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Alternative Microbial Routes to Homoserine Activation and Sulfuration: Critical Steps in Methionine Biosynthesis

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Authors' contributions

This work was carried out in collaboration between both authors. Author BT conducted the literature review and wrote the initial manuscript draft. Both authors corrected, modified and approved the final manuscript.

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ABSTRACT

Background: Methionine is a sulfur-containing amino acid that is synthesized *via* a branch point in the aspartate metabolic pathway. *S*-adenosyl methionine is subsequently synthesized from methionine, and plays a critical role in the transfer of methyl groups to various biomolecules, including DNA, proteins and small-molecule secondary metabolites. The branch point that leads to the synthesis of methionine and *S*-adenosyl methionine starts with the activation of the hydroxyl group of homoserine.
Species Variation: The mode of activation of homoserine differs from plants to microorganisms, as well as within different microbial systems. At this point, depending on the species, at least three different activation routes have been identified. In addition, the route of sulfur assimilation in these systems can also vary from species to species.
Enzymology: Homoserine acyl transferases catalyze the primary routes to homoserine activation in microbes, and these enzymes are members of the α/β hydrolase superfamily. While the two different families of homoserine acyl transferases use the same kinetic and chemical mechanisms

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to catalyze this related reaction, they do so by using significantly different overall structures, as well as subtle differences in their closely related active site structures.
Conclusion: Since this pathway produces metabolites that play a number of critical biochemical roles in microorganisms, and because of the complete absence of related enzymes in mammals, the enzymes in this pathway represent novel targets for future antibiotic drug development.

Keywords: Methionine biosynthesis; homoserine activation; homoserine sulfuration; homoserine acyltransferases; enzyme mechanisms; enzyme evolution; enzyme structures.

ABBREVIATIONS

AdoMet, S-adenosyl-L-methionine; APS, adenosine 5'-phosphosulfate; CBL, cystathionine β-lyase; CGS, cystathionine γ-synthase; HK, homoserine kinase; HMT, homoserine methyltransferase; HTA, homoserine transacetylase; HTS, homoserine transsuccinylase; Mtb, Mycobacterium tuberculosis; OAH, O-acetylhomoserine; OAHS, O-acetylhomoserine sulfhydrylase; OPH, O-phosphohomoserine; OSHS, O-succinylhomoserine sulfhydrylase; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; PFE, Pseudomonias fluorescens esterase; PLP, pyridoxal phosphate; STA, L-serine O-acetyltransferase.

1. INTRODUCTION

The vast majority of metabolic pathways proceed in a sequential fashion, where a sequence of enzyme-catalyzed reactions is used to convert metabolic precursors to the final end products. The introduction of branch points, where two different enzymes will utilize the same substrate to yield different products, allows common precursors to lead to different final products. It is highly unusual for multiple enzymes, with different co-substrates and different structures, to be involved in the conversion of a common metabolic intermediate to the same final product. However, such is the case for the conversion of the common intermediate L-homoserine to the final product, L-methionine. In this report we examine the diversity of enzyme-catalyzed reactions that are involved in the activation and sulfuration of L-homoserine in different microbial species, leading to the ultimate production of the amino acid L-methionine and the important methyl donor, *S*-adenosyl-L-methionine (AdoMet). The pathway that synthesizes these essential amino acid products is found only in plants and microbes such as bacteria and fungi, but is absent in mammals. Therefore the microbial enzymes involved in this pathway are attractive targets for the development of new antibiotic and antifungal compounds with novel modes of action.

2. THE ASPARTATE PATHWAY OF AMINO ACID BIOSYNTHESIS

The aspartate metabolic pathway (Fig. 1) leads to the biosynthesis of the essential amino acids, threonine, lysine, methionine and isoleucine in most plants, bacteria, and fungi [1,2]. This pathway also produces a number of important metabolites that are critical for the growth and survival of microbes. Dipicolinate, a metabolite produced from the pathway intermediate dihydrodipicolinate, is required for sporulation in Gram-positive bacteria. The UDP-N-acetylmuraminic acid pentapeptide, produced from either diaminopimelate or from lysine, is the crosslinking component in bacterial cell walls. 4,5-dihydroxy-2,3-pentanedione and acyl homoserine lactones which are produced from AdoMet are the primary signaling molecules used in bacterial quorum sensing. The methylation reactions which are crucial for cell growth and viability are also mediated by AdoMet, one of the key products of the aspartate pathway [3-5].

2.1 The Roles of Methionine and S-Adenosylmethionine

Methionine, a sulfur-containing amino acid, is one of the eight essential dietary amino acids in mammals, with four of these essential compounds produced by the aspartate pathway. Methionine biosynthesis occurs as one of the branches in the aspartate pathway. Both microorganisms and plants synthesize methionine by utilizing aspartic acid as the source of the carbon skeleton and cysteine as the primary sulfur source (Fig. 1). Methionine holds a critical position in cellular metabolism, where the processes of protein synthesis, polyamine biosynthesis and methyl group transfers are each interconnected through

AdoMet [6-8]. AdoMet is synthesized from methionine and ATP by *S*-adenosylmethionine synthetase, and AdoMet serves as the methyl donor for a wide variety of acceptors catalyzed by many different methyltransferases [3]. Methylation reactions are involved in numerous central biochemical processes that are essential for both microbes and mammals, including key steps in cell development and maturation [9]. More recent studies have shown that the methionine/AdoMet products are also involved in the synthesis of the inducers acylhomoserine lactones and pentanediones, groups of intercellular signaling molecules that facilitate the bacterial quorum sensing and communication phenomenon [7,10-12].

2.2 Variation in and Control of Methionine Synthesis

2.2.1 General metabolic routes to methionine

Methionine biosynthesis (Fig. 2) is tightly controlled, with the initial point of regulation located at the first biosynthetic step in this branch of the pathway [6,13]. In addition, microorganisms also employ different synthetic and regulatory paths for methionine biosynthesis that vary from species to species [14-17]. The primary species variations are the different methods for the activation of homoserine and the different routes in the mode of assimilation of the sulfur atom into the carbon backbone [18]. The activation of homoserine produced in the aspartate pathway occurs at the branch point leading to methionine synthesis (Fig. 1) [19]. Acylation of homoserine produces different *O*-acyl homoserines in different species [20-22]. The hydroxyl group at carbon-4 of homoserine is activated in this commitment step, with the subsequent nucleophilic attack by cysteine to generate cystathionine. This reaction is catalyzed by the pyridoxal phosphate (PLP)-containing enzyme cystathionine Υ-synthase (CGS), coded by the *metB* gene [23]. In the next step cystathionine is broken down to homocysteine, catalyzed by cystathionine β-lyase (CBL), coded by the *metC gene*. Finally, the transfer of a methyl group from 5'-methyl-THF is catalyzed by homocysteine methyltransferase to yield L-methionine (Fig. 2).

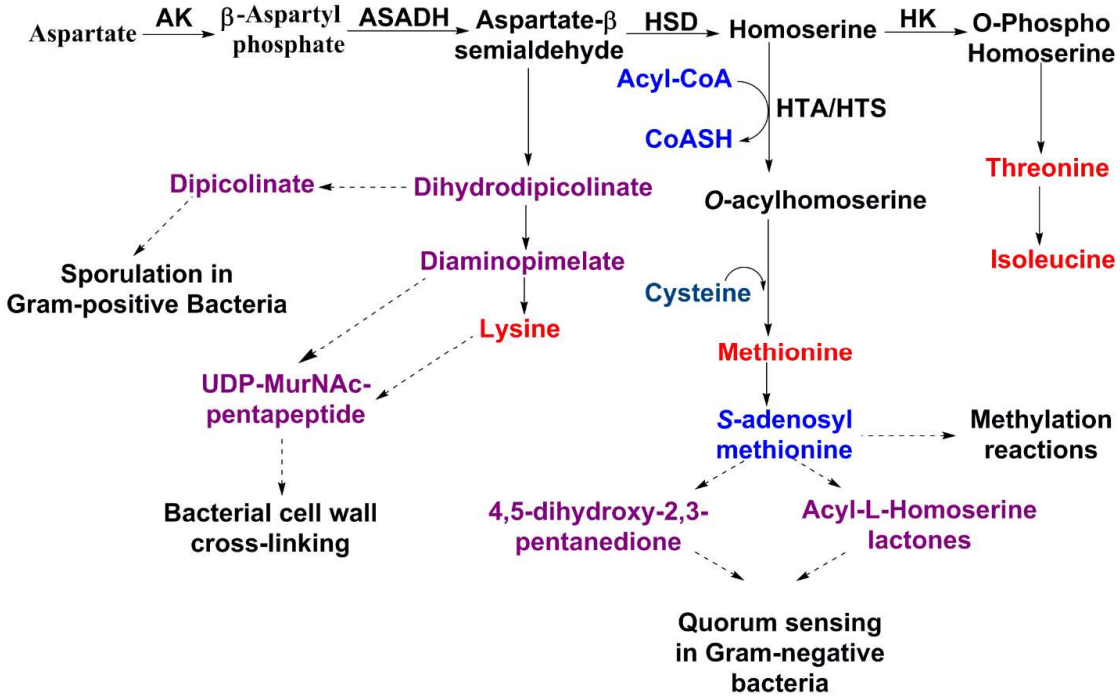


Fig. 1. The Aspartate metabolic pathway leading to four essential amino acids (shown in red) and to the synthesis of several important metabolites (shown in purple)
(Figure adapted from Viola [1])

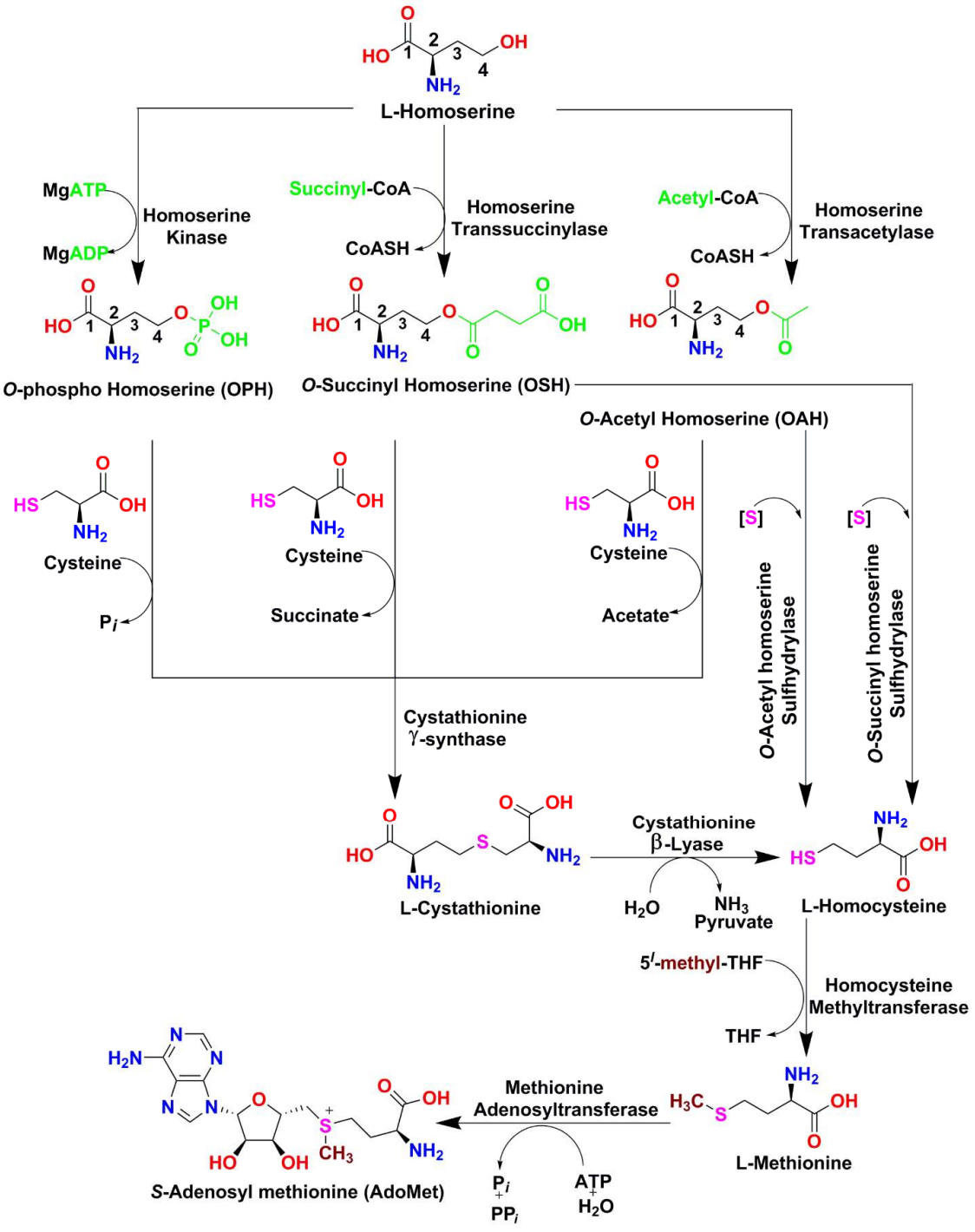


Fig. 2. The diverse pathways leading to the synthesis of methionine, with the activation step catalyzed by homoserine kinase, homoserine transsuccinylase or homoserine transacetylase in various microbes and plants. Also shown are the different pathways to homocysteine, either *via* transsulfuration with cysteine as the sulfur source, or by direct sulfhydrylation

2.2.2 Variations in the mode of homoserine activation

While straightforward in principle, the mode of homoserine activation can be quite diverse, and shows significant species to species variation. Two different families of acyltransferases have been found to catalyze the acylation of homoserine. In most enteric bacteria, including *E. coli* and other Gram-negative facultative and anaerobic bacteria, a succinyl group is transferred to homoserine from succinyl-CoA to form O-succinylhomoserine (OSH) catalyzed by homoserine transsuccinylase (HTS) (Fig. 2), with this enzyme coded by the *metA* gene [24]. In all fungi, and in many Gram-positive bacterial species such as *Bacillus, Brevibacterium* and *Corynebacterium*, an acetyl group is transferred to homoserine from acetyl-CoA to form *O*-acetyl homoserine (OAH) catalyzed by homoserine transacetylase (HTA) and coded by the *met2* gene [25]. However, in plants neither of these families of acyltransferases are involved in this activation step. In these species homoserine is esterified with a phosphoryl group from ATP to generate *O*-phospho-homoserine (OPH) catalyzed by homoserine kinase (HK) [26,27] and coded by the *hsk* gene [28]. The catalytic activity of both HTS and HTA has been shown to be regulated by coordinated feedback inhibition by both L-methionine and AdoMet [29]. No organism has been identified to date that contains the genes for both acyltransferases. In addition to their variant in species distribution, these two enzymes display virtually no primary sequence similarity (Fig. 3). This suggests that each of these proteins evolved independently to catalyze a nearly identical reaction utilizing the same amino acid substrate, but a different acyl CoA substrate.

2.2.3 Variations in the assimilation of sulfur

The metabolic routes where cysteine plays the role as the sulfur donor is called transsulfuration and is the predominant incorporation mechanism seen is most microorganisms. Alternatively, in yeast such as *Saccharomyces cerevisiae* and *Candida albicans*, and in bacteria such as *Brevibacterium flavum*, *Bacillus subtilis*, *Streptococcus pneumonia* and *Leptospira meyeri*, the sulfur donor is sulfide instead of cysteine. Here sulfur is incorporated into the homoserine ester by *O*-acetylhomoserine sulfhydrylase (OAHS), coded by the *metY* (alternatively, *met17* or *met25*) gene, to form homocysteine (Fig. 2) [30,31]. In *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* and in all enteric bacteria, including *E. coli,* the same function is carried out by *O*-succinylhomoserine sulfhydrylase (OSHS), coded by the *met*Z gene [33,34]. This route is called direct sulfhydrylation. Methylation of homocysteine by homocysteine methyltransferase then produces L-methionine, and the subsequent adenosylation of methionine by methionine adenosyltransferase leads to the formation of AdoMet (Fig. 2). In some bacteria and fungi both routes for sulfur atom assimilation, transsulfuration and direct sulfhydrylation, can occur [26]. The evolutionary basis behind the choice of the routes for sulfur assimilation in different organisms is likely linked to the intermediates that are available in their core metabolic network, and also depends on their natural habitat [35].

2.2.4 The lack of variation in cystathionine production

Now that homoserine has been shown to be activated by at least three different routes depending on the species involved, it becomes necessary for these organisms to develop a means to merge these different metabolites back to a common metabolic intermediate that will lead to methionine as the final product. That task falls to a single enzyme in these species. The most intriguing feature of this enzyme, cystathionine Υ-synthase (CGS), is its remarkable substrate flexibility. CGS can accept any of the three esterified homoserine variants (OAH, OSH or OPH) as a substrate (Fig. 2), but it does so with different affinities and with different catalytic efficiencies [36]. While each of the various microbial organisms have evolved to produce only a single homoserine ester, each of their cysteine-condensing enzymes retains the ability to utilize the other esters as well. In contrast, for the related CGS enzyme from plants only OPH is utilized as a substrate [37]. Arabidopsis CGS has been shown to be capable of using all three homoserine esters as substrates with similar efficiency, but in nature it only utilizes OPH. In a broader study, about 75 bacterial species were screened *in vitro* and the majority of the CGS bacterial enzymes were shown to be capable of using both OSH and OAH [38,39]. Since the different precursors, homoserine, acetyl-CoA, succinyl-CoA and ATP are each available in bacterial, fungal and plant cells, it is very likely that each of these homoserine esters can potentially be produced in these organisms. Although one of the homoserine esters is preferred in a particular organism, the alternate sources are available to

be used, albeit with lower proficiency. The controlling factor at this juncture is most likely the availability of a particular substrate and the specific regulation of the metabolic network of that species, but not the specificity of the enzyme.

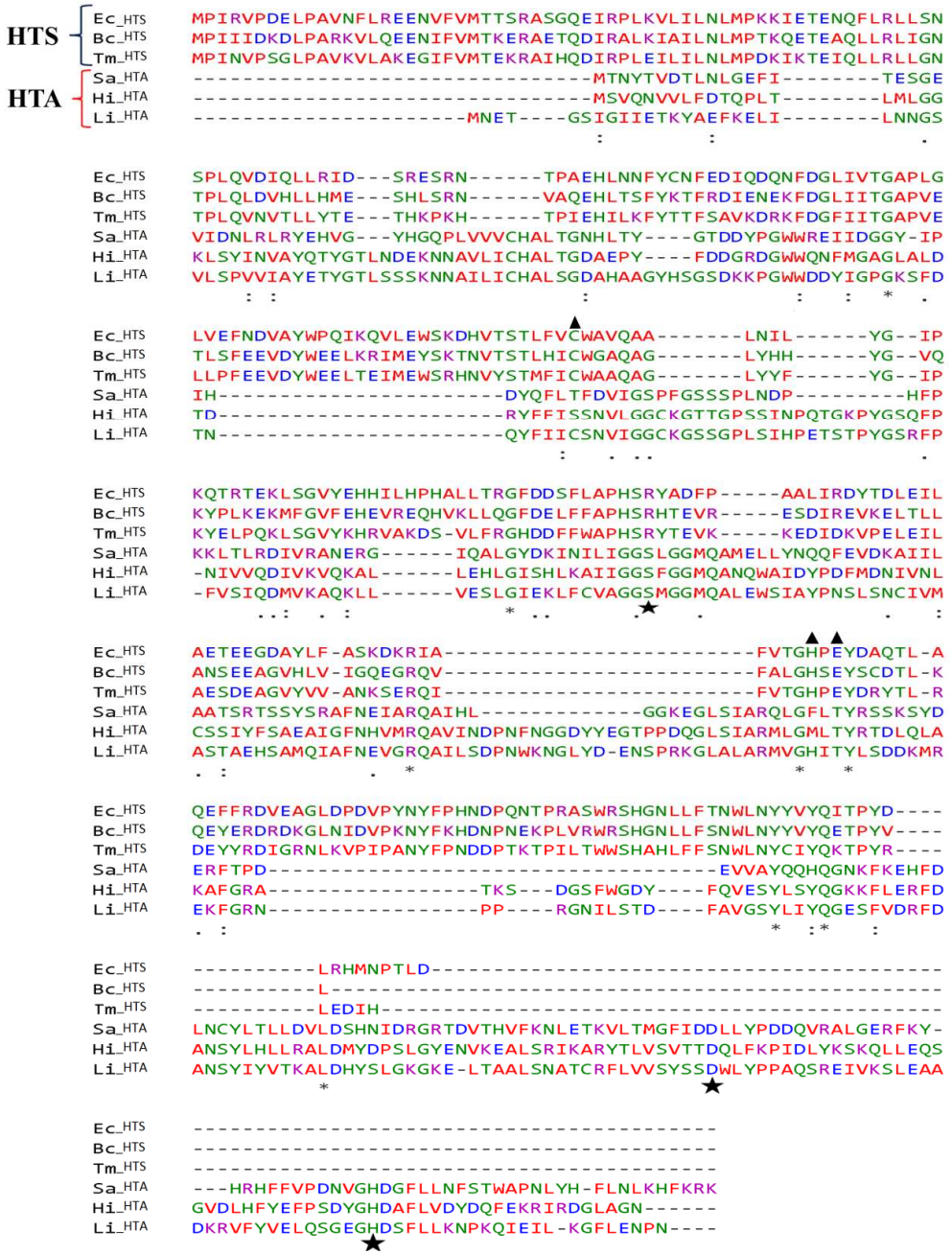


Fig. 3. Sequence alignment between homoserine transsuccinylases and homoserine transacetylases, showing the poor sequence similarity between these two mechanistically related enzyme families. The catalytic triad residues of HTA are indicated by stars (★) and those of HTS by triangles (▲)

This figure was generated by using Clustal Omega [32]

This multi-substrate affinity with different homoserine esters possessed by these enzymes is also complemented by their capability to use different sulfur sources. CGS enzymes from plants and bacterial species were shown to substitute cysteine for sulfide as their source of sulfur in methionine synthesis. Further *in vitro* studies showed that these enzymes can, if necessary, utilize various thiol compounds as the source for sulfur in methionine synthesis [37,40]. Thus, these CGS enzymes possess a remarkable ability to change mechanisms by acting through transsulfuration as well as through direct sulfhydrylation. The occurrence of this phenomenon is further supported by examination of this enzyme-catalyzed reaction in *Mycobacterium tuberculosis* and *S. cerevisiae* [15], and in *Niesseria meningitidis* and *Corynebacterium glutamicum* [23,41]. However, it is still unclear whether these different pathways are each physiologically active in these organisms.

C. glutamicum has been shown to utilize both the transsulfuration and the direct sulfhydrylation pathways with nearly the same efficiency [41]. However, in yeast direct sulfhydrylation is found to be the most active pathway [15]. *P. aeruginosa* and *P. putida* can synthesize methionine both by transsulfuration and by the direct sulfhydrylation pathways, but preferentially proceeds *via* direct sulfhydrylation in these species [33,34]. It is possible that the direct sulfhydrylation pathway could exist in all species of plants, bacteria and fungi, while in some bacterial and fungal species only a sulfhydrylase is present and in other species CGS catalyzes the sulfhydrylase reaction [40,42,43]. It is also likely that early organisms relied exclusively on OAH/OSH sulfhydrylase for methionine synthesis using sulfide as a source of sulfur, and that over time this enzyme evolved to function as a CGS, synthesizing methionine by utilizing cysteine as the sulfur source. However, CGS can still utilize sulfide as the source of sulfur and act as sulfhydrylase, consequently maintaining the substrate flexibility of its progenitor.

2.2.5 An alternative route to direct homocysteine production

Until fairly recently the mechanism utilized by sulfide transferase in the direct sulfhydrylation pathway to form homocysteine from OAH was not well characterized. A subsequent study of this pathway in *W. succinogenes*, a Gram-positive bacterium, identified a protein thiocarboxylate as a likely sulfur donor [44,45]. A gene cluster was found in this species encoding a putative sulfur-carrier protein (HcyS), a putative metalloprotease (HcyD), and an adenylating enzyme (HcyF), that appear to be involved in sulfur-assimilation and in methionine biosynthesis. This pathway involves an ATP sulfurylase that converts sulfate to adenosine 5'-phosphosulfate (APS), followed by phosphorylation at the 3'-hydroxyl group by APS kinase to generate 3'-phosphoadenosine 5'-phosphosulfate (PAPS). The sulfate is reduced to sulfite by PAPS reductase and then to sulfide by a ferrodoxin-containing sulfite reductase. The sulfide is now transferred to the C-terminal carboxylate of the carrier protein HcyS to form HcyS thiocarboxylate (HcySCOSH). OAH sulfhydrylase then catalyzes the pyridoxal 5'-phosphate (PLP)-dependent condensation of HcyS-COSH and OAH, followed by an S, N-acyl shift, to form HcyS-homocysteine (HcyS-Hcy), which is then cleaved by the HcyD protease to form homocysteine. Thus, through the use of this sulfur-carrier protein, sulfur is activated, reduced and then assimilated into homocysteine, rather than directly from sulfide as has been observed for the other OAHSs [31].

2.2.6 Regulation of methionine biosynthesis

Methionine biosynthesis is tightly regulated both at the level of gene expression and at the enzyme activity level, and these regulatory mechanisms are also closely connected with sulfur assimilation [46]. Because methionine belongs to the aspartate family of amino acids, its biosynthesis is also partly controlled by other products of this pathway, including lysine and threonine. Aspartate kinase, the enzyme that catalyzes the initial conversion of aspartate to β-aspartyl phosphate, plays an important role in controlling the overall biosynthesis of the aspartate family of amino acids (Fig. 1). Different species have multiple aspartate kinase isoenzyme forms, with both the catalytic activity and the gene expression levels of the individual forms differentially regulated by the end product amino acids [16]. The homoserine branch of this pathway represents the commitment step in the biosynthesis of methionine, with multiple additional modes of regulation identified to control methionine levels [47].

In *E. coli* expression of the entire *met* gene cluster, with the exception of *met*H, is repressed by exogenous methionine. In that case a separate apo-repressor, MetJ which binds

AdoMet for activation, is responsible for this gene repression [48-50]. Additionally, a positive regulator MetR is involved in the activation of homocysteine methyltransferase (HMT), the enzyme that catalyzes the methylation of homocysteine to methionine. When the binding of MetR is disrupted the activity of HMT is impaired [51]. Methionine has also been shown to represses the expression levels of the enzymes involved in the activation of homoserine (HTA), the production of cystathionine (CGS), and the synthesis of homocysteine (CBL and OAHS) [23,41,52,53]. Furthermore, HTS in *E. coli* and HTA from *H. influenzae* and *B. polymyxa* have each been shown to be feedback-inhibited by methionine /AdoMet [24,25,54].

In addition to these classical repression and feedback inhibition regulatory mechanisms, the HTA from *B. polymyxa* was also found to be inactivated at elevated temperatures [54,55], by undergoing an energy-dependent proteolysis at these higher temperatures [55-57]. This thermal instability can serve as an effective mode of controlling cell growth rates, by limiting the availability of methionine which is necessary for initiation and continuation of protein synthesis, biosynthesis of purines and polyamines and various methylation reactions. The presence of a control system of this kind has already been demonstrated in *E. coli*, where the availability of methionine provides a mechanism for a sensitive response to elevated temperatures, leading to control of cell growth [56,57]. This observation suggests a possible role for the *met*A gene product in a global growth regulatory system, and therefore in controlling cell physiology [46].

3. ENZYME STRUCTURAL HOMOLOGY AND DIVERSITY

Numerous enzymes, including lipases, proteases, esterases, dehalogenases, peroxidases and epoxide hydrolases share a similar overall structural organization and topology, known as the α/β hydrolase superfamily [58-61]. In spite of the similar overall structural features, and in many cases similar active site residues and active site architectures, these different classes of enzymes catalyze a wide range of reactions involving a variety of different substrates and a diversity of products. The acyltransferase enzymes that are responsible for the activation of homoserine in the methionine branch of the aspartate pathway are members of this α/β hydrolase superfamily.

3.1 Structural Features of the α/β Hydrolases

The canonical α/β hydrolase fold which includes the HTA family (Fig. 4A) consists of an eight-stranded β-sheet with strands that are predominately parallel, surrounded on both sides by α-helices. The HTS enzyme family consists of a modified α/β hydrolase fold composed of a nine-stranded, predominately parallel β-sheet with six interspersed α-helices (Fig. 4B), but is missing the lid domain found in the HTA structures. This α/β hydrolase fold provides a stable scaffold upon which to display the active site residues of a range of different enzymes. The catalytic triad in these different enzymes consists of a conserved nucleophile, an acidic residue and a histidine catalytic base. In the HTA enzyme family the nucleophilic residue (serine) is located on a loop between strand β5 and helix αC, the acidic residue (aspartic acid) between strand β7 and helix αE, and the histidine base is always found after the last β-strand. In the HTS family the catalytic triad residues are located in different regions of the secondary structure (Fig. 4B), but surprisingly, the three-dimensional positions of the members of these catalytic triads are similar among these diverse classes of enzymes.

The nucleophile in each enzyme family is positioned at a sharp turn, called the 'nucleophile elbow', which can be identified from the sequence, consisting of Sm-X-Nu-X-Sm (Sm=small residue, X=any residue and Nu=nucleophile). The tightness of this motif causes the backbone atoms at the nucleophile position to adopt an energetically unfavorable conformation with highly strained torsional angles [62,63]. The geometry of this nucleophile elbow also provides the structure of the oxyanion-binding site. As has been seen in the well-characterized serine proteases, this site stabilizes the high energy, negatively-charged tetrahedral transition state that occurs during catalysis. Usually two backbone nitrogen atoms, one from the residue following the nucleophile and the other from the residue located between β3 and helix αA make up the oxyanion-binding site.

The family of α/β hydrolase-fold enzymes has evolved to accommodate a wide variety of different substrates. Loops are inserted in the protein structure between different beta strands to allow the formation of the proper geometry for the different substrate-binding domains. These

insertions may be as short as only a few residues or may be long enough to form a complete domain that encloses the catalytic cavity. The entrance to the active site is typically a narrow tunnel with a flexible lid that controls the entry and exit of substrates and products. This lid domain is a distinct feature of most of the enzymes of this structural class, suggesting the need for a conformational change to make the active site accessible to the substrates, and the capability to restrict access to the active site once substrates are bound.

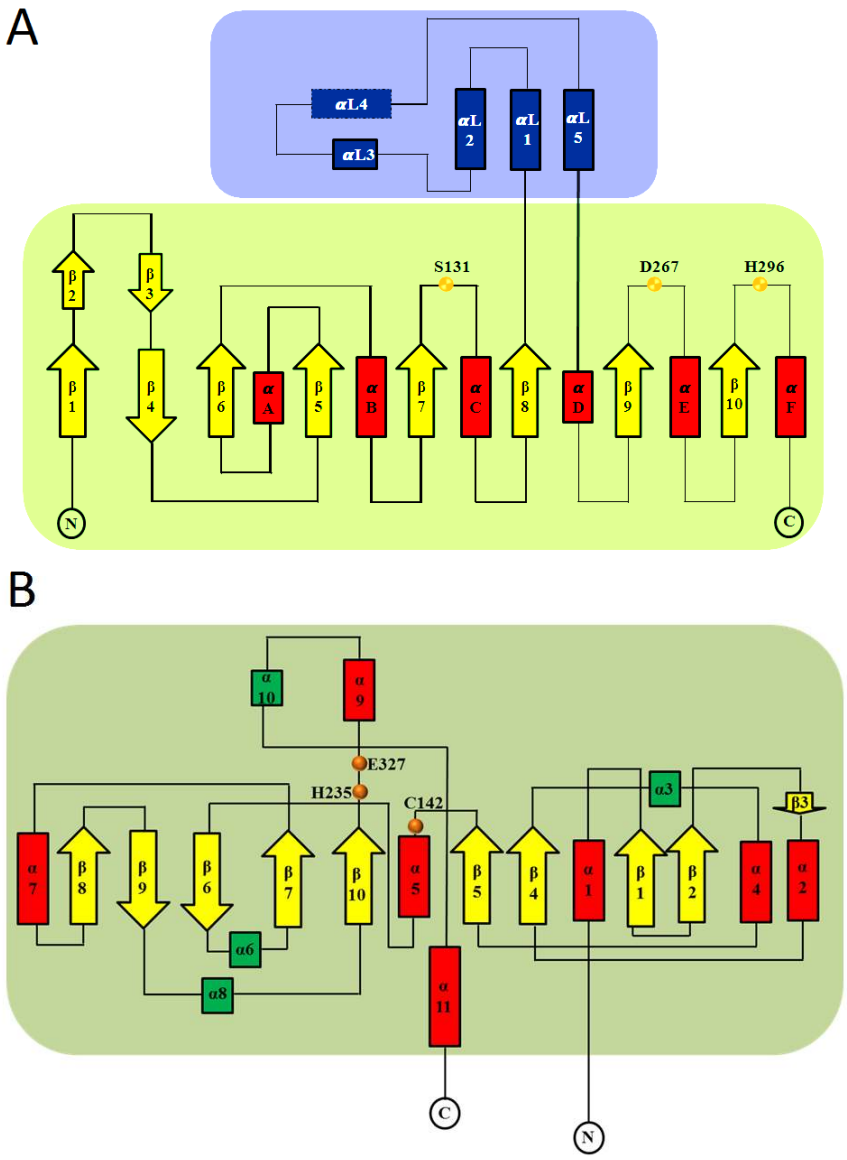


Fig. 4. (A) Schematic diagram of the topology of HTA, showing the canonical α/β hydrolase fold consisting of eight-stranded β sheets (yellow) surrounded on both sides by α helices (red) in the central domain and 5 α helices (blue) in the helical domain. The positions of the catalytic triad residues (Ser131, Asp267, His296) are noted on the loops between a β-sheet and an α-helix. (B) Schematic diagram of the α/β hydrolase topology of HTS, consisting of 11 β-strands (yellow), 7 α-helices (red) and 4 3_{10}-helices (green). The positions of the catalytic triad residues (Cys142, His235, Glu237) are noted

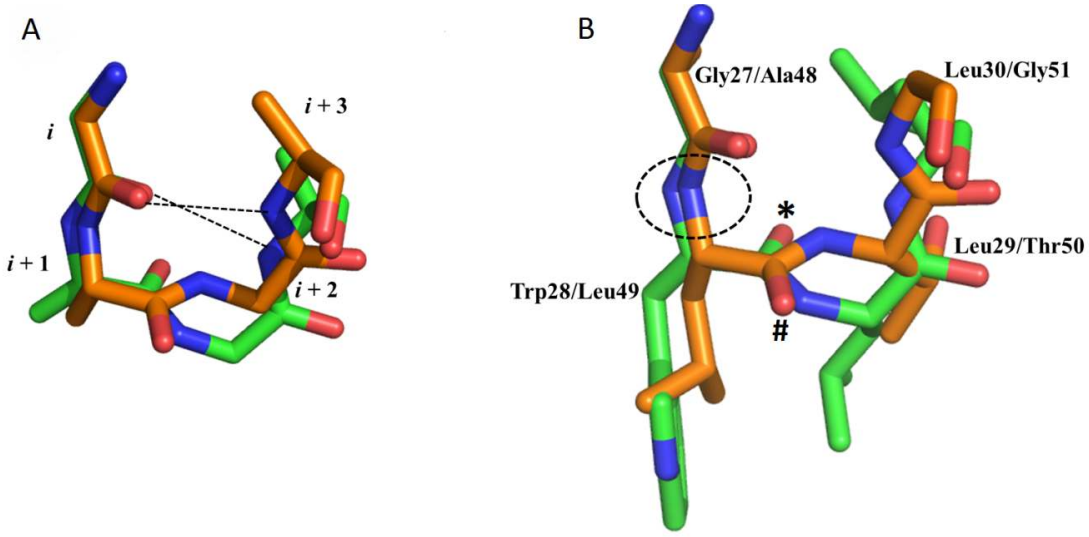
(Figure from Thangavelu, et al. [80])

3.2 Differences in Oxyanion-loop Orientation

So, with a range of enzymes that have very similar overall topologies and nearly identical active site functional groups, how do the different enzyme classes in this structural family achieve substrate recognition specificity and unique mechanistic identities? The acyltransferases of interest for methionine synthesis catalyze the transfer of an acyl group from a donor (a thioester) to an acceptor (an alcohol or amine) through an acyl-enzyme intermediate [64]. This reaction is similar to the hydrolysis reaction catalyzed by esterases and lipases; the only difference is that the acceptor in the latter cases is water. The overlapping catalytic activity between these acyltransferases and the esterases/lipases is a consequence of their similar three-dimensional structures and catalytic machinery [58,65]. Both adopt the α/β-hydrolase fold and contain an identical catalytic triad of Ser-His-Asp/Glu. Both also involve an oxyanion hole for intermediate stabilization and use a ping-pong reaction mechanism through an acyl-enzyme intermediate. The step which differs between these enzyme classes is the nature of the attack on the acyl-enzyme intermediate once it is formed. In the esterases/lipases water attacks the acyl-enzyme intermediate leading to hydrolysis, while in the acyltransferases an alcohol nucleophile attacks the acyl-enzyme intermediate leading to acyl group transfer. So, how can the acyltransferases favor acyl transfer over hydrolysis in the presence of a vast excess of water? The most straightforward solutions would be to either decrease the ability of water to act as the nucleophile or to increase the likelihood of alcohol as the nucleophile. There is crystallographic evidence to suggest that the hydrophobic active site found in many acyltransferases acts to exclude water from the active site [65-68], while some acyltransferase active site structures suggest the presence of a specific binding interaction between an alcohol and the acyl-enzyme intermediate that eliminates the possibility for the hydrolysis reaction to occur [69,70].

In an attempt to resolve this issue, representative structures of each class of enzymes were compared to identify differences in the active site region that could serve to discriminate between the binding of an alcohol or a nucleophilic water molecule. An interesting difference was revealed in the main-chain oxyanion loop orientation that could potentially explain the mechanism of deactivation of water as a potential nucleophile in the acyltransferases. Structural comparison between the homoserine transacetylase from *Hemophilus influenza* (*Hi*HTA) and the *Pseudomonas fluorescens* esterase (PFE) showed an important difference in the oxyanion loop orientation that occurs after strand β3 in the α/β hydrolase fold. A β-turn is composed of four amino acid residues, with a hydrogen bond formed between the backbone carbonyl of the first residue and the backbone amide of the last residue (Fig. 5A). The two intervening residues do not participate in backbone hydrogen bonding and this feature is critical for catalysis in both classes of enzymes. This is because the backbone amide of the second residue functions as a hydrogen bond donor to stabilize the oxyanion intermediate. In *Hi*HTA this position corresponds to Leu49, while in PFE the corresponding residue is Trp28. This loop in *Hi*HTA adopts a type-I β-turn, while in PFE this loop adopts a type-II β-turn, with the Φ and Ψ angles between the two interior residues determining the type of β-turn (Fig. 5C). The outcome of the different types of β-turns in these two enzyme types causes the backbone carbonyl groups of Trp28 and Leu49 to orient in opposite directions (Fig. 5B). The carbonyl of Trp28 in PFE (*) points into the active site, while the carbonyl of Leu49 in *Hi*HTA (#) points away from the active site. The change in the orientation of this structural feature plays a significant role in the types of reactions catalyzed by each enzyme.

In the hydrolases a bridging water molecule interacts with the carbonyl oxygen of Trp28 and help to establish its role as a base. By serving as a hydrogen bond donor, the partial negative charge of the bridging water molecule is increased, thus allowing it to accept a hydrogen bond from the nucleophilic water molecule. An additional role for the bridging water molecule is to help position the nucleophilic water molecule in the correct orientation for attack. In the case of the acyltransferases this scenario is unlikely because of the incorrect orientation of the backbone carbonyl at the corresponding position. Also, the larger size of the alcohol (acyl acceptor) would displace the bridging water molecule from the active site and eliminate any possible role for water in catalysis.



C

Type of β turn	Φ_{i+1}	ψ_{i+1}	Φ_{i+2}	ψ_{1+2}
Type I	-60	-30	-90	0
Type II	-60	120	80	0

Fig. 5. (A) Idealized type-I β-turn (orange) overlaid on an idealized type-II β-turn (green). The amino acids are XxxAlaAlaXxx, where Xxx indicates an incompletely specified residue. Cα of each residue is labeled; dotted lines indicate hydrogen bonds between the carbonyl of residue i and the amide of residue i+3. (B) Superimposed structures of the oxyanion loop regions of PFE (green, a type-II β-turn) and *Hi*HTA (orange, a type-I β-turn). The carbonyl of Trp28 in PFE (*) points toward the active site, whereas for *Hi*HTA the carbonyl of Leu49 (#) points away from the active site. In both cases the backbone amide of residue i+1 (dashed circle) points toward the active site because it forms a hydrogen bond with the oxyanion intermediate. (C) Table showing the ideal angles for different types of beta-turns

(Figure derived from Jiang, et al. [64])

4. EVOLUTIONARY ASPECTS OF HOMOSERINE ACYLTRANSFERASES

4.1 Convergent Evolution of Homoserine Acyltransferases

The homoserine acyltransferases have evolved in different organisms to show a preference for either acetyl CoA or succinyl CoA as the acyl group donor. However, in some bacterial species, including *Thermotoga maritima*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus cereus* and some *Clostridium* species, genes that have been annotated to code for a HTS actually will use acetyl-CoA preferentially over succinyl-CoA [35,71] as the acyl group donor. Evidence for this change in donor preference comes from the structure of HTS from *Bacillus subtilis* (*Bs*HTA), which reveals that this enzyme has a conformationally flexible active site that is consistent with the use of the shorter acetyl group for catalysis [71]. It is quite likely that early organisms relied exclusively on a single transacylase with little acyl donor specificity. Gradual mutations could have then evolved the enzyme into forms which utilized either of the two substrates more efficiently [72]. Thus, although one substrate is preferred, the alternative donor source can be still utilized, although less efficiently.

4.2 Changes in Acyl Group Specificity

A study of the structural basis for these specificity differences showed that the identity of a single amino acid is responsible for the substrate specificity of a homoserine transsuccinylase [71]. The *Bacillus cereus metA* (*Bc*MetA) has been annotated as an HTS on the basis of sequence similarity with other HTS enzymes. However, biochemical characterization showed that this enzyme utilizes succinyl-CoA as a substrate quite poorly, but shows good catalytic efficiency with acetyl-CoA. This enzyme is not quite a fully optimized HTA, since the turnover number with this substrate is still 100-fold lower when compared to that of the *H. influenzae* HTA. The availability of a structure of *Bc*MetA enzyme complexed with homoserine provides an opportunity to examine this acyltransferase with a bound ligand [71]. Functional analysis of *Bc*MetA revealed some interesting features of this acyltransferase that helps to determine its substrate specificity. *Bc*MetA shares 51% amino acid sequence identity with *E. coli* HTS, but has only 10% identity with HTA from bacteria or fungi, suggesting that this enzyme should be properly classified as a succinyltransferase. Biochemical and structural characterization of *Bc*MetA identified Cys142, His235 and Glu237 as the catalytic triad, and a sequence comparison with *Ec*HTS is consistent with this assignment. Mutations of the Cys142 nucleophile to either alanine or serine, and His235 to alanine each produced mutant enzyme forms with no catalytic activity. These mutagenesis results with *Bc*MetA are similar to the reported loss of activity observed with the same changes in the catalytic triad of both HTA from yeast and HTS from bacteria [73].

Modeling studies on the *Bc*MetA acetyl-enzyme intermediate shows that the side chain of Glu111 is oriented towards the active site cysteine, placing it in a position that would sterically clash with the binding of succinyl-CoA, but would still allow acetyl-CoA binding (Fig. 6A). An NCBI database Blast search shows that each of the metA enzymes that function as an HTS have a glycine at this position, while in the case of HTA this residue is glutamate. To test the possible role of Glu111 as a gatekeeper of acyl donor selectivity an E111G mutant of *Bc*MetA was prepared. While this mutant enzyme showed no detectable acyltransferase activity with acetyl-CoA, it could now catalyze this reaction with succinyl-CoA as the acyl donor, with a comparable rate to that of a native HTS (Fig. 6B) [64].

Enzymes of the same superfamily share a common three-dimensional framework and closely related amino acid sequences. Changing substrate binding groups while preserving the active site functional groups and architecture affords an efficient means of evolving novel enzyme activity by altering substrate specificity [74,75]. These results with *Bc*MetA demonstrate the limitations in the use of bioinformatics alone as a means of assigning the function of a protein, and emphasize the importance of biochemical characterization of proteins as the ultimate criteria of its function.

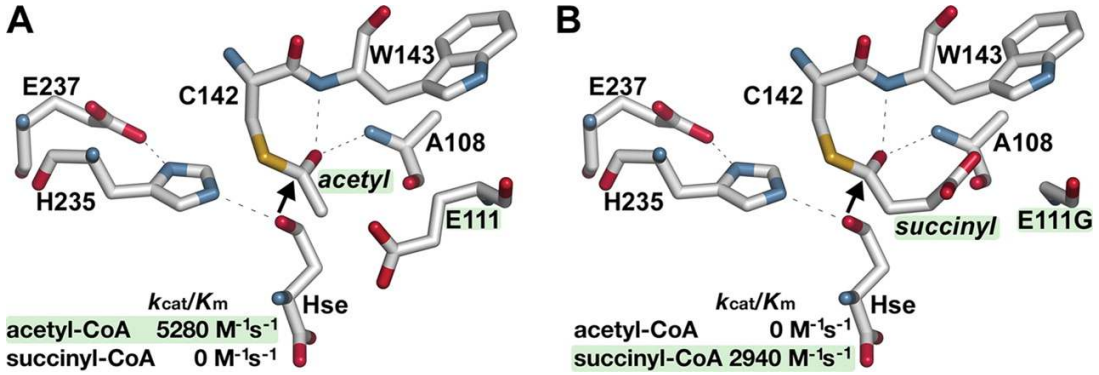


Fig. 6. (A) Model of homoserine addition (→) to the acetyl-enzyme intermediate in *Bc*MetA. The native enzyme shows no activity with succinyl-CoA. (B) Model of homoserine addition (→) to the succinyl-enzyme intermediate in the E111G mutant of *Bc*MetA. This mutant enzyme form shows no detectable acyltransferase activity with acetyl-CoA

(Figure from Zubieta, et al. [71])

4.3 Convergent Evolution of a Serine Acetyltransferase

O-acetyl-L-serine is an intermediate in the D-cycloserine biosynthetic pathway and is generated from L-serine in a reaction catalyzed by L-serine *O*-acetyltransferase (STA) [76,77]. The DcsE gene codes for an acetyltransferase that prefers L-serine as its substrate over L-homoserine [78]. Remarkably, this DcsE protein displays no homology with the other STAs, but does show high sequence homology with HTA. Also, the structure of DcsE is quite similar to those of other HTAs from *H. influenza*, *L. interrogans* and *S. aureus* (Fig. 7). The overall structural organization of DcsE with two domains is identical to that of the HTAs; an α/β hydrolase domain, consisting of residues 1 to 176 and 289 to 374, and a helical domain, composed of residues 179 to 286. Like in the HTAs, the helical domain is involved in the formation of the dimeric structure of DcsE, and a deep tunnel is formed by the juxtaposition of the two domains.

Despite these structural similarities, an intriguing feature of this enzyme is a small difference in the turn region forming the oxyanion hole that appears to dictate the different in substrate specificity between these two enzymes. Similar to that of Leu49 in *Hi*HTA, the turn region containing Leu53 in DcsE is proposed to form the oxyanion hole that stabilizes the tetrahedral intermediate [62]. This turn region was found to be structurally distinct from that of the HTAs. The first and last residues that constitute this turn, Gly52 and Pro55, respectively, in DcsE are different from those in the HTA family, which are Ala and Gly. This unusual turn at the active site of DscE plays an important role in dictating the substrate specificity differences between these enzymes.

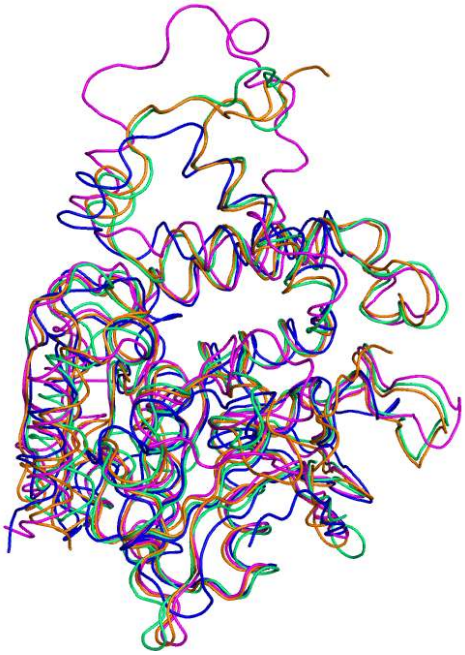


Fig. 7. Ribbon diagram showing the superimposition of DcsE (Orange), *Hi*HTA (green), *Li*HTA (magenta) and *Sa*HTA (blue)
(Figure was generated by using VMD program [79])

Mutation studies in this region of DcsE were carried out by replacing either of the amino acids at the beginning and end of this loop region with those found in HTA. Native DcsE has about a 20-fold preference for serine as its acyl acceptor over homoserine, while in the P55G mutant this specificity preference has been reduced to only 2-fold (Table 1).

The G52A has its specificity reversed with over a 200-fold preference for homoserine, and the double mutant G52A/P55G has a dramatically enhanced preference for homoserine as the

Table 1. Substrate utilization and specificity of native and mutant serine acetyltransferase[a]

Enzyme form	L-Serine			L-Homoserine			Specificity	
	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})	L-Serine	L-Homoserine
wild type	4.9	95	19	98	88	0.90	21	---
P55G	0.50	37	74	1.0	38	38	2	---
G52A	ND	ND	0.0022	25	13	0.52	---	240
G52A/P55G	ND	ND	0.0029	2	47	24	---	8300

[a] *data are from Oda, et al. [78]*
[b] *ratio of k_{cat}/K_m values for the preferred over the non-preferred substrate*
[c] *not determined*

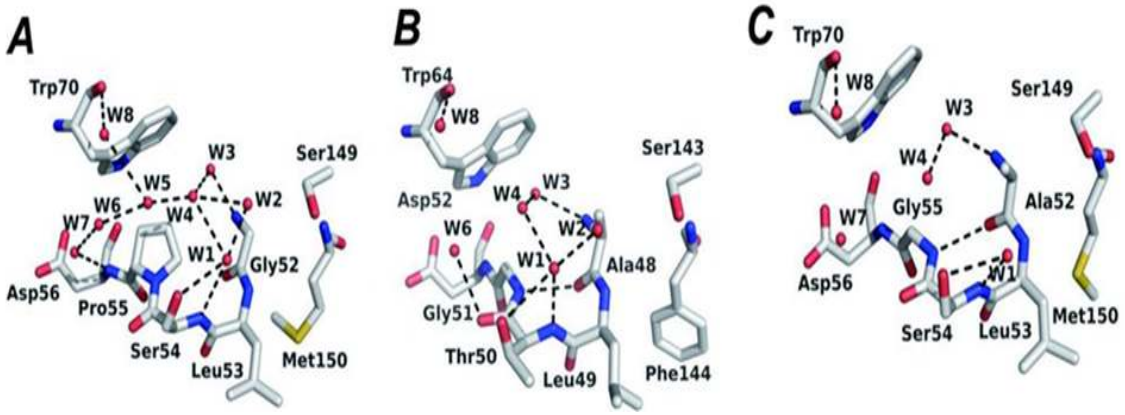


Fig. 8. Changes in the orientation of the β turn in the active site of (A) native DcsE, (B) *Hi*HTA and (C) G52A/P55G mutant of DcsE

(Figure from Oda, et al. [78])

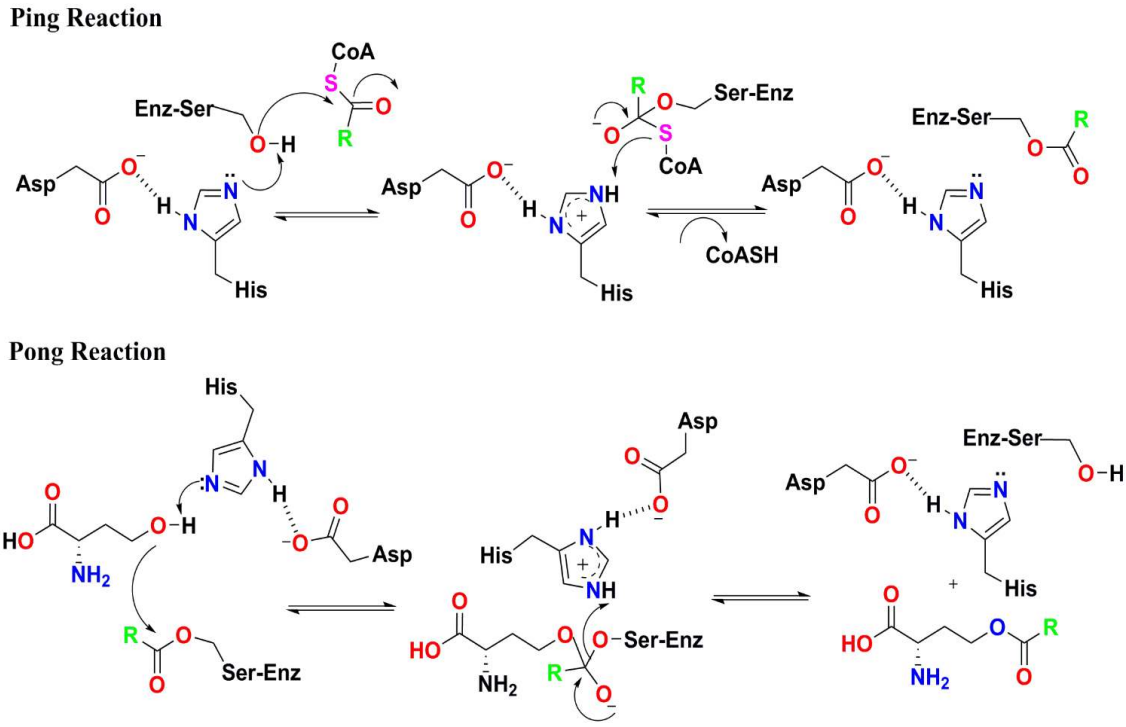


Fig. 9. Ping pong catalytic mechanism of HTA and HTS. The active site nucleophile (serine in HTA or cysteine in HTS) is activated by histidine to form the acyl-enzyme intermediate in the ping reaction. This acyl-enzyme intermediate is then attacked by the substrate hydroxyl group leading to formation of product in the pong reaction

(Mechanism adapted from Born, et al. [24,25])

substrate for acetylation and now can barely utilize its native substrate, serine [78]. Structural support for this change in substrate specificity comes from the structure of the G52A/P55G mutant of DcsE (Fig. 8C), in which the turn structure in this mutant is very similar to that of *Hi*HTA (Fig. 8B), and is now altered from that of the native DcsE structure (Fig. 8A). Additionally, the electron densities of the side chains of Arg218 and Asp353, residues that are involved in binding the serine substrate, are significantly weaker in the mutant enzyme form. This is likely

due to the enhanced flexibility of these side chain residues as a consequence of the disrupted water-mediated hydrogen bonding network near the turn region. This is a classic example of divergent evolution, where structurally similar enzymes have evolved to catalyze the same acyl transfer reaction, but with different substrates.

5. MECHANISM OF HOMOSERINE ACYLTRANSFERASES

Initial mechanistic studies on HTS demonstrated that it exhibits a ping-pong kinetic mechanism [24] wherein the succinyl group from succinyl-CoA is transferred to an active site cysteine, then subsequently transferred to the γ-hydroxyl group of the substrate L-homoserine to generate the product. The HTA and HTS enzymes each catalyze an acyl transfer by this ping-pong mechanism (Fig. 9), facilitated by a catalytic triad consisting of cysteine/serine, histidine and aspartic acid/glutamic acid [25]. The hydroxyl group of serine or the thiol group of cysteine are each activated for nucleophilic attack on acyl-CoA by the histidine, leading to the formation of an acyl-enzyme intermediate with the release of coenzyme A (Ping reaction). The next step involves hydroxyl group attack by the substrate homoserine on the acylated enzyme intermediate leading to the formation of the product, *O*-acylhomoserine, and liberating the enzyme nucleophile (Pong reaction). This reaction mechanism is similar to that employed by hydrolases and thioesterases, except that the acyl moiety from the acyl-enzyme intermediate is not transferred to water but instead specifically to homoserine. In addition to the conserved catalytic triad, a few other active site residues have also been found to be highly conserved. These include an arginine and an aspartic acid, which are involved in substrate binding by interacting with the carboxylate and amine moieties of the homoserine substrate. Additionally, the oxyanion hole formed from the backbone amide nitrogens of leucine and phenylalanine/methionine help to stabilize the tetrahedral transition state. As a result these residues position the hydroxyl group of homoserine for acyl transfer from the acyl-enzyme intermediate. A water molecule attempting to bind at this site lacks this stabilizing effect from these amino acid residues, thereby disfavoring a hydrolysis reaction.

6. STRUCTURES OF HOMOSERINE ACYLTRANSFERASES

6.1 Homoserine Transacetylases

Structural studies to date have determined the high resolution structures of HTAs from *Hemophilus influenza* (*Hi*HTA) [62] (PDB ID 2B61), *Leptospira interrogans* (*Li*HTA) [63] (PDB ID 2PL5) and *S. aureus* (*Sa*HTA) [80] (PDB ID 4QLO). Each of these enzymes shares the same overall structural features. HTA is organized into two distinct domains: the core α/β domain and a helical bundle lid domain forming a canopy over the core domain (Fig. 10A). The core domain consist of an eight-stranded, predominantly parallel β-sheet (in red), with the connectivity of the β-sheet achieved by the five α-helices on one side and one on the opposite side (in yellow). Two short antiparallel beta strands, β2 and β3, provide the connection between β1 and β4. The lid domain is organized into five α-helices (in blue). This domain is connected with the core domain *via* links between αL1 to β8 and αL5 to αD with the intervening residues lacking defined secondary structure. The region constituting the αL4 lid domain is proposed to be quite flexible and would allow structural changes in response to substrate binding.

HTA crystallizes with only one protein molecule in the asymmetric unit. However, these enzymes have been shown by gel filtration and by sedimentation velocity analysis to exist as dimers in solution, with the dimerization occurring through interactions between the two all-α-helical domains [62,63]. Collectively, they form a typical four-helix bundle arrangement in which the core is entirely hydrophobic (Fig. 10B). In *Sa*HTA the four helix bundle is found to be left-handed similar to that seen in *Hi*HTA, while in the *Li*HTA structure this bundle is right-handed. In the *Li*HTA apo-enzyme structure the active site histidine was found to exist in two conformations with similar occupancy, and in the *Sa*HTA structure His296 is also somewhat disordered. In contrast, in the *Hi*HTA structure the active site histidine has well-defined density and exists in a single conformation [62]. This observed conformational flexibility of this active site histidine in the HTAs is consistent with its proposed role in catalysis, allowing this side chain to move into position to extract a proton to generate the enzyme nucleophile.

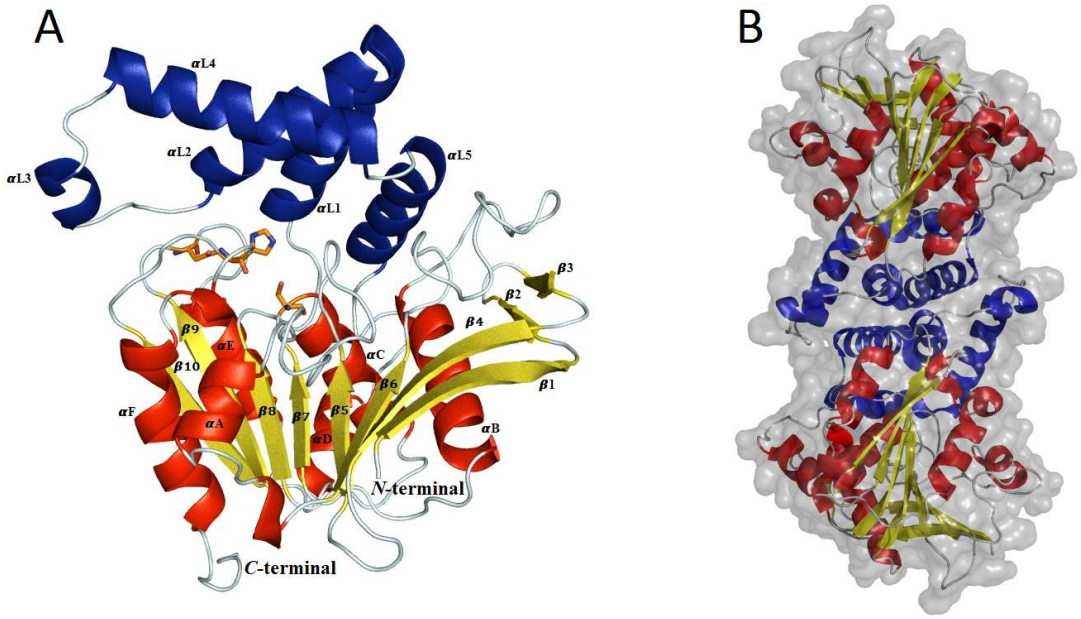


Fig. 10. (A) Structure of *Sa*HTA in ribbon representation. The β-sheets colored yellow and the α-helices colored red comprise the core domain, while the α-helices of the lid domain are colored blue. The catalytic triad constituting Ser, His and Asp are shown by orange sticks. (B) Quaternary structure of HTA showing a typical four-helix bundle arrangement in which the dimerization interface is entirely hydrophobic

(Figure from Thangavelu, et al. [80])

Despite the many similarities, there are some significant structural differences among HTAs. *Sa*HTA is a shorter protein (322 amino acids) when compared to *Li*HTA (366 amino acids) and *Hi*HTA (377 amino acids), but these deletions are primarily located in the loop regions while the structure of the α/β core is preserved (Fig. 11). The loop connecting β6 and αB is shorter in *Sa*HTA [62,63,80]. Similarly, the 15 amino acid loop connecting αL1 and αL2 has been essentially eliminated, as has the 15 amino acid loop between αL3 and αL4, when compared to those regions in *Li*HTA and *Hi*HTA. The most distinct structural feature of the HTAs is the deep tunnel which is formed at the junction of the two domains. The active site serine lies at the end of this tunnel, and this extended substrate binding tunnel serves as the channel for the entry of substrates and exit of the products.

6.2 Homoserine Transsuccinylases

To date only a single HTS structure from *Bacillus cereus* [71,81] (PDB ID 2VDJ/2GHR) has been determined. Like HTA, *Bacillus cereus* HTS (*Bc*HTS) crystallizes with only one subunit in the asymmetric unit. However, *Bc*HTS is organized into a single-domain with a Rossmann fold and the overall topology is different from that of HTA (Fig. 4A). HTS is composed of 11 β-strands, 7 α-helices and 4 3_{10}-helices, with the core protein composed of parallel β-sheet sandwiched by α-helices (Fig. 4B). Analytical size exclusion chromatography and light scattering experiments support the dimeric form of HTS as the functional unit. The other structural feature differentiating HTS from HTA is the mode of dimerization. The *N*-terminal end of the *Bc*HTS monomer exhibits an extended conformation (Fig. 12A) and forms the primary dimerization interface driven by β-strand exchange between the two monomers (Fig. 12B). The Rossmann fold regions of HTA and HTS are comparable, but the lid domain is completely absent in HTS. The catalytic triad constitutes of a classical Cys-His-Glu set of amino acids, and this triad is conserved within the HTS enzyme family. Although the structures of HTA and HTS are quite different from one another, these scaffolds still present a nearly identical active site constellation through convergent evolution that allows proteins with distinctly different structures to catalyze mechanistically identical chemical reactions with the same substrate as the acyl group acceptor.

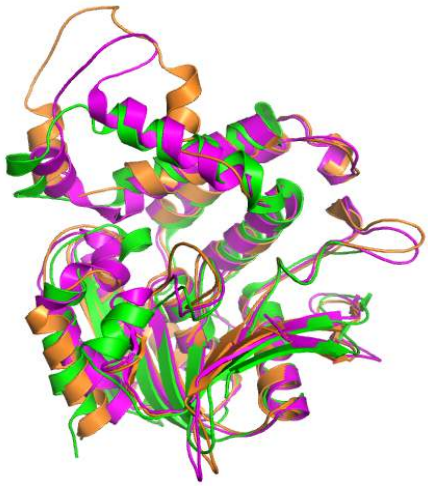


Fig. 11. Ribbon diagram showing superimposition of *Sa*HTA (green), *Hi*HTA (magenta) and *Li*HTA (orange), with the nearly identical α/β core structures but with differences in the size and orientation of the surface loops

(Figure adapted from Thangavelu, et al. [80])

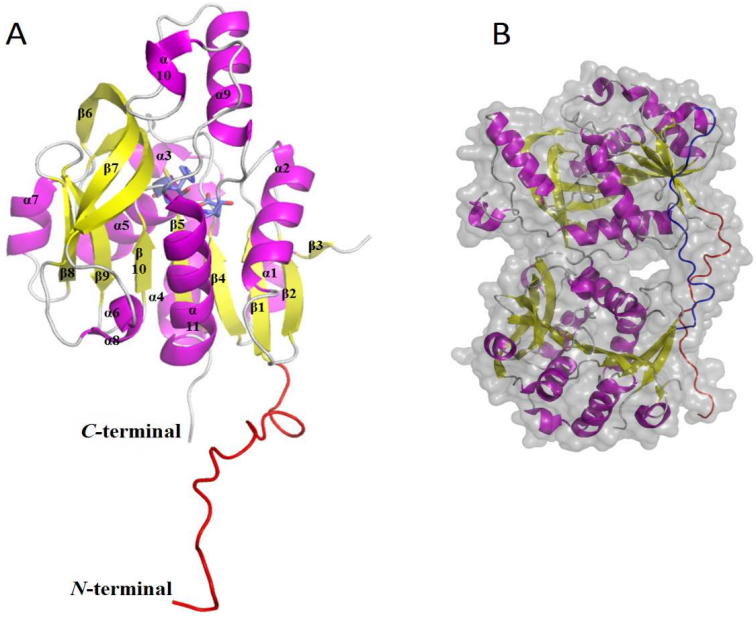


Fig. 12. (A) Structure of the *Bc*HTS subunit organized into a single domain. Alpha helices are colored in magenta, beta sheets are yellow and the *N*-terminal region is shown in red. The catalytic triad constituting Cys, His and Glu are shown by blue sticks. (B) Quaternary structure of *Bc*HTS showing *N*-terminal beta-strand exchange between the monomers. The *N*-terminal region of one monomer is colored red and the other is blue

(Figure adapted from Zubieta, et al. [81])

7. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

There is a growing demand for novel antifungal and antibacterial drug targets that acts through new mechanisms of action to combat the increasing threats of drug resistant microorganisms [82] and the emergence of new pathogens. The critical biochemical roles for the metabolic intermediates and final products, and the absence of a homologous pathway in humans, makes the enzymes [83-86] of the

aspartate pathway attractive targets for drug design [73,80,87]. Gene disruption studies have confirmed the importance of these enzyme-catalyzed reactions in pathogenesis in several models of virulence [84,88]. Close regulation of these enzymes *in vivo*, feedback control by the end products and their absence in mammals are important features that validate the prospects of these enzymes to serve as drug development targets. Several enzymes of this pathway are already being targeted to screen for small molecule inhibitors [89,90]. The uniquely characteristic elongated tunnel in HTA has been identified for the development of potent inhibitors. β-Lactones [87] which acts like β-lactams, are shown to inactivate the HTA enzyme by covalent modification of the active site serine. This strategy is being pursued to develop novel and selective HTA inhibitors [80]. Elaboration and optimization of these initial inhibitor structural scaffolds could provide promising new compounds in the development of specific and potent inhibitors against this target enzyme.

The latest work on *Mycobacterium tuberculosis* (Mtb), has demonstrates this pathogenic organism's dependence on the methionine/ AdoMet biosynthetic pathway for successful infection and survival in host tissues [91]. AdoMet is absolutely required for the biosynthesis of mycolic acid, an essential cell wall component in several bacteria including Mtb. Depletion of methionine and AdoMet in Mtb leads to the complete loss of viability *in vivo* and the inability to proliferate in the host's macrophages. Blockade of the methionine pathway results in the impairment of several essential functions required for growth and for continued viability. Thus, the aspartate pathway provides a number of excellent targets for the development of drugs that can overcome the developing antibiotic resistance of this bacterium and lead to more effective treatment options.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Viola RE. The central enzymes of the aspartate family of amino acid biosynthesis. Acc Chem Res. 2001;34: 339-349.
2. Viola RE, Faehnle CR, Blanco J. Moore RA, Liu X, Arachea BT, et al. The catalytic machinery of a key enzyme in amino Acid biosynthesis. J Amino Acids. 2011;352-538.
3. Borchardt RT. S-Adenosyl-L-methionine-dependent macromolecule methyltransferases: Potential targets for the design of chemotherapeutic agents. J Med Chem. 1980;23:347-357.
4. Zano SP, Bhansali P, Luniwal A, Viola RE. Alternative substrates selective for S-adenosylmethionine synthetases from pathogenic bacteria. Arch Biochem Biophys. 2013;536:64-71.
5. Arevalo-Rodriguez M, Pan X, Boeke JD, Heitman J. FKBP12 controls aspartate pathway flux in Saccharomyces cerevisiae to prevent toxic intermediate accumulation. Eukaryot Cell. 2004;3:1287-1296.
6. Saint-Girons I, Parsot C, Zakin MM, Barzu O, Cohen GN. Methionine biosynthesis in Enterobacteriaceae: Biochemical, regulatory, and evolutionary aspects. CRC Crit Rev Biochem. 1988;23(Suppl 1):S1-42.
7. Val DL, Cronan JE, Jr. *In vivo* evidence that S-adenosylmethionine and fatty acid synthesis intermediates are the substrates for the LuxI family of autoinducer synthases. J Bacteriol. 1998;180:2644-2651.
8. Raybin D, Flavin M. An enzyme tyrosylating alpha-tubulin and its role in microtubule assembly. Biochem Biophys Res Commun. 1975;65:1088-1095.
9. Yang Z, Pascon RC, Alspaugh A, Cox GM, McCusker JH, Molecular and genetic analysis of the Cryptococcus neoformans MET3 gene and a met3 mutant. Microbiology. 2002;148:2617-2625.
10. Hanzelka BL, Greenberg EP. Quorum sensing *in vibrio* fischeri: Evidence that S-adenosylmethionine is the amino acid substrate for autoinducer synthesis. J Bacteriol. 1996;178:5291-5294.
11. Schaefer AL, Hanzelka BL, Eberhard A. Greenberg EP, Quorum sensing *in vibrio* fischeri: Probing autoinducer-LuxR interactions with autoinducer analogs. J Bacteriol. 1996;178:2897-2901.

12. Huisman GW, Kolter R. Sensing starvation: A homoserine lactone--dependent signaling pathway in *Escherichia coli.* Science. 1994;265:537-539.
13. Marzluf GA. Genetics and molecular genetics of sulfur assimilation in the fungi. Adv Genet. 1994;31:187-206.
14. Brzywczy J, Sienko M, Kucharska A, Paszewski A. Sulphur amino acid synthesis in Schizosaccharomyces pombe represents a specific variant of sulphur metabolism in fungi. Yeast. 2002;19:29-35.
15. Marzluf GA. Molecular genetics of sulfur assimilation in filamentous fungi and yeast. Annu Rev Microbiol. 1997;51:73-96.
16. Umbarger HE. Amino acid biosynthesis and its regulation. Annu Rev Biochem. 1978;47:532-606.
17. Thomas D, Surdin-Kerjan Y. Metabolism of sulfur amino acids in Saccharomyces cerevisiae. Microbiol Mol Biol Rev. 1997;61:503-532.
18. Gophna U, Bapteste E, Doolittle WF, Biran D, Ron EZ. Evolutionary plasticity of methionine biosynthesis. Gene. 2005;355: 48-57.
19. Nagai S, Flavin M. Acetylhomoserine. An intermediate in the fungal biosynthesis of methionine. J Biol Chem. 1967;242:3884-3895.
20. Rowbury RJ. The accumulation of o-succinylhomoserine by *Escherichia coli* and Salmonella Typhimurium. J Gen Microbiol. 1964;37:171-180.
21. Rowbury RJ. Synthesis of cystathionine and Its control in salmonella typhimurium. Nature. 1964;203:977-978.
22. Rowbury RJ, Woods DD, o-succinylhomoserine as an intermediate in the synthesis of cystathionine by *Escherichia coli.* J Gen Microbiol. 1964;36:341-358.
23. Hwang BJ, Kim Y, Kim HB, Hwang HJ, Kim JH, Lee HS. Analysis of Corynebacterium glutamicum methionine biosynthetic pathway: Isolation and analysis of metB encoding cystathionine gamma-synthase. Molecules and Cells. 1999;9:300-308.
24. Born TL, Blanchard JS. Enzyme-catalyzed acylation of homoserine: Mechanistic characterization of the *Escherichia coli* metA -Encoded homoserine transsuccinylase. Biochemistry. 1999; 38:14416-14423.
25. Born TL, Franklin M, Blanchard JS. Enzyme-catalyzed aclyation of homoserine: Mechanistic characterization of the hemophilus influenzae met2 - encoded homoserine transacetylase. Biochemistry. 2000;39:8556-8564.
26. Ravanel S, Gakiere B, Job D, Douce R. The specific features of methionine biosynthesis and metabolism in plants. Proc Natl Acad Sci USA. 1998;95:7805-7812.
27. Curien G, Job D, Douce R, Dumas R. Allosteric activation of Arabidopsis threonine synthase by S-adenosylmethionine. Biochemistry. 1998; 37:13212-13221.
28. Lee M, Leustek T. Identification of the gene encoding homoserine kinase from Arabidopsis thaliana and characterization of the recombinant enzyme derived from the gene. Arch Biochem Biophys. 1999;372:135-142.
29. Lee LW, Ravel JM, Shive W. Multimetabolite control of a biosynthetic pathway by sequential metabolites. J Biol Chem. 1966;241:5479-5480.
30. Yamagata S. O-acetylhomoserine sulfhydrylase of the fission yeast schizosaccharomyces-pombe - partial-purification, characterization, and its probable role in homocysteine biosynthesis. Journal of Biochemistry. 1984;96:1511-1523.
31. Yamagata S, Roles of O-Acetyl-L-Homoserine sulfhydrylases in Microorganisms. Biochimie. 1989;71:1125-1143.
32. Goujon M, McWilliam H, Li WZ, Valentin F, Squizzato S, Paern J, et al. A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Res. 2010; 38:W695-W699.
33. Foglino M, Borne F, Bally M, Ball G, Patte JC. A direct sulfhydrylation pathway is used for methionine biosynthesis in *Pseudomonas aeruginosa.* Microbiology. 1995;141(Pt 2):431-439.
34. Vermeij P, Kertesz MA. Pathways of assimilative sulfur metabolism in Pseudomonas putida. J Bacteriol. 1999;181:5833-5837.
35. Goudarzi M, Born TL, Purification and characterization of *Thermotoga maritima* homoserine transsuccinylase indicates it is a transacetylase. Extremophiles. 2006;10: 469-478.

36. Hacham Y, Gophna U, Amir R. *In vivo* analysis of various substrates utilized by cystathionine gamma-synthase and O-acetylhomoserine sulfhydrylase in methionine biosynthesis. Mol Biol Evol. 2003;20:1513-1520.
37. Ravanel S, Gakiere B, Job D, Douce R. The specific features of methionine biosynthesis and metabolism in plants. Proc Natl Acad Sci USA. 1998;95:7805-7812.
38. Kanzaki H, Kobayashi M, Nagasawa T, Yamada H. Distribution of 2 Kinds of Cystathionine Gamma-Synthase in Various Bacteria. Fems Microbiology Letters. 1986;33:65-68.
39. Kanzaki H, Nagasawa T, Yamada H. Highly efficient production of L-cystathionine from o-succinyl-l-homoserine and l-cysteine by streptomyces cystathionine gamma-lyase. Applied Microbiology and Biotechnology. 1986;25:97-100.
40. Ravanel S, Gakiere B, Job D, Douce R. Cystathionine gamma-synthase from Arabidopsis thaliana: Purification and biochemical characterization of the recombinant enzyme overexpressed in *Escherichia coli.* Biochemical Journal. 1998;331:639-648.
41. Hwang BJ, Yeom HJ, Kim Y, Lee HS. Corynebacterium glutamicum utilizes both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis. J Bacteriol. 2002;184:1277-1286.
42. Auger S, Yuen WH, Danchin A, Martin-Verstraete I. The metIC operon involved in methionine biosynthesis in Bacillus subtilis is controlled by transcription antitermination. Microbiology-Sgm. 2002; 148:507-518.
43. Simon M, Hong JS. Direct homocysteine biosynthesis from o-succinylhomoserine in *Escherichia coli* - An Alternate Pathway That Bypasses Cystathionine. J Bacteriol. 1983;153:558-561.
44. Krishnamoorthy K, Begley TP, Protein thiocarboxylate-dependent methionine biosynthesis in Wolinella succinogenes. J Am Chem Soc. 2011;133:379-386.
45. Tran TH, Krishnamoorthy K, Begley TP. Ealick SE. A novel mechanism of sulfur transfer catalyzed by o-acetylhomoserine sulfhydrylase in the methionine-biosynthetic pathway of Wolinella succinogenes. Acta Crystallogr D Biol Crystallogr. 2011;67:831-838.
46. Lee HS, Hwang BJ. Methionine biosynthesis and its regulation in Corynebacterium glutamicum: Parallel pathways of transsulfuration and direct sulfhydrylation. Applied Microbiology and Biotechnology. 2003;62:459-467.
47. Cremer J, Treptow C, Eggeling L, Sahm H. Regulation of enzymes of lysine biosynthesis in corynebacterium-glutamicum. J Gen Microbiol. 1988;134: 3221-3229.
48. Phillips SEV, Stockley PG. Structure and function of *Escherichia coli* met repressor: Similarities and contrasts with trp repressor. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences. 1996;351:527-535.
49. Weissbach H, Brot N. Regulation of methionine synthesis in *Escherichia-coli.* Molecular Microbiology. 1991;5:1593-1597.
50. Wild CM, McNally T, Phillips SEV, Stockley PG. Effects of systematic variation of the minimal *Escherichia coli* met consensus operator site: *In vivo* and *in vitro* met repressor binding. Molecular Microbiology. 1996;21:1125-1135.
51. Urbanowski ML, Stauffer LT, Plamann LS, Stauffer GV. A new methionine locus, metr, that encodes a trans-acting protein required for activation of mete and meth in *Escherichia-coli* and salmonella-typhimurium. J Bacteriol. 1987;169:1391-1397.
52. Kase H, Nakayama K. Production of o-Acetyl-L-Homoserine by methionine analog-resistant mutants and regulation of Homoserine-O-Transacetylase in corynebacterium-glutamicum. Agricultural and Biological Chemistry. 1974;38:2021-2030.
53. Ozaki H, Shiio I. Methionine biosynthesis in brevibacterium-flavum - properties and essential role of O-Acetylhomoserine sulfhydrylase. Journal of Biochemistry. 1982;91:1163-1171.
54. Wyman A, Paulus H. Purification and properties of homoserine transacetylase from bacillus-polymyxa. Journal of Biological Chemistry. 1975;250:3897-3903.
55. Wyman A, Shelton E, Paulus H. Regulation of homoserine transacetylase in whole cells of bacillus-polymxa. Journal of Biological Chemistry. 1975;250:3904-3908.
56. Ron EZ, Shani M, Growth rate of *Escherichia-coli* at elevated temperatures -

reversible inhibition of homoserine trans-succinylase. J Bacteriol. 1971;107:397.
57. Ron EZ. Growth-rate of *enterobacteriaceae* at elevated-temperatures - limitation by methionine. J Bacteriol. 1975;124:243-246.
58. Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, et al. The alpha/beta hydrolase fold. Protein Eng. 1992;5:197-211.
59. Nardini M, Dijkstra BW. Alpha/beta hydrolase fold enzymes: The family keeps growing. Curr Opin Struct Biol. 1999;9:732-737.
60. Heikinheimo P, Goldman A, Jeffries C, Ollis DL. Of barn owls and bankers: A lush variety of alpha/beta hydrolases. Structure. 1999;7:R141-146.
61. Holmquist M. Alpha/Beta-hydrolase fold enzymes: Structures, functions and mechanisms. Curr Protein Pept Sci. 2000;1:209-235.
62. Mirza IA, Nazi I, Korczynska M, Wright GD, Berghuis AM. Crystal structure of homoserine transacetylase from Haemophilus influenzae reveals a new family of alpha/beta-hydrolases. Biochemistry. 2005;44:15768-15773.
63. Wang M, Liu L, Wang Y, Wei Z, Zhang P, Li Y, et al. Crystal structure of homoserine O-acetyltransferase from Leptospira interrogans. Biochem Biophys Res Commun. 2007;363:1050-1056.
64. Jiang Y, Morley KL, Schrag JD, Kazlauskas RJ. Different active-site loop orientation in serine hydrolases versus acyltransferases. Chembiochem. 2011; 12:768-776.
65. Brumlik MJ, Buckley JT. Identification of the catalytic triad of the lipase/acyltransferase from Aeromonas hydrophila. J Bacteriol. 1996;178:2060-2064.
66. Milkowski C, Strack D. Serine carboxypeptidase-like acyltransferases. Phytochemistry. 2004;65:517-524.
67. Frenken LG, Egmond MR, Batenburg AM, Bos JW, Visser C, Verrips CT. Cloning of the Pseudomonas glumae lipase gene and determination of the active site residues. Appl Environ Microbiol. 1992;58:3787-3791.
68. Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem. 1997;272:27218-27223.
69. Muller J, Sowa MA, Fredrich B, Brundiek H, Bornscheuer UT. Enhancing the acyltransferase activity of candida antarctica lipase a by rational design. Chembiochem. 2015;16:1791-1796.
70. Rauwerdink A, Kazlauskas RJ. How the same core catalytic machinery catalyzes 17 different reactions: The serine-histidine-aspartate catalytic triad of alpha/beta-hydrolase fold enzymes. Acs Catalysis. 2015;5:6153-6176.
71. Zubieta C, Arkus KA, Cahoon RE, Jez JM. A single amino acid change is responsible for evolution of acyltransferase specificity in bacterial methionine biosynthesis. J Biol Chem. 2008;283:7561-7567.
72. Ziegler K, Yusupov M, Bishop B, Born TL. Substrate analysis of homoserine acyltransferase from Bacillus cereus. Biochem Biophys Res Commun. 2007;361:510-515.
73. Nazi I, Wright GD, Catalytic mechanism of fungal homoserine transacetylase. Biochemistry. 2005;44:13560-13566.
74. Jensen RA, Enzyme recruitment in evolution of new function. Annu Rev Microbiol. 1976;30:409-425.
75. Penning TM, Jez JM. Enzyme redesign. Chem Rev. 2001;101:3027-3046.
76. Johnson CM, Roderick SL, Cook PF. The serine acetyltransferase reaction: Acetyl transfer from an acylpantothenyl donor to an alcohol. Arch Biochem Biophys. 2005;433:85-95.
77. Pye VE, Tingey AP, Robson RL, Moody PC. The structure and mechanism of serine acetyltransferase from Escherichia coli. J Biol Chem. 2004;279:40729-40736.
78. Oda K, Matoba Y, Kumagai T, Noda M, Sugiyama M. Crystallographic study to determine the substrate specificity of an L-serine-acetylating enzyme found in the D-cycloserine biosynthetic pathway. J Bacteriol. 2013;195:1741-1749.
79. Humphrey W, Dalke A, Schulten K. VMD: Visual molecular dynamics. Journal of Molecular Graphics & Modelling. 1996;14:33-38.
80. Thangavelu B, Pavlovsky AG, Viola R, Structure of homoserine O-acetyltransferase from Staphylococcus aureus: The first Gram-positive ortholog structure. Acta Crystallogr F Struct Biol Commun. 2014;70:1340-1345.

81. Zubieta C, Krishna SS, McMullan D, Miller MD, Abdubek P, Agarwalla S, et al. Crystal structure of homoserine O-succinyltransferase from Bacillus cereus at 2.4 angstrom resolution. Proteins-Structure Function and Bioinformatics. 2007;68:999-1005.
82. Walsh C. Where will new antibiotics come from? Nat Rev Microbiol. 2003;1:65-70.
83. Ejim L, Mirza IA, Capone C, Nazi I, Jenkins S, Chee GL, et al. New phenolic inhibitors of yeast homoserine dehydrogenase. Bioorg Med Chem. 2004;12:3825-3830.
84. Pascon RC, Ganous TM, Kingsbury JM, Cox GM, McCusker JH. Cryptococcus neoformans methionine synthase: Expression analysis and requirement for virulence. Microbiology. 2004;150:3013-3023.
85. Yamaguchi H, Uchida K, Hiratani T, Nagate T, Watanabe N, Omura S. RI-331, a new antifungal antibiotic. Ann N Y Acad Sci. 1988;544:188-190.
86. Ejim LJ, D'Costa VM, Elowe NH, Loredo-Osti JC, Malo D, Wright GD. Cystathionine beta-lyase is important for virulence of *Salmonella enterica* serovar typhimurium. Infect Immun. 2004;72:3310-3314.
87. De Pascale G, Nazi I, Harrison PH, Wright GD. Beta-lactone natural products and derivatives inactivate homoserine transacetylase, a target for antimicrobial agents. J Antibiot (Tokyo). 2011;64:483-487.
88. Nazi I, Scott A, Sham A, Rossi L, Williamson PR, Kronstad JW, et al. Role of homoserine transacetylase as a new target for antifungal agents. Antimicrob Agents Chemother. 2007;51:1731-1736.
89. Gao G, Liu X, Pavlovsky A, Viola RE, Identification of selective enzyme inhibitors by fragment library screening. J Biomol Screen. 2010;15:1042-1050.
90. Thangavelu B, Bhansali P, Viola RE, Elaboration of a fragment library hit produces potent and selective aspartate semialdehyde dehydrogenase inhibitors. Bioorg Med Chem. 2015;23:6622-6631.
91. Berney M, Berney-Meyer L, Wong KW, Chen B, Chen M, Kim J, et al. Essential roles of methionine and s-adenosylmethionine in the autarkic lifestyle of Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2015;112:10008-10013.

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