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Review

Bacterial methionine biosynthesis

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Methionine is essential in all organisms, as it is both a proteinogenic amino acid and a component of the cofactor, *S*-adenosyl methionine. The metabolic pathway for its biosynthesis has been extensively characterized in *Escherichia coli*; however, it is becoming apparent that most bacterial species do not use the *E. coli* pathway. Instead, studies on other organisms and genome sequencing data are uncovering significant diversity in the enzymes and metabolic intermediates that are used for methionine biosynthesis. This review summarizes the different biochemical strategies that are employed in the three key steps for methionine biosynthesis from homoserine (i.e. acylation, sulfurylation and methylation). A survey is presented of the presence and absence of the various biosynthetic enzymes in 1593 representative bacterial species, shedding light on the non-canonical nature of the *E. coli* pathway. This review also highlights ways in which knowledge of methionine biosynthesis can be utilized for biotechnological applications. Finally, gaps in the current understanding of bacterial methionine biosynthesis are noted. For example, the paper discusses the presence of one gene (*metC*) in a large number of species that appear to lack the gene encoding the enzyme for the preceding step in the pathway (*metB*), as it is understood in *E. coli*. Therefore, this review aims to move the focus away from *E. coli*, to better reflect the true diversity of bacterial pathways for methionine biosynthesis.

Introduction

Methionine is a proteinogenic amino acid, best known for its role in the initiation of translation. It possesses an unbranched, hydrophobic side chain and it is the only amino acid that contains a thioether (i.e. C–S–C bonding). In general, methionine is assumed to play a simple structural role in the hydrophobic cores of proteins, in a similar way to the other hydrophobic amino acids (valine, leucine and isoleucine). Additionally, S/π interactions between the side chain sulfur atom and aromatic amino acids have recently been identified as prevalent and important stabilizing interactions in one-third of all known protein structures (Valley *et al.*, 2012). In a handful of proteins, methionine also plays a functional role as a redox sensor (Bigelow & Squier, 2005).

In all organisms, including bacteria, methionine is one of the less abundant amino acids in proteins (Pasamontes & Garcia-Vallve, 2006). However, it is also the key component of the cofactor *S*-adenosyl methionine (SAM), which is the main cellular carrier of methyl groups (Chiang *et al.*, 1996). The intracellular concentrations of SAM and free methionine in *Escherichia coli*, growing exponentially on glucose, have been estimated at 0.18 and 0.15 mM, respectively (Bennett *et al.*, 2009).

Almost all bacterial species possess biosynthetic pathways for methionine. Exceptions include endosymbiotic species with degraded genomes, such as the insect endosymbiont *Wolbachia* (Foster *et al.*, 2005; McCutcheon & Moran, 2012). Methionine is synthesized from homoserine, which in turn is derived from aspartate by two consecutive reductions of the terminal carboxyl group (Blanco *et al.*, 2003). The conserved biochemical logic for converting homoserine to methionine is: (i) to activate homoserine by acylating it; (ii) to effect replacement of the side chain hydroxyl group with a thiol group, giving homocysteine; and (iii) to transfer a methyl group to the thiol, yielding methionine. The most well-studied biosynthetic pathway is the one from *E. coli* (Fig. 1). However, studies on other species and the rapidly expanding catalogue of complete genome sequences are revealing alternative enzymes and intermediates throughout the pathway. It is now apparent that the *E. coli* methionine biosynthesis pathway is far from canonical, although it remains useful as the basis for comparisons with other, more common, pathways.

In this review, we summarize the diversity of bacterial methionine biosynthesis pathways. In addition, we highlight the ways in which this information can shed light on bacterial physiology, ecology and evolution, as well as on aspects of microbial biotechnology.

Abbreviations: PLP, pyridoxal-5′-phosphate; SAM, *S*-adenosyl methionine.

Methionine biosynthesis in *E. coli*

The pathway by which *E. coli* synthesizes methionine from homoserine is shown in Fig. 1. The first step is the activation of homoserine, which is done by transferring a

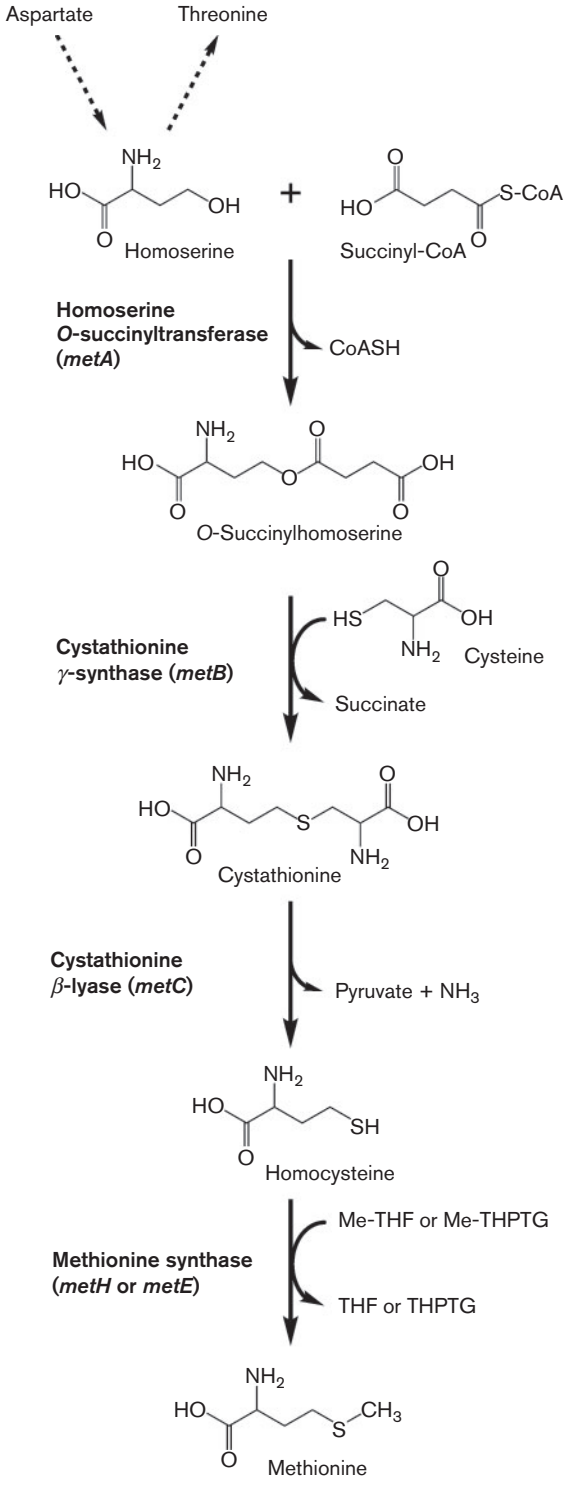

Fig. 1. Methionine biosynthesis in *E. coli*. The enzymes catalysing each step, and the genes that encode them, are indicated in bold type. Me-THF, 5-methyl-tetrahydrofolate; Me-THPTG, 5-methyltetrahydropteroyl tri-L-glutamate; THF, tetrahydrofolate; THPTG, tetrahydropteroyl tri-L-glutamate.

succinyl group from succinyl-CoA to the γ-hydroxyl group, resulting in *O*-succinylhomoserine (Flavin & Slaughter, 1967; Born & Blanchard, 1999). The enzyme that catalyses this reaction is homoserine *O*-succinyltransferase and it is encoded by the *metA* gene (Rowbury & Woods, 1964; Duclos *et al.*, 1989). The reaction is an ordered ping-pong reaction, in which the first half-reaction is the substitution of the CoA group from succinyl-CoA with a cysteine residue of the enzyme, resulting in a succinyl-enzyme intermediate. This is followed by transfer of the succinyl group from the enzyme to homoserine, resulting in the formation of *O*-succinylhomoserine (Born & Blanchard, 1999).

Activation of homoserine by succinylation allows trans-sulfurylation to occur. This involves the transfer of a thiol group from cysteine to homoserine, forming homocysteine in two enzyme-catalysed steps (Fig. 1). The first step is catalysed by cystathionine γ-synthase (encoded by *metB*), which produces cystathionine and succinate from *O*-succinylhomoserine and cysteine. The second step is catalysed by cystathionine β-lyase (encoded by *metC*), which cleaves cystathionine to form homocysteine, pyruvate and ammonia (Clausen *et al.*, 1996). The *metB* and *metC* genes are homologues.

The final step in methionine biosynthesis is the *S*-methylation of homocysteine (Fig. 1). *E. coli* can carry out this step using either one of two non-homologous enzymes: cobalamin-dependent methionine synthase, encoded by *metH*; or cobalamin-independent methionine synthase, encoded by *metE*. The *metH*-encoded synthase catalyses the transfer of a methyl group from 5-methyl-tetrahydrofolate to homocysteine, with its cobalamin cofactor serving as the acceptor and donor of the methyl group (Koutmos *et al.*, 2009). However, *E. coli* has lost the pathway for synthesizing cobalamin and instead the cofactor is scavenged (Lawrence & Roth, 1996). Therefore, the *metE*-encoded synthase is also present, to enable methionine production in the absence of exogenous cobalamin (*metE* expression is repressed in the presence of cobalamin). The cobalamin-independent methionine synthase catalyses direct transfer of a methyl group from the triglutamate derivative of 5-methyl-tetrahydrofolate to homocysteine (Whitfield *et al.*, 1970), albeit with a turnover number that is ~50-fold lower than that for the cobalamin-dependent enzyme (Gonzalez *et al.*, 1996).

Studies of the *E. coli* methionine biosynthesis pathway have provided paradigmatic examples of enzyme structure and function. However, the use of *O*-succinylhomoserine as the activated metabolite, and the presence of a single trans-sulfurylation route to homocysteine, appears to be a pathway that is conserved solely within the parent order of *E. coli*, the *Enterobacteriales*. In the following sections, we will discuss the species distributions of alternative biosynthetic strategies, before considering biochemical and evolutionary aspects of selected enzymes in more detail.

Acylation

In *E. coli*, an *O*-succinyl group is used to activate homoserine; however, in many other bacteria, an *O*-acetyl group derived from acetyl-CoA is used instead (Fig. 2). Based on studies in *E. coli*, it was generalized that all *metA* homologues encoded homoserine *O*-succinyltransferases. For example, in the NCBI database the homology group of *metA* (COG1897) is annotated as 'homoserine *O*-succinyltransferase' (Tatusov *et al.*, 2003). However, the *metA*-encoded enzymes from *Thermotoga maritima* (Goudarzi & Born, 2006), *Bacillus cereus* (Ziegler *et al.*, 2007) and *Agrobacterium tumefaciens* (Rotem *et al.*, 2013) have all been experimentally validated as homoserine *O*-acetyltransferases (and not homoserine *O*-succinyltransferases), thereby complicating the picture.

The crystal structure of the *B. cereus metA*-encoded homoserine *O*-acetyltransferase revealed the presence of a key specificity-determining residue, Glu111, in the active site (Zubieta *et al.*, 2008). Mutation of this residue to glycine was sufficient to switch substrate preference from acetyl-CoA to succinyl-CoA. The authors classified ~70 additional *metA*-encoded enzymes as either homoserine *O*-succinyltransferases or homoserine *O*-acetyltransferases, based solely on the presence of glycine or glutamate, respectively, at the specificity-determining position (Zubieta *et al.*, 2008). We have updated this analysis to include almost 900 species. A FASTA file of all *metA*-encoded proteins, annotated as homoserine *O*-succinyltransferases (family IPR005697), was downloaded from the InterPro server (Hunter *et al.*, 2012). A custom Perl script (available upon request) was used to align each of the protein sequences with the *E. coli* homoserine *O*-succinyltransferase using MUSCLE (Edgar, 2004), and then to identify the residue that was equivalent to Gly111 in the *E. coli* sequence. The dataset was pruned so that it included a single strain for each species in the NCBI summary table of sequenced prokaryotic genomes (Sayers *et al.*, 2009), and only species with *metA* were retained. The results (Table 1) show that over 60 % of *metA*-encoded enzymes have glutamate at the specificity-determining position, suggesting that they are *O*-acetyltransferases. Only 20 % of the enzymes are predicted to be *O*-succinyltransferases (with glycine instead of glutamate), while the remainder have neither glutamate nor glycine at the specificity-determining position. Furthermore, this analysis emphasizes that homoserine *O*-succinyltransferases are common amongst the *Gammaproteobacteria* (including *E. coli*), but extremely infrequent in other taxa.

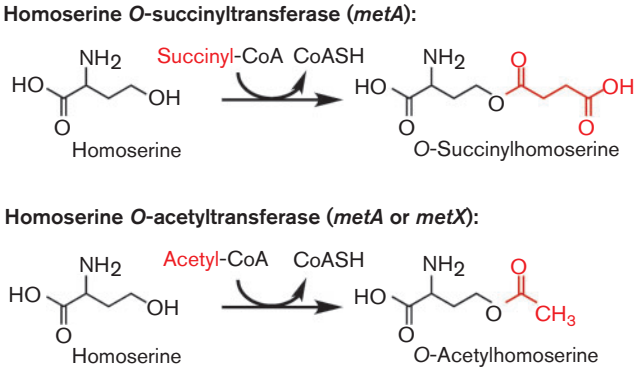

Fig. 2. Alternative reactions for the *O*-acylation of homoserine.

An alternative homoserine *O*-acetyltransferase is encoded by the *metX* gene. The *metX*- and *metA*-encoded enzymes show no sequence or structural homology. Instead, they have arisen through convergent evolution, and both classes of enzyme use the same ping-pong mechanism (Born & Blanchard, 1999; Born *et al.*, 2000). Unlike the *metA*-encoded enzymes, *metX*-encoded enzymes have been found to use acetyl-CoA exclusively (Rowbury, 1983; Hacham *et al.*, 2003; Hwang *et al.*, 2007; Tran *et al.*, 2011). To date, the only possible exception concerns the *metX* gene from *Pseudomonas aeruginosa* strain PAO1. Indirect complementation tests were used to infer that it encoded a homoserine *O*-succinyltransferase, and therefore the gene was originally annotated as *metA* (Foglino *et al.*, 1995). Genome sequencing revealed that it was, in fact, a *metX* (Stover *et al.*, 2000), in turn suggesting that it was a unique example of a *metX*-encoded *O*-succinyltransferase. However, the closely related *metX* genes from *Pseudomonas putida* and *Pseudomonas syringae* encode homoserine *O*-acetyltransferases (Alaminos & Ramos, 2001; Andersen *et al.*, 1998). On balance, it seems likely that the *P. aeruginosa* homologue is also specific for acetyl-CoA, but *in vitro* assays will be required to verify this hypothesis.

The phylogenetic distribution of *metX* genes, as matched by InterPro (Hunter *et al.*, 2012), is summarized in Table 1. Overall, we tabulated 905 occurrences of *metX* (InterPro family IPR008220) in the 1593 representative species that we analysed. This is slightly more than the total number of *metA* sequences (884) that we observed. Of the major classes, *metX* is particularly prevalent in the *Actinobacteria* and *Betaproteobacteria*, while it is comparatively uncommon in the *Bacilli* and *Clostridia*. The *metX* and *metA* genes are employed at approximately equal frequencies by alphaproteobacterial and gammaproteobacterial species. Our analysis also revealed 101 cases in which both genes were present, such as in *B. cereus*, illustrating the complexity and robustness associated with methionine biosynthesis in many species.

Like methionine, the bacterial biosynthetic route to threonine also begins with homoserine. In threonine biosynthesis, homoserine is activated by phosphorylation rather than by acylation. This is carried out by the *thrB*-encoded homoserine kinase and yields *O*-phosphohomoserine (Chassagnole *et al.*, 2001). Plants use *O*-phosphohomoserine as the activated intermediate for both methionine and threonine biosynthesis (Bartlem *et al.*, 2000). Neither a bacterial nor an archaeal route to methionine via

Table 1. Phylogenetic distribution of genes for homoserine acylation enzymes

Phylum	Class	No. of species	No. of species with *metA*			No. with *metX*
			Succinyl-transferase*	Acetyl-transferase†	Not classified‡	
Actinobacteria	*Actinobacteria*	237	2	38	4	201
Bacteroidetes/Chlorobi	*Bacteroidetes*	103	0	68	7	38
	Calditrix	1	0	0	0	1
	Chlorobi	11	0	0	0	11
	Ignavibacteriae	1	0	0	0	1
Chlamydiae/ Verrucomicrobia	*Lentisphaerae*	1	0	0	0	1
	Verrucomicrobia	4	0	0	0	4
Chloroflexi	*Anaerolineae*	1	0	1	0	0
	Caldilineae	1	0	0	0	1
	Chloroflexi	7	0	0	0	7
	Dehalococcoidia	1	0	0	0	1
	Ktedonobacteria	1	0	0	0	1
	Thermomicrobia	2	0	0	0	2
Chrysiogenetes	*Chrysiogenetes*	1	0	0	0	1
Cyanobacteria	*Nostocales*	5	0	0	0	5
	Oscillatoriophycideae	5	0	1	0	4
	Prochlorales	1	0	0	1	0
Deferribacteres	*Deferribacteres*	5	0	0	0	5
Deinococcus–Thermus	*Deinococci*	17	0	0	0	17
Elusimicrobia	*Elusimicrobia*	1	0	1	0	0
	Acidobacteria	4	0	1	0	3
	Fibrobacteres	1	0	0	0	1
Firmicutes	*Bacilli*	139	1	140	37	40
	Clostridia	165	0	130	12	31
	Erysipelotrichia	8	0	6	2	0
	Negativicutes	27	0	25	1	1
	Unclassified	1	0	1	0	0
Fusobacteria	*Fusobacteria*	6	0	8	4	0
Gemmatimonadetes	*Gemmatimonadetes*	1	0	0	0	1
Planctomycetes	*Phycisphaerae*	1	0	0	0	1
	Planctomycetia	7	0	0	0	7
Proteobacteria	*Alphaproteobacteria*	194	6	80	28	113
	Betaproteobacteria	125	1	3	1	123
	Deltaproteobacteria/Epsilonproteobacteria	67	0	15	0	56
	Gammaproteobacteria	389	169	7	46	200
	Zetaproteobacteria	1	1	0	0	0
Spirochaetes	*Spirochaetia*	37	0	12	16	21
Synergistetes	*Synergistia*	7	0	1	0	6
Thermotogae	*Thermotogae*	7	0	7	0	0
Total		1593	180	545	159	905

*Predicted based on the presence of a glycine at the specificity-determining position that was identified by Zubieta *et al.* (2008).
†Predicted based on the presence of a glutamate at the specificity-determining position.
‡Amino acid sequences that contain neither glycine nor glutamate at the specificity-determining position.

O-phosphohomoserine has been reported to date; however, our bioinformatics analysis suggests that *Haliangium ochraceum* is a bacterial candidate for possessing the plant-like pathway. Its genome (Ivanova *et al.*, 2010) appears to lack *metA* and *metX* genes, but it possesses *thrB* and a gene encoding a cystathionine γ-synthase that is a close homologue of the enzyme from *Arabidopsis thaliana* (which synthesizes cystathionine from *O*-phosphohomoserine; Ravanel *et al.*, 1998). Therefore, there may be as many as three different ways by which bacterial species synthesize activated homoserine derivatives for subsequent sulfurylation.

It is unclear which of the different homoserine activation routes was present in the primordial pathway for methionine biosynthesis. Hacham *et al.* (2003) concluded that the use of an acyl group was ancestral: of the three downstream enzymes (cystathionine γ-synthases) that were tested, only the one from *A. thaliana* could accept *O*-phosphohomoserine; while all of them had some activity towards *O*-succinylhomoserine. In contrast, another study concluded that the most parsimonious explanation was that the primordial enzyme was a *thrB*-encoded homoserine kinase (Gophna *et al.*, 2005).

Sulfurylation

After activation of homoserine, the next step is to exchange its acylated hydroxyl group for a thiol group, generating homocysteine. In *E. coli*, homocysteine is synthesized from *O*-succinylhomoserine in two steps (Fig. 1), catalysed by cystathionine γ-synthase (encoded by *metB*) and cystathionine β-lyase (encoded by *metC*). The phylogenetic distributions of these two enzymes (families IPR011821 and IPR006233, respectively), as matched by InterPro (Hunter *et al.*, 2012), are shown in Table 2. This analysis demonstrates that the trans-sulfurylation route to

Table 2. Phylogenetic distribution of genes for sulfurylation enzymes

Phylum	Class	No. of species	No. with *metB*	No. with *metC*	No. with *metY*	No. with *metZ*
Actinobacteria	*Actinobacteria*	237	4	0	233	88
Bacteroidetes/Chlorobi	*Bacteroidetes*	103	0	0	103	1
	Calditrix	1	0	0	1	0
	Chlorobi	11	0	0	11	0
	Ignavibacteriae	1	0	0	1	0
Chlamydiae/Verrucomicrobia	*Lentisphaerae*	1	0	0	1	0
	Verrucomicrobia	4	0	0	4	0
Chloroflexi	*Anaerolineae*	1	0	0	1	0
	Caldilineae	1	0	0	1	0
	Chloroflexi	7	0	0	7	0
	Dehalococcoidia	1	0	0	1	0
	Ktedonobacteria	1	0	0	1	0
	Thermomicrobia	2	0	0	2	0
Chrysiogenetes	*Chrysiogenetes*	1	0	0	1	0
Cyanobacteria	*Nostocales*	5	0	0	5	0
	Oscillatoriophycideae	5	0	0	5	0
	Prochlorales	1	0	0	1	0
Deferribacteres	*Deferribacteres*	5	0	0	5	0
Deinococcus–Thermus	*Deinococci*	17	0	0	17	0
Elusimicrobia	*Elusimicrobia*	1	0	0	1	0
	Acidobacteria	4	0	1	4	0
	Fibrobacteres	1	0	0	1	0
Firmicutes	*Bacilli*	139	0	0	139	0
	Clostridia	165	0	0	165	0
	Erysipelotrichia	8	0	0	8	0
	Negativicutes	27	0	1	27	0
	Unclassified	1	0	0	1	0
Fusobacteria	*Fusobacteria*	6	0	0	6	0
Gemmatimonadetes	*Gemmatimonadetes*	1	1	0	1	0
Planctomycetes	*Phycisphaerae*	1	0	0	1	0
	Planctomycetia	7	0	0	7	0
Proteobacteria	*Alphaproteobacteria*	194	19	154	159	157
	Betaproteobacteria	125	0	76	93	98
	Deltaproteobacteria/Epsilonproteobacteria	67	0	4	67	0
	Gammaproteobacteria	389	199	210	218	138
	Zetaproteobacteria	1	0	0	1	0
Spirochaetes	*Spirochaetia*	37	0	0	37	1
Synergistetes	*Synergistia*	7	0	0	7	0
Thermotogae	*Thermotogae*	7	0	0	7	0
Total		1593	223	446	1351	483

homocysteine, via the thioether intermediate cystathionine, is almost exclusively utilized by gammaproteobacterial species. Outside the *Gammaproteobacteria*, only a handful of alphaproteobacterial species contain both a cystathionine γ-synthase and a cystathionine β-lyase.

Most bacteria utilize a direct sulfurylation step instead of, or as well as, the trans-sulfurylation route. Direct sulfurylation involves the replacement of the *O*-acyl group of activated homoserine with free hydrogen sulfide, generating homocysteine in a single step (Fig. 3b). Two homologous groups of enzymes, encoded by the *metY* and *metZ* genes, are thought to catalyse this reaction.

Unsurprisingly, species that inhabit environments with abundant hydrogen sulfide – such as *Thermus thermophilus*, from thermal vents – possess the direct sulfurylation route (Iwama *et al.*, 2004). Moreover, enzymes encoded by *metY* (InterPro family IPR006235) are prevalent in all classes of bacteria (Table 2), illustrating that this is the predominant route for homocysteine synthesis. The *metY*-encoded enzymes catalyse the direct sulfurylation of *O*-acetylhomoserine. Historically, and most commonly, they are referred to as *O*-acetylhomoserine thiolases (e.g. Kerr, 1971; Aitken & Kirsch, 2005). Occasionally, they are also referred to as *O*-acetylhomoserine (thiol)-lyases, based on their original classification by the Enzyme Commission (EC 4.2.99.10). However, the currently accepted name is the *O*-acetylhomoserine aminocarboxypropyltransferases, as they have now been reclassified as transferases (EC 2.5.1.49) by the Enzyme Commission (Bairoch, 2000). We refer to them herein as *O*-acetylhomoserine thiolases.

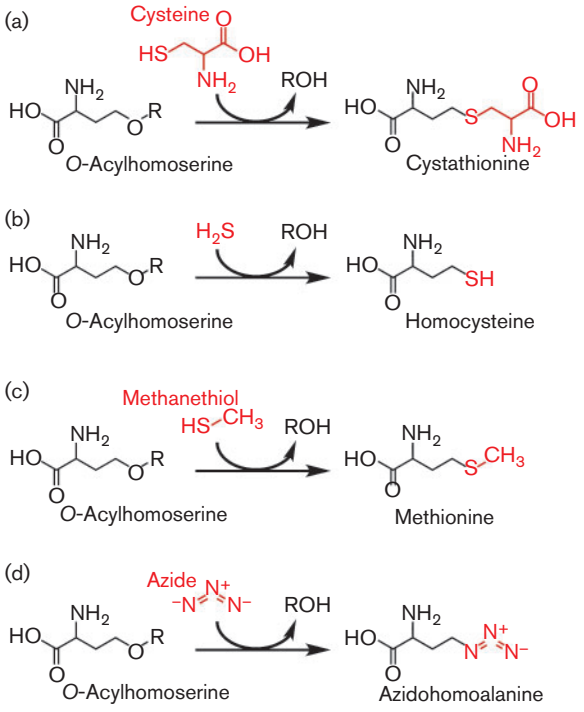

Fig. 3. Alternative attacking nucleophiles in the γ-replacement reaction. (a) Cystathionine γ-synthase (encoded by *metB*) catalyses the γ-replacement of the succinyl/acetyl group from acylated homoserine with cysteine, resulting in cystathionine. (b) *O*-Acylhomoserine thiolases (encoded by *metY* and *metZ*) catalyse γ-replacement with hydrogen sulfide, resulting in homocysteine. The *B. subtilis metI*-encoded enzyme can catalyse γ-replacement with either cysteine or hydrogen sulfide. (c) *O*-Acetylhomoserine thiolase (*metY*-encoded) can accept methanethiol as the attacking nucleophile, resulting in the direct synthesis of methionine. (d) The *C. glutamicum O*-acetylhomoserine thiolase has been shown to have relaxed specificity for the non-natural substrate azide, providing a biosynthetic route to azidohomoalanine.

The *metZ*-encoded enzymes (InterPro family IPR006234) are annotated as *O*-succinylhomoserine thiolases (Hunter *et al.*, 2012), presumed to be responsible for the one-step conversion of *O*-succinylhomoserine to homocysteine (Gophna *et al.*, 2005). In contrast to *metY*, *metZ* is found infrequently in bacterial genomes (Table 2). Its presence in *P. aeruginosa* and *P. putida* has been discussed (Foglino *et al.*, 1995; Alaminos & Ramos, 2001), and an unpublished structure of a *Mycobacterium tuberculosis O*-succinylhomoserine thiolase has been deposited in the Protein Data Bank (PDB ID 3NDN). However, no *metZ*-encoded enzyme has yet been characterized biochemically. In the previous section, we suggested that *P. aeruginosa metX* may encode a homoserine *O*-acetyltransferase, instead of an *O*-succinyltransferase (as originally proposed; Foglino *et al.*, 1995). A corollary is that we would then expect the *P. aeruginosa metZ*-encoded enzyme to encode an *O*-acetylhomoserine thiolase. Consistent with this conjecture, of the 483 *metZ* occurrences that we identified (Table 2), only 28 were in species that also contained a *metA*-encoded *O*-succinyltransferase (i.e. an enzyme with a glycine in the specificity-determining position; Zubieta *et al.*, 2008). It appears likely that, in most cases, *metY* and *metZ* encode functionally equivalent *O*-acetylhomoserine thiolases; however, further biochemical characterization of *metZ*-encoded enzymes will be required to shed light on the physiological roles of these two homologues.

O-Acetylhomoserine thiolase can catalyse an even more direct route to methionine. In organisms in which methanethiol is produced as a catabolic by-product, the enzyme can use not only hydrogen sulfide, but also this methanethiol, as a substrate. The result is a one-step synthesis of methionine from *O*-acetylhomoserine (Fig. 3c). This method of methionine biosynthesis was first discovered in *Saccharomyces cerevisiae* (Yamagata, 1971) and is particularly important for bacterioplankton, such as species of *Roseobacter*, that can obtain methanethiol from degradation of the abundant algal osmolyte, dimethylsulfoniopropionate (Kiene *et al.*, 1999). Recently, *Rhodospirillum rubrum* has also been shown to recycle methanethiol into methionine using a dedicated *O*-acetylhomoserine thiolase (Erb *et al.*, 2012). Furthermore, and in a biotechnological context, *Corynebacterium glutamicum* can use its *metY*-encoded enzyme to synthesize high levels of methionine

when it is fed exogenous methanethiol (Kromer *et al.*, 2006; Bolten *et al.*, 2010).

The trans-sulfurylation route and the direct sulfurylation routes are not mutually exclusive. In many species (160 of those analysed for Table 2) both pathways are found together, presumably because they offer metabolic flexibility. Using cysteine as the source of the thiol group is more costly metabolically, but it is often more available than free sulfide, which is highly volatile. Examples of organisms in which both routes have been studied are *C. glutamicum* (Hwang *et al.*, 2002) and *Leptospira meyeri* (Hwang *et al.*, 2002; Picardeau *et al.*, 2003), although in *C. glutamicum* the cystathionine β-lyase is encoded not by a *metC* gene, but by the more distant structural homologue, *aecD* (Ruckert *et al.*, 2003).

A final variation in the sulfurylation step is observed in *Bacillus subtilis*. This species contains a dual-specificity enzyme encoded by *metI*, which can catalyse the γ-replacement of *O*-acetylhomoserine either with sulfide (i.e. direct sulfurylation, the same as *O*-acetylhomoserine thiolase) or with cysteine to yield cystathionine, akin to the step catalysed by cystathionine γ-synthase in *E. coli* (Auger *et al.*, 2002).

Methylation

While there are several alternatives for the sulfurylation steps that generate homocysteine, the final step in methionine biosynthesis – *S*-methylation – is highly conserved. The two methionine synthases, encoded by *metE* (cobalamin-independent; InterPro families IPR013215 and IPR022921) and *metH* (cobalamin-dependent; InterPro families IPR017215 and IPR011822), are present together in a large number of bacteria (Table 3), although some species have one but not the other. For example, *Haemophilus influenzae* possesses only *metE*, while *L. meyeri* possesses only *metH* (Gophna *et al.*, 2005). A minor variation in cyanobacteria, including *Synechocystis* species (Tanioka *et al.*, 2009) and *Spirulina platensis* (Tanioka *et al.*, 2010), is the use of adeninylcobamide as the cofactor for the *metH*-encoded methionine synthase, instead of cobalamin. Adeninylcobamide differs from cobalamin in that the axial group is an adeninyl moiety, as opposed to a 5,6-dimethylbenzimidazole ribonucleotyl moiety.

Another methylation route that is independent of 5-methyl-tetrahydrofolate and 5-methyl-tetrahydropteroyl tri-glutamate is also known. This involves the enzyme betaine-homocysteine *S*-methyltransferase (InterPro family IPR017226, encoded by *bhmT* or *gbt*), which is from choline catabolism and catalyses the conversion of betaine and homocysteine to dimethylglycine and methionine. The gene for this transmethylase is known to be present alongside *metE* and *metH* in *P. aeruginosa* (Serra *et al.*, 2002) and in *Sinorhizobium meliloti* (Barra *et al.*, 2006). It is known to provide the sole route to methionine in *Oceanobacillus iheyensis* (Rodionov *et al.*, 2004) and in *Pelagibacter ubique* (Sun *et al.*, 2011); however, its occurrence is rare (Table 3).

Parallels with cysteine metabolism

The biosynthesis of cysteine from serine is highly analogous to the synthesis of homocysteine from homoserine. Unsurprisingly, similar biochemical strategies and homologous enzymes are employed in the two pathways. Cysteine is synthesized by first activating serine with an *O*-acetyl group, and then replacing it with a thiol group. As with methionine biosynthesis, there is both a direct sulfurylation route and a reverse trans-sulfurylation route from *O*-acetylserine to cysteine (Fig. 4).

Direct sulfurylation (Fig. 4a) is catalysed by cysteine synthase, which is also known as *O*-acetylserine (thiol)-lyase and *O*-acetylserine thiolase (Rabeh & Cook, 2004). Many bacterial species, including *E. coli*, possess two cysteine synthase isozymes that are encoded by the *cysK* and *cysM* genes (Rabeh & Cook, 2004). In the reverse trans-sulfurylation route (Fig. 4b), cystathionine β-synthase catalyses the transfer of the sulfur from homocysteine to *O*-acetylserine, generating cystathionine. Cystathionine γ-lyase cleaves cystathionine to yield cysteine, α-ketobutyrate and ammonia, in a reaction that is highly similar to the one catalysed by cystathionine β-lyase (Fig. 1). In *Klebsiella pneumoniae*, the genes encoding cystathionine β-synthase and cystathionine γ-lyase have been labelled *mtcB* and *mtcC*, respectively (Seiflein & Lawrence, 2006), while in *B. subtilis* they have been named *mccA* and *mccB* (Hullo *et al.*, 2007).

Biochemistry of sulfurylation

Much of the diversity in bacterial pathways of methionine biosynthesis is attributable to differences in the sulfurylation steps. Nevertheless, the enzymes involved in homoserine sulfurylation (encoded by *metB*, *metC*, *metY* and *metZ*), as well as the cystathionine γ-lyases (encoded by *mtcC* and *mccB*) from cysteine biosynthesis, are all homologous and all require pyridoxal-5′-phosphate (PLP) for activity. PLP is a cofactor that forms a Schiff base with the substrate and acts as an electron sink to stabilize carbanion intermediates (Eliot & Kirsch, 2004).

Cystathionine γ-synthase (encoded by *metB*) and the *O*-acylhomoserine thiolases (encoded by *metY* and *metZ*) all catalyse γ-replacement reactions, with the mechanism of *E. coli* cystathionine γ-synthase having been particularly well studied (Aitken *et al.*, 2003; Aitken & Kirsch, 2005). The γ-replacement reaction begins with the substrate, *O*-succinylhomoserine or *O*-acetylhomoserine, forming a Schiff base with PLP and thereby allowing the acetyl or succinyl group to be eliminated. This results in a PLP-bound intermediate with a vinyl side chain, which is then attacked by the second substrate (cysteine, hydrogen sulfide or methanethiol) to yield cystathionine, homocysteine or methionine, respectively (Fig. 3).

Table 3. Phylogenetic distribution of genes for methylation enzymes

Phylum	Class	No. of species	No. with *metE*	No. with *metH*	No. with *bhmT*
Actinobacteria	*Actinobacteria*	237	152	147	2
Bacteroidetes/Chlorobi	*Bacteroidetes*	103	23	90	0
	Caldithrix	1	0	1	0
	Chlorobi	11	1	11	0
	Ignavibacteriae	1	0	1	0
Chlamydiae/Verrucomicrobia	*Lentisphaerae*	1	0	1	0
	Verrucomicrobia	4	1	3	0
Chloroflexi	*Anaerolineae*	1	0	1	0
	Caldilineae	1	0	0	0
	Chloroflexi	7	1	7	0
	Dehalococcoidia	1	0	0	0
	Ktedonobacteria	1	1	1	0
	Thermomicrobia	2	1	0	0
Chrysiogenetes	*Chrysiogenetes*	1	1	1	0
Cyanobacteria	*Nostocales*	5	0	5	0
	Oscillatoriophycideae	5	0	5	0
	Prochlorales	1	0	1	0
Deferribacteres	*Deferribacteres*	5	1	5	0
Deinococcus–Thermus	*Deinococci*	17	3	16	0
Elusimicrobia	*Elusimicrobia*	1	0	0	0
	Acidobacteria	4	0	3	0
	Fibrobacteres	1	1	1	0
Firmicutes	*Bacilli*	139	105	62	0
	Clostridia	165	32	117	0
	Erysipelotrichia	8	2	5	0
	Negativicutes	27	2	22	0
	Unclassified	1	0	1	0
Fusobacteria	*Fusobacteria*	6	0	5	0
Gemmatimonadetes	*Gemmatimonadetes*	1	0	1	0
Planctomycetes	*Phycisphaerae*	1	0	1	0
	Planctomycetia	7	1	7	0
Proteobacteria	*Alphaproteobacteria*	194	39	158	3
	Betaproteobacteria	125	87	103	0
	Deltaproteobacteria/Epsilonproteobacteria	67	36	44	0
	Gammaproteobacteria	389	281	344	4
	Zetaproteobacteria	1	0	1	0
Spirochaetes	*Spirochaetia*	37	9	29	0
Synergistetes	*Synergistia*	7	0	1	0
Thermotogae	*Thermotogae*	7	5	5	0
Total		1593	785	1206	9

Cystathionine β-lyase (encoded by *metC*) is a close homologue of cystathionine γ-synthase, and acts on its product, cystathionine (Fig. 1). Instead of a γ-replacement reaction, however, it catalyses a β-elimination. In the first step, cystathionine forms a Schiff base with PLP, allowing homocysteine to be eliminated. The remaining PLP-bound substrate, aminoacrylate, is released and hydrolyses spontaneously (due to enamine–ketimine tautomerism) to pyruvate and ammonia (Clausen *et al.*, 1996). Cystathionine is pseudo-symmetrical about the sulfur atom (Fig. 3a), but it is orientated oppositely within the active sites of cystathionine γ-synthase and cystathionine β-lyase. The structures of the two *E. coli* enzymes (as well as the *S. cerevisiae* cystathionine γ-lyase) are almost identical, having been superimposed with a root-mean-square deviation of only 1.5 Å over 350 C_α positions (Messerschmidt *et al.*, 2003). Therefore, several studies have attempted to map the determinants of specificity in the two enzymes (reviewed by Aitken & Kirsch, 2005; and in Aitken *et al.*, 2011). This is achieved not by a single residue, but by an interplay of several residues that influence the protonation state of the active site and the hydrophobic environment leading to it (Hopwood *et al.*, 2014).

From the current understanding of the trans-sulfurylation route in methionine biosynthesis, *metC* should always be found together with *metB* (or at least with the bi-functional *metI*): the *metB*-encoded cystathionine γ-synthase is required to synthesize cystathionine, which in turn is the

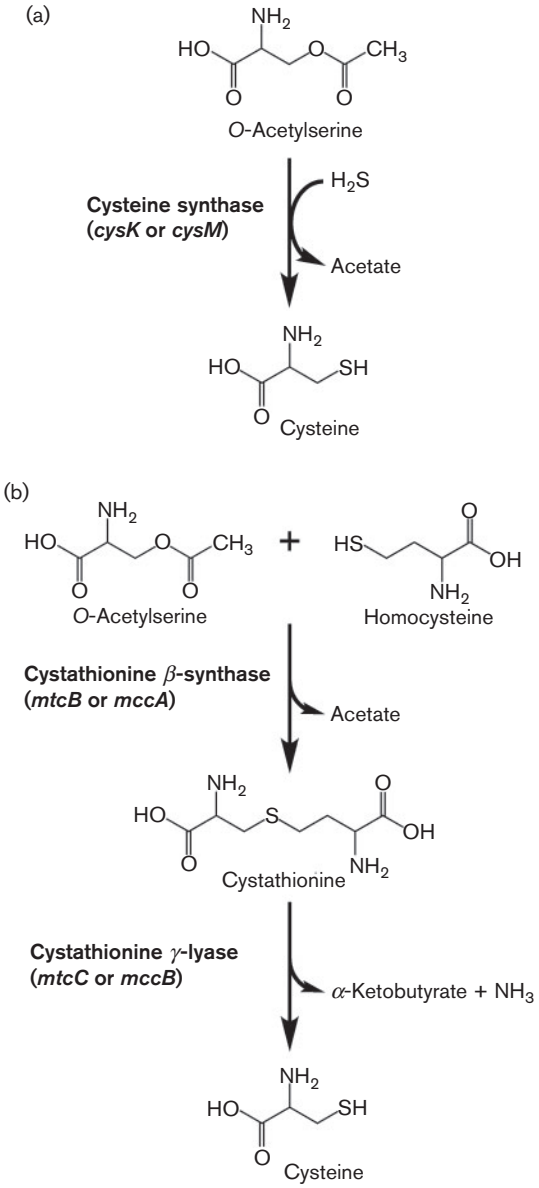

Fig. 4. Cysteine biosynthesis. (a) In the direct sulfurylation pathway, cysteine is synthesized in a single step from *O*-acetylserine and hydrogen sulfide. (b) Reverse trans-sulfurylation is a two-step synthesis, involving cystathionine as an intermediate metabolite. The enzymes catalysing each step, and the genes that encode them, are indicated in bold type.

substrate of the *metC*-encoded cystathionine β-lyase (Fig. 1). However, our analysis of InterPro clusters shows that this is not always the case (Fig. 5). Assuming no annotation bias, *metC* is found equally as commonly without *metB* as with it. One putative explanation is that the *metC*-encoded enzyme could act as a bi-functional cystathionine γ-synthase/cystathionine β-lyase. To date, however, no enzyme has been identified that is able to catalyse both reactions. Overexpression of *metC* did not rescue the methionine auxotrophy of an *E. coli* Δ*metB* strain, and nor

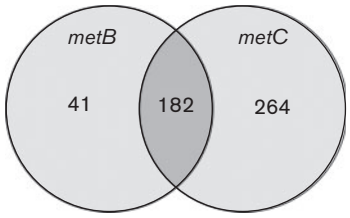

Fig. 5. Venn diagram showing the co-occurrence of cystathionine γ-synthase (encoded by *metB*; InterPro family IPR011821) and cystathionine β-lyase (encoded by *met*C; InterPro family IPR006233) in bacterial genomes.

did overexpression of *metB* rescue *E. coli* Δ*metC* (Patrick *et al.*, 2007). More recently, a series of *metB–metC* chimeras were designed and constructed, but none of the expressed enzymes possessed both activities (Manders *et al.*, 2013). Another possibility is that the *metY*-encoded enzymes in these species may be able to accept cysteine as an alternative substrate (in addition to hydrogen sulfide), thus providing a route to cystathionine that is independent of a *metB*-encoded synthase (as is seen with *metI* in *B. subtilis*). However, this remains to be tested and the role of *metC* in species without *metB* therefore remains unresolved.

Phylogeny of the sulfurylation enzymes

As discussed above, the majority of the sulfurylation enzymes are homologous. They possess PLP-dependent enzyme fold type I, making them members of the aspartate aminotransferase family (Eliot & Kirsch, 2004), which is labelled as clan CL0061 in Pfam (Punta *et al.*, 2012). Sulfurylation enzymes that are not in this clan are cysteine synthase (encoded by *cysK* and *cysM*) and cystathionine β-synthase (*mtcB* and *mccA*), from the reverse trans-sulfurylation route for cysteine biosynthesis (Fig. 4), which belong to the PLP-dependent enzyme fold type II.

Within clan CL0061, the sulfurylation enzymes are found in two families. The minor one of these is Pfam family PF00155 (Punta *et al.*, 2012), which contains three members that have been shown to possess varying amounts of cystathionine β-lyase activity. Of these enzymes, only AecD from *C. glutamicum* acts physiologically as a cystathionine β-lyase (Ruckert *et al.*, 2003). PatB from *B. subtilis* (Auger *et al.*, 2005) and MalY from *E. coli* (Zdych *et al.*, 1995) can catalyse cystathionine β-elimination poorly, while their native activities and physiological roles are unknown.

The major clade of sulfurylation enzymes is Pfam family PF01053, which is named the 'Cys/Met metabolism PLP-dependent enzyme family' in Pfam (Punta *et al.*, 2012) and the 'γ-subfamily' elsewhere (Alexander *et al.*, 1994). Sequence identities between members of this family with different functions are relatively low (20–30 % identity at the amino acid level). We have constructed a phylogenetic

network of representative sequences from this family. The result (Fig. 6) emphasizes that several enzymes with the same activity are not closely related. Instead, each enzyme activity (β-elimination, γ-replacement and γ-elimination) appears to have arisen multiple times during evolution.

In the case of cystathionine β-lyase (encoded by *metC*), β-elimination activity may have arisen in three different lineages (Fig. 6). One sequence cluster is composed of most bacterial sequences, a second contains eukaryotic sequences and a third contains the *B. subtilis* sequence. The other clusters in Fig. 6 comprise enzymes that act on the γ-carbon, but the sequences do not fall into single groups for each activity. The cystathionine γ-lyases fall into three separate clusters: one with the enzyme encoded by *K. pneumoniae mtcC*, another with the *B. subtilis mccB*-encoded enzyme and a third with the eukaryotic cystathionine γ-lyase sequences. Similarly, the bacterial cystathionine γ-synthases (encoded by *metB*) cluster away from the *O*-phosphohomoserine-utilizing cystathionine γ-synthases from plants.

Overall, the phylogeny of the sulfurylation enzymes confirms that the PLP-dependent enzyme fold type I is highly plastic with respect to β-elimination, γ-replacement and γ-elimination activities. In spite of this phylogenetic evidence, however, it has not yet proven possible to interconvert enzymes with these activities by using rational mutagenesis approaches (Farsi *et al.*, 2009; Manders *et al.*, 2013).

In light of the fact that direct sulfurylation provides the simplest, one-step route to homocysteine (or methionine itself, when methanethiol is available), it is likely that the ancestral gene encoded an *O*-acetylhomoserine thiolase. This has also been proposed by others (Hacham *et al.*, 2003; Aitken & Kirsch, 2005), based on the observation that the cystathionine γ-synthases from both *E. coli* and *A. thaliana* possess the vestigial ability to accept sulfide (in place of cysteine), and thus catalyse direct sulfurylation at low levels.

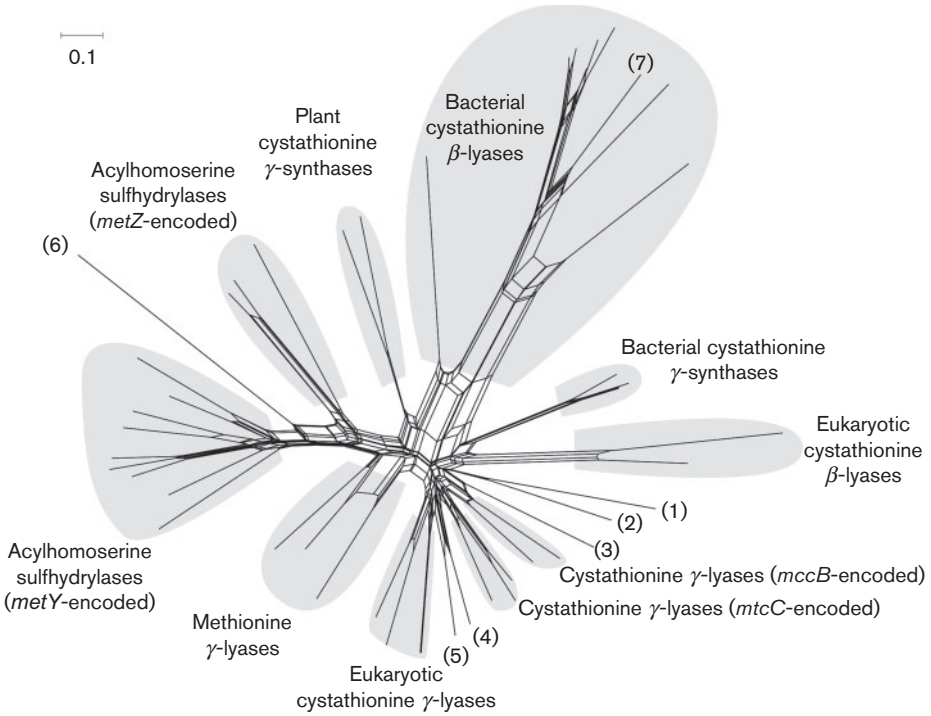

Fig. 6. Protein phylogenetic network of the major clade of sulfurylation enzymes (Pfam family PF01053; Punta *et al.*, 2012). A dataset was made by manually selecting protein sequences, aligning them in MUSCLE (Edgar, 2004), and trimming in Gblocks set to its most permissive settings and to allow half-filled columns (Talavera & Castresana, 2007). The Splitstree package (Bryant & Moulton, 2004) was used to infer from this dataset a neighbour-net tree with maximum-likelihood distances calculated with a Dayhoff substitution matrix. The major clusters are annotated. In addition, there are several other clusters numbered, each of which is represented by a single protein in this analysis. These are: (1) *Lactobacillus fermentum* cystathionine γ-lyase (Smacchi & Gobbetti, 1998); (2) *B. subtilis* MetI (Auger *et al.*, 2002); (3) *B. subtilis* cystathionine β-lyase (Auger *et al.*, 2002); (4) *Mycobacterium tuberculosis* cystathionine γ-lyase (Saha *et al.*, 2009); (5) *C. glutamicum* cystathionine γ-synthase (Hwang *et al.*, 1999); (6) *Thermus thermophilus* acetylhomoserine thiolase (Iwama *et al.*, 2004); (7) a *Zymomonas mobilis* cystathionine β-lyase homologue that is fused to a sulfurtransferase domain.

Methionine and biotechnology

Methionine is widely used as a feed additive in the poultry, swine and fish farming industries. It is produced as a racemic mixture from petrochemical feedstocks, with global production capacities in the hundreds of thousands of tonnes per annum. A microbial fermentation process would offer a valuable alternative for sustainable production. However, methionine biosynthesis is tightly regulated in bacteria (Rowbury & Woods, 1961), meaning that there are no known species which are natural overproducers. Instead, research efforts have focused on isolating or engineering mutated strains with deregulated methionine biosynthesis pathways (reviewed by Kumar & Gomes, 2005).

As an illustrative example, a point mutation in the *E. coli* methionine repressor protein, MetJ, was sufficient to derepress the *met* regulon and give a strain that accumulated low levels of methionine in liquid culture (Nakamori *et al.*, 1999). However, the enzymes of methionine biosynthesis are also feedback regulated. For example, the *E. coli* homoserine *O*-succinyltransferase is allosterically inhibited both by methionine and by SAM (Lee *et al.*, 1966). Selection for feedback-resistant mutants has yielded strains that excrete up to 240 mg methionine l^{-1} (Usuda & Kurahashi, 2005). Similar studies have been carried out in numerous other species (Kumar & Gomes, 2005); however, no commercially viable fermentation process has been implemented to date.

Bio-orthogonal approaches for incorporating non-natural amino acids into proteins have attracted considerable attention for their potential to probe protein structure and function, as well as to yield proteins with modified activities (Sletten & Bertozzi, 2009). Recently, the *C. glutamicum* *O*-acetylhomoserine thiolase (encoded by *metY*) has been found to possess a promiscuous activity that allows it to use azide as the attacking nucleophile (Fig. 3d), rather than hydrogen sulfide or methanethiol. Expression of *C. glutamicum metY* in an *E. coli* methionine auxotroph that was fed *O*-acetylhomoserine and sodium azide led to the biosynthesis of azidohomoalanine (Ma *et al.*, 2014). This methionine analogue was stably incorporated into proteins *in vivo*, allowing them to be site-specifically derivatized with an alkyne-containing fluorophore by 'click chemistry'. This proof-of-principle study illustrates how our increasing understanding of methionine biosynthesis may lead to novel biotechnological applications.

Concluding remarks

E. coli has proven to be a reliable model organism for the study of most metabolic pathways. Its pathway for the biosynthesis of methionine from homoserine, via an *O*-succinylated intermediate and trans-sulfurylation, is well characterized (Fig. 1). However, studies on species including *C. glutamicum* (Hwang *et al.*, 2002), *B. subtilis* (Auger *et al.*, 2002) and *P. putida* (Vermeij & Kertesz, 1999) have demonstrated that the *E. coli* pathway is not ubiquitous. In this review, we have attempted to summarize the range of biochemical strategies that bacteria employ for synthesizing methionine. Our bioinformatics analyses (Tables 1–3) update a previous survey (Gophna *et al.*, 2005) and emphasize that the *E. coli* pathway appears to be confined to the *Enterobacteriales*. In other taxa, the canonical pathway involves the activation of homoserine by *O*-acetylation, and then a direct sulfurylation route to homocysteine. These different strategies reflect past or present adaptations to different ecological niches, such as those where hydrogen sulfide or methanethiol are abundant. Finally, we note that our understanding of bacterial methionine metabolism has grown dramatically in the past decade, yet we have highlighted some of the questions that remain to be addressed.

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References

Aitken, S. M. & Kirsch, J. F. (2005). The enzymology of cystathionine biosynthesis: strategies for the control of substrate and reaction specificity. *Arch Biochem Biophys* **433**, 166–175.

Aitken, S. M., Kim, D. H. & Kirsch, J. F. (2003). *Escherichia coli* cystathionine γ-synthase does not obey ping-pong kinetics. Novel continuous assays for the elimination and substitution reactions. *Biochemistry* **42**, 11297–11306.

Aitken, S. M., Lodha, P. H. & Morneau, D. J. (2011). The enzymes of the transsulfuration pathways: active-site characterizations. *Biochim Biophys Acta* **1814**, 1511–1517.

Alaminos, M. & Ramos, J. L. (2001). The methionine biosynthetic pathway from homoserine in *Pseudomonas putida* involves the *metW*, *metX*, *metZ*, *metH* and *metE* gene products. *Arch Microbiol* **176**, 151–154.

Alexander, F. W., Sandmeier, E., Mehta, P. K. & Christen, P. (1994). Evolutionary relationships among pyridoxal-5′-phosphate-dependent enzymes. Regio-specific α, β and γ families. *Eur J Biochem* **219**, 953–960.

Andersen, G. L., Beattie, G. A. & Lindow, S. E. (1998). Molecular characterization and sequence of a methionine biosynthetic locus from *Pseudomonas syringae*. *J Bacteriol* **180**, 4497–4507.

Auger, S., Yuen, W. H., Danchin, A. & Martin-Verstraete, I. (2002). The *metIC* operon involved in methionine biosynthesis in *Bacillus subtilis* is controlled by transcription antitermination. *Microbiology* **148**, 507–518.

Auger, S., Gomez, M. P., Danchin, A. & Martin-Verstraete, I. (2005). The PatB protein of *Bacillus subtilis* is a C-S-lyase. *Biochimie* **87**, 231–238.

Bairoch, A. (2000). The ENZYME database in 2000. *Nucleic Acids Res* **28**, 304–305.

Barra, L., Fontenelle, C., Ermel, G., Trautwetter, A., Walker, G. C. & Blanco, C. (2006). Interrelations between glycine betaine catabolism and methionine biosynthesis in *Sinorhizobium meliloti* strain 102F34. *J Bacteriol* **188**, 7195–7204.

Bartlem, D., Lambein, I., Okamoto, T., Itaya, A., Uda, Y., Kijima, F., Tamaki, Y., Nambara, E. & Naito, S. (2000). Mutation in the threonine synthase gene results in an over-accumulation of soluble methionine in *Arabidopsis*. *Plant Physiol* **123**, 101–110.

Bennett, B. D., Kimball, E. H., Gao, M., Osterhout, R., Van Dien, S. J. & Rabinowitz, J. D. (2009). Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*. *Nat Chem Biol* **5**, 593–599.

Bigelow, D. J. & Squier, T. C. (2005). Redox modulation of cellular signaling and metabolism through reversible oxidation of methionine sensors in calcium regulatory proteins. *Biochim Biophys Acta* **1703**, 121–134.

Blanco, J., Moore, R. A., Kabaleeswaran, V. & Viola, R. E. (2003). A structural basis for the mechanism of aspartate-β-semialdehyde dehydrogenase from *Vibrio cholerae*. *Protein Sci* **12**, 27–33.

Bolten, C. J., Schröder, H., Dickschat, J. & Wittmann, C. (2010). Towards methionine overproduction in *Corynebacterium glutamicum*–methanethiol and dimethyldisulfide as reduced sulfur sources. *J Microbiol Biotechnol* **20**, 1196–1203.

Born, T. L. & Blanchard, J. S. (1999). Enzyme-catalyzed acylation of homoserine: mechanistic characterization of the *Escherichia coli metA*-encoded homoserine transsuccinylase. *Biochemistry* **38**, 14416–14423.

Born, T. L., Franklin, M. & Blanchard, J. S. (2000). Enzyme-catalyzed acylation of homoserine: mechanistic characterization of the *Haemophilus influenzae met2*-encoded homoserine transacetylase. *Biochemistry* **39**, 8556–8564.

Bryant, D. & Moulton, V. (2004). Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Mol Biol Evol* **21**, 255–265.

Chassagnole, C., Raïs, B., Quentin, E., Fell, D. A. & Mazat, J. P. (2001). An integrated study of threonine-pathway enzyme kinetics in *Escherichia coli*. *Biochem J* **356**, 415–423.

Chiang, P. K., Gordon, R. K., Tal, J., Zeng, G. C., Doctor, B. P., Pardhasaradhi, K. & McCann, P. P. (1996). *S*-Adenosylmethionine and methylation. *FASEB J* **10**, 471–480.

Clausen, T., Huber, R., Laber, B., Pohlenz, H. D. & Messerschmidt, A. (1996). Crystal structure of the pyridoxal-5′-phosphate dependent cystathionine β-lyase from *Escherichia coli* at 1.83 Å. *J Mol Biol* **262**, 202–224.

Duclos, B., Cortay, J. C., Bleicher, F., Ron, E. Z., Richaud, C., Saint Girons, I. & Cozzone, A. J. (1989). Nucleotide sequence of the *metA* gene encoding homoserine trans-succinylase in *Escherichia coli*. *Nucleic Acids Res* **17**, 2856.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792–1797.

Eliot, A. C. & Kirsch, J. F. (2004). Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. *Annu Rev Biochem* **73**, 383–415.

Erb, T. J., Evans, B. S., Cho, K., Warlick, B. P., Sriram, J., Wood, B. M. K., Imker, H. J., Sweedler, J. V., Tabita, F. R. & Gerlt, J. A. (2012). A RubisCO-like protein links SAM metabolism with isoprenoid biosynthesis. *Nat Chem Biol* **8**, 926–932.

Farsi, A., Lodha, P. H., Skanes, J. E., Los, H., Kalidindi, N. & Aitken, S. M. (2009). Interconversion of a pair of active-site residues in *Escherichia coli* cystathionine γ-synthase, *E. coli* cystathionine β-lyase, and *Saccharomyces cerevisiae* cystathionine γ-lyase and development of tools for the investigation of their mechanisms and reaction specificity. *Biochem Cell Biol* **87**, 445–457.

Flavin, M. & Slaughter, C. (1967). Enzymatic synthesis of homocysteine or methionine directly from *O*-succinyl-homoserine. *Biochim Biophys Acta* **132**, 400–405.

Foglino, M., Borne, F., Bally, M., Ball, G. & Patte, J. C. (1995). A direct sulfhydrylation pathway is used for methionine biosynthesis in *Pseudomonas aeruginosa*. *Microbiology* **141**, 431–439.

Foster, J., Ganatra, M., Kamal, I., Ware, J., Makarova, K., Ivanova, N., Bhattacharyya, A., Kapatral, V., Kumar, S. & other authors (2005). The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. *PLoS Biol* **3**, e121.

González, J. C., Peariso, K., Penner-Hahn, J. E. & Matthews, R. G. (1996). Cobalamin-independent methionine synthase from *Escherichia coli*: a zinc metalloenzyme. *Biochemistry* **35**, 12228–12234.

Gophna, U., Bapteste, E., Doolittle, W. F., Biran, D. & Ron, E. Z. (2005). Evolutionary plasticity of methionine biosynthesis. *Gene* **355**, 48–57.

Goudarzi, M. & Born, T. L. (2006). Purification and characterization of *Thermotoga maritima* homoserine transsuccinylase indicates it is a transacetylase. *Extremophiles* **10**, 469–478.

Hacham, Y., Gophna, U. & Amir, R. (2003). *In vivo* analysis of various substrates utilized by cystathionine γ-synthase and *O*-acetylhomoserine sulfhydrylase in methionine biosynthesis. *Mol Biol Evol* **20**, 1513–1520.

Hopwood, E. M., Ahmed, D. & Aitken, S. M. (2014). A role for glutamate-333 of *Saccharomyces cerevisiae* cystathionine γ-lyase as a determinant of specificity. *Biochim Biophys Acta* **1844**, 465–472.

Hullo, M. F., Auger, S., Soutourina, O., Barzu, O., Yvon, M., Danchin, A. & Martin-Verstraete, I. (2007). Conversion of methionine to cysteine in *Bacillus subtilis* and its regulation. *J Bacteriol* **189**, 187–197.

Hunter, S., Jones, P., Mitchell, A., Apweiler, R., Attwood, T. K., Bateman, A., Bernard, T., Binns, D., Bork, P. & other authors (2012). InterPro in 2011: new developments in the family and domain prediction database. *Nucleic Acids Res* **40** (D1), D306–D312.

Hwang, B. J., Kim, Y., Kim, H. B., Hwang, H. J., Kim, J. H. & Lee, H. S. (1999). Analysis of *Corynebacterium glutamicum* methionine biosynthetic pathway: isolation and analysis of *metB* encoding cystathionine γ-synthase. *Mol Cells* **9**, 300–308.

Hwang, B. J., Yeom, H. J., Kim, Y. & Lee, H. S. (2002). *Corynebacterium glutamicum* utilizes both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis. *J Bacteriol* **184**, 1277–1286.

Hwang, B. J., Park, S. D., Kim, Y., Kim, P. & Lee, H. S. (2007). Biochemical analysis on the parallel pathways of methionine biosynthesis in *Corynebacterium glutamicum*. *J Microbiol Biotechnol* **17**, 1010–1017.

Ivanova, N., Daum, C., Lang, E., Abt, B., Kopitz, M., Saunders, E., Lapidus, A., Lucas, S., Glavina Del Rio, T. & other authors (2010). Complete genome sequence of *Haliangium ochraceum* type strain (SMP-2). *Stand Genomic Sci* **2**, 96–106.

Iwama, T., Hosokawa, H., Lin, W., Shimizu, H., Kawai, K. & Yamagata, S. (2004). Comparative characterization of the *oah2* gene homologous to the *oah1* of *Thermus thermophilus* HB8. *Biosci Biotechnol Biochem* **68**, 1357–1361.

Kerr, D. S. (1971). *O*-Acetylhomoserine sulfhydrylase from *Neurospora*. Purification and consideration of its function in homocysteine and methionine synthesis. *J Biol Chem* **246**, 95–102.

Kiene, R. P., Linn, L. J., González, J., Moran, M. A. & Bruton, J. A. (1999). Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. *Appl Environ Microbiol* **65**, 4549–4558.

Koutmos, M., Datta, S., Pattridge, K. A., Smith, J. L. & Matthews, R. G. (2009). Insights into the reactivation of cobalamin-dependent methionine synthase. *Proc Natl Acad Sci U S A* **106**, 18527–18532.

Krömer, J. O., Wittmann, C., Schröder, H. & Heinzle, E. (2006). Metabolic pathway analysis for rational design of L-methionine production by *Escherichia coli* and *Corynebacterium glutamicum*. *Metab Eng* **8**, 353–369.

Kumar, D. & Gomes, J. (2005). Methionine production by fermentation. *Biotechnol Adv* **23**, 41–61.

Lawrence, J. G. & Roth, J. R. (1996). Evolution of coenzyme B12 synthesis among enteric bacteria: evidence for loss and reacquisition of a multigene complex. *Genetics* **142**, 11–24.

Lee, L. W., Ravel, J. M. & Shive, W. (1966). Multimetabolite control of a biosynthetic pathway by sequential metabolites. *J Biol Chem* **241**, 5479–5480.

Ma, Y., Biava, H., Contestabile, R., Budisa, N. & di Salvo, M. L. (2014). Coupling bioorthogonal chemistries with artificial metabolism: intracellular biosynthesis of azidohomoalanine and its incorporation into recombinant proteins. *Molecules* **19**, 1004–1022.

Manders, A. L., Jaworski, A. F., Ahmed, M. & Aitken, S. M. (2013). Exploration of structure–function relationships in *Escherichia coli* cystathionine γ-synthase and cystathionine β-lyase via chimeric constructs and site-specific substitutions. *Biochim Biophys Acta* **1834**, 1044–1053.

McCutcheon, J. P. & Moran, N. A. (2012). Extreme genome reduction in symbiotic bacteria. *Nat Rev Microbiol* **10**, 13–26.

Messerschmidt, A., Worbs, M., Steegborn, C., Wahl, M. C., Huber, R., Laber, B. & Clausen, T. (2003). Determinants of enzymatic specificity in the Cys-Met-metabolism PLP-dependent enzymes family: crystal structure of cystathionine γ-lyase from yeast and intrafamiliar structure comparison. *Biol Chem* **384**, 373–386.

Nakamori, S., Kobayashi, S., Nishimura, T. & Takagi, H. (1999). Mechanism of L-methionine overproduction by *Escherichia coli*: the replacement of Ser-54 by Asn in the MetJ protein causes the derepression of L-methionine biosynthetic enzymes. *Appl Microbiol Biotechnol* **52**, 179–185.

Pasamontes, A. & Garcia-Vallve, S. (2006). Use of a multi-way method to analyze the amino acid composition of a conserved group of orthologous proteins in prokaryotes. *BMC Bioinformatics* **7**, 257.

Patrick, W. M., Quandt, E. M., Swartzlander, D. B. & Matsumura, I. (2007). Multicopy suppression underpins metabolic evolvability. *Mol Biol Evol* **24**, 2716–2722.

Picardeau, M., Bauby, H. & Saint Girons, I. (2003). Genetic evidence for the existence of two pathways for the biosynthesis of methionine in the *Leptospira* spp. *FEMS Microbiol Lett* **225**, 257–262.

Punta, M., Coggill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G. & other authors (2012). The Pfam protein families database. *Nucleic Acids Res* **40** (D1), D290–D301.

Rabeh, W. M. & Cook, P. F. (2004). Structure and mechanism of *O*-acetylserine sulfhydrylase. *J Biol Chem* **279**, 26803–26806.

Ravanel, S., Gakière, B., Job, D. & Douce, R. (1998). The specific features of methionine biosynthesis and metabolism in plants. *Proc Natl Acad Sci U S A* **95**, 7805–7812.

Rodionov, D. A., Vitreschak, A. G., Mironov, A. A. & Gelfand, M. S. (2004). Comparative genomics of the methionine metabolism in Gram-positive bacteria: a variety of regulatory systems. *Nucleic Acids Res* **32**, 3340–3353.

Rotem, O., Biran, D. & Ron, E. Z. (2013). Methionine biosynthesis in *Agrobacterium tumefaciens*: study of the first enzyme. *Res Microbiol* **164**, 12–16.

Rowbury, R. J. (1983). Methionine biosynthesis and its regulation. In *Amino Acids: Biosynthesis and Genetic Regulation*, pp. 191–211. Edited by K. M. Hermann & R. L. Somerville. Reading, MA: Addison-Wesley.

Rowbury, R. J. & Woods, D. D. (1961). Further studies on the repression of methionine synthesis in *Escherichia coli*. *J Gen Microbiol* **24**, 129–144.

Rowbury, R. J. & Woods, D. D. (1964). *O*-Succinylhomoserine as an intermediate in the synthesis of cystathionine by *Escherichia coli*. *J Gen Microbiol* **36**, 341–358.

Rückert, C., Pühler, A. & Kalinowski, J. (2003). Genome-wide analysis of the L-methionine biosynthetic pathway in *Corynebacterium glutamicum* by targeted gene deletion and homologous complementation. *J Biotechnol* **104**, 213–228.

Saha, B., Mukherjee, S. & Das, A. K. (2009). Molecular characterization of *Mycobacterium tuberculosis* cystathionine gamma synthase–Apo- and holoforms. *Int J Biol Macromol* **44**, 385–392.

Sayers, E. W., Barrett, T., Benson, D. A., Bryant, S. H., Canese, K., Chetvernin, V., Church, D. M., DiCuccio, M., Edgar, R. & other authors (2009). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* **37** (Database), D5–D15.

Seiflein, T. A. & Lawrence, J. G. (2006). Two transsulfurylation pathways in *Klebsiella pneumoniae*. *J Bacteriol* **188**, 5762–5774.

Serra, A. L., Mariscotti, J. F., Barra, J. L., Lucchesi, G. I., Domenech, C. E. & Lisa, A. T. (2002). Glycine betaine transmethylase mutant of *Pseudomonas aeruginosa*. *J Bacteriol* **184**, 4301–4303.

Sletten, E. M. & Bertozzi, C. R. (2009). Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. *Angew Chem Int Ed Engl* **48**, 6974–6998.

Smacchi, E. & Gobbetti, M. (1998). Purification and characterization of cystathionine γ-lyase from *Lactobacillus fermentum* DT41. *FEMS Microbiol Lett* **166**, 197–202.

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S., Hufnagle, W. O., Kowalik, D. J. & other authors (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**, 959–964.

Sun, J., Steindler, L., Thrash, J. C., Halsey, K. H., Smith, D. P., Carter, A. E., Landry, Z. C. & Giovannoni, S. J. (2011). One carbon metabolism in SAR11 pelagic marine bacteria. *PLoS ONE* **6**, e23973.

Talavera, G. & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol* **56**, 564–577.

Tanioka, Y., Yabuta, Y., Yamaji, R., Shigeoka, S., Nakano, Y., Watanabe, F. & Inui, H. (2009). Occurrence of pseudovitamin B12 and its possible function as the cofactor of cobalamin-dependent methionine synthase in a cyanobacterium *Synechocystis* sp. PCC6803. *J Nutr Sci Vitaminol (Tokyo)* **55**, 518–521.

Tanioka, Y., Miyamoto, E., Yabuta, Y., Ohnishi, K., Fujita, T., Yamaji, R., Misono, H., Shigeoka, S., Nakano, Y. & other authors (2010). Methyladeninylcobamide functions as the cofactor of methionine synthase in a Cyanobacterium, *Spirulina platensis* NIES-39. *FEBS Lett* **584**, 3223–3226.

Tatusov, R. L., Fedorova, N. D., Jackson, J. D., Jacobs, A. R., Kiryutin, B., Koonin, E. V., Krylov, D. M., Mazumder, R., Mekhedov, S. L. & other authors (2003). The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**, 41.

Tran, T. H., Krishnamoorthy, K., Begley, T. P. & Ealick, S. E. (2011). A novel mechanism of sulfur transfer catalyzed by *O*-acetylhomoserine sulfhydrylase in the methionine-biosynthetic pathway of *Wolinella succinogenes*. *Acta Crystallogr D Biol Crystallogr* **67**, 831–838.

Usuda, Y. & Kurahashi, O. (2005). Effects of deregulation of methionine biosynthesis on methionine excretion in *Escherichia coli*. *Appl Environ Microbiol* **71**, 3228–3234.

Valley, C. C., Cembran, A., Perlmutter, J. D., Lewis, A. K., Labello, N. P., Gao, J. & Sachs, J. N. (2012). The methionine-aromatic motif plays a unique role in stabilizing protein structure. *J Biol Chem* **287**, 34979–34991.

Vermeij, P. & Kertesz, M. A. (1999). Pathways of assimilative sulfur metabolism in *Pseudomonas putida*. *J Bacteriol* **181**, 5833–5837.

Whitfield, C. D., Steers, E. J., Jr & Weissbach, H. (1970). Purification and properties of 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase. *J Biol Chem* **245**, 390–401.

Yamagata, S. (1971). Homocysteine synthesis in yeast. Partial purification and properties of *O*-acetylhomoserine sulfhydrylase. *J Biochem* **70**, 1035–1045.

Zdych, E., Peist, R., Reidl, J. & Boos, W. (1995). MalY of *Escherichia coli* is an enzyme with the activity of a β C-S lyase (cystathionase). *J Bacteriol* **177**, 5035–5039.

Ziegler, K., Yusupov, M., Bishop, B. & Born, T. L. (2007). Substrate analysis of homoserine acyltransferase from *Bacillus cereus*. *Biochem Biophys Res Commun* **361**, 510–515.

Zubieta, C., Arkus, K. A., Cahoon, R. E. & Jez, J. M. (2008). A single amino acid change is responsible for evolution of acyltransferase specificity in bacterial methionine biosynthesis. *J Biol Chem* **283**, 7561–7567.

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