Silke Leimkühler

The biosynthesis of the molybdenum cofactors in Escherichia coli

Suggested citation referring to the original publication:
Environmental microbiology 22 (2020) 6, pp. 2007 - 2026
DOI https://doi.org/10.1111/1462-2920.15003
ISSN 1462-2912, 1462-2920

Journal article | Version of record

Secondary publication archived on the Publication Server of the University of Potsdam:
Zweitveröffentlichungen der Universität Potsdam : Mathematisch-Naturwissenschaftliche Reihe 1433
ISSN: 1866-8372
https://nbn-resolving.org/urn:nbn:de:kobv:517-opus4-516559
DOI: https://doi.org/10.25932/publishup-51655

environmental microbiology

Environmental Microbiology (2020) **22**(6), 2007–2026 doi:10.1111/1462-2920.15003

Minireview

The biosynthesis of the molybdenum cofactors in *Escherichia coli*

Silke Leimkühler *

Department of Molecular Enzymology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany.

Summary

The biosynthesis of the molybdenum cofactor (Moco) is highly conserved among all kingdoms of life. In all molybdoenzymes containing Moco, the molybdenum atom is coordinated to a dithiolene group present in the pterin-based 6-alkyl side chain of molybdopterin (MPT). In general, the biosynthesis of Moco can be divided into four steps in in bacteria: (i) the starting point is the formation of the cyclic pyranopterin monophosphate (cPMP) from 5′-GTP, (ii) in the second step the two sulfur atoms are inserted into cPMP leading to the formation of MPT, (iii) in the third step the molybdenum atom is inserted into MPT to form Moco and (iv) in the fourth step bis-Mo-MPT is formed and an additional modification of Moco is possible with the attachment of a nucleotide (CMP or GMP) to the phosphate group of MPT, forming the dinucleotide variants of Moco. This review presents an update on the well-characterized Moco biosynthesis in the model organism *Escherichia coli* including novel discoveries from the recent years.

Introduction into general aspects of the molybdenum cofactor and its biosynthesis

The transition element molybdenum is an important trace element for bacteria, archaea and eukaryotes. In its biologically active form molybdate (MoO_4^{2-}), it enters the cell by active transport systems (Aguilar-Barajas *et al*., 2011). In the cell, molybdenum is either coordinated to the molybdenum cofactors (Moco) which contain a dithiolene group on the 6-alkyl side chain of a pterin called molybdopterin (MPT) (Rajagopalan and Johnson, 1992), or in some bacteria to the Fe-S cluster based iron-molybdenum cofactor (FeMoco) which forms the active site of nitrogenase (Schwarz *et al*., 2009; Ribbe *et al*., 2013), an enzyme which is not covered in this review. Molybdenum forms the active site of molybdoenzymes to which the substrate is bound, and these enzymes execute key transformations in the metabolism of nitrogen, sulfur and carbon compounds (Hille, 1996; Hille *et al*., 2014). Molybdenum has a chemical versatility that is useful to biological systems: it is redox-active under physiological conditions (ranging between the oxidation states VI and IV); since the V oxidation state is also accessible, the metal can act as transducer between obligatory two-electron and one-electron oxidation–reduction systems and it can exist over a wide range of redox potentials (Hille, 1996, 2002).

The catalyzed reactions are in most cases oxo-transfer reactions, e.g. the hydroxylation of carbon centres, however, also C-H bond cleavage reactions are catalyzed (Hille *et al*., 2014). Evolutionary studies indicate that molybdoenzymes can be divided into ‘ancient’ members already present in LUCA and into recently evolved enzymes (Baymann *et al*., 2003; Schoepp-Cothenet *et al*., 2012). By comparative genomics analyses, it was shown that 74% of bacteria from almost all phyla harbour enzymes containing Moco (Leimkühler and Iobbi-Nivol, 2016). The number of molybdoenzymes synthetized varies from one organism to another and the largest number of molybdoenzymes was identified in *Desulfitobacterium hafniense* (Kim *et al*., 2012). This organism contains about 60 different molybdoenzymes, while in contrast the well-studied model organism *Escherichia coli* harbours 19 different molybdoenzymes most of which have been characterized to date. In the redox reactions catalyzed, electron transfer from or to Moco is mediated by additional redox centres like Fe-S centres, cytochromes or FAD/FMN cofactors that are bound to the protein (Iobbi-Nivol and Leimkühler, 2013; Yokoyama and Leimkühler, 2015). During these redox reactions, the molybdenum

Received 10 February, 2020; revised 10 March, 2020; accepted 11 March, 2020. *For correspondence. E-mail sleim@uni-potsdam.de; Tel. +49-331-977-5603; Fax +49-331-977-5128.

atom can exist in the oxidation states IV, V and VI and couples proton transfer to electron transfer (Hille, 2002).

The chemical nature of Moco has been determined by Rajagopalan and Johnson (1992). In general, the structure of Moco is a tricyclic pyranopterin with a unique dithiolene group coordinating the molybdenum atom. Moco biosynthesis is a highly conserved pathway which has been best studied in *E. coli* by using a combination of biochemical, genetic and structural approaches (Leimkühler *et al*., 2011). In all prokaryotes, Moco biosynthesis can be divided into four general steps based on stable intermediates that were isolated (Rajagopalan, 1996; Mendel and Leimkühler, 2015): (i) formation of cyclic pyranopterin monophosphate (cPMP), (ii) formation of MPT from cPMP by the insertion of two sulfur atoms, (iii) insertion of molybdenum into MPT to form Mo-MPT and (iv) formation of bis-Mo-MPT from two Mo-MPT moieties in addition to possible further modifications of Moco by the attachment of different nucleotides like CMP or GMP, forming the MPT cytosine dinucleotide cofactor (MCD) and bis-molybdopterin guanine dinucleotide (bis-MGD) cofactors respectively. These dinucleotide modifications are not present in eukaryotic molybdoenzymes.

In *E. coli*, five gene loci are directly involved in the biosynthesis of Moco that were named *moa (moaABCDE)*, *mob (mobAB)*, *moc (mocA)*, *moe (moeAB)* and *mog (mogA)* (Shanmugam *et al*., 1992). These loci are organized in operons and code for 11 proteins in total with 9 of those having an essential role in Moco biosynthesis (Table 1) (Shanmugam *et al*., 1992; Rajagopalan, 1996). Each of the steps of Moco biosynthesis will be described in detail in the following sections.

Different modifications in the ligand sphere of the molybdenum atom or additional modifications at the pterin backbone have been identified. According to these differences, molybdoenzymes have been classified into three families: the xanthine oxidase (XO) family, the sulfite oxidase (SO) family and the dimethyl sulfoxide (DMSO) reductase family (Hille, 1996) (Fig. 1). Newer classifications also include the tungsten-containing enzymes in one enzyme family (Hille, 2002; Magalon *et al*., 2011). Since *E. coli* does not contain tungsten-containing enzymes, we will stick to the original classification with three enzyme families in this review. Enzymes of the XO family contain in general a Moco with an MPT (or MCD)-$Mo^{VI}OS(OH)$ ligand sphere, and typical members in bacteria are xanthine dehydrogenases and aldehyde oxidoreductases (Hille *et al*., 2014). Enzymes of the SO family contain a Moco with an MPT-$Mo^{VI}O_2$Cys core, and this family comprises bacterial sulfite dehydrogenases and methionine sulfoxide reductase (MsrP). Enzymes of the DMSO reductase family are exclusively found in prokaryotes. These enzymes comprise a Moco with an MGD_2-$Mo^{VI}XY$ core with X being either a sulfur or an oxygen ligand and Y either being a hydroxo or amino acid ligand (Ser, Cys, Sec and Asp ligands were identified so far). In contrast, mainly anaerobic archaea and some bacteria require tungsten (W) instead of molybdenum and bind the W in a similar manner to the dithiolene group of MPT, forming the tungsten cofactor (Wco) (Hagen, 2011). While the biosynthesis of Moco is well characterized, the biosynthesis of Wco has not been studied in detail so far. In general, it is believed that the basic mechanisms of Moco and Wco synthesis are similar, because most genes with an assigned function in Moco biosynthesis are also present in the genome of organisms that use tungsten in their metabolism.

This review will focus on the biosynthesis of Moco in bacteria, and its incorporation into specific target proteins with a particular focus on the one in *E. coli*, which represents the best studied model organism for Moco biosynthesis and molybdoenzymes so far. For an overview on tungsten-containing enzymes, the reader is referred to other reviews (Johnson *et al*., 1996; Kletzin and Adams, 1996; Romao, 2009; Hagen, 2011).

Table 1. Proteins for Moco biosynthesis in bacteria, plants and humans and the catalyzed reactions.

Bacteria	Reaction catalyzed
MoaA	Formation of (3′,8-cH_2GTP) from 5′-GTP
MoaB	Unknown function
MoaC	Formation of cPMP from (3′,8-cH_2GTP)
MoaD	Sulfur transfer to cPMP, formation of MPT
MoaE	Binding of cPMP, formation of MPT
MoeB	Adenylation of MPT synthase small subunit
MogA	Formation of MPT-AMP
MoeA	Molybdate insertion into MPT-AMP, Mo-MPT formation
MobA	Formation of bis-Mo-MPT, GMP transfer to bis-Mo-MPT, formation of bis-MGD
MobB	Not essential role in Moco biosynthesis
MocA	CMP transfer to Mo-MPT, formation of MCD
IscS	L-cysteine desulfurase
TusA	Persulfide-containing sulfur transferase additionally involved in s^2U34 tRNA modification

First step: Conversion of 5′-GTP to cPMP

The biosynthesis of Moco starts with 5′-GTP which is converted to cPMP (Fig. 2). cPMP is the first stable intermediate in Moco biosynthesis that was identified in 1993, initially named Precursor Z and later identified to be a 6-alkyl pterin with a cyclic phosphate group at the C2′ and C4′ atoms (Wuebbens and Rajagopalan, 1993, 1995; Santamaria-Araujo *et al*., 2004). The synthesis of cPMP is catalyzed by two enzymes, and the reaction was studied in detail using the bacterial enzymes from *Staphylococcus aureus* (Hover *et al*., 2013; Mehta *et al*., 2013). In bacteria, the *moaA* and *moaC* gene products are involved in this reaction (Wuebbens and Rajagopalan, 1993, 1995)

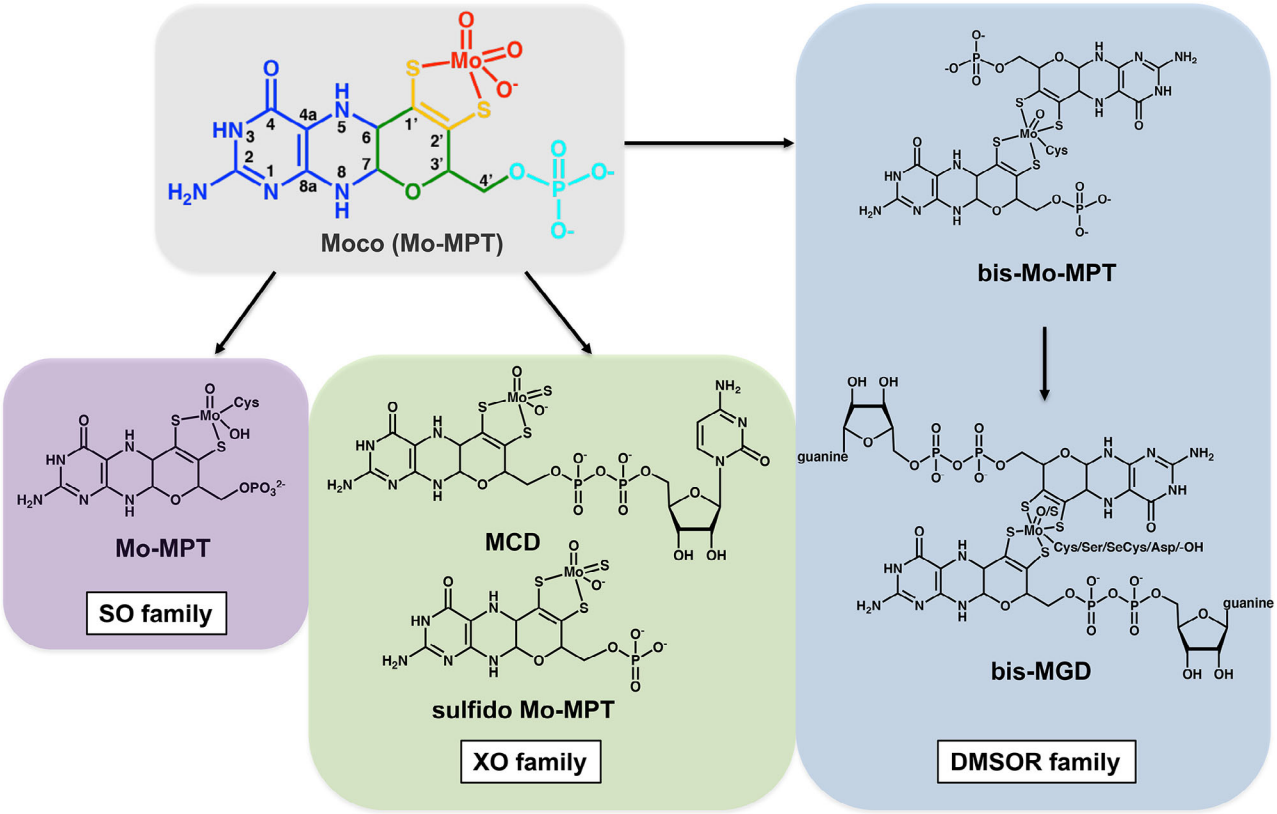

Fig. 1. Different structures of Moco in bacteria.
The basic form of Moco (Mo-MPT) is a 5,6,7,8-tetrahydropyranopterin (MPT) with a dithiolene group coordinating the molybdenum atom in a trioxo form.
The molybdenum atom is coloured in red, the characteristic dithiolene group is coloured in orange, the pterin group is coloured in blue, the pyrano-ring is coloured in green and the phosphate group is coloured in turquois.
Moco exists in different variants and is divided into three enzyme families according to the coordination at the molybdenum atom: the SO family, the XO family and the DMSO reductase family.
The SO family is characterized by a molybdenum ligation with one oxo, one hydroxide and one cysteine ligand from the protein backbone. In *Escherichia coli*, the XO family contains the sulfurated MCD, while in other bacteria also the sulfurated form of Mo-MPT without an additional nucleotide has been identified.
The DMSO reductase family contains two MPTs (bis-Mo-MPT) or two MGDs (bis-MGD) ligated to one molybdenum atom with additional ligands being an O/S, and a sixth ligand which can be a serine, a cysteine, a selenocysteine, an aspartate or a hydroxide ligand.

(Fig. 2, Table 1). MoaA belongs to the superfamily of S-adenosyl methionine (SAM)-dependent radical enzymes (Sofia *et al*., 2001; Hänzelmann *et al*., 2004). The X-ray structure of MoaA from *S. aureus* showed that MoaA is a homodimer (Hänzelmann and Schindelin, 2004) and each monomer contains two [4Fe-4S] clusters. It was shown that the N-terminal [4Fe-4S] cluster binds SAM and generates the 5′-deoxyadenosyl radical, which subsequently initiates the transformation of 5′-GTP bound by the C-terminal [4Fe-4S] cluster (Hänzelmann *et al*., 2004; Hänzelmann and Schindelin, 2006). In this reaction, the C8 of GTP is inserted between the C2′ and C3′ carbons of the ribose (Hover *et al*., 2013; Mehta *et al*., 2013). An (8S)-3′,8-cyclo-7,8-dihydroguanosine 5′-triphosphate (3′,8-cH$_2$GTP) intermediate is formed during the reaction (Fig. 2). This intermediate is then converted by MoaC to cPMP, a reaction which involves pyrophosphate cleavage in addition to the formation of the cyclic phosphate group (Hover *et al*., 2013). The structure of MoaC from *Thermus thermophilus* HB8 with bound 5′-GTP has been solved (Kanaujia *et al*., 2010a), which revealed that the protein forms an $(\alpha_2)_3$ complex (Wuebbens *et al*., 2000; Kanaujia *et al*., 2010b).

Second step: Formation of MPT by sulfur transfer to cPMP

In the next step of Moco biosynthesis, cPMP is converted to MPT in a reaction catalyzed by MPT synthase (Pitterle and Rajagopalan, 1989; Pitterle *et al*., 1990, 1993; Pitterle and Rajagopalan, 1991, 1993). In this reaction two sulfur atoms are inserted to the C1′ and C2′ positions of cPMP (Daniels *et al*., 2007). The purification of MPT

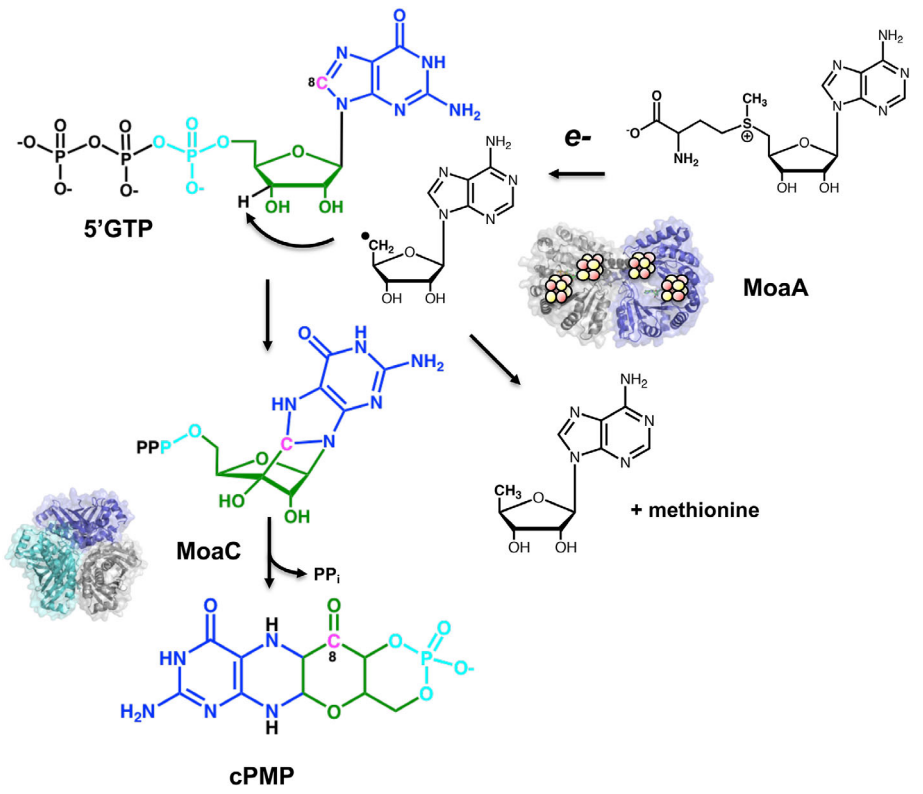

Fig. 2. Synthesis of cPMP from 5′-GTP.
The conversion of 5′-GTP to cPMP is catalyzed by MoaA (PDB: 1TV8) and MoaC (PDB: 1EKR) in bacteria.
All carbon atoms of the 5′-GTP are found within cPMP. The C8 atom (coloured in pink) from the 5′-GTP is inserted between the C2′ and C3′ atoms of the ribose (coloured in green), forming the 3′,8-cH$_2$GTP intermediate. The guanine is coloured in blue and the α-phosphate group is coloured in turquois. This reaction is catalyzed by MoaA, which belongs to the superfamily of SAM-dependent enzymes.
In the reaction catalyzed by MoaC, the pyrophosphate group is cleaved, forming cPMP. The [4Fe4S] clusters present on MoaA are shown schematically to highlight them.

synthase identified a heterotetrameric enzyme, consisting of two small (~8750 Da) and two large subunits (~16,850 Da) (Fig. 3), encoded by *moaD* and *moaE* respectively (Pitterle and Rajagopalan, 1989) (Table 1). Pitterle *et al*. (1993) were able to establish an in vitro system for MPT synthesis, consisting solely of MPT synthase and cPMP. By using sulfhydryl reagents, it was shown that the small subunit of MPT synthase carries the sulfur atoms in form of a thiocarboxylate group at the C-terminus of MoaD (Gutzke *et al*., 2001; Leimkühler *et al*., 2003). The X-ray structures of the *E. coli* and *S. aureus* MPT synthases showed that the central dimer is formed by two MoaE subunits containing one MoaD at each end (Rudolph *et al*., 2001; Daniels *et al*., 2007). Both MoaE–MoaD dimers act independently during the reaction, and each MoaE subunit thereby binds one cPMP molecule (Wuebbens and Rajagopalan, 2003). Studies on the reaction mechanism showed that the first sulfur is added by one MoaD molecule at the C2′ position of cPMP, forming a hemisulfurated intermediate (Fig. 3). This reaction is coupled to the hydrolysis of the cPMP cyclic phosphate group (Daniels *et al*., 2007). After transfer of its thiocarboxylate sulfur to cPMP, the first MoaD subunit dissociates from the MPT synthase complex (Wuebbens and Rajagopalan, 2003; Daniels *et al*., 2007) and a new MoaD molecule containing a thiocarboxylate group (MoaD-SH) associates with the complex and attacks the C1′ atom. MPT is then formed and released from the MPT synthase heterotetramer (Fig. 3).

The regeneration of sulfur at the C-terminal glycine of MoaD is catalyzed by MoeB (Fig. 3; Leimkühler *et al*., 2001; Schindelin, 2005). The X-ray structure of the (MoaD-MoeB)$_2$ complex showed that the C-terminus of MoaD is first activated by the formation of an acyl-adenylate group at its terminal glycine (Lake *et al*., 2001; Leimkühler *et al*., 2001; Schmitz *et al*., 2007). MoaD-AMP then receives the sulfur from a sulfur transferase and MoaD-SH is formed. The sulfur is directly transferred to MoaD in the (MoaD-MoeB)$_2$ complex, additionally releasing (MoeB)$_2$ and AMP. MoaD-SH dissociates from the complex and reassociates with MoaE to form the active MPT synthase heterotetramer (Fig. 3) (Tong *et al*., 2005; Schmitz *et al*., 2007). It was determined that in *E. coli*, L-cysteine serves as the origin of the MPT dithiolene sulfurs (Leimkühler and Rajagopalan, 2001). In the sulfur transfer reactions to MoaD, the proteins IscS and TusA are involved, forming a sulfur relay system in which a persulfide-sulfur is formed on a conserved cysteine residue of these proteins (Dahl *et al*., 2013; Zhang *et al*., 2010; Fig. 3). IscS has been initially described to have a role in the assembly of house-keeping Fe-S clusters (Zheng *et al*., 1998; Hidese *et al*., 2011). In addition, a second L-cysteine desulfurase is present in *E. coli*, named SufS, with a role under iron-limiting conditions or

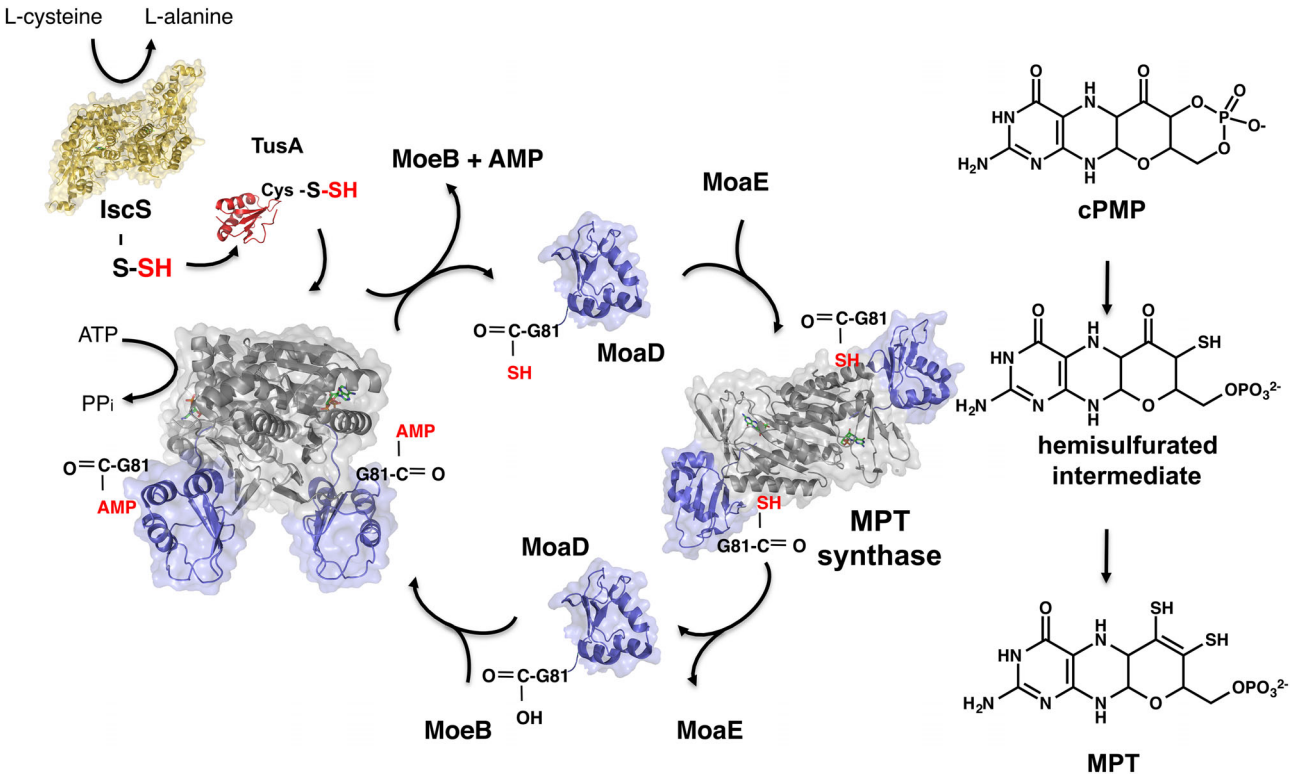

Fig. 3. Synthesis of MPT from cPMP.
The MPT synthase tetramer is built of two MoaE and two MoaD subunits. In the MPT synthase mechanism, cPMP is bound to the MoaE subunit and the sulfur is transferred from the C-terminal thiocarboxylate group of MoaD to cPMP. A hemisulfurated intermediate is formed during the reaction.
MoaD is regenerated and a new MoaD-SH thiocarboxylate is formed on MoeB, where MoaD is first activated under ATP consumption to form an activated MoaD-acyl adenylate. MoaD-AMP is then sulfurated by a protein-bound persulfide, which is transferred from IscS via TusA to MoaD.
After the formation of the thiocarboxylate group, MoaD-SH dissociates from the MoeB dimer and reassociates with MoaE, forming the active MPT synthase.

under oxidative stress (Outten *et al*., 2004). It was shown SufS cannot replace IscS in its ability to mobilize the sulfur from L-cysteine, based on the fact that SufS is unable to interact with TusA (Buhning *et al*., 2017). It is believed that the sulfur of one of these persulfide-containing proteins interacts with the $(MoaD\text{-}MoeB)_2$ complex and attacks the MoaD-AMP bond, releasing AMP and creating a transient MoaD perthiocarboxylate intermediate with the sulfur transferase, which further is reductively cleaved (Iobbi-Nivol and Leimkühler, 2013).

Overall, Moco biosynthesis is closely linked to Fe-S cluster assembly by sharing the protein IscS for sulfur mobilization from L-cysteine (Leimkühler, 2014; Yokoyama and Leimkühler, 2015). The IscS protein has been identified as major player that mobilizes the sulfur not only for Fe-S cluster assembly but also for the biosynthesis of Moco in addition to other sulfur-containing biomolecules such as thiamin, biotin, lipoic acid and thionucleosides in transfer RNA (tRNA) (Leimkühler *et al*., 2017). In total, there is a complex protein–protein interaction network around IscS, transferring the sulfur in the form of a persulfide further to specific sulfur acceptor proteins (Leimkühler *et al*., 2017). It remains, however, still elusive how the preference for the specific interaction partner is regulated and which factors direct the sulfur transfer towards the synthesis of a specific biomolecule.

In addition to IscS, also TusA has been described to be involved in a second cellular sulfur transfer pathway. TusA was initially described to be involved in the formation of mnm^5s^2U34 modified tRNA for Lys, Gln, Glu (Ikeuchi *et al*., 2006). Thiomodifications of tRNAs were shown to be important for proper function of prokaryotic and eukaryotic organisms (Suzuki, 2005; Shigi, 2014). For the formation of mnm^5s^2U in tRNA for Lys, Gln, Glu in *E. coli*, a sulfur-relay system was identified including the initial sulfur mobilization by the L-cysteine desulfurase IscS and the proteins TusA, TusBCD, TusE and MnmA (Ikeuchi *et al*., 2006). Among them, *tusB*, *tusC* and *tusD* are encoded on a single operon and their gene products form the TusBCD complex composed as a dimer of a heterotrimer $(\alpha\beta\gamma)_2$. Efficient 2-thiouridine formation can be achieved in vitro using purified tRNA incubated with

purified proteins IscS, TusA, the TusBCD complex, TusE and MnmA (Ikeuchi *et al*., 2006). TusA thereby directly interacts with IscS, stimulates its desulfurase activity and directs the sulfur flow to 2-thiouridine formation. In conclusion, the IscS/TusA interaction presents a way to coordinate Fe-S cluster assembly, Moco biosynthesis and translation efficiency in the cell.

Third step: Molybdate transport and insertion into MPT

The introduction of molybdate to the dithiolene moiety in MPT completes the formation of the chemical backbone for binding and coordination of the molybdenum atom (Grunden and Shanmugam, 1997). Molybdenum enters the cell as the soluble oxyanion molybdate for which high-affinity transporters have been described in bacteria (Grunden and Shanmugam, 1997; Self *et al*., 2001; Aguilar-Barajas *et al*., 2011). The first high-affinity molybdate transport mutant, *E. coli modC* (Shanmugam *et al*., 1992), was identified because molybdoenzyme activity could be restored by addition of molybdenum at a 1000-fold higher concentration (>0.1 mM) than that is sufficient for wild-type cells (Shanmugam *et al*., 1992). The *modC* mutant phenotype is pleiotropic for all molybdoenzymes. *Escherichia coli* ModC is the ATP-binding protein of a high-affinity molybdenum uptake system belonging to the ATP-binding cassette (ABC) superfamily of transporters that is responsible for the transport of a wide variety of molecules in prokaryotes and eukaryotes (Higgins, 1992; Davidson and Chen, 2004). In their most common form in bacteria, ABC transporters consist of two integral membrane proteins (ModB for molybdate transport in *E. coli*), and two hydrophilic peripheral membrane proteins (the ATP-binding protein ModC for molybdate transport in *E. coli*). In Gram-negative bacteria, a periplasmic binding protein captures the substrate and delivers it to the transporter complex in the inner membrane (ModA for the molybdate transport system in *E. coli*). Both molybdate and tungstate bind to the *E. coli* ModA protein in the periplasm as a tetrahedral complex which is held by seven hydrogen bonds formed between the oxygen of the bound anion and the protein groups from two domains (Hu *et al*., 1997). For molybdate-dependent regulation, the *modABC* operon expression is controlled by the ModE protein, which binds to the operator region of the *modABC* operon in its molybdate-bound form (McNicholas *et al*., 1997). ModE also enhances the transcription of molybdenum-dependent enzymes like DMSO reductase (*dmsABC*) (McNicholas *et al*., 1998) or nitrate reductase (*narGHI*) (Self *et al*., 1999) and also of the Moco biosynthesis operon *moaABCDE* (Anderson *et al*., 2000). ModE is a homodimer and each monomer can be subdivided into four structural domains: the N-terminal DNA-binding domain, a linker domain and two molybdate-binding (Mop) domains (Schüttelkopf *et al*., 2003). ModE binds two molybdate molecules per dimer, and molybdate binding subsequently results in extensive conformational changes of the domain thus enabling DNA binding (Schüttelkopf *et al*., 2003). For the synthesis of molybdoenzymes, the cell needs to activate the molybdate after transport into the cell to an appropriate form for incorporation into MPT.

In the step of molybdenum insertion into MPT, the gene products of *moeA* and *mogA* are involved (Table 1). MogA was the first protein of Moco biosynthesis for which the crystal structure was solved (Liu *et al*., 2000). The crystal structure of MoeA showed a two-domain structure, with one domain being structurally related to MogA (Schrag *et al*., 2001; Xiang *et al*., 2001). It was suggested that *E. coli* MoeA and MogA proteins are both essential for the incorporation of molybdenum in the cofactor, indicating a multistep reaction of molybdenum chelation (Nichols and Rajagopalan, 2002).

During the reaction, MogA first forms an MPT-AMP intermediate under ATP consumption (Kuper *et al*., 2004), and this intermediate is further transferred to MoeA, which mediates molybdenum ligation at low concentrations of MoO_4^{2-} (Fig. 4; Nichols and Rajagopalan, 2002, 2005). The selection of molybdenum over tungsten for insertion into the MPT backbone has been proposed to occur by MogA and MoeA (Llamas *et al*., 2006). Some organisms that contain tungstoenzymes have duplicated versions of MogA or MoeA, suggesting a metal-specific role for each protein pair (Bevers *et al*., 2008). In a medium containing high tungstate concentrations, tungsten has been shown to be inserted instead of molybdenum into various enzymes expressed in *E. coli*; thus, the control system by MogA and MoeA can be bypassed (Neumann and Leimkühler, 2008).

Also, under high molybdate concentrations (>1 mM), MPT-AMP formed by MogA has been revealed to be not essential for the molybdate insertion step, since molybdate was directly inserted into MPT without the aid of the MogA and MoeA proteins (Neumann and Leimkühler, 2008). This shows that the ATP-dependent activation of MPT and molybdenum is only required under physiological concentrations. The end product of the MoeA and MogA reaction is Mo-MPT in a tri-oxo form, the basic form of the Moco which can be further modified by nucleotide addition in the next step (Reschke *et al*., 2013). Alternatively, the Mo-MPT cofactor can be directly inserted into enzymes of the sulfite oxidase family, where Moco is coordinated by a cysteine ligand which is provided by the polypeptide chain of the protein, forming an MPT-$Mo^{VI}O_2$-Cys core in its oxidized state (Hille *et al*., 2014; Loschi *et al*., 2004; Fig. 1). In *E. coli*, the MsrP protein is so far the only protein of the SO family for which a

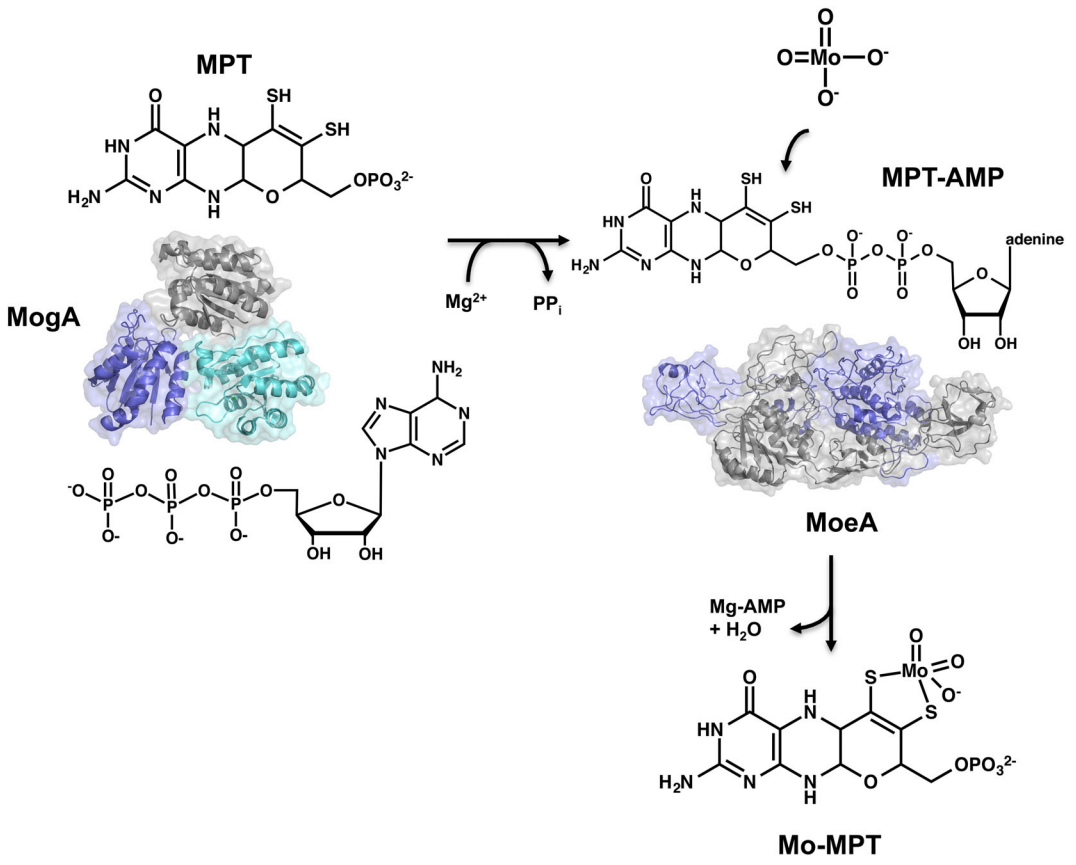

Fig. 4. Insertion of molybdate into MPT.
The bacterial MogA (PDB: 1DI6) and MoeA (PDB: 1G8I) proteins catalyze the specific incorporation of molybdenum into MPT in a multistep reaction with an adenylated MPT intermediate (MPT-AMP).
MogA forms the MPT-adenylate intermediate from Mo-MPT and Mg-ATP and transfers it to MoeA, which mediates molybdate ligation to MPT in an Mg^{2+}-dependent manner.

binding of the Mo-MPT cofactor has been confirmed (Table 2; Gennaris *et al*., 2015).

Fourth step: Further modification of Moco

Formation of bis-Mo-MPT and bis-MGD

In most prokaryotes, the majority of molybdoenzymes belong to the DMSO reductase family (Hille *et al*., 2014; Fig. 1, Table 2). These molybdoenzymes contain two dithiolene moieties from two MPT backbones ligated to the molybdenum atom and most often a GMP group at the terminal phosphate group of each MPT moiety, forming the bis-MGD cofactor. The synthesis of bis-MGD was shown to occur in a two-step reaction which solely requires Mo-MPT, MobA and Mg-GTP (Reschke *et al*., 2013; Table 1, Fig. 5). In the first reaction, the bis-Mo-MPT intermediate is formed on MobA with Mo-MPT as substrate (directly delivered from MoeA; Fig. 5). For this reaction the ligation of molybdenum to MPT is essential (Temple and Rajagopalan, 2000), but no further cofactors or molecules are required. Recently, the YdhV protein has been identified in *E. coli* to bind the bis-Mo-MPT cofactor (Reschke *et al*., 2019). The identification of bis-Mo-MPT to be used as cofactor in a molybdoenzyme represented a novel discovery, since this form of Moco has not been identified earlier. So far, enzymes containing the bis-cofactor without a nucleotide were all described to be tungstoenzymes, harbouring tungsten instead of molybdenum at the dithiolenes of MPT (Culka *et al*., 2017). In YdhV, also the bis-W-MPT could be inserted; however, the enzyme was shown to have a preference of molybdenum over tungsten, so that YdhV was classified as a molybdoenzyme (Reschke *et al*., 2019). The bis-Mo-MPT cofactor of YdhV was revealed to be redox-active, despite the fact that the functional substrate for YdhV still remains elusive. The failure to identify any substrate for YdhV may be explained by the fact that an inactive enzyme was purified due to the lack of a functional [4Fe-4S] cluster in proximity to the bis-Mo-MPT cofactor. According to a bioinformatic analysis, YdhV has been grouped to an enzyme class containing a so-called

Table 2. Overview of the molybdoenzymes in *Escherichia coli*, their substrates, subunit composition, localization and form of Moco present.

Mo-enzyme (substrate/product)	Subunits	Oligomerization	Moco	Localization	Tat-leader	Chaperone
DMSO reductase family						
Nitrate reductase A (NO_3/NO_2)	NarGHI	$(\alpha\beta\gamma)_2$	bis-MGD	Cytosol	-[a]	NarJ
Nitrate reductase Z (NO_3/NO_2)	NarZYV	$(\alpha\beta\gamma)_2$	bis-MGD	Cytosol	-	NarW
Periplasmic nitrate reductase (NO_3/NO_2)	NapAB	$\alpha\beta$	bis-MGD	Periplasm	NapA	NapD
TMAO reductase A ($(CH_3)_3NO/(CH_3)_3N$)	TorAC	$\alpha\beta$	bis-MGD(S)[b]	Periplasm	TorA	TorD
TMAO reductase Z ($(CH_3)_3NO/(CH_3)_3N$)	TorZY	$\alpha\beta$	bis-MGD	Periplasm	TorZ	?[c]
DMSO reductase ($(CH_3)_2SO/(CH_3)_2S$)	DmsABC	$\alpha\beta\gamma$	bis-MGD	Periplasm	DmsA	DmsD
Formate dehydrogenase N ($HCOO^-/CO_2$)	FdnGHI	$(\alpha\beta\gamma)_3$	bis-MGD	Periplasm	FdnG	FdhD
Formate dehydrogenase O ($HCOO^-/CO_2$)	FdoGHI	$(\alpha\beta\gamma)_3$	bis-MGD	Periplasm	FdoG	FdhD
Formate dehydrogenase H ($HCOO^-/CO_2$)	FdhF	α	bis-MGD(S)[b]	Cytosol	-	FdhD
Biotin sulfoxide reductase (biotin sulfoxide/biotin)	BisC	α	bis-MGD	Cytosol	-	-
Selenate reductase (selenite/selenite)	YnfEFGH	$\alpha\beta\gamma$	bis-MGD	Periplasm	YnfE/YnfF	DmsD
? ?	YdhYVUX	?	bis-Mo-MPT	Periplasm	YdhX	YdhW
? ?	YdeP	?	bis-MGD	Cytosol	-	?
Xanthine oxidase family						
Aldehyde oxidoreductase (aldehydes/carboxylic acids)	PaoABC	$\alpha\beta\gamma$	MCD (S)[b]	Periplasm	PaoA	PaoD
Xanthine dehydrogenase (xanthine/uric acid)	$(\alpha\beta\gamma)_2$		MCD (S)[b]	Cytosol	-	YqeB?
? ?	XdhD	?	MCD	Cytosol	-	YqeB?
Sulfite oxidase family						
Methionine sulfoxide reductase (protein-methionine sulfoxides/protein-methionine)	MsrPQ	$\alpha\beta$	Mo-MPT	Periplasm	MrsP	-
MOSC family enzyme (6-N-hydroxylaminopurine?)	YcbX	?	Mo-MPT?	Cytosol	-	-
MOSC family enzyme (6-N-hydroxylaminopurine?)	YiiM	α_2	Mo-MPT?	Cytosol	-	-

a. No Tat translocation/no chaperone required.
b. The (S) group indicates the presence of a sulfido ligand at the Mo active site.
c. Role not known/protein not identified.

'hyperactive' cysteine residue that contributes to [4Fe-4S]-cluster instability during the purification of YdhV even under strict anaerobic conditions (Wang *et al*., 2018). The unexpected presence of a bis-Mo-MPT cofactor in an enzyme in *E. coli* opened an additional route for Moco biosynthesis and expanded the canon of the structurally highly versatile Mocos.

After bis-Mo-MPT formation by MobA, for most enzymes in *E. coli*, two GMP moieties from GTP are added to the C4′ phosphates of bis-Mo-MPT, forming the bis-MGD cofactor (Palmer *et al*., 1996; Lake *et al*., 2000). While MobA was shown to catalyze both reactions, bis-Mo-MPT formation and the addition of the nucleotides the phosphate groups of both MPTs (Fig. 5), the molecular mechanism of bis-Mo-MPT formation and its binding mode to MobA has not been completely resolved to date (Reschke *et al*., 2013). The crystal structure of MobA showed that the protein is a monomer containing two domains, in which the N-terminal domain of the molecule adopts a nucleotide (GTP)-binding Rossmann fold, and the second domain at the C-terminus harbours a possible MPT binding site (Lake *et al*., 2000; Stevenson *et al*., 2000). Since for bis-Mo-MPT formation two Mo-MPT moieties have to be bound to monomeric MobA, it was suggested that this occurs by using both the MPT and predicted GTP binding sites on one MobA monomer (Reschke *et al*., 2013). During this reaction, one molecule of molybdate has to be released when two Mo-MPT molecules are combined. The bis-MGD cofactor can either be inserted into enzymes of the DMSO reductase family

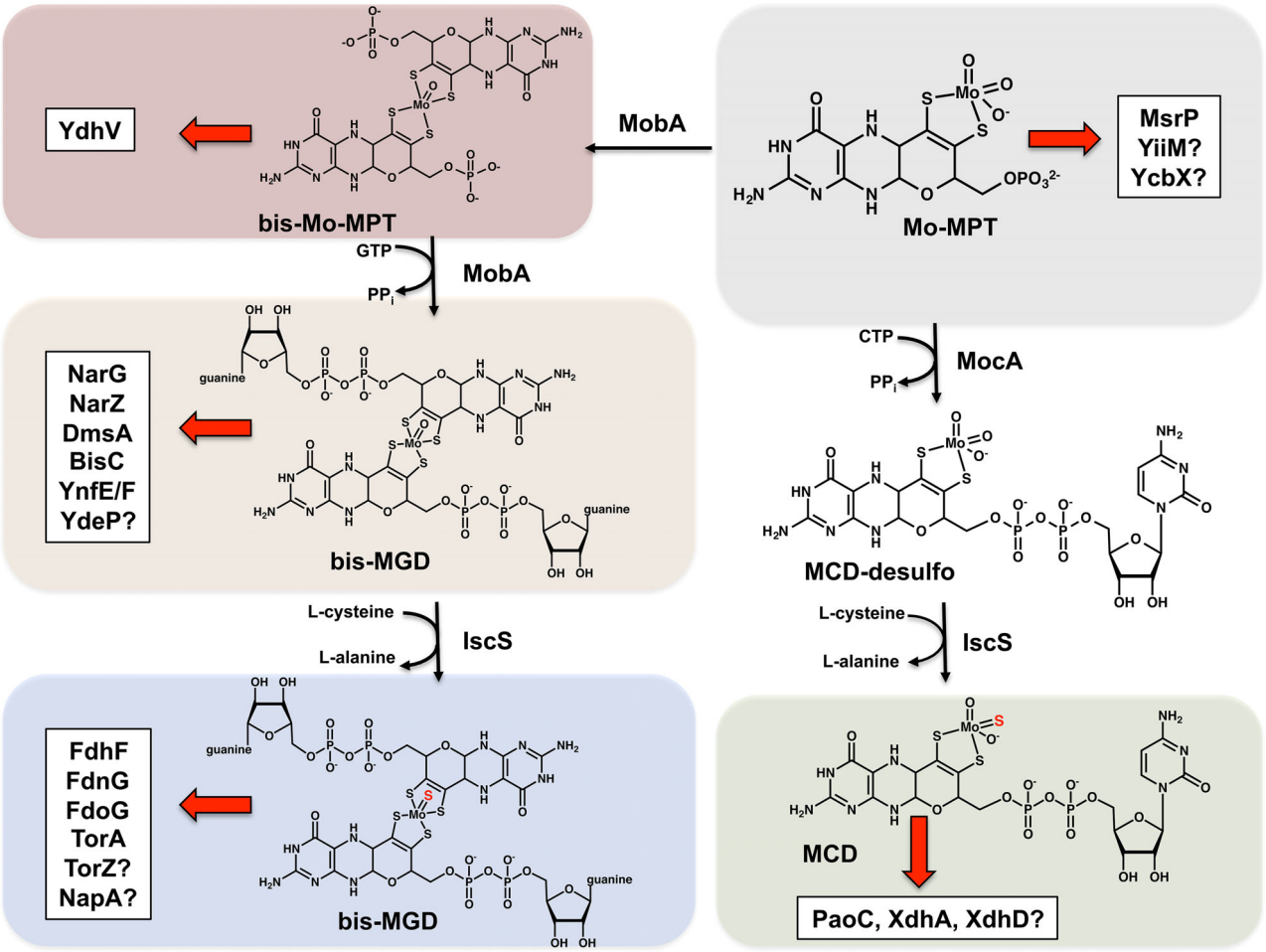

Fig. 5. Formation of dinucleotide and sulfurated forms of Moco.
After its synthesis, Mo-MPT is inserted into enzymes of the sulfite oxidase family, like MsrP, YiiM (predicted) and YcbX (predicted).
Alternatively, Mo-MPT can be further modified in prokaryotes by the addition of GMP or CMP to the C4′ phosphate of MPT via a pyrophosphate bond.
For enzymes of the XO family, MCD is formed by the MocA protein, which acts specifically on CTP. For PaoABC, MCD is further modified in *Escherichia coli* by exchange of the equatorial oxygen to a sulfido ligand, forming sulfurated MCD. This step is carried out by the PaoD protein in conjunction with IscS.
After sulfuration, MCD is inserted by the aid of PaoD into PaoC. XdhA and XdhD are also expected receive the MCD cofactor.
For enzymes of the DMSO reductase family, the bis-MGD cofactor is formed by the MobA protein, which specifically binds GTP, in a two-step reaction via the formation of a bis-Mo-MPT intermediate. Bis-Mo-MPT is the cofactor that is inserted into YdhV. The bis-MGD cofactor without further modification is inserted into the subunits of enzymes like NarG, NarZ, DmsA, BisC, YnfE/F and likely YdeP.
Bis-MGD can be further modified by sulfuration. In the reaction for bis-MGD sulfuration IscS is involved. Sulfido-containing bis-MGD is inserted into FdhF, FdnG, FdoG, TorA and likely TorZ (predicted) and NapA (predicted).

or in some enzymes is further modified by the insertion of a sulfido ligand to the molybdenum atom (see below).

Formation of the MCD cofactor

For enzymes of the XO family in bacteria, Mo-MPT can be further modified by formation of the MCD (Neumann *et al*., 2009b; Iobbi-Nivol and Leimkühler, 2013). However, some bacteria directly bind Mo-MPT after sulfuration without the additional attachment of a nucleotide (Leimkühler *et al*., 1998). The addition of CMP to Mo-MPT is catalyzed by the *E. coli* MocA protein (Table 1, Fig. 5), which displays a high level of amino acid sequence conservation when compared with MobA (Neumann *et al*., 2011). In contrast to MobA, MocA is specific for the pyrimidine nucleotide CTP. The overall catalytic reaction of MocA is similar to the second part of the reaction of MobA, in which MocA acts as a MPT-CTP transferase and covalently links MPT and CMP with the concomitant release of pyrophosphate (Neumann *et al*., 2009b). However, in this reaction CMP is added to Mo-MPT and not to bis-Mo-MPT. The existence of bis-Mo-MCD in bacterial molybdoenzymes has not been identified to date. In a follow-up reaction, the MCD cofactor for all enzymes of

the XO family is further modified and contains an equatorial sulfido ligand at its active site which is essential for enzyme activity (see below).

Moco sulfuration of MCD and bis-MGD cofactors

Sulfurated Moco is the form of the cofactor that is present in all members of the XO family (Hille, 1996; Hille *et al*., 2014). In *E. coli*, the aldehyde oxidoreductase PaoABC was shown to bind the sulfurated form of MCD (Fig. 5; Neumann *et al*., 2009a). For Moco sulfuration, the cofactor is handed over to Moco binding chaperones, which belong to the XdhC-family of chaperones (Neumann and Leimkühler, 2011; Iobbi-Nivol and Leimkühler, 2013). In *E. coli*, PaoD and YqeB were described to belong to this family (Table 2). PaoD is the specific Moco-binding chaperone for the periplasmic aldehyde oxidoreductase PaoABC (Iobbi-Nivol and Leimkühler, 2013). These chaperones were shown to bind Moco to protect it from oxidation during the sulfur insertion reaction. Furthermore, an interaction with an L-cysteine desulfurase has been demonstrated, the protein that replaces the equatorial oxygen ligand of Mo-MPT by a sulfido ligand (Neumann *et al*., 2007b; Fig. 5). After the Moco sulfuration reaction, the matured cofactor is inserted into the apo-target molybdoenzyme (Neumann *et al*., 2007a, 2007b). Since Moco is deeply buried in these prefolded proteins, XdhC-like proteins were suggested to perform several chaperone reactions: first to ensure that Moco is sulfurated by the interaction with an L-cysteine desulfurase (Neumann *et al*., 2007b) and second, after sulfuration they have to interact with a specific target protein for insertion of sulfurated Moco. Third, it has also been suggested that these chaperones are involved in proper folding of the target protein after Moco insertion (Leimkühler and Klipp, 1999). This model implies that apo-molybdoenzymes exist in a Moco-competent 'open' conformation until the insertion of sulfurated Moco and that after the insertion reaction, the protein adopts the final active 'closed' conformation, which is incapable to accept Moco (Leimkühler and Klipp, 1999).

For enzymes of the XO family, the Mo-sulfido group has long been established to be a characteristic modification of this class of molybdoenzymes. However, also enzymes of the DMSO reductase family are modified with a sulfido-ligand after formation of the bis-MGD cofactor.

For the *E. coli* FdhD protein, it was reported that it binds bis-MGD and acts as a sulfur transferase between the L-cysteine desulfurase IscS and the formate dehydrogenase FdhF, a mechanism which is essential to yield active FdhF (Table 2; Thome *et al*., 2012). FdhF was inactive when FdhD was absent during expression but could be activated by chemical sulfuration. For sulfuration of bis-MGD, FdhD first binds bis-MGD and then interacts with the L-cysteine desulfurase IscS. It has been suggested that IscS transfers the sulfur from L-cysteine to FdhD in form of a persulfide. In the sulfur transfer process from IscS to bis-MGD, the cysteines 121 and 124 in a conserved CXXC motif of FdhD were proposed to be involved, which are present on a disordered loop that facilitates the interaction with both IscS and the bis-MGD cofactor (Arnoux *et al*., 2015). Cysteine to alanine variants in these residues were shown to produce an inactive FdhF enzyme. However, the cysteine residues in the motif CXXC are not conserved among FdhD-like proteins, so that also other mechanisms for sulfur transfer to bis-MGD have to exist (Schwanhold *et al*., 2018). *Escherichia coli* FdhD was co-crystallized in complex with GDP (Arnoux *et al*., 2015), and direct binding of bis-MGD to FdhD was confirmed later (Bohmer *et al*., 2014; Schwanhold *et al*., 2018).

In addition to formate dehydrogenases, the DMSO reductase family includes other members for which an additional sulfur ligand of the molybdenum atom has been reported at the catalytic site. The X-ray crystal structure of the periplasmic nitrate reductase (Nap) of *Cupriavidus necator* showed the presence of a terminal sulfur ligand at the molybdenum coordination sphere (Coelho *et al*., 2011). Similar data were obtained for the homologous NapA protein from *Desulfovibrio desulfuricans* ATCC 27774, for which the crystal structure showed a unique coordination sphere of six sulfur ligands bound to the molybdenum atom (Najmudin *et al*., 2008). These observations might suggest that sulfuration of bis-MGD is more common in this group of enzymes than previously expected. This hypothesis was further confirmed by the identification of a sulfido ligand present in the coordination sphere of *E. coli* TMAO reductase (TorA; Kaufmann *et al*., 2018). This sulfido ligand at the bis-MGD cofactor was shown to be sensitive to oxygen exposure in TorA. The specific mechanism of Moco sulfuration in these enzymes has not been studied so far.

Consequently, it has to be considered also for other enzymes of the DMSO reductase family that a sulfido ligand might be present at the molybdenum atom, since TMAO reductase represents the third enzyme in addition to nitrate reductase and formate dehydrogenase for which a sulfido ligand has been identified (Raaijmakers and Romao, 2006; Coelho *et al*., 2011; Hartmann *et al*., 2016). Anaerobic expression and purification might be obligatory for conserving the intact Mo coordination environment (Kaufmann *et al*., 2018).

Molecular chaperones for bis-MGD insertion into target enzymes

The last step of Moco modification, including the formation of bis-Mo-MPT, the addition of GMP and the insertion

of a sulfido ligand are modifications that occur before the insertion of the bis-MGD variants into the specific apoenzymes. Until now it is not completely understood how bis-MGD is inserted into the folded proteins. The crystal structures of several molybdoenzymes revealed that Moco is deeply buried inside the proteins, at the end of a funnel-shaped passage giving access only to the substrate (Kisker *et al*., 1997). The insertion step is catalyzed by bis-MGD-binding molecular chaperones, which bind the respective Moco variant and insert it into the target molybdoenzyme (Genest *et al*., 2009). Most of the molybdoenzymes of the DMSO reductase family in *E. coli* have a specific chaperone for Moco insertion (Table 2; Iobbi-Nivol and Leimkühler, 2013): NarJ is the chaperone for nitrate reductase A NarGHI (Blasco *et al*., 1998), NarW is the chaperone for nitrate reductase Z NarZYV (Blasco *et al*., 1992) and NapD the chaperone for NapA. However, exceptions exist, since DmsD is the chaperone for the two enzymes DmsABC (Ray *et al*., 2003) and YnfE/F (Guymer *et al*., 2009) and FdhD is the chaperone for the three enzymes FdhF (Thome *et al*., 2012), FdnGHI and FdoGHI. Also other exceptions exists, since no defined specific chaperone has been identified so far for the cytoplasmic molybdoenzymes BisC or the periplasmic molybdoenzyme TorZ (Ilbert *et al*., 2004; Turner *et al*., 2004).

The step of Moco insertion into target molybdoenzymes has been best studied for the TorD/TorA pair of TMAO reductase in *E. coli*. TorD was shown to be the specific chaperone for TorA (Genest *et al*., 2005) and plays a direct role in the insertion of Moco into apo-TorA (Genest *et al*., 2006). During this reaction, TorD interacts with both MobA and apoTorA and further stabilizes apoTorA for Moco insertion to avoid a proteolytic attack of the latter. This is consistent with its role as 'facilitator' of the bis-MGD insertion and maturation of the apoenzyme (Genest *et al*., 2008; Genest *et al*., 2009; Iobbi-Nivol and Leimkühler, 2013). For the chaperone TorD, it was described that it is able to bind to the signal peptide of apo-TMAO reductase until the bis-MGD is inserted and TMAO reductase is correctly folded (Genest *et al*., 2008). Pre-TorA is then translocated to the periplasm where the active TorA enzyme is finally generated after cleavage of the signal peptide (Iobbi-Nivol and Leimkühler, 2013).

Targeting of molybdoenzymes to the periplasm

After Moco insertion and final maturation of the molybdoenzymes, the enzymes are targeted to their final destination. Being part of respiratory systems, most molybdoenzymes in *E. coli* are either membrane-associated or located in the periplasm (Iobbi-Nivol and Leimkühler, 2013). It was shown that the insertion of Moco occurs before the translocation of molybdoenzymes either to the membrane or the periplasmic space. The Tat pathway (twin-arginine translocation) is dedicated to the transport of folded proteins (Jack *et al*., 2004). Proteins are targeted to the Tat translocase by N-terminal signal peptides harbouring consecutive, essentially invariant, arginine residues within an SRRxFLK consensus motif (Berks, 1996). This signal sequence has been identified in *E. coli* on subunits of DMSO reductase and a homologue (DmsA and DmsB, YnfE/F and YnfG), formate dehydrogenases (FdnG and FdnH, FdoG and FdoH), periplasmic nitrate reductase and its electron donor protein (NapA and NapG), TMAO reductase and a homologue (TorA and TorZ), one subunit of the bis-Mo-MPT aldehyde ferredoxin oxidoreductase-like enzyme (YdhX), the MsrP and the periplasmic aldehyde oxidoreductase (PaoA; Table 2) (Berks *et al*., 2005; Ize *et al*., 2009).

Using the TorA/TorD pair as example, TorD has a dual role in binding to the Tat-leader peptide of TorA and preventing its translocation by the Tat system until bis-MGD is inserted into TorA and final folding has been accomplished (Leimkühler and Iobbi-Nivol, 2016). By binding to the core of the apoTorA as second binding site of TorD, TorD induces a conformational change of apoTorA that becomes thereby competent for Moco insertion (Genest *et al*., 2008). After bis-MGD insertion into apoTorA, TorD is released from the complex and TorA has to be targeted to the Tat machinery. This step is facilitated by the fact that insertion of Moco into the catalytic site of TorA modifies the affinity between the interacting region of TorA and TorD allowing the release of TorD (Pommier *et al*., 1998). Consequently, the Tat leader peptide is exposed after TorD release and mature TorA can be targeted to the Tat-machinery.

On overview of the roles of molybdoenzymes in E. coli

In *E. coli*, 19 molybdoenzymes are present, to which a short overview is presented on what is known about their roles and cellular localization. Overall, reduction of nitrate can be carried out by at least three respiratory systems (Cole, 1996) (Fig. 6): at high concentrations of nitrate, only the membranous NarGHI system is synthesized (Stewart, 1988), whereas at very low concentrations the periplasmic Nap system is produced (Brondijk *et al*., 2004). The operon encoding a third system, *narZYWV* (Table 2), is expressed during the early stationary phase independent of the presence of nitrate (Chang *et al*., 1999). Furthermore, the formate dehydrogenase FdhGHI is a component of the nitrate respiratory pathway, where, under anaerobic conditions, formate oxidation is coupled to nitrate reduction (NarG) via lipid-soluble quinone (Jormakka *et al*., 2003). FdoGHI and NarZYV are the corresponding isoenzymes that are additionally present

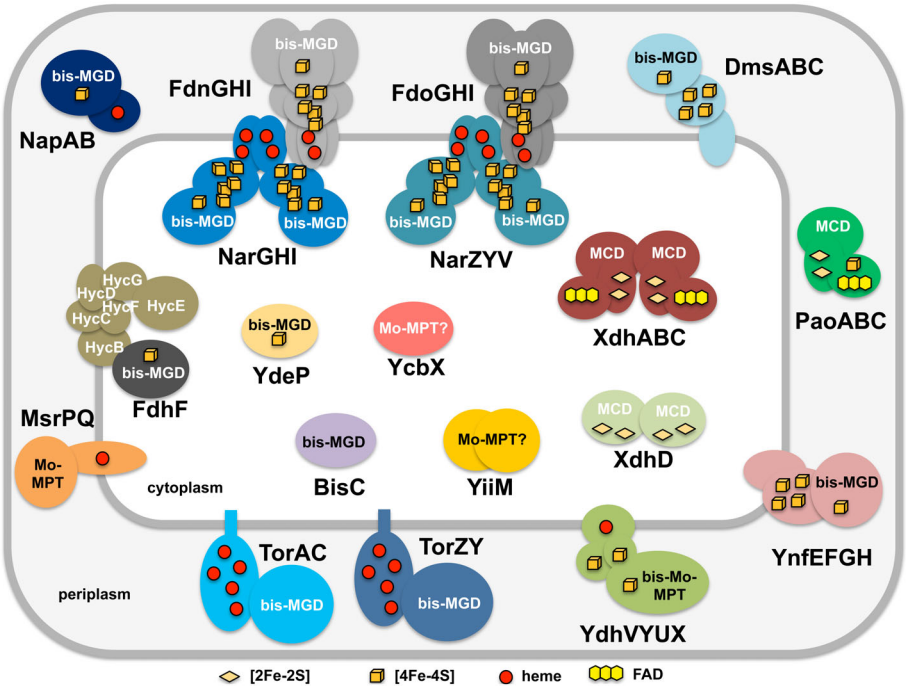

Fig. 6. Proposed localization of *Escherichia coli* molybdoenzymes.
FdnGHI/FdoGHI are components of the nitrate respiratory pathway, in which formate oxidation is coupled to nitrate reduction (NarGHI/NarVYZ). The periplasmic nitrate reductase NapAB is produced under nitrate-limiting conditions. TMAO is reduced to TMA by at least two respiratory systems, TorCA and TorYZ which are located in the periplasm.
The DMSO reductase DmsABC reduces DMSO to DMS and is located in the periplasm. FdhF is part of the formate/hydrogen lyase system oxidizing formate and producing hydrogen. The cofactors of the Hyc-subunits are not shown.
BisC is the cytoplasmic biotin sulfoxide reductase. The role of YdeP is also not characterized yet but is expected to be a cytoplasmic protein. The selenite reductase YnfEFGH is located in the periplasm. The substrates for YdhVYUX have not been identified yet, but a periplasmic localization is proposed, since YdhX contains a Tat-leader peptide.
PaoABC is localized in the periplasm and detoxifies aromatic aldehydes. XdhABC and XdhD are located in the cytoplasm. The role of XdhD is so far unclear.
The MsrQP protein is localized in the periplasm and uses whole proteins as substrates to reduce oxidized methionine sulfoxides to methionine. The role of the two cytoplasmic MOSC proteins YcbX and YiiM have not been identified in detail to date. The cofactors present in these proteins are schematically shown.

under aerobic conditions to ensure rapid adaptation during a shift from aerobiosis to anaerobiosis (Table 2, Fig. 6) (Abaibou *et al*., 1995). A third formate dehydrogenase is present in *E. coli*, namely FdhF, which is facing the cytoplasm and forms the formate hydrogen lyase (FHL) complex together with the hydrogenase-3 (encoded by *hycBCDEFG*) (Sawers, 1994). The FHL complex is membrane-bound and produces H_2 and CO_2 under fermentative conditions (Rossmann *et al*., 1991). The three *E. coli* formate dehydrogenases have in common that their catalytic subunits coordinate the bis-MGD cofactor with a selenocysteine ligand and a sulfido ligand, and as additional cofactor a [4Fe-4S] cluster that is present in vicinity (Hartmann *et al*., 2015). FdnGHI expression is induced in the presence of nitrate under anaerobic conditions. In contrast, FdoGHI is also present at low levels under aerobiosis, during fermentative conditions and during nitrate respiration. The so far uncharacterized YdeP protein has amino acid sequence homologies to FdhF, FdnG and FdoG (Masuda and Church, 2002, 2003). Deletion of *ydeP* has been described to decrease the acid resistance of cells in the exponential phase (Masuda and Church, 2002).

A further respiratory molybdoenzyme under anaerobic conditions is DMSO reductase, encoded by *dmsABC* in *E. coli* (Rothery and Weiner, 1993). DMSO reductase reduces DMSO to DMS, and the enzyme from *E. coli* and some other species is a αβγ-heterotrimer that is located in the periplasm (Bilous and Weiner, 1988). *Escherichia coli* DMSO reductase has a broad substrate specificity, being able to reduce S- and N-oxides.

The chaperone for DMSO reductase, DmsD, is located in the *ynfEFGHdmsD* operon encoding selenate reductase. On the basis of sequence similarity, the *ynfEFGH* gene cluster is predicted to encode an oxidoreductase complex closely related to the dimethyl sulfoxide reductase heterotrimer DmsABC (Lubitz and Weiner, 2003). Genetic analysis of *E. coli ynfE* and *ynfF* null mutants

has implicated the cluster in selenate reduction (Guymer *et al*., 2009). The exact role of this enzyme still has to be elucidated.

Another alternative electron acceptor that is used under anaerobic conditions by *E. coli* is TMAO (McCrindle *et al*., 2005). TMAO reductase is encoded by the *torCAD* operon in *E. coli* (Mejean *et al*., 1994). The TMAO respiratory proteins face the periplasm and have a high substrate specificity towards TMAO as substrate. *Escherichia coli* harbours a second TMAO reductase, encoded by *torYZ* (Gon *et al*., 2000). In contrast to *torCAD*, the expression of *torYZ* is very low and is not induced by TMAO or DMSO.

Escherichia coli also produces a biotin sulfoxide/MsrP encoded by the *bisC* gene (Pierson and Campbell, 1990; Ezraty *et al*., 2005). BisC is a cytoplasmic enzyme (Mejean *et al*., 1994) and was first identified as a biotin sulfoxide reductase that reduces a spontaneous oxidation product of biotin, biotin-*d*-sulfoxide, back to biotin. BisC also exhibits methionine-*S*-sulfoxide reductase activity, acting specifically on the *S* enantiomer in the free, but not the protein-bound form. It thus plays a role in assimilation of oxidized methionines (Ezraty *et al*., 2005).

The YdhV protein has been described to bind bis-Mo-MPT as novel cofactor form in *E. coli* (Reschke *et al*., 2019). The YdhV protein is encoded by the *ydhYVWXUT*, and YdhV, YdhY, YdhU and YdhX are expected to form the oxidoreductase enzyme system that is located in the periplasm (Fig. 6). An *ydhV* mutant was reported to be impaired in the anaerobic utilization of certain sulfur sources, e.g. L-cysteine, D-cysteine, L-cysteic acid, hypotaurine and butane sulfonic acid (Partridge *et al*., 2008).

Escherichia coli also contains three enzymes annotated to belong to the XO family that bind the MCD cofactor. The periplasmic aldehyde oxidoreductase, PaoABC, is an oxidoreductase with a possible role in the detoxification of aldehydes. Purified PaoABC oxidizes a broad spectrum of aldehydes with a preference for aromatic aldehydes such as vanillin and cinnemaldehyde (Neumann *et al*., 2009a). PaoABC was revealed to use molecular oxygen as terminal electron acceptor (unpublished results).

E. coli also contains the coding region for a predicted xanthine dehydrogenase, *xdhABC*. The enzyme has not been characterized yet, but from amino acid sequence alignments, it is predicted to bind the MCD cofactor (Neumann *et al*., 2009a). An *xdhA* mutant was shown to exhibit sensitivity to adenine, which is indicative of a defect in purine salvage (Xi *et al*., 2000).

A third gene in *E. coli* was identified, the *xdhD* gene, which is predicted to code for a protein with homologies to xanthine dehydrogenases. This putative enzyme has also not been characterized yet. It was reported that an *xdhD* mutant also exhibited sensitivity to adenine (Xi *et al*., 2000). However, no xanthine dehydrogenase activity could be detected so far (Neumann *et al*., 2009a).

Also enzymes belonging to the SO family of molybdoenzymes have been identified in *E. coli*. MsrP (formerly designated as YedY (Loschi *et al*., 2004; Brokx *et al*., 2005)) was characterized as a periplasmic MsrP (Gennaris *et al*., 2015). MrsP interacts with MsrQ, an inner membrane heme-binding protein. The MsrPQ system was described to act on whole proteins as substrates. The main role of MsrP is to protect periplasmic proteins from oxidative damage by acting as an MsrP at the N-terminus (Gennaris *et al*., 2015).

Two further proteins are predicted to belong to the SO family of molybdoenzymes in *E. coli*, named YcbX and YiiM (Kozmin *et al*., 2008). Both proteins contain a MOSC (Moco sulfurase C-terminal) domain, based on sequence homologies to the C-terminal domain of Moco sulfurase (Anantharaman and Aravind, 2002). The presence of Moco has not been identified in these proteins yet. YcbX contains three domains, an N-terminal β-barrel domain, a central MOSC domain and a C-terminal [2Fe-2S]-ferredoxin-like domain (Kozmin *et al*., 2008). YcbX functions in the detoxification of *N*-hydroxylated base analogues, an activity that was shown to depend on the Moco–biosynthesis pathway (Kozmin *et al*., 2008). The CysJ flavin reductase functions as a partner of YcbX, potentially by providing the reducing equivalents required for an YcbX-catalyzed detoxification reaction.

YiiM contains an N-terminal MOSC domain and a C-terminal helical domain (Anantharaman and Aravind, 2002) (Kozmin *et al*., 2008). Crystal structures of YiiM have been solved (Namgung *et al*., 2018); however, Moco could not be detected in the crystallized protein. YiiM also has a role in the Moco-dependent pathway for detoxification of *N*-hydroxylated base analogues (Kozmin *et al*., 2008; Kozmin and Schaaper, 2013).

The regulation of Moco biosynthesis

Genetic analysis of the regulation of Moco biosynthesis revealed that mainly the *moaABCDE* operon is the target for transcriptional and translational regulation (Anderson *et al*., 2000). It was shown that the expression of *moaABCDE* is enhanced under anaerobic conditions and in the presence of sufficient amounts of molybdate, but repressed in strains which are to synthesize an excess of Moco. When molybdate is available, molybdate-bound ModE acts as a positive regulator and binds to the *moa* promotor region, thereby enhancing the transcription of the operon (Anderson *et al*., 2000). In addition, a regulation of the *moaABCDE* operon by the carbon storage regulator CsrA has been described (Patterson-Fortin *et al*., 2013). CsrA is a global regulator that represses stationary phase metabolism and activates central carbon

metabolism (Sabnis *et al*., 1995). Two binding sites of CsrA were identified within the *moaA* promoter region (Patterson-Fortin *et al*., 2013). For Moco biosynthesis, CsrA was shown to activate the transcription, and it has been suggested that CsrA enhances Moco biosynthesis under conditions of high demand (Zupok *et al*., 2019b).

In *E. coli*, transcription of the *moaABCDE* operon is also upregulated by FNR (fumarate and nitrate reduction regulatory protein) (Anderson *et al*., 2000; Zupok *et al*., 2019a). FNR is a transcriptional regulator that is essential for expressing anaerobic respiratory processes (Unden *et al*., 2002). FNR senses the oxygen concentration directly via the disassembly and reassembly of its [4Fe-4S] clusters (Unden and Schirawski, 1997). In addition, the *moeAB* operon was also shown to be regulated by FNR in addition to ArcA (Hasona *et al*., 2001). Surprisingly, FNR represses the nitrate-dependent transcription of the *moe* operon, but it is expected that under anaerobic conditions, an intermediate level of transcription is ensured of the *moeAB* operon by the antagonistic effects of FNR and ArcA-P (Hasona *et al*., 2001). FNR further regulates the expression of most molybdoenzymes in *E. coli*, like the *narGHJI* operon (Melville and Gunsalus, 1996; Lamberg and Kiley, 2000), the *dmsABC* operon (Lamberg and Kiley, 2000), the *napFDAGHBC* operon (Stewart *et al*., 2003), the *xdhABC* operon (Salmon *et al*., 2003), the *ynfEFGH* operon (Kang *et al*., 2005; Xu *et al*., 2009), the *fdhF* gene (Salmon *et al*., 2003), the *ydhYVWXUT* operon (Kang *et al*., 2005), the *fdnGHI* operon (Li and Stewart, 1992) and the *torCAD* operon (Zupok et al., unpublished).

For the *moa*-operon, an additional level of regulation was identified, since expression of this operon is prevented, when Moco is present in excess (Anderson *et al*., 2000). A highly conserved RNA motif located upstream of the *moa* operon was identified that controls gene expression in response to Moco production (Regulski *et al*., 2008). It was suggested that this structured RNA represents a riboswitch that senses Moco (Regulski *et al*., 2008). A regulatory region responding to the availability of Moco upstream of the *moaA* start codon was first described by Anderson *et al*. (2000). The architectural features of Moco RNA, which spans 138 nt and forms at least five conserved stem-loop elements, strongly suggested that Moco RNA representatives are metabolite-sensing riboswitches where Moco regulates translation of the downstream operon by binding to the RNA element without a protein factor (Regulski *et al*., 2008). It has also been suggested that only Moco can bind to the RNA region and not the tungsten derivative of the cofactor, implying that Moco RNAs are able to distinguish between these two nearly identical cofactors (Regulski *et al*., 2008). It was shown that translation of the *moa* operon is prevented when the Moco RNA structure and Moco are present (Regulski *et al*., 2008). A Moco sensing riboswitch would ensure that the translation of the proteins encoded by the *moaABCDE* operon is downregulated when Moco is present in sufficient amounts or when molybdoenzymes are not needed. This would allow a rapid and efficient response to the changing demand for Moco within the cell. However, due to instability of Moco and its intermediates, so far, it was impossible to perform direct mRNA binding experiments, as direct proof of Moco binding to RNA without the involvement of proteins.

Outlook

In general, the biosynthesis of Moco is conserved in all organisms which contain molybdoenzymes. Although a wide range of variations on the basic Moco structure exists in bacteria, no dinucleotide forms of Moco have been identified in eukaryotes to date. Additionally, bacteria contain a large variety of molybdoenzymes that catalyze specific, usually non-essential, redox-reactions. Often, molybdoenzymes contribute to anaerobic respiration. In humans, however, only four molybdoenzymes have been identified, and a defect in Moco biosynthesis is lethal due to the loss of sulfite oxidase activity.

In the last few years it became obvious that Moco biosynthesis in *E. coli* is more diverse than initially expected. Novel forms of Moco have been identified recently, like the bis-Mo-MPT cofactor without a nucleotide attachment, which so far has been described only for enzymes containing tungsten. Furthermore, also a sulfido ligand was identified to be present on enzymes of the DMSO reductase family, which initially has been a characteristic ligand only for enzymes of the XO family. In enzymes like formate dehydrogenase and TMAO reductase, this sulfido ligand was shown to be lost upon oxygen exposure. This suggests that anaerobic purification of enzymes containing the sulfido-ligand is mandatory to keep the enzyme intact.

In the upcoming years, it is expected that more details on Moco biosynthesis and the versatile roles of molybdoenzymes will be revealed. High-resolution X-ray and cryo-EM structures will unravel the molecular details of these enzymes even further.

Acknowledgements

The author thanks all current and former members of the research group in addition to collaboration partners who were involved in the work over the past years. The research of the author's laboratory was supported by continuous individual grants from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) (LE1171/15-2, LE1171/11-2) including funding for coordinated research projects by the DFG under Germany's Excellence Strategy –

EXC 2008/1 – 390540038 (gefördert durch die Deutsche Forschungsgemeinschaft (DFG) im Rahmen der Exzellenzstrategie des Bundes und der Länder – EXC 2008/1 – 390540038) and the Priority Programme SPP1927 'FeS for Life'.

References

Abaibou, H., Pommier, J., Benoit, S., Giordano, G., and Mandrand-Berthelot, M.A. (1995) Expression and characterization of the *Escherichia coli* fdo locus and a possible physiological role for aerobic formate dehydrogenase. *J Bacteriol* **177**: 7141–7149.

Aguilar-Barajas, E., Diaz-Perez, C., Ramirez-Diaz, M.I., Riveros-Rosas, H., and Cervantes, C. (2011) Bacterial transport of sulfate, molybdate, and related oxyanions. *Biometals* **24**: 687–707.

Anantharaman, V., and Aravind, L. (2002) MOSC domains: ancient, predicted sulfur-carrier domains, present in diverse metal-sulfur cluster biosynthesis proteins including molybdenum cofactor sulfurases. *FEMS Microbiol Lett* **207**: 55–61.

Anderson, L.A., McNairn, E., Lubke, T., Pau, R.N., and Boxer, D.H. (2000) ModE-dependent molybdate regulation of the molybdenum cofactor operon moa in *Escherichia coli*. *J Bacteriol* **182**: 7035–7043.

Arnoux, P., Ruppelt, C., Oudouhou, F., Lavergne, J., Siponen, M.I., Toci, R., *et al*. (2015) Sulphur shuttling across a chaperone during molybdenum cofactor maturation. *Nat Commun* **6**: 6148.

Baymann, F., Lebrun, E., Brugna, M., Schoepp-Cothenet, B., Giudici-Orticoni, M.T., and Nitschke, W. (2003) The redox protein construction kit: pre-last universal common ancestor evolution of energy-conserving enzymes. *Biol Sci* **358**: 267–274.

Berks, B.C. (1996) A common export pathway for proteins binding complex redox cofactors? *Mol Microbiol* **22**: 393–404.

Berks, B.C., Palmer, T., and Sargent, F. (2005) Protein targeting by the bacterial twin-arginine translocation (Tat) pathway. *Curr Opin Microbiol* **8**: 174–181.

Bevers, L.E., Hagedoorn, P.L., Santamaria-Araujo, J.A., Magalon, A., Hagen, W.R., and Schwarz, G. (2008) Function of MoaB proteins in the biosynthesis of the molybdenum and tungsten cofactors. *Biochemistry* **47**: 949–956.

Bilous, P.T., and Weiner, J.H. (1988) Molecular cloning and expression of the *Escherichia coli* dimethyl sulfoxide reductase operon. *J Bacteriol* **170**: 1511–1518.

Blasco, F., Pommier, J., Augier, V., Chippaux, M., and Giordano, G. (1992) Involvement of the narJ or narW gene product in the formation of active nitrate reductase in *Escherichia coli*. *Mol Microbiol* **6**: 221–230.

Blasco, F., Dos Santos, J.P., Magalon, A., Frixon, C., Guigliarelli, B., Santini, C.L., and Giordano, G. (1998) NarJ is a specific chaperone required for molybdenum cofactor assembly in nitrate reductase A of *Escherichia coli*. *Mol Microbiol* **28**: 435–447.

Bohmer, N., Hartmann, T., and Leimkühler, S. (2014) The chaperone FdsC for Rhodobacter capsulatus formate dehydrogenase binds the bis-molybdopterin guanine dinucleotide cofactor. *FEBS Lett* **588**: 531–537.

Brokx, S.J., Rothery, R.A., Zhang, G., Ng, D.P., and Weiner, J.H. (2005) Characterization of an *Escherichia coli* sulfite oxidase homologue reveals the role of a conserved active site cysteine in assembly and function. *Biochemistry* **44**: 10339–10348.

Brondijk, T.H., Nilavongse, A., Filenko, N., Richardson, D.J., and Cole, J.A. (2004) NapGH components of the periplasmic nitrate reductase of *Escherichia coli* K-12: location, topology and physiological roles in quinol oxidation and redox balancing. *Biochem J* **379**: 47–55.

Buhning, M., Valleriani, A., and Leimkühler, S. (2017) The Role of SufS Is Restricted to Fe-S Cluster Biosynthesis in *Escherichia coli*. *Biochemistry* **56**: 1987–2000.

Chang, L., Wei, L.I., Audia, J.P., Morton, R.A., and Schellhorn, H.E. (1999) Expression of the *Escherichia coli* NRZ nitrate reductase is highly growth phase dependent and is controlled by RpoS, the alternative vegetative sigma factor. *Mol Microbiol* **34**: 756–766.

Coelho, C., Gonzalez, P.J., Moura, J.G., Moura, I., Trincao, J., and Joao Romao, M. (2011) The crystal structure of Cupriavidus necator nitrate reductase in oxidized and partially reduced states. *J Mol Biol* **408**: 932–948.

Cole, J. (1996) Nitrate reduction to ammonia by enteric bacteria: redundancy, or a strategy for survival during oxygen starvation? *FEMS Microbiol Lett* **136**: 1–11.

Culka, M., Huwiler, S.G., Boll, M., and Ullmann, G.M. (2017) Breaking benzene aromaticity-computational insights into the mechanism of the tungsten-containing benzoyl-CoA reductase. *J Am Chem Soc* **139**: 14488–14500.

Dahl, J.U., Radon, C., Bühning, M., Nimtz, M., Leichert, L.I., Denis, Y., *et al*. (2013) The sulfur carrier protein TusA has a pleiotropic role in *Escherichia coli* that also affects molybdenum cofactor biosynthesis. *J Biol Chem* **288**: 5426–5442.

Daniels, J.N., Wuebbens, M.M., Rajagopalan, K.V., and Schindelin, H. (2007) Crystal structure of a molybdopterin synthase-precursor Z complex: insight into its sulfur transfer mechanism and its role in molybdenum cofactor deficiency. *Biochemistry* **47**: 615–626.

Davidson, A.L., and Chen, J. (2004) ATP-binding cassette transporters in bacteria. *Annu Rev Biochem* **73**: 241–268.

Ezraty, B., Bos, J., Barras, F., and Aussel, L. (2005) Methionine sulfoxide reduction and assimilation in *Escherichia coli*: new role for the biotin sulfoxide reductase BisC. *J Bacteriol* **187**: 231–237.

Genest, O., Ilbert, M., Mejean, V., and Iobbi-Nivol, C. (2005) TorD, an essential chaperone for TorA molybdoenzyme maturation at high temperature. *J Biol Chem* **280**: 15644–15648.

Genest, O., Seduk, F., Theraulaz, L., Mejean, V., and Iobbi-Nivol, C. (2006) Chaperone protection of immature molybdoenzyme during molybdenum cofactor limitation. *FEMS Microbiol Lett* **265**: 51–55.

Genest, O., Neumann, M., Seduk, F., Stöcklein, W., Mejean, V., Leimkühler, S., and Iobbi-Nivol, C. (2008) Dedicated metallochaperone connects apoenzyme and molybdenum cofactor biosynthesis components. *J Biol Chem* **283**: 21433–21440.

Genest, O., Mejean, V., and Iobbi-Nivol, C. (2009) Multiple roles of TorD-like chaperones in the biogenesis of molybdoenzymes. *FEMS Microbiol Lett* **297**: 1–9.

Gennaris, A., Ezraty, B., Henry, C., Agrebi, R., Vergnes, A., Oheix, E., *et al*. (2015) Repairing oxidized proteins in the bacterial envelope using respiratory chain electrons. *Nature* **528**: 409–412.

Gon, S., Patte, J.C., Mejean, V., and Iobbi-Nivol, C. (2000) The torYZ (yecK bisZ) operon encodes a third respiratory trimethylamine N-oxide reductase in *Escherichia coli*. *J Bacteriol* **182**: 5779–5786.

Grunden, A.M., and Shanmugam, K.T. (1997) Molybdate transport and regulation in bacteria. *Arch Microbiol* **168**: 345–354.

Gutzke, G., Fischer, B., Mendel, R.R., and Schwarz, G. (2001) Thiocarboxylation of molybdopterin synthase provides evidence for the mechanism of dithiolene formation in metal-binding pterins. *J Biol Chem* **276**: 36268–36274.

Guymer, D., Maillard, J., and Sargent, F. (2009) A genetic analysis of in vivo selenate reduction by *Salmonella enterica* serovar Typhimurium LT2 and *Escherichia coli* K12. *Arch Microbiol* **191**: 519–528.

Hagen, F. (2011) Cellular uptake of molybdenum and tungsten. *Coord Chem Rev* **255**: 1117–1128.

Hänzelmann, P., and Schindelin, H. (2004) Crystal structure of the S-adenosylmethionine-dependent enzyme MoaA and its implications for molybdenum cofactor deficiency in humans. *Proc Natl Acad Sci U S A* **101**: 12870–12875.

Hänzelmann, P., and Schindelin, H. (2006) Binding of 5′-GTP to the C-terminal FeS cluster of the radical S-adenosylmethionine enzyme MoaA provides insights into its mechanism. *Proc Natl Acad Sci U S A* **103**: 6829–6834.

Hänzelmann, P., Hernandez, H.L., Menzel, C., Garcia-Serres, R., Huynh, B.H., Johnson, M.K., *et al*. (2004) Characterization of MOCS1A, an oxygen-sensitive iron-sulfur protein involved in human molybdenum cofactor biosynthesis. *J Biol Chem* **279**: 34721–34732.

Hartmann, T., Schwanhold, N., and Leimkühler, S. (2015) Assembly and catalysis of molybdenum or tungsten-containing formate dehydrogenases from bacteria. *Biochim Biophys Acta* **1854**: 1090–1100.

Hartmann, T., Schrapers, P., Utesch, T., Nimtz, M., Rippers, Y., Dau, H., *et al*. (2016) The molybdenum active site of formate dehydrogenase is capable of catalyzing C-H bond cleavage and oxygen atom transfer reactions. *Biochemistry* **55**: 2381–2389.

Hasona, A., Self, W.T., and Sahanmugam, K.T. (2001) Transcriptional regulation of moe (molybdate metabolism) operon of *Escherichia coli*. *Arch Microbiol* **175**: 178–188.

Hidese, R., Mihara, H., and Esaki, N. (2011) Bacterial cysteine desulfurases: versatile key players in biosynthetic pathways of sulfur-containing biofactors. *Appl Microbiol Biotechnol* **91**: 47–61.

Higgins, C.F. (1992) ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* **8**: 67–113.

Hille, R. (1996) The mononuclear molybdenum enzymes. *Chem Rev* **96**: 2757–2816.

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

Hille, R., Hall, J., and Basu, P. (2014) The mononuclear molybdenum enzymes. *Chem Rev* **114**: 3963–4038.

Hover, B.M., Loksztejn, A., Ribeiro, A.A., and Yokoyama, K. (2013) Identification of a cyclic nucleotide as a cryptic intermediate in molybdenum cofactor biosynthesis. *J Am Chem Soc* **135**: 7019–7032.

Hu, Y., Rech, S., Gunsalus, R.P., and Rees, D.C. (1997) Crystal structure of the molybdate binding protein ModA. *Nat Struct Biol* **4**: 703–707.

Ikeuchi, Y., Shigi, N., Kato, J., Nishimura, A., and Suzuki, T. (2006) Mechanistic insights into sulfur relay by multiple sulfur mediators involved in thiouridine biosynthesis at tRNA wobble positions. *Mol Cell* **21**: 97–108.

Ilbert, M., Mejean, V., and Iobbi-Nivol, C. (2004) Functional and structural analysis of members of the TorD family, a large chaperone family dedicated to molybdoproteins. *Microbiology* **150**: 935–943.

Iobbi-Nivol, C., and Leimkühler, S. (2013) Molybdenum enzymes, their maturation and molybdenum cofactor biosynthesis in *Escherichia coli*. *Biochim Biophys Acta* **1827**: 1086–1101.

Ize, B., Coulthurst, S.J., Hatzixanthis, K., Caldelari, I., Buchanan, G., Barclay, E.C., *et al*. (2009) Remnant signal peptides on non-exported enzymes: implications for the evolution of prokaryotic respiratory chains. *Microbiology* **155**: 3992–4004.

Jack, R.L., Buchanan, G., Dubini, A., Hatzixanthis, K., Palmer, T., and Sargent, F. (2004) Coordinating assembly and export of complex bacterial proteins. *EMBO J* **23**: 3962–3972.

Johnson, M.K., Rees, D.C., and Adams, M.W. (1996) Tungstoenzymes. *Chem Rev* **96**: 2817–2840.

Jormakka, M., Byrne, B., and Iwata, S. (2003) Formate dehydrogenase – a versatile enzyme in changing environments. *Curr Opin Struct Biol* **13**: 418–423.

Kanaujia, S.P., Jeyakanthan, J., Nagawaka, N., Balasubramaniam, S., Shinkai, A., Kuramitsu, S., *et al*. (2010a) Sructure of apo and GTP-bound molydenum cofactor biosynthesis protein MoaC from *Thermus thermophilus* HB8. *Acta Crystallogr D Biol Crystallogr* **66**: 821–833.

Kanaujia, S.P., Jeyakanthan, J., Nakagawa, N., Balasubramaniam, S., Shinkai, A., Kuramitsu, S., *et al*. (2010b) Structures of apo and GTP-bound molybdenum cofactor biosynthesis protein MoaC from Thermus thermophilus HB8. *Acta Crystallogr* **66**: 821–833.

Kang, Y., Weber, K.D., Qiu, Y., Kiley, P.J., and Blattner, F.R. (2005) Genome-wide expression analysis indicates that FNR of *Escherichia coli* K-12 regulates a large number of genes of unknown function. *J Bacteriol* **187**: 1135–1160.

Kaufmann, P., Duffus, B.R., Mitrova, B., Iobbi-Nivol, C., Teutloff, C., Nimtz, M., *et al*. (2018) Modulating the molybdenum coordination sphere of *Escherichia coli* trimethylamine N-oxide reductase. *Biochemistry* **57**: 1130–1143.

Kim, S.H., Harzman, C., Davis, J.K., Hutcheson, R., Broderick, J.B., Marsh, T.L., and Tiedje, J.M. (2012) Genome sequence of *Desulfitobacterium hafniense* DCB-2, a Gram-positive anaerobe capable of dehalogenation and metal reduction. *BMC Microbiol* **12**: 21.

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

Kletzin, A., and Adams, M.W. (1996) Tungsten in biological systems. *FEMS Microbiol Rev* **18**: 5–63.

Kozmin, S.G., and Schaaper, R.M. (2013) Genetic characterization of moaB mutants of *Escherichia coli*. *Res Microbiol* **164**: 689–694.

Kozmin, S.G., Leroy, P., Pavlov, Y.I., and Schaaper, R.M. (2008) YcbX and yiiM, two novel determinants for resistance of *Escherichia coli* to N-hydroxylated base analogues. *Mol Microbiol* **68**: 51–65.

Kuper, J., Llamas, A., Hecht, H.J., Mendel, R.R., and Schwarz, G. (2004) Structure of the molybdopterin-bound Cnx1G domain links molybdenum and copper metabolism. *Nature* **430**: 803–806.

Lake, M.W., Temple, C.A., Rajagopalan, K.V., and Schindelin, H. (2000) The crystal structure of the *Escherichia coli* MobA protein provides insight into molybdopterin guanine dinucleotide biosynthesis. *J Biol Chem* **275**: 40211–40217.

Lake, M.W., Wuebbens, M.M., Rajagopalan, K.V., and Schindelin, H. (2001) Mechanism of ubiquitin activation revealed by the structure of a bacterial MoeB-MoaD complex. *Nature* **414**: 325–329.

Lamberg, K.E., and Kiley, P.J. (2000) FNR-dependent activation of the class II dmsA and narG promoters of *Escherichia coli* requires FNR-activating regions 1 and 3. *Mol Microbiol* **38**: 817–827.

Leimkühler, S. (2014) The biosynthesis of the molybdenum cofactor in *Escherichia coli* and its connection to FeS cluster assembly and the thiolation of tRNA. *Adv Biol* **2014**: 808569.

Leimkühler, S., and Iobbi-Nivol, C. (2016) Bacterial molybdoenzymes: old enzymes for new purposes. *FEMS Microbiol Rev* **40**: 1–18.

Leimkühler, S., and Klipp, W. (1999) Role of XDHC in molybdenum cofactor insertion into xanthine dehydrogenase of *Rhodobacter capsulatus*. *J Bacteriol* **181**: 2745–2751.

Leimkühler, S., and Rajagopalan, K.V. (2001) An *Escherichia coli* NifS-like sulfurtransferase is required for the transfer of cysteine sulfur in the *in vitro* synthesis of molybdopterin from precursor Z. *J Biol Chem* **276**: 22024–22031.

Leimkühler, S., Kern, M., Solomon, P.S., McEwan, A.G., Schwarz, G., Mendel, R.R., and Klipp, W. (1998) Xanthine dehydrogenase from the phototrophic purple bacterium *Rhodobacter capsulatus* is more similar to its eukaryotic counterparts than to prokaryotic molybdenum enzymes. *Mol Microbiol* **27**: 853–869.

Leimkühler, S., Wuebbens, M.M., and Rajagopalan, K.V. (2001) Characterization of *Escherichia coli* MoeB and its involvement in the activation of molybdopterin synthase for the biosynthesis of the molybdenum cofactor. *J Biol Chem* **276**: 34695–34701.

Leimkühler, S., Freuer, A., Araujo, J.A., Rajagopalan, K.V., and Mendel, R.R. (2003) Mechanistic studies of human molybdopterin synthase reaction and characterization of mutants identified in group B patients of molybdenum cofactor deficiency. *J Biol Chem* **278**: 26127–26134.

Leimkühler, S., Wuebbens, M.M., and Rajagopalan, K.V. (2011) The history of the discovery of the molybdenum cofactor and novel aspects of its biosynthesis in bacteria. *Coord Chem Rev* **255**: 1129–1144.

Leimkühler, S., Bühning, M., and Beilschmidt, L. (2017) Shared sulfur mobilization routes for tRNA thiolation and molybdenum cofactor biosynthesis in prokaryotes and eukaryotes. *Biomolecules* **7**: 5.

Li, J., and Stewart, V. (1992) Localization of upstream sequence elements required for nitrate and anaerobic induction of fdn (formate dehydrogenase-N) operon expression in *Escherichia coli* K-12. *J Bacteriol* **174**: 4935–4942.

Liu, M.T., Wuebbens, M.M., Rajagopalan, K.V., and Schindelin, H. (2000) Crystal structure of the gephyrin-related molybdenum cofactor biosynthesis protein MogA from *Escherichia coli*. *J Biol Chem* **275**: 1814–1822.

Llamas, A., Otte, T., Multhaup, G., Mendel, R.R., and Schwarz, G. (2006) The mechanism of nucleotide-assisted molybdenum insertion into molybdopterin. A novel route toward metal cofactor assembly. *J Biol Chem* **281**: 18343–18350.

Loschi, L., Brokx, S.J., Hills, T.L., Zhang, G., Bertero, M.G., Lovering, A.L., *et al*. (2004) Structural and biochemical identification of a novel bacterial oxidoreductase. *J Biol Chem* **279**: 50391–50400.

Lubitz, S.P., and Weiner, J.H. (2003) The *Escherichia coli* ynfEFGHI operon encodes polypeptides which are paralogues of dimethyl sulfoxide reductase (DmsABC). *Arch Biochem Biophys* **418**: 205–216.

Magalon, A., Fedor, J.G., Walburger, A., and Weiner, J.A. (2011) Molybdenum enzymes in bacteria and their maturation. *Coord Chem Rev* **255**: 1159–1178.

Masuda, N., and Church, G.M. (2002) *Escherichia coli* gene expression responsive to levels of the response regulator EvgA. *J Bacteriol* **184**: 6225–6234.

Masuda, N., and Church, G.M. (2003) Regulatory network of acid resistance genes in *Escherichia coli*. *Mol Microbiol* **48**: 699–712.

McCrindle, S.L., Kappler, U., and McEwan, A.G. (2005) Microbial dimethylsulfoxide and trimethylamine-N-oxide respiration. *Adv Microb Physiol* **50**: 147–198.

McNicholas, P.M., Rech, S.A., and Gunsalus, R.P. (1997) Characterization of the ModE DNA-binding sites in the control regions of modABCD and moaABCDE of *Escherichia coli*. *Mol Microbiol* **23**: 515–524.

McNicholas, P.M., Mazzotta, M.M., Rech, S.A., and Gunsalus, R.P. (1998) Functional dissection of the molybdate-responsive transcription regulator, ModE, from *Escherichia coli*. *J Bacteriol* **180**: 4638–4643.

Mehta, A.P., Abdelwahed, S.H., and Begley, T.P. (2013) Molybdopterin biosynthesis: trapping an unusual purine ribose adduct in the MoaA-catalyzed reaction. *J Am Chem Soc* **135**: 10883–10885.

Mejean, V., Iobbi-Nivol, C., Lepelletier, M., Giordano, G., Chippaux, M., and Pascal, M.C. (1994) TMAO anaerobic respiration in *Escherichia coli*: involvement of the tor operon. *Mol Microbiol* **11**: 1169–1179.

Melville, S.B., and Gunsalus, R.P. (1996) Isolation of an oxygen-sensitive FNR protein of *Escherichia coli*: interaction at activator and repressor sites of FNR-controlled genes. *Proc Natl Acad Sci U S A* **93**: 1226–1231.

Mendel, R.R., and Leimkühler, S. (2015) The biosynthesis of the molybdenum cofactors. *J Biol Inorg Chem* **20**: 337–347.

Najmudin, S., Gonzalez, P.J., Trincao, J., Coelho, C., Mukhopadhyay, A., Cerqueira, N.M., *et al*. (2008) Periplasmic nitrate reductase revisited: a sulfur atom

completes the sixth coordination of the catalytic molybdenum. *J Biol Inorg Chem* **13**: 737–753.

Namgung, B., Kim, J.H., Song, W.S., and Yoon, S.I. (2018) Crystal structure of the hydroxylaminopurine resistance protein, YiiM, and its putative molybdenum cofactor-binding catalytic site. *Sci Rep* **8**: 3304.

Neumann, M., and Leimkühler, S. (2008) Heavy metal ions inhibit molybdoenzyme activity by binding to the dithiolene moiety of molybdopterin in *Escherichia coli*. *FEBS J* **275**: 5678–5689.

Neumann, M., and Leimkühler, S. (2011) The role of system-specific molecular chaperones in the maturation of molybdoenzymes in bacteria. *Biochem Res Int* **2011**: 850924.

Neumann, M., Stöcklein, W., and Leimkühler, S. (2007a) Transfer of the molybdenum cofactor synthesized by rhodobacter capsulatus MoeA to XdhC and MobA. *J Biol Chem* **282**: 28493–28500.

Neumann, M., Stöcklein, W., Walburger, A., Magalon, A., and Leimkühler, S. (2007b) Identification of a Rhodobacter capsulatus L-cysteine desulfurase that sulfurates the molybdenum cofactor when bound to XdhC and before its insertion into xanthine dehydrogenase. *Biochemistry* **46**: 9586–9595.

Neumann, M., Mittelstädt, G., Iobbi-Nivol, C., Miguel Saggu, M., Lendzian, F., Hildebrandt, P., and Leimkühler, S. (2009a) A periplasmic aldehyde oxidoreductase represents the first molybdopterin cytosine dinucleotide cofactor containing molybdo-flavoenzyme from *Escherichia coli*. *FEBS J* **276**: 2762–2774.

Neumann, M., Mittelstädt, G., Seduk, F., Iobbi-Nivol, C., and Leimkühler, S. (2009b) MocA is a specific cytidylyltransferase involved in molybdopterin cytosine dinucleotide biosynthesis in *Escherichia coli*. *J Biol Chem* **284**: 21891–21898.

Neumann, M., Seduk, F., Iobbi-Nivol, C., and Leimkühler, S. (2011) Molybdopterin dinucleotide biosynthesis in *Escherichia coli*: identification of amino acid residues of molybdopterin dinucleotide transferases that determine specificity for binding of guanine or cytosine nucleotides. *J Biol Chem* **286**: 1400–1408.

Nichols, J., and Rajagopalan, K.V. (2002) *Escherichia coli* MoeA and MogA. Function in metal incorporation step of molybdenum cofactor biosynthesis. *J Biol Chem* **277**: 24995–25000.

Nichols, J.D., and Rajagopalan, K.V. (2005) In vitro molybdenum ligation to molybdopterin using purified components. *J Biol Chem* **280**: 7817–7822.

Outten, F.W., Djaman, O., and Storz, G. (2004) A suf operon requirement for Fe-S cluster assembly during iron starvation in *Escherichia coli*. *Mol Microbiol* **52**: 861–872.

Palmer, T., Santini, C.-L., Iobbi-Nivol, C., Eaves, D.J., Boxer, D.H., and Giordano, G. (1996) Involvement of the *narJ* and *mob* gene products in the biosynthesis of the molybdoenzyme nitrate reductase in *Escherichia coli*. *Mol Microbiol* **20**: 875–884.

Partridge, J.D., Browning, D.F., Xu, M., Newnham, L.J., Scott, C., Roberts, R.E., *et al*. (2008) Characterization of the *Escherichia coli* K-12 ydhYVWXUT operon: regulation by FNR, NarL and NarP. *Microbiology* **154**: 608–618.

Patterson-Fortin, L.M., Vakulskas, C.A., Yakhnin, H., Babitzke, P., and Romeo, T. (2013) Dual posttranscriptional regulation via a cofactor-responsive mRNA leader. *J Mol Biol* **425**: 3662–3677.

Pierson, D.E., and Campbell, A. (1990) Cloning and nucleotide sequence of bisC, the structural gene for biotin sulfoxide reductase in *Escherichia coli*. *J Bacteriol* **172**: 2194–2198.

Pitterle, D.M., and Rajagopalan, K.V. (1989) Two proteins encoded at the *chlA* locus constitute the converting factor of *Escherichia coli chlA1*. *J Bacteriol* **171**: 3373–3378.

Pitterle, D.M., and Rajagopalan, K.V. (1991) Purification and characterization of the converting factor from *E. coli chlA1*. *FASEB J* **5**: A468.

Pitterle, D.M., and Rajagopalan, K.V. (1993) The biosynthesis of molybdopterin in *Escherichia coli*. Purification and characterization of the converting factor. *J Biol Chem* **268**: 13499–13505.

Pitterle, D.M., Johnson, J.L., and Rajagopalan, K.V. (1990) Molybdopterin formation by converting factor of *E. coli chlA1*. *FASEB J* **4**: A1957.

Pitterle, D.M., Johnson, J.L., and Rajagopalan, K.V. (1993) *In vitro* synthesis of molybdopterin from precursor Z using purified converting factor. Role of protein-bound sulfur in formation of the dithiolene. *J Biol Chem* **268**: 13506–13509.

Pommier, J., Mejean, V., Giordano, G., and Iobbi-Nivol, C. (1998) TorD, a cytoplasmic chaperone that interacts with the unfolded trimethylamine N-oxide reductase enzyme (TorA) in *Escherichia coli*. *J Biol Chem* **273**: 16615–16620.

Raaijmakers, H.C., and Romao, M.J. (2006) Formate-reduced *E. coli* formate dehydrogenase H: the reinterpretation of the crystal structure suggests a new reaction mechanism. *J Biol Inorg Chem* **11**: 849–854.

Rajagopalan, K.V. (1996) Biosynthesis of the molybdenum cofactor. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, Neidhardt, F.C. (ed). Washington, DC: ASM Press, pp. 674–679.

Rajagopalan, K.V., and Johnson, J.L. (1992) The pterin molybdenum cofactors. *J Biol Chem* **267**: 10199–10202.

Ray, N., Oates, J., Turner, R.J., and Robinson, C. (2003) DmsD is required for the biogenesis of DMSO reductase in *Escherichia coli* but not for the interaction of the DmsA signal peptide with the Tat apparatus. *FEBS Lett* **534**: 156–160.

Regulski, E.E., Moy, R.H., Weinberg, Z., Barrick, J.E., Yao, Z., Ruzzo, W.L., and Breaker, R.R. (2008) A widespread riboswitch candidate that controls bacterial genes involved in molybdenum cofactor and tungsten cofactor metabolism. *Mol Microbiol* **68**: 918–932.

Reschke, S., Sigfridsson, K.G., Kaufmann, P., Leidel, N., Horn, S., Gast, K., *et al*. (2013) Identification of a Bis-molybdopterin intermediate in molybdenum cofactor biosynthesis in *Escherichia coli*. *J Biol Chem* **288**: 29736–29745.

Reschke, S., Duffus, B.R., Schrapers, P., Mebs, S., Teutloff, C., Dau, H., *et al*. (2019) Identification of YdhV as the first molybdoenzyme binding a Bis-Mo-MPT cofactor in *Escherichia coli*. *Biochemistry* **58**: 2228–2242.

Ribbe, M.W., Hu, Y., Hodgson, K.O., and Hedman, B. (2013) Biosynthesis of nitrogenase metalloclusters. *Chem Rev* **114**: 4063–4080.

Romao, M.J. (2009) Molybdenum and tungsten enzymes: a crystallographic and mechanistic overview. *Dalton Trans* **21**: 4053–4068.

Rossmann, R., Sawers, G., and Bock, A. (1991) Mechanism of regulation of the formate-hydrogenlyase pathway by oxygen, nitrate, and pH: definition of the formate regulon. *Mol Microbiol* **5**: 2807–2814.

Rothery, R.A., and Weiner, J.H. (1993) Topological characterization of *Escherichia coli* DMSO reductase by electron paramagnetic resonance spectroscopy of an engineered [3Fe-4S] cluster. *Biochemistry* **32**: 5855–5861.

Rudolph, M.J., Wuebbens, M.M., Rajagopalan, K.V., and Schindelin, H. (2001) Crystal structure of molybdopterin synthase and its evolutionary relationship to ubiquitin activation. *Nat Struct Biol* **8**: 42–46.

Sabnis, N.A., Yang, H., and Romeo, T. (1995) Pleiotropic regulation of central carbohydrate metabolism in *Escherichia coli* via the gene csrA. *J Biol Chem* **270**: 29096–29104.

Salmon, K., Hung, S.P., Mekjian, K., Baldi, P., Hatfield, G. W., and Gunsalus, R.P. (2003) Global gene expression profiling in *Escherichia coli* K12. The effects of oxygen availability and FNR. *J Biol Chem* **278**: 29837–29855.

Santamaria-Araujo, J.A., Fischer, B., Otte, T., Nimtz, M., Mendel, R.R., Wray, V., and Schwarz, G. (2004) The tetrahydropyranopterin structure of the sulfur- and metal-free molybdenum cofactor precursor. *J Biol Chem* **279**: 15994–15999.

Sawers, G. (1994) The hydrogenases and formate dehydrogenases of *Escherichia coli*. *Antonie Van Leeuwenhoek* **66**: 57–88.

Schindelin, H. (2005) Evolutionary origin of the activation step during ubiquitin-dependent protein degradation. In *Protein Degradation: Ubiquitin and the Chemistry of Life*, Mayer, R.J., Ciechanover, A., and Rechsteiner, M. (eds). Weinheim, Germany: Wiley-VCH, pp. 21–43.

Schmitz, J., Wuebbens, M.M., Rajagopalan, K.V., and Leimkühler, S. (2007) Role of the C-terminal Gly-Gly motif of *Escherichia coli* MoaD, a molybdenum cofactor biosynthesis protein with a ubiquitin fold. *Biochemistry* **46**: 909–916.

Schoepp-Cothenet, B., van Lis, R., Philippot, P., Magalon, A., Russell, M.J., and Nitschke, W. (2012) The ineluctable requirement for the trans-iron elements molybdenum and/or tungsten in the origin of life. *Sci Rep* **2**: 263.

Schrag, J.D., Huang, W., Sivaraman, J., Smith, C., Plamondon, J., Larocque, R., *et al*. (2001) The crystal structure of *Escherichia coli* MoeA, a protein from the molybdopterin synthesis pathway. *J Mol Biol* **310**: 419–431.

Schüttelkopf, A.W., Boxer, D.H., and Hunter, W.N. (2003) Crystal structure of activated ModE reveals conformational changes involving both oxyanion and DNA-binding domains. *J Mol Biol* **326**: 761–767.

Schwanhold, N., Iobbi-Nivol, C., Lehmann, A., and Leimkühler, S. (2018) Same but different: comparison of two system-specific molecular chaperones for the maturation of formate dehydrogenases. *PLoS One* **13**: e0201935.

Schwarz, G., Mendel, R.R., and Ribbe, M.W. (2009) Molybdenum cofactors, enzymes and pathways. *Nature* **460**: 839–847.

Self, W.T., Grunden, A.M., Hasona, A., and Shanmugam, K. T. (1999) Transcriptional regulation of molybdoenzyme synthesis in *Escherichia coli* in response to molybdenum: ModE-molybdate, a repressor of the modABCD (molybdate transport) operon is a secondary transcriptional activator for the hyc and nar operons. *Microbiology* **145**: 41–55.

Self, W.T., Grunden, A.M., Hasona, A., and Shanmugam, K. T. (2001) Molybdate transport. *Res Microbiol* **152**: 311–321.

Shanmugam, K.T., Stewart, V., Gunsalus, R.P., Boxer, D.H., Cole, J.A., Chippaux, M., *et al*. (1992) Proposed nomenclature for the genes involved in molybdenum metabolism in *Escherichia coli* and *Salmonella typhimurium*. *Mol Microbiol* **6**: 3452–3454.

Shigi, N. (2014) Biosynthesis and functions of sulfur modifications in tRNA. *Front Genet* **5**: 67.

Sofia, H.J., Chen, G., Hetzler, B.G., Reyes-Spindola, J.F., and Miller, N.E. (2001) Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. *Nucleic Acids Res* **29**: 1097–1106.

Stevenson, C.E., Sargent, F., Buchanan, G., Palmer, T., and Lawson, D.M. (2000) Crystal structure of the molybdenum cofactor biosynthesis protein MobA from *Escherichia coli* at near-atomic resolution. *Structure* **8**: 1115–1125.

Stewart, V. (1988) Nitrate respiration in relation to facultative metabolism in enterobacteria. *Microbiol Rev* **52**: 190–232.

Stewart, V., Bledsoe, P.J., and Williams, S.B. (2003) Dual overlapping promoters control napF (periplasmic nitrate reductase) operon expression in *Escherichia coli* K-12. *J Bacteriol* **185**: 5862–5870.

Suzuki, T. (2005) Biosynthesis and function of tRNA wobble modifications. *Top Curr Genet* **12**: 23–69.

Temple, C.A., and Rajagopalan, K.V. (2000) Mechanism of assembly of the bis(molybdopterin guanine dinucleotide) molybdenum cofactor in *Rhodobacter sphaeroides* dimethyl sulfoxide reductase. *J Biol Chem* **275**: 40202–40210.

Thome, R., Gust, A., Toci, R., Mendel, R., Bittner, F., Magalon, A., and Walburger, A. (2012) A sulfurtransferase is essential for activity of formate dehydrogenases in *Escherichia coli*. *J Biol Chem* **287**: 4671–4678.

Tong, Y., Wuebbens, M.M., Rajagopalan, K.V., and Fitzgerald, M.C. (2005) Thermodynamic analysis of subunit interactions in *Escherichia coli* molybdopterin synthase. *Biochemistry* **44**: 2595–2601.

Turner, R.J., Papish, A.L., and Sargent, F. (2004) Sequence analysis of bacterial redox enzyme maturation proteins (REMPs). *Can J Microbiol* **50**: 225–238.

Unden, G., and Schirawski, J. (1997) The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signals and reactions. *Mol Microbiol* **25**: 205–210.

Unden, G., Achebach, S., Holighaus, G., Tran, H.G., Wackwitz, B., and Zeuner, Y. (2002) Control of FNR function of *Escherichia coli* by O2 and reducing conditions. *J Mol Microbiol Biotechnol* **4**: 263–268.

Wang, H., Chen, X., Li, C., Liu, Y., Yang, F., and Wang, C. (2018) Sequence-based prediction of cysteine reactivity using machine learning. *Biochemistry* **57**: 451–460.

Wuebbens, M.M., and Rajagopalan, K.V. (1993) Structural characterization of a molybdopterin precursor. *J Biol Chem* **268**: 13493–13498.

Wuebbens, M.M., and Rajagopalan, K.V. (1995) Investigation of the early steps of molybdopterin biosynthesis in *Escherichia coli* through the use of *in vivo* labeling studies. *J Biol Chem* **270**: 1082–1087.

Wuebbens, M.M., and Rajagopalan, K.V. (2003) Mechanistic and mutational studies of *Escherichia coli* molybdopterin synthase clarify the final step of molybdopterin biosynthesis. *J Biol Chem* **278**: 14523–14532.

Wuebbens, M.M., Liu, M.T., Rajagopalan, K., and Schindelin, H. (2000) Insights into molybdenum cofactor deficiency provided by the crystal structure of the molybdenum cofactor biosynthesis protein MoaC. *Structure* **8**: 709–718.

Xi, H., Schneider, B.L., and Reitzer, L. (2000) Purine catabolism in Escherichia coli and function of xanthine dehydrogenase in purine salvage. *J Bacteriol* **182**: 5332–5341.

Xiang, S., Nichols, J., Rajagopalan, K.V., and Schindelin, H. (2001) The crystal structure of *Escherichia coli* MoeA and its relationship to the multifunctional protein gephyrin. *Structure* **9**: 299–310.

Xu, M., Busby, S.J., and Browning, D.F. (2009) Activation and repression at the *Escherichia coli* ynfEFGHI operon promoter. *J Bacteriol* **191**: 3172–3176.

Yokoyama, K., and Leimkühler, S. (2015) The role of FeS clusters for molybdenum cofactor biosynthesis and molybdoenzymes in bacteria. *Biochim Biophys Acta* **1853**: 1335–1349.

Zhang, W., Urban, A., Mihara, H., Leimkühler, S., Kurihara, T., and Esaki, N. (2010) IscS functions as a primary sulfur-donating enzyme by interacting specifically with MoeB and MoaD in the biosynthesis of molybdopterin in *Escherichia coli*. *J Biol Chem* **285**: 2302–2308.

Zheng, L., Cash, V.L., Flint, D.H., and Dean, D.R. (1998) Assembly of iron-sulfur clusters. Identification of an iscSUA-hscBA-fdx gene cluster from *Azotobacter vinelandii*. *J Biol Chem* **273**: 13264–13272.

Zupok, A., Gorka, M., Siemiatkowska, B., Skirycz, A., and Leimkühler, S. (2019a) Iron-dependent regulation of molybdenum cofactor biosynthesis genes in *Escherichia coli*. *J Bacteriol* **201**: e00382-00319.

Zupok, A., Iobbi-Nivol, C., Mejean, V., and Leimkühler, S. (2019b) The regulation of Moco biosynthesis and molybdoenzyme gene expression by molybdenum and iron in bacteria. *Metallomics* **11**: 1602–1624.