2011). The Ub/Ubl systems are involved in protein conjugation in eukaryotes and also in the sulfur transfers for MoCo biosynthesis in bacteria and eukaryotes (Stallmeyer *et al.*, 1999; Lake *et al.*, 2001; Wuebbens and Rajagopalan, 2003; Matthies *et al.*, 2004; Marelja *et al.*, 2008), s^2U biosynthesis in eukaryotes (Pedrioli *et al.*, 2008;

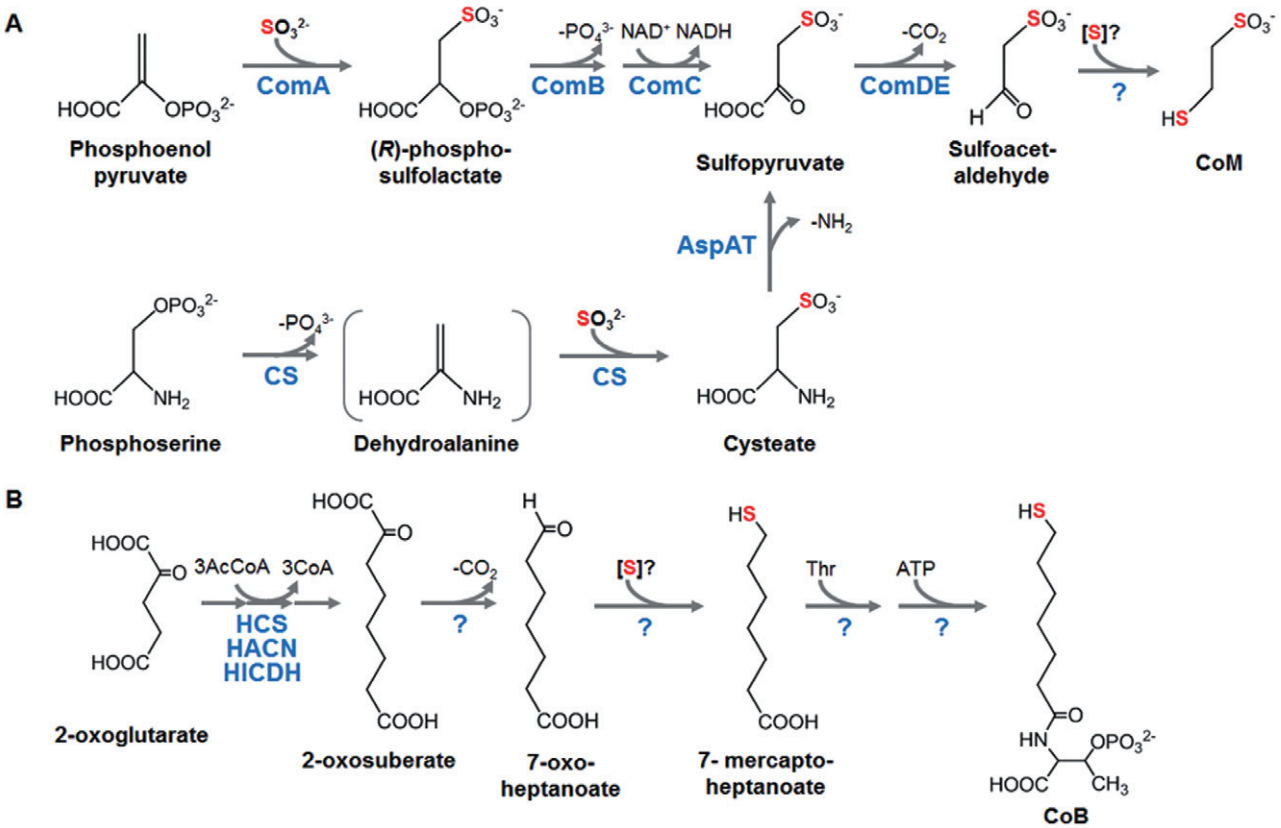

Fig. 7. The coenzyme M (CoM) (A) and coenzyme B (CoB) (B) biosynthetic pathways in methanogens.
A. For CoM biosynthesis, two different pathways are used to produce the intermediate sulfopyruvate. The orders *Methanococcales*, *Methanobacteriales* and *Methanopyrales* use the upper pathway catalysed by ComA, B and C; and the orders *Methanosarcinales* and *Methanomicrobiales* use the lower pathway catalysed by cysteate synthase (CS) and aspartate aminotransferase (AspAT). Sulfur (in red) is incorporated in two steps, with sulfite and an unknown molecule as sulfur donors respectively.
B. For CoB biosynthesis, sulfur is incorporated with an unknown sulfur donor.
ComA, phosphosulfolactate synthase; ComB, 2-phosphosulfolactate phosphatase; ComC, sulfolactate dehydrogenase; ComDE, sulfopyruvate decarboxylase; CoA, coenzyme A; AcCoA, acetyl-CoA; HCS, homocitrate synthase; HACN, homoaconitase; HICDH, homoisocitrate dehydrogenase.

Schlieker *et al*., 2008; Leidel *et al*., 2009; Mullick Chowdhury *et al*., 2012), cysteine biosynthesis in bacteria (O'Leary *et al*., 2008), thiamine biosynthesis in bacteria (Taylor *et al*., 1998) and thioquinolobactin siderophore biosynthesis in bacteria (Godert *et al*., 2007). In these sulfur transfers, the Ubl proteins are first activated by adenylation catalysed by E1-like proteins. They then receive a sulfur atom from a persulfide, which is covalently bound to a cysteinyl residue of the rhodanese-domain of the E1-like protein (e.g. Uba4 and MOSC3 in eukaryotes) or a separate rhodanese-domain containing protein (e.g. ThiI that is involved in thiamine biosynthesis in *E. coli*). The rhodanese-domain is persulfurated by cysteine desulfurases (Nfs1 in eukaryotes and IscS in bacteria) (Kessler, 2006). As a consequence, the Ub/Ubl protein forms a carboxyl-terminus thiocarboxylate or a covalent complex with the E1-like protein (through an acyl disulfide bond) and provides sulfur for subsequent reactions (Fig. 6). Given the similar structures and cellular functions of SAMPs and Ub/Ubl proteins, the SAMP pathway presumably operates in a similar pattern as the ubiquitin system. However, although most archaea contain SAMPs and E1-like proteins (except several methanogens including *Methanocaldococcus* spp., *Methanothermococcus okinawensis*, *Methanococcus aeolicus* and *Methanopyrus kandleri*) (Darwin and Hofmann, 2010; Makarova and Koonin, 2010), cysteine desulfurases are not conserved across archaea; therefore, the thiolation of SAMPs may require novel components.

Coenzyme M and coenzyme B

The thiol-containing cofactors coenzyme M (CoM; 2-mercaptoethanesulfonic acid) (McBride and Wolfe, 1971; Taylor *et al*., 1974) and coenzyme B (CoB; 7-mercaptoheptanoylthreonine phosphate) (Noll *et al*., 1986) are involved in methanogenesis and anaerobic methane oxidation (the reverse methanogenesis) in

archaea (Scheller *et al*., 2010). In addition, CoM is also involved in alkene oxidation in a few bacteria, including mycobacteria, *Xanthobacter* and *Rhodococcus* (Allen *et al*., 1999; Krishnakumar *et al*., 2008). CoM and CoB biosynthesis have been extensively studied in methanogens (reviewed in Fahey, 2001; White, 2001; Graham and White, 2002; Graham, 2011), and both cofactors arise from 2-oxoacids through biosynthetic pathways that recruit enzymes from amino acid and 2-oxoacid metabolism.

Methanogens use two different pathways to synthesize sulfopyruvate, a precursor of CoM (Fig. 7A). The first pathway is utilized by the orders *Methanococcales*, *Methanobacteriales* and *Methanopyrales* and involves three enzymes (ComA, B and C). ComA incorporates sulfite into phosphoenolpyruvate to form phosphosulfolactate, which is then converted to sulfopyruvate (Graham *et al*., 2002). The second pathway is utilized by the orders *Methanosarcinales* and *Methanomicrobiales* and involves two pyridoxal 5'-phosphate (PLP)-dependent enzymes (cysteate synthase and a paralogue of aspartate aminotransferase). Cysteate synthase incorporates sulfite into phosphoserine to form cysteate, which is then converted to sulfopyruvate (Graham *et al*., 2009). The source of sulfite is not known for either of these pathways. Following the formation of sulfopyruvate, all methanogens use the enzyme composed of ComD and ComE to produce sulfoacetaldehyde, which is then thiolated to form CoM by an undefined mechanism. Cysteine serves as a sufficient sulfur source for this final step *in vitro* (White, 1988); however, the *in vivo* sulfur donor is still under investigation.

CoB is proposed to be synthesized through the extension of 2-oxoglutarate to form 2-oxosuberate, the decarboxylation and thiolation of 2-oxosuberate to form 7-mercaptoheptanoate (White, 1989a,b), followed by the addition and phosphorylation of a threonine head group (Solow and White, 1997) (Fig. 7B). Three enzymes involved in the formation of 2-oxosuberate, which is also an intermediate of biotin biosynthesis, have been identified (Graham, 2011). However, the enzymes responsible for the subsequent steps are not yet known. The thiolation step requires the replacement of an aldehyde with a thiol, in a reaction analogous to the final step of CoM synthesis. However, in this case cysteine does not serve as a direct sulfur source *in vitro* (White, 1989b). Possibly, a protein bound persulfide or thiocarboxylate serves as the physiological sulfur donor in a mechanism similar to sulfur transfers for Fe-S cluster or MoCo biosynthesis respectively.

Concluding remarks

Recent discoveries have indicated a number of unique enzymes and pathways involved in sulfur metabolism in archaea. However, many questions still exist for both dissimilatory and assimilatory sulfur metabolism. For example, how do sulfur molecules (e.g. S^0, SO_4^{2-}, SO_3^{2-} and $S_2O_3^{2-}$) get into the cell; what are the missing enzymes and cofactors that couple sulfur oxidation and reduction with energy conservation; what are the iron and sulfur donors for Fe-S cluster biogenesis; does the ubiquitin-like SAMP system have additional physiological functions; how widely is each process distributed; and what are their environmental and evolutionary relevance? Future investigations taking advantage of genomic sequences and in-depth omics analysis, together with developed genetic tools are necessary to uncover additional components and novel pathways to fully elucidate archaeal sulfur metabolism.

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