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The Radical SAM Superfamily

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ABSTRACT The radical *S*-adenosylmethionine (SAM) superfamily currently comprises more than 2800 proteins with the amino acid sequence motif CxxxCxxC unaccompanied by a fourth conserved cysteine. The charcteristic three-cysteine motif nucleates a [4Fe–4S] cluster, which binds SAM as a ligand to the unique Fe not ligated to a cysteine residue. The members participate in more than 40 distinct biochemical transformations, and most members have not been biochemically characterized. A handful of the members of this superfamily have been purified and at least partially characterized. Significant mechanistic and structural information is available for lysine 2,3-aminomutase, pyruvate formate-lyase, coproporphyrinogen III oxidase, and MoaA required for molybdopterin biosynthesis. Biochemical information is available for spore photoproduct lyase, anaerobic ribonucleotide reductase activation subunit, lipoyl synthase, and MiaB involved in methylthiolation of isopentenyladenine-37 in tRNA. The radical SAM enzymes biochemically characterized to date have in common the cleavage of the $[4Fe–4S]^{1+}$–SAM complex to $[4Fe–4S]^{2+}$–Met and the 5′-deoxyadenosyl radical, which abstracts a hydrogen atom from the substrate to initiate a radical mechanism.

KEYWORDS radical SAM superfamily, S-adenosylmethionine, iron-sulfur clusters, 5′-deoxyadenosyl radical

DISCOVERY OF THE RADICAL SAM SUPERFAMILY

Lysine 2,3-Aminomutase

Early research on lysine metabolism in *Clostridium subterminale* revealed the interconversion of L-lysine and L-β-lysine–hereafter lysine and β-lysine–catalyzed by the enzyme, lysine 2,3-aminomutase (LAM) (Chirpich *et al.*, 1970; Zappia & Barker, 1970). The enzyme was sensitive to air and was initially purified and assayed under a blanket of argon at the laboratory bench. Like many amino acid metabolizing enzymes, LAM was found to contain pyridoxal-5′-phosphate (PLP), and addition of PLP to purified LAM enhanced the activity. LAM also contained traces of iron, and activity was enhanced by addition of ferrous ions. Most significantly, the activity absolutely depended on the addition of dithionite and *S*-adenosylmethionine (SAM).

The coenzyme-dependence of LAM was surprising at the time. The reaction proceeded according to Eq. (1), with the α-amino group of lysine migrating to the β-carbon in β-lysine. Careful stereochemical experiments showed the stereochemistry to be as in Eq. (1), the amino group migrating with inversion of configuration at C3 and the 3-pro-*R* hydrogen of

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lysine migrating to the 2-pro-*R* position of β-lysine (Aberhart *et al.*, 1983).

(1)

Unlike other PLP-dependent enzymes (Frey & Hegeman, 2007a; Eliot & Kirsch, 2004), LAM did not catalyze the exchange of substrate-protons with solvent-protons; thus, in D_2O there was no incorporation of deuterium into lysine or β-lysine. The activity was enhanced when the enzyme was subjected to reductive pre-incubation with glutathione, ferrous ions, and PLP for more than 1 hour before assaying. Even then, no activity could be detected unless both SAM and dithionite were added with lysine to start the reaction, and the apparently homogeneous enzyme then displayed activities between 3 and 6 U mg^{-1} at pH 8 and 37°C. Unlike other SAM-dependent reactions, LAM employed SAM as a true coenzyme to facilitate catalysis, not as a substrate as in methyltransferases (Frey & Hegeman, 2007b; Tagusagawa *et al.*, 1998).

The reaction of Eq. (1) was typical of adenosylcobalamin-dependent rearrangements (Frey & Hegeman, 2007a; Reed, 2004), in which a functional group underwent a 1,2-migration concomitant with the cross-2,1-migration of a hydrogen atom, but the reaction of LAM did not require a vitamin B_{12} coenzyme. Should the 5′-deoxyadenosyl moiety of SAM function in the same way in the action of LAM as it does in reactions of adenosylcobalamin in coenzyme B12-dependent reactions, then SAM might be an evolutionary predecessor of adenosylcobalamin. A predecessor could be expected to exist because the biosynthesis of adenosylcobalamin required some thirty steps (Scott & Roessner, 2002). The end-product of such a long biosynthetic pathway could not be expected to arise solely from the survival benefits of organisms acquiring it for metabolism unless the biosynthetic intermediates were themselves beneficial to life. The very early heme and cobalamin precursor uroporphyrinogen III would have been beneficial, but subsequent cobalamin intermediates were not known to be biologically important. A predecessor such as SAM could provide a biochemical context for radical mechanisms involving the 5′-deoxyadenosyl radical: It was pointed out that "…lysine 2,3-aminomutase may also shed light on the evolutionary origin of coenzymes that potentiate free radical formation" (Frey & Moss, 1987). Within such a context, a much more complex molecule than SAM, such as adenosylcobalamin, could arise through evolution in incremental steps (Frey, 1993).

Furthermore, the requirement of LAM for PLP could not be explained on the basis of the traditional role of PLP in catalysis, namely the stabilization of amino acid carbanions (Frey & Hegeman, 2007a; Eliot & Kirsch, 2004). A new mechanistic principle for the action of PLP was required. The ability of PLP to facilitate radical rearrangements came to light in the mechanism of action of LAM.

The foregoing considerations stimulated interest in mechanistic analysis, leading to the discovery that the 5′-deoxyadenosyl moiety of SAM mediated hydrogen transfer in the action of LAM (Moss & Frey, 1987; Baraniak *et al.*, 1989), just as the 5′-deoxyadenosyl moiety of adenosylcobalamin had been found to mediate hydrogen transfer in coenzyme B_{12}-dependent enzymatic reactions. The postulate was put forward that SAM served as a source of the 5′-deoxyadenosyl radical in the action of LAM.

When purified in an anaerobic chamber, the activity of LAM was more than tenfold higher than when purified at the bench under Ar. The resulting protein displayed a visible absorption spectrum typical of iron-sulfur proteins, and it released hydrogen sulfide when treated with acid. EPR analysis revealed the presence a [4Fe–4S] cluster (Petrovich *et al.*, 1991; 1992). Thus, LAM functioned with SAM and a [4Fe–4S] cluster to catalyze a process analogous to those facilitated by adenosylcobalamin.

Pyruvate Formate-Lyase Activase.

Under conditions of anaerobic growth, *Escherichia coli* was found to metabolize pyruvate differently than in aerobic growth. Aerobically, the pyruvate dehydrogenase complex catalyzed the NAD- and CoA-dependent decarboxylation and dehydrogenation of pyruvate into CO_2, NADH, and acetyl-CoA. However, in anaerobic growth pyruvate was converted with CoA into acetyl-CoA and formate in a reaction catalyzed by pyruvate-formate lyase (PFL). PFL appeared to be activated by SAM (Knappe & Schmitt, 1976), and this was traced to the action of a PFL activase (Knappe *et al.*, 1984), which catalyzed the Fe^{2+}-dependent cleavage of SAM into methionine and 5′-deoxyadenosine concomitant with the generation of a free radical signal in PFL (Knappe

et al., 1984). The PFL activase was postulated to act by generating the 5′-deoxyadenosyl radical from SAM, with the PFL-radical arising from abstraction of a C_2(H) from Gly734 by the 5-deoxyadenosyl radical according to Eq. 2 (Wagner *et al.*, 1992).

$$\text{PFL - Gly}^{734} - \text{H} + \text{SAM} \longrightarrow \text{PFL - Gly}^{734}\cdot + \text{Met} + 5'\text{-dAdo} \quad (2)$$

PFL was the first glycyl-radical enzyme, and the glycyl radical participated in catalyzing the cleavage of pyruvate into acetyl-CoA and formate. The PFL activase proved to be the first SAM-dependent activase in a family of activases for glycyl-radical enzymes. When cloned, overexpressed in *E. coli* and purified, PFL activase turned out to be an iron-sulfur protein (Broderick *et al.*, 1997; Kulzer *et al.*, 1998; Broderick *et al.*, 2000; Krebs *et al.*, 2000).

Biotin Synthase

Biotin synthase was discovered as the product of *bioB*, a gene required for the biosynthesis of biotin from dethiobiotin (Sanyal *et al.*, 1994; Florentin, *et al.*, 1994). The purified protein contained iron and acid-labile sulfide. Studies were made difficult by the inability to observe catalytic turnover of biotin synthase and actual catalysis of the formation of D-biotin from dethiobiotin. However, the slow transformation of dethiobiotin into D-biotin could be observed in less than one turnover of biotin synthase in a reaction that required the presence of SAM and led to the production of methionine and 5′-deoxyadenosine. Biotin synthase was found to be a [4Fe–4S] protein (Duin *et al.*, 1997; Ollagnier-De Choudens *et al.*, 2000).

Ribonucleoside Triphosphate Reductase III

E. coli grown under anaerobic conditions could not use the Class I ribonucleotide reductase to produce deoxyribonucleotides because of the requirement for molecular oxygen to produce the tyrosyl radical in the Class I reductase (Nordlund & Reichard, 2006). Under anaerobic growth conditions, *E. coli* produced a different reductase, an anaerobic ribonucleotide reductase (ARR), a Class III reductase, which turned out to be a glycyl radical enzyme (Eliasson *et al.*, 1990; ibid, 1992; Harder *et al.*, 1992). One subunit of the ARR proved to be an ARR activase, which following the precedent of PFL activase, catalyzed the radicalization of Gly681 in the catalytic subunit in a SAM-dependent process according to Eq. (3) (King & Reichard, 1995). ARR activase also contained a [4Fe–4S] cluster (Mulliez *et al.*, 1993; Ollagnier *et al.*, 1996; Ollagnier *et al.*, 1997; Tamarit *et al.*, 1999; 2000; Liu & Graslund, 2000).

$$\text{ARR - Gly}^{681} - \text{H} + \text{SAM} \longrightarrow \text{ARR - Gly}^{681}\cdot + \text{Met} + 5\text{'-dAdo} \quad (3)$$

As in the action of PFL activase, the 5′-deoxyadenosyl radical from reductive cleavage of SAM was implicated in the abstraction of an α-hydrogen from Gly681.

Lipoyl Synthase

The discovery of *lipA* as required for the biosynthesis of lipoic acid opened the way for the identification of its product, lipoyl synthase or LipA, as the enzyme that catalyzed the transformation of an activated, octanoyl form of octanate into the dihydrolipoyl group (Hayden *et al.*, 1993; Reed & Cronan, 1993; Miller *et al.*, 2000). Lipoyl synthase proved to be an iron-sulfur protein as well (Busby *et al.*, 1999; Ollangier-De Choudens & Fontecave, 1999). Like biotin synthase, lipoyl synthase catalyzed the insertion of sulfur into unreactive C–H bonds. Unlike biotin synthase, two sulfur atoms were inserted, one each into the C6–H and C8–H bonds of the octanoyl group.

The foregoing SAM-dependent enzymes catalyzed diverse reactions but had mechanistic properties in common (Frey & Magnusson, 2003). All contained iron-sulfur clusters, all cleaved SAM into methionine and 5′-deoxyadenosine, and all appeared to use the 5′-deoxyadenosyl radical in a hydrogen abstraction role. By the year 2000, the amino acid sequences of these enzymes were available from translation of the cloned genes and could be compared. Inspection of these sequences led to the identification of a common cysteine motif, CxxxCxxC shown below,

LAM	...CSMYCRHCTRR...
PFL activase	...CLMRCLYCHNR...
ARR	...CVHECPGCYNK...
BioB	...CPEDCYKCPQS...
LipA	...CTRRCPFCDVA...

comprising three conserved cysteine residues separated by three and two nonconserved residues, respectively,

with no fourth conserved cysteine (Ruzicka *et al.*, 2000; Wong *et al.*, 1993; Reed & Cronan, 1993). This motif was consistent with and explained the physicochemical properties of the [4Fe–4S] cluster in LAM, which seemed to include a ligand other than cysteine for one iron (Petrovich *et al.*, 1992). A search of the protein sequence database for this motif, together with a SAM binding motif, netted more than 580 proteins in 2001, and this group was named the radical SAM superfamily (Sofia *et al.*, 2001). Some of the original members were species variants of LAM, PFL activase, biotin synthase, ARR, and lipoyl synthase. Nevertheless, the superfamily included many other proteins that had not before been related to SAM, nor to iron-sulfur proteins, nor to radical biochemistry. Since the discovery of the superfamily, new members have been found through the widespread recognition of the significance of the motif CxxxCxxC and the release of new genomic sequences. At this writing, at least 2845 proteins with the motif CxxxCxxC can be identified in 781 microbial genomes.

Currently recognized members of the radical SAM superfamily are listed in Table 1, together with what is known of their biological functions. The members are engaged in promoting a remarkably diverse collection of biochemical processes, including aminomutase-action; radicalization of glycyl residues in glycyl radical enzymes; repair of thymine dimers in DNA, formylglycine formation in sulfatases; biosynthesis of vitamins and coenzymes such as thiamine, lipoic acid, biotin, molybdopterin, pyrroloquinoline quinone, and cysteinyltopaquinone; the thiomethylation of N^6-isopentenyladenine in tRNA; the biosynthesis of a number of antibiotics, and the maturation of metallocofactors associated with enzymes. More than 40 distinct enzymatic functions are currently attributed to members of the radical SAM superfamily.

STRUCTURES OF RADICAL SAM ENZYMES

Structures of four Radical SAM enzymes are currently available, those of HemN, biotin synthase (BioB), MoaA, and LAM (Figure 1). All four display subunit-folds related to the β-barrel or TIM barrel, in which β-strands are arranged in a barrel-like array, with peripheral helices intervening between β-strands. The [4Fe–4S] clusters and substrates are bound within the barrels, as is typical of TIM barrel enzymes. Of the available structures, only biotin synthase has a complete TIM barrel, with eight β,α-pairs, shown in a side-view in Figure 1. HemN, MoaA, and LAM incorporate 3/4-barrels resembling crescents when viewed along axes down the center, as shown in Figure 1. The structures of several members have been compared (Layer *et al.*, 2005; Marsh *et al.*, 2004).

In a correlation of fold with amino acid sequences among family members, it was concluded that most members would be likely to be 3/4-barrels, $(\alpha\beta)6$ (Nicolet & Drennan, 2004), as in HemN, MoaA, & LAM (Figure 1). The most primitive members were predicted to have 1/2-barrel structures, $(\alpha\beta)4$, with a few recently evolved members displaying full TIM barrels, $(\alpha\beta)8$. The analysis indicated that the activating subunit of ARR would be a very primitive 1/2-barrel, $(\alpha\beta)4$-enzyme and an evolutionary precursor of other radical SAM enzymes. Because of the requirement for 2′-deoxyribonucleotides in the DNA world, ARR might have been required for DNA biosynthesis in anaerobic evolution, and if so it could have been the origin of free radical mechanisms involving SAM. Thus, ARR might have established the biochemical context for the evolution of enzymes catalyzing reactions initiated by the 5′-deoxyadenosyl radical. In this way, SAM could have been a true predecessor of adenosylcobalamin.

MEMBERS THAT USE SAM CATALYTICALLY AS A COENZYME

Radical SAM enzymes use SAM either catalytically or as an oxidizing substrate. In its catalytic reactions, SAM participates chemically as a reversible source of the 5′-deoxyadenosyl radical; and SAM is regenerated in each catalytic cycle. In these reactions, the 5′-deoxyadenosyl radical from SAM is a reversible oxidizing agent. SAM also functions noncatalytically as a substrate and net oxidant in many reactions of Radical SAM enzymes (Wang & Frey, 2007). This section deals with enzymes that use SAM as a reversible source of the 5′-deoxyadenosyl radical.

Aminomutases

Aminomutases catalyze the reversible intramolecular migration of an amino group between adjacent carbon atoms of a substrate. Lysine 2,3-aminomutase, a founding member of the Radical SAM superfamily, is the most intensively studied and most thoroughly characterized aminomutase. Originally described as a hexameric enzyme (Chirpich *et al.*, 1970), based on analysis

TABLE 1 **Radical SAM enzymes and associated biological functions**

Protein	Function	Reference
LAM	Lysine 2,3aminomutase	Ruzicka *et al.*, 2000
BlsG	Arginine 2,3-aminomutase	Cone *et al.*, 2003
Eam	Glutamate 2,3-aminomutase	Ruzicka & Frey, 2007
SplB	Spore photoproduct lyase	Rebeil *et al.*, 1998
DesII	Desosamine biosynthesis	Trefzer *et al.*,1999
Littorine mutase	Alkaloid biosynthesis	Ollagnier *et al.*, 1998
PFL activase	Glycyl radicalization	Wong *et al.*, 1993
ARR activase	Glycyl radicalization	Eliasson *et al.*, 1990
BssD	Glycyl radicalization	Heider *et al.*, 1999
Glycerol dehydratase activase	Glycyl radicalization	O'Brien *et al.*, 2004
Hydroxyphenylacetate decarboxylase activase	Glycyl radicalization	Yu *et al.*, 2004
BioB	Biotin synthase	Duin *et al.*, 1997
LipA	Lipoyl synthase	Reed & Cronan, 1993
BchE	Bacteriochlorophyll biosynthesis	Suzuki *et al.*, 1997
HemN	Coproporphyrinogen III oxidase	Akhtar, 1994
MoaA	Molybdopterin biosynthesis	Rieder *et al.*, 1998
MiaB	Methylthiolation of tRNA	Esberg *et al.*, 1999
TYW1	Wybusine biosynthesis in $tRNA^{Phe}$	Nona *et al..*, 2006
ThiH	Biogenesis of thiazole in thiamine	Begley *et al.*, 1999
PqqE	Pyrroloquinoline quinone biosynthesis	Goodwin & Anthony, 1998
NifB	Nitrogenase FeMoCo maturation	Allen *et al.*, 1995
AtsB	Formylglycine formation in sulfatases	Fang *et al.*, 2004
ExsD	Succinoglycan production	Becker *et al.*, 1995
SpcY	Spectinomycin biosynthesis	Lyutzkanova *et al.*, 1997
AlbA	Subtilosin biosynthesis	Zhang *et al.*, 1999
SanA	Nicomycin biosynthesis	Möhrle *et al.*, 1995
BcpD	Bialaphos biosynthesis	Thompson & Seto, 1995
MitD/MmcD	Mitomycin C biosynthesis	Mao *et al.*, 1999
OxsB	Oxetanocin biosynthesis	Morita *et al.*, 1999
Fms7	Fortimicin biosynthesis	Kuzuyama *et al.*, 1995
Fom3	Fosfomycin biosynthesis	Kuzuyama *et al.*, 1992
CloN6	Clorobicin biosynthesis	Westrich *et al..*, 2003
Nclk-binding	Cdk5 activator binding	Ching *et al.*, 2000
Best5	Interferon inducible protein	Grewal *et al.*, 200
HydE/HydG	Cofactor maturation/[FeFe] hydrogenase	Posewitz *et al.*, 2004
AviX12	Epimerization in Avilamycin A	Boll *et al.*, 2006
ORF2	Cofactor maturation/amine dehydrogenase	Ono *et al.*, 2006
Elp3	Elongator complex function	Paraskevopoulou *et al.*, 2006
CofG/CofH	Coenzyme F(420) biosynthesis	Graham *et al.*, 2003
PcfB	Maturation of propionicin F	Brede *et al.*, 2004

by SDS-PAGE (47 kDa) and ultracentrifugation (280 kDa), it crystallizes as a tetramer composed of two intimate dimers in the unit cell (Lepore *et al.*, 2005). The tetrameric unit cell is not inconsistent with alternative structures in solution, which are suggested by the original ultracentrifugation data. The specific activity of LAM declines at high concentrations, which is attributed to aggregation (Ballinger *et al.*, 1992a), and cross-linking experiments reveal the presence of dimers, tetramers, and hexamers in solution (Song &

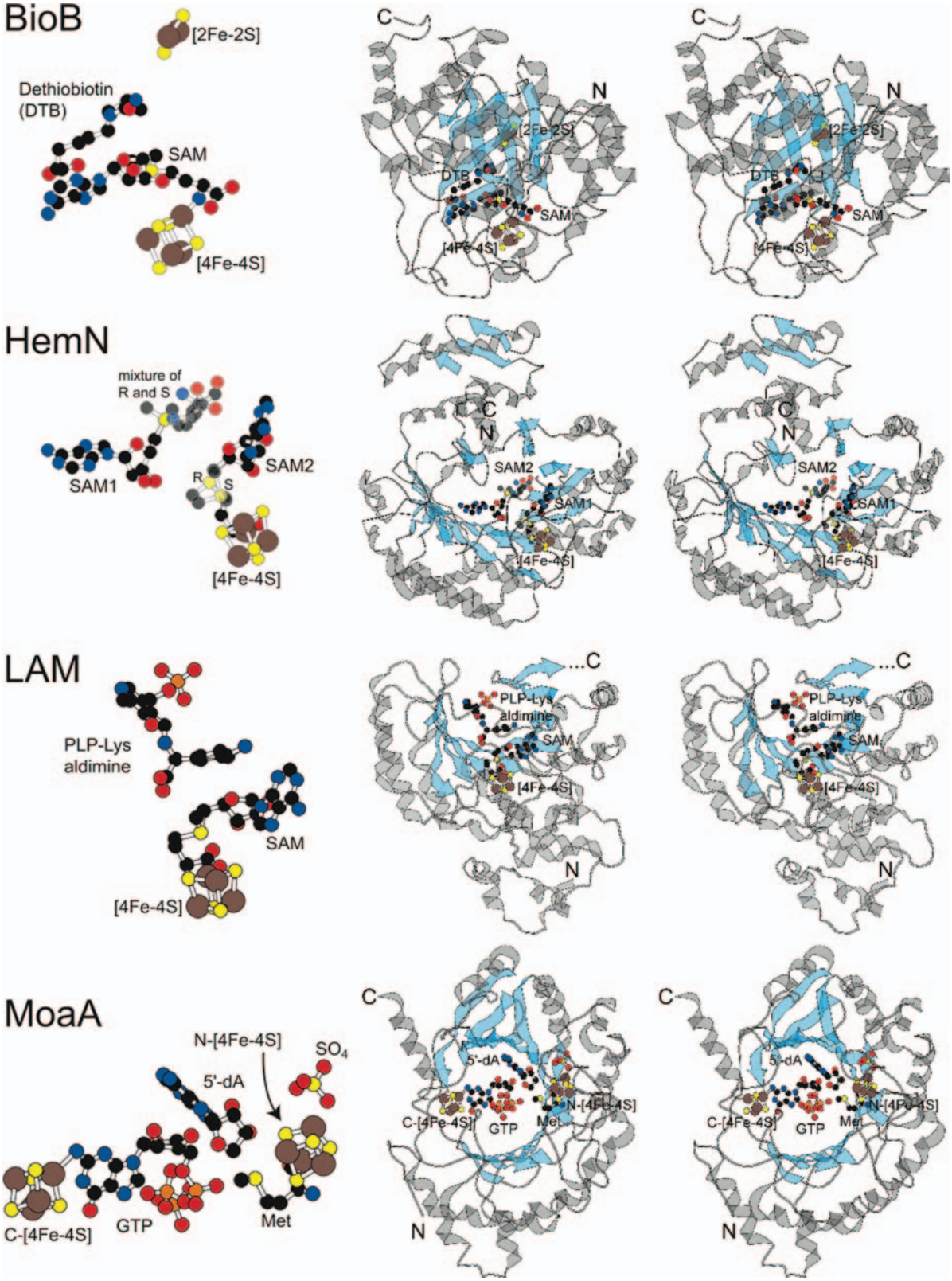

FIGURE 1 **Subunit structures of four Radical SAM enzymes. BioB is biotin synthase, and the subunit is folded in a $(\alpha\beta)8$ (TIM barrel), shown in a side view. The images are created from PDB 1R30 (Berkovitch *et al.*, 2004). HemN is coproporphyrinogen oxidase III, and the subunit fold is $(\alpha\beta)6$ (3/4-barrel), shown down the barrel axis. The images are created from PDB 1OLT (Layer *et al.*, 2003). LAM is lysine 2,3-aminomutase, and the subunit fold is $(\alpha\beta)6$ (3/4-barrel), shown down the barrel axis. The images are created from PDB 2A5H (Lepore *et al.*, 2005). MoaA is required in molybdopterin biosynthesis, and the subunit fold is $(\alpha\beta)6$ (3/4-barrel), shown down the barrel axis. The images are created from PDB 2FB3 (Hänzelmann *et al.*, 2006).**

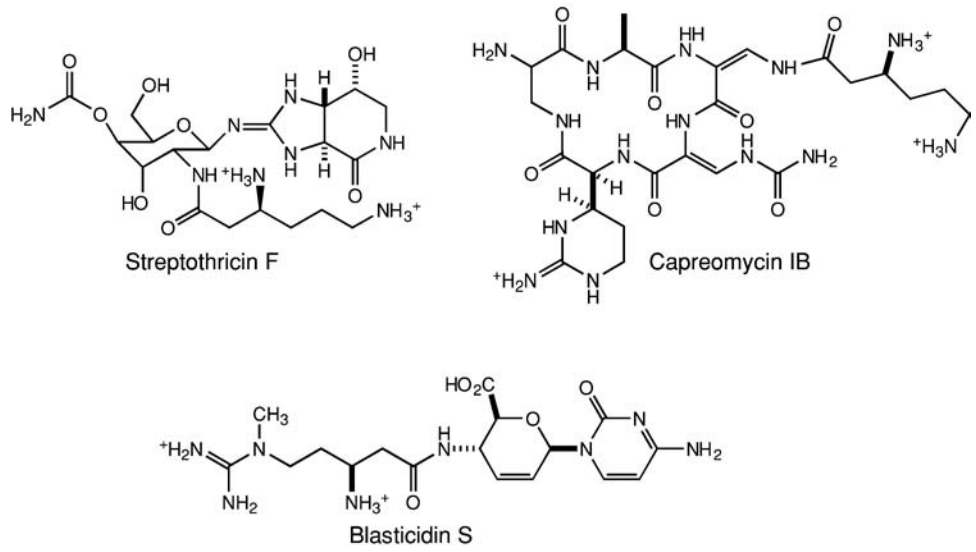

FIGURE 2 **Structures of three antibiotics incorporating β-aminoacyl groups. Streptothricin F and capreomycin IB contain β-lysyl groups in amide linkages to the core structures (Thiruvengadam *et al.*, 1983; DeMong & Williams, 2003), and blastcidin S contains a β-arginyl group (Prabhakaran *et al.*, 1988; Cone *et al.*, 2003).**

Frey, 1991). All things considered, the basic functional unit is likely to be the tetramer found in the unit cell.

The biological role of LAM is twofold, the metabolism of lysine in anaerobes (Stadtman, 1973) and the production of β-lysine for antibiotic biosynthesis (Figure 2). The metabolism of lysine in eukaryotes is a complex affair, requiring a dozen steps for its degradation to acetyl CoA and succinyl CoA. In anaerobes, degradation to acetyl CoA is simplified by the action of LAM. Lysine is sufficiently functionalized to allow for metabolism; however, the placement of amino groups is not optimal for degradation to acetyl CoA. Transformation into β-lysine overcomes the chemical barrier to acetyl CoA-formation. With the amino group in the β-position, the molecule is in condition for dehydrogenation or transamination to a β-ketoacid, activation to the CoA ester, and β-ketothiolase-like breakdown into acetyl CoA. The biological roles of β-lysine and β-arginine, produced by lysine and arginine 2,3-aminomutases, in antibiotic biosynthesis is illustrated by the structures in Figure 2, showing three antibiotics incorporating β-aminoacyl groups. The exact role of β-lysine and β-arginine in the actions of the antibiotics is not known; however, we note that these β-aminoacyl groups contribute positive charges to the core structures and may thereby facilitate their cellular absorption. It might be that β-aminoacyl derivatives are less susceptible to hydrolytic breakdown by enzymes in the cell than the corresponding α-aminoacyl substituents would be.

The mechanism of action of LAM is well established through chemical, radiochemical, spectroscopic, crystallographic, mutagenic, and electrochemical experimentation. The mechanism of hydrogen and amino transfer is as illustrated in Figure 3, as originally put forward on the basis of tritium and deuterium tracer experiments (Moss & Frey, 1987; Baraniak *et al.*, 1989). The product-related radical 3 in Figure 3 is the dominant radical in the steady state and is readily observed and characterized by electron paramagnetic resonance (EPR) spectroscopy (Ballinger *et al.*, 1992a,b). Radical 3 in Figure 3 is stabilized by delocalization of the unpaired electron into the carboxylate group and so is the most stable radical in the mechanism, and rapid-mix-freeze-quench EPR experiments verify its kinetic competence as an intermediate (Chang *et al.*, 1996). The other radical intermediates in Figure 3 are not stabilized by spin delocalization and cannot be observed in the steady state. However, analogs of lysine or/and SAM that can be synthesized incorporate functional groups that delocalize the unpaired electron, and they are often reactive as substrates or coenzymes. In such cases, the free radical intermediates stabilized by spin delocalization can be observed and characterized by EPR in the steady state. Thus, the 4-thia-analog of radical 1 in Figure 3 is an intermediate in the reaction of L-4-thialysine as a substrate, and as the dominant radical it is observable by EPR in the steady state (Wu *et al.*, 1995; Miller *et al.*, 2001). The 5′-deoxyadenosyl radical (H_2C–Ado in Figure 3) is too high in energy to be observed by EPR. However,

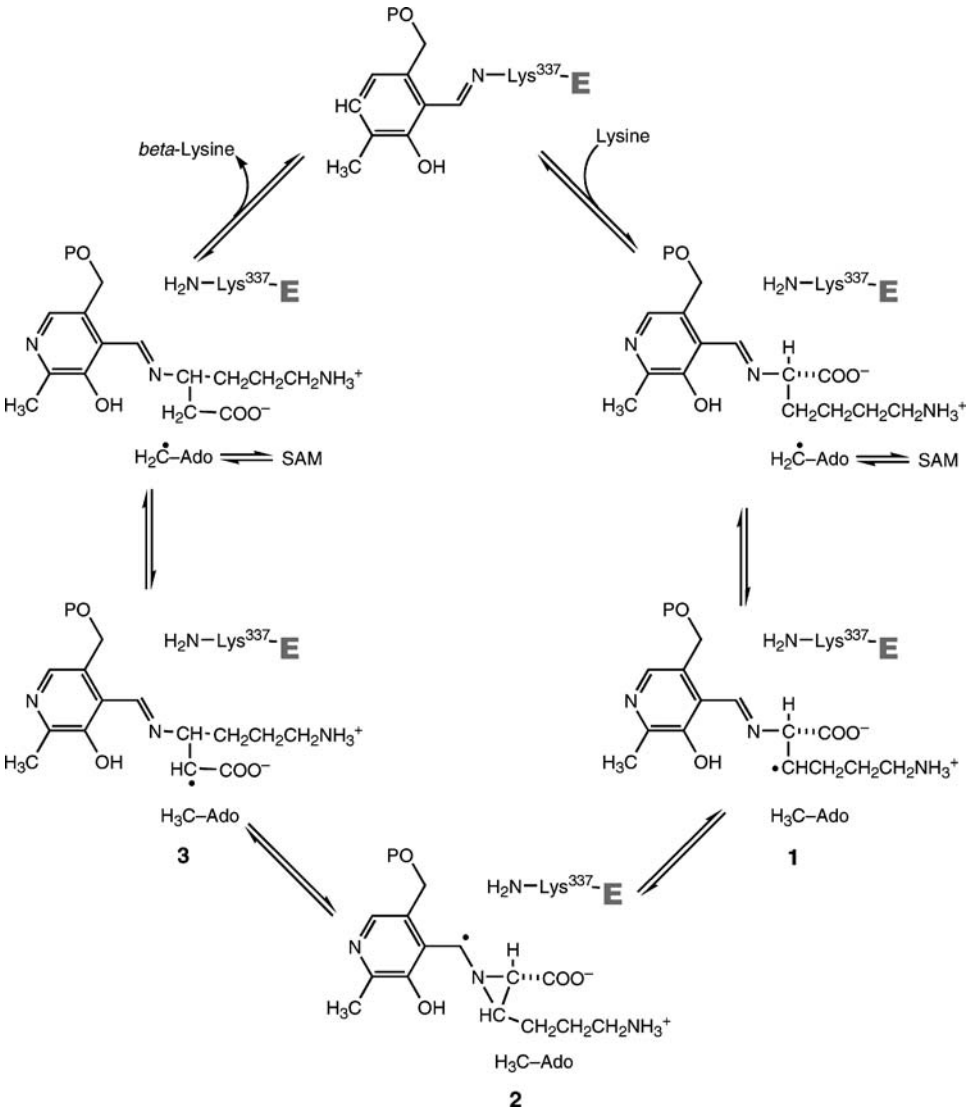

FIGURE 3 **The mechanism of radical isomerization in the reaction of LAM. Reaction begins with the internal PLP-aldimine and proceeds clockwise. Reaction with lysine produces the external PLP-aldimine of lysine. The reversible reductive cleavage of SAM produces the 5′-deoxyadenosyl radical, which abstracts the 3-pro-*R* hydrogen from the lysyl side chain to form the substrate-related radical intermediate 1. Isomerization proceeds by internal cyclization to the azacyclopropylcarbinyl radical 2, and ring opening to the product-related radical intermediate 3. Abstraction of hydrogen from the methyl group of 5-deoxyadenosine by radical 3 generates the external PLP-aldimine of β-lysine, and transaldimination releases β-lysine and restores the internal PLP-aldimine**

the SAM-analog 3′,4′-anhydroadenosyl-L-methionine (*an*SAM), with a double bond between ribosyl-C3′ and C4′, is a true coenzyme; and the resultant allylic 3′,4′-anhydro-5′-deoxyadenosyl radical is spin delocalized. It is the dominant radical in the steady state of the reaction with *an*SAM as the coenzyme, and it is readily observed and characterized by EPR as a kinetically competent intermediate (Magnusson *et al.*, 1999; 2001).

The most likely mechanism by which SAM is cleaved reversibly to the 5′-deoxyadenosyl radical is shown in Figure 4 (Chen *et al.*, 2003). Alternative mechanisms have been reviewed elsewhere (Fontecave *et al.*, 2001; Jarrett, 2003). The mechanism in Figure 4 is consistent with the original characterization of the [4Fe–4S] cluster in LAM as one with a unique iron lacking a cysteinyl

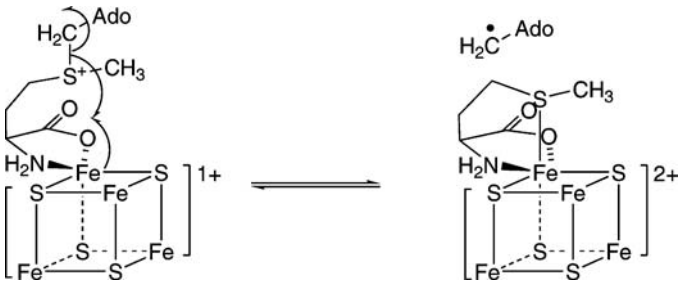

FIGURE 4 **The mechanism of reductive cleavage of SAM to the 5′-deoxyadenosyl radical by LAM.**

ligand (Petrovich *et al.*, 1992) and with the observation of $[4Fe–4S]^{1+}$ as the oxidation state for the active enzyme (Lieder *et al.*, 1998). Experiments employing selenium x-ray absorption spectroscopy (XAS) directly support the mechanism (Cosper *et al.*, 2000). *Se*-adenosyl-L-methionine (*Se*SAM) is a good coenzyme in place of SAM. Using *Se*SAM as the coenzyme, XAS experiments show that Se in *Se*SAM is not ligated to atoms other than carbon when bound to LAM. However, whenever *Se*SAM is cleaved into *seleno*-L-methionine and 5′-deoxyadensine at the active site, selenium is ligated to iron in the [4Fe–4S] cluster. In one method of cleavage, reaction of the complex of *Se*SAM and LAM with dithionite and the suicide inactivator *trans*-4,5-dehydro-L-lysine cleaves *Se*SAM to *seleno*-L-methionine and the 5′-deoxyadenosyl radical, which in turn abstracts a hydrogen atom from C4 of the suicide inactivator to form the ultra-stable allylic analog of radical 1 in Figure 3 (Wu *et al.*, 2000). XAS data show that selenium in the cleavage complex is ligated to iron at a distance of 2.7 Å. Cleaved *Se*SAM can be simulated by adding *seleno*-L-methinonine and 5′-deoxyadenosine directly to LAM, and selenium XAS again shows the selenium to be ligated to iron (at 2.7 Å). Experiments employing ^{15}N- and ^{17}O-electron nuclear double resonance (ENDOR) spectroscopy, patterned after research on PFL activase (Walsby *et al.*, 2002b), implicate the amino and carboxylate groups of the methionyl moiety in SAM as ligands to the unique iron whenever SAM is present with LAM, as illustrated in Figure 4 (Chen *et al.*, 2003). The x-ray crystal structure of the complex of LAM with the [4Fe–4S] cluster, PLP, *Se*SAM, and lysine consolidates the structural aspects of the mechanism in Figure 4 (Lepore *et al.*, 2005). The crystal structure shows the unique iron of the [4Fe–4S] cluster to be the nearest neighbor to selenium in *Se*SAM at a distance of 3.2 Å. The close approach of selenium to iron is illustrated by the ball and stick model in the structure of LAM in Figure 1.

The energetics in the reductive cleavage of SAM to the 5′-deoxyadenosyl radical and methionine is an important issue in the field of Radical SAM enzymes. The midpoint reduction potential for $[4Fe–4S]^{2+/1+}$ in LAM varies from -516 mV to -430 mV, depending on the ligand to the unique iron. With SAM as the ligand, the midpoint potential is -430 mV (Hinckley & Frey, 2006). The potential required to reductively cleave a generic trialkylsulfonium ion (e.g., SAM) in aqueous solution is ~ -1.8 V (Colichman & Love, 1953; Grimshaw, 1981; Saeva & Morgan, 1984). The difference of $\sim$1.4 V corresponds to 32 kcal mol^{-1} ($\Delta G^\circ = -n\mathcal{F}\,\Delta E^\circ$). The barrier must be decreased at the enzymatic site for the reaction to occur: were it not decreased, the equilibrium constant for electron transfer would be so small that the rate constant governing the slowest step in the mechanism would have to be $k \sim 10^{22}$ s^{-1}, many orders of magnitude larger than a bond vibrational frequency. Recent experiments indicate that the binding of lysine to LAM lowers the midpoint reduction potential of $[4Fe–4S]^{2+/1+}$by $\sim$170 mV, and the binding of SAM to the [4Fe–4S] cluster raises the reduction potential required for reductive cleavage by $\sim$810 mV (Wang & Frey, 2007b).

Binding of SAM to Fe^{3+} in the [4Fe–4S] cluster can be expected raise the potential purely on electrostatic grounds; however, the mechanism in Figure 4 further drives electron transfer because of the favorable change in coordination about the unique iron, from pentavalent to the favored hexavalent coordination. The combined effects of lysine binding to LAM and SAM binding to the iron-sulfur cluster in the configuration of Figure 4 dramatically decrease ΔE° to $\sim$ 0.4 V and facilitate electron transfer.

In any enzymatic radical mechanism, a question that arises is how side reactions of the radical with enzymatic groups are avoided. In the case of LAM, the 5′-deoxyadenosyl radical is highly reactive and can be expected to abstract a hydrogen atom from any alkyl groups with which it may adventitiously come in contact within the protein structure, thereby inactivating the enzyme. LAM is not subject to inactivation in its reaction with lysine. Results of ^{13}C-ENDOR experiments answer the question of how destructive side reactions are prevented in the case of LAM. The experiments show that the reacting radical atoms of intermediates 1 and 3 in Figure 3 are held in van der Waals contact with the methyl group of 5′-deoxyadenosine (Lees *et al.*, 2006). It is reasonable to expect that the the 5′-methylene group of the 5′-deoxyadenosyl radical is also held in van der Waals contact with the substrate by substrate and coenzyme binding interactions. Such immobilization would prevent side reactions from interfering with catalysis.

More than 280 species variants of 2,3-aminomutases appear in the genomic database, all of which show that the essential active site residues of LAM from *Clostridium subterminale* SB4 are conserved. These include Lys337, which binds PLP in the internal aldimine; Cys125, Cys129, and Cys132 in the cysteine motif; and

Arg134, which binds the lysyl carboxylate group. The residues Asp293 and Asp330 bind the ε-aminium group of lysine in clostridial LAM, and corresponding residues are found in more than 275 currently known variants. Mutation of any of these residues completely abolishes activity (Chen *et al.*, 2006; Chen & Frey, 2001). A few very close homolog of LAM lack one or both of Asp293 and Asp330 and cannot act on lysine. They are now known to be glutamate and arginine 2,3-aminomutases and are new members of the radical SAM superfamily (Cone *et al.*, 2003; Ruzicka & Frey, 2007). In arginine 2,3-aminomutase, an aspartate corresponding to Asp293 of clostridial LAM is conserved, but Asp330 is replaced by threonine. In the glutamate 2,3-aminomutases, Asp293 and Asp330 of clostridial LAM are replaced by Lys and Asn residues, respectively. Sequence alignments in Figure 5 show the differences and similarities in the active site residues of the 2,3-aminomutases.

β-Lysine mutase and ornithine 4,5-aminomutase are adenosylcobalamin- and PLP-dependent enzymes and are not members of the radical SAM superfamily (Baker *et al.*, 1973; Chen *et al.*, 2001). The adenosylcobalamin-dependent aminomutases function similarly to the radical SAM-aminomutases, except that adenosylcobalamin is the source of the 5′-deoxyadenosyl radical, which serves as the initiator in the mechanism.

Other aminomutases that are not radical SAM enzymes include tyrosine and phenylalanine 2,3-aminomutases, which incorporate the amino acid-based 4-methylideneimidazole-5-one (MIO) moiety within their amino acid sequences (Christenson *et al.*, 2003; Walker *et al.*, 2004). Tyrosine and phenylalanine 2,3-aminomutases catalyze aminomutation by a completely different mechanism, in that the MIO moiety within the amino acid sequence facilitates the elimination of the α-amino group from the substrate, as it does in phenylalanine ammonia-lyase (PAL) (Frey & Hegeman, 2007a; Langer *et al.*, 2001). Unlike PAL, tyrosine and phenylalanine aminomutases do not allow the ammonia to escape but instead catalyze its re-addition to the β-carbon to produce the β-amino acid.

Spore Photoproduct Lyase.

Certain lesions in DNA are thymine dimers of the type 5-thyminyl-5,6-dihydrothymine, in which the bases are covalently linked through a methylene bridge originating with the methyl group of one thymine ring. These lesions are repaired by the action of spore photoproduct lyase, a Radical SAM enzyme (Rebeil *et al.*, 1998; Rebeil & Nicholson, 2001). There is one optical center in the 5-thyminyl-5,6-dihydrothymine-moiety in the dihydrothymine ring, and only the 5*S*-steroisomer is repaired (Friedel *et al.*, 2006). The enzyme from *Bacillus subtilis* is monomeric and contains a [4Fe–4S] cluster typical of Radical SAM enzymes (Buis *et al.*, 2006).

Figure 6A shows the overall reaction and an attractive repair mechanism. The process begins with binding the 5-thyminyl-5,6-dihydrothymine moiety of the DNA lesion at the active site adjacent to SAM ligated to the $[4Fe–4S]^{1+}$ cluster. Reversible electron transfer from the $[4Fe–4S]^{1+}$ cluster to SAM concomitant with cleavage of the C5′–S bond generates the 5′-deoxyadenosyl radical, which abstracts a hydrogen atom C6(H) from the dihydrothymine ring to form 5′-deoxyadenosine and the substrate-related radical 5-thyminyl-5,6-dihydrothymin-6-yl. Radical fragmentation generates one thymine moiety and a thymine-5-carbinyl radical. Abstraction of a hydrogen atom from the methyl group of 5′-deoxyadenosine generates the methyl group of the second thymine moiety. Experimental evidence supporting the mechanism in Figure 6A includes the observation that enzymatic repair of a substrate containing 5-thyminyl-5,6-dihydro[6-^{3}H]thymine leads to incorporation of tritium into SAM, and repair using [5′-^{3}H]SAM leads to incorporation of tritium into repaired thymine (Cheek & Broderick, 2002). These experiments directly support the mechanism and would be very difficult to explain in any other way.

A simple substrate for spore photoproduct lyase is the dinucleoside-3,5-monophosphate shown in Figure 6B (Chandor *et al.*, 2006). Repair of the dinucleoside monophosphate shows that the enzyme does not require the structural context of DNA be active. Activity on the simple substrate is interpreted to imply that the lesion is flipped out of the DNA double helix when bound to the repair enzyme.

MEMBERS THAT USE SAM STOICHIOMETRICALLY AS A SUBSTRATE

Glycyl Radical Activases

SAM is an oxidizing substrate in the transformation of active site-glycyl residues to glycyl radicals in enzymes such as PFL, ARR, benzylsuccinate

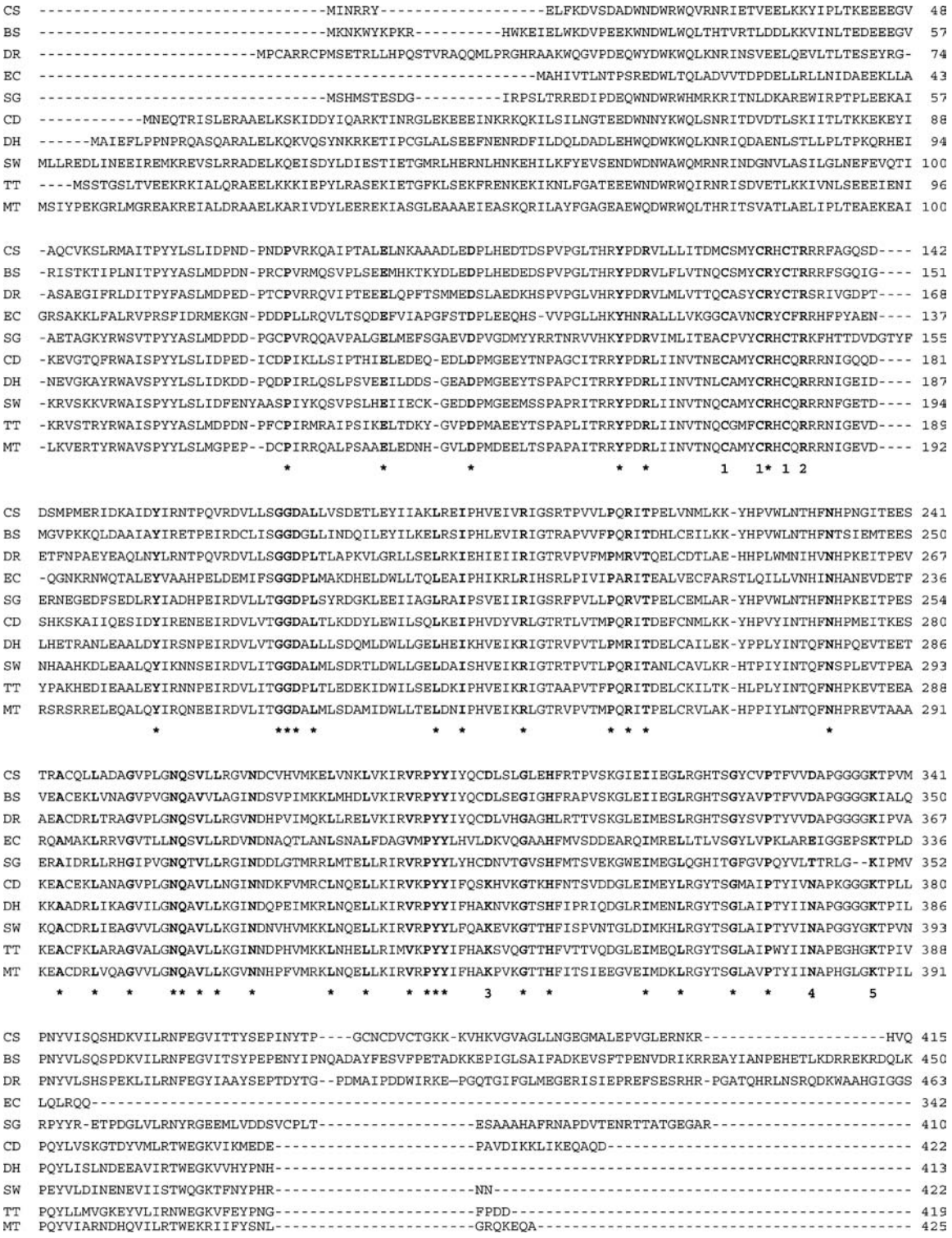

FIGURE 5 **Amino acid sequence relationships among amino acid 2,3-aminomutases. The amino acid sequences of four of the 276 species of LAM are shown at the top encoded in the left column as: CS, *Clostridium subterminale* SB4; BS, *Bacillus subtilis*; DR, *Deinococcus radiodurans*; and EC, *Escherichia coli*. The amino acid sequence of arginine 2,3-aminomutase is fifth from the top and encoded as SG, *Streptomyces griseochromogenes*. The five sequences at the bottom are variants of glutamate 2,3-aminomutase encoded in the left column as: CD, *Clostridium difficile*; DH, *Desulfitobacterium hafniense* DCB-2; SW, *Syntrophomonas wolfei* str Göttingen; TT, *Thermoanaerobacter tengcongensis* MB4; and MT, *Moorella thermoacetica*. The residues conserved among all amino acid 2,3-aminomutases are marked with asterisks. The active site residues are marked with the following numerals: 1, for the conserved cysteine motif CxxxCxxC; 2, for R134 in Clostridial LAM that binds the α-carboxyl group; 3 and 4, for D293 and D330 in Clostidial LAM that bind the lysyl side chain; and 5, for K337 in Clostridial LAM that binds PLP. In arginine 2,3-aminomutase (SG), 3 and 4 are Asp and Thr, respectively; and in all glutamate 2,3-aminomutases 3 and 4 are Lys and Asn, respectively. The C-terminal sequences of CS, BS, and DR are truncated (1 to 30 residues).**

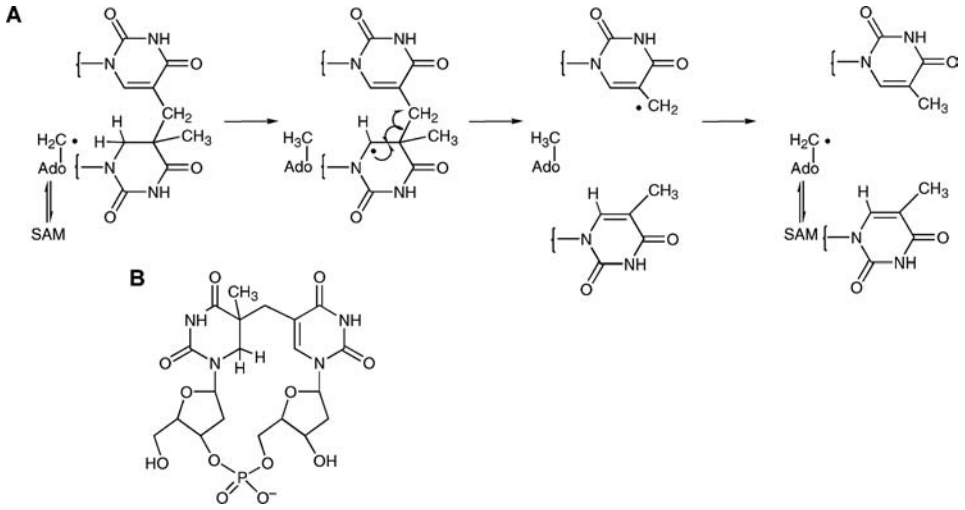

FIGURE 6 ***A*** **radical mechanism for SAM-mediated repair of a thymine dimer in DNA. A. Spore photoproduct lyase reductively cleaves SAM to the 5′-deoxyadenosyl radical, which abstracts a hydrogen atom from C6 of the dihydrothymine ring of the bridged dimer to form 5′-deoxyadenosine and a substrate-radical. Radical fragmentation proceeds to cleave the dimer to thymine and a thymine-carbinyl radical, which abstracts a hydrogen atom from the methyl group of 5′-deoxyadenosine.** ***B.*** **A simple dinucleoside substrate for spore photoproduct lyase.**

synthase, B12-independent glycerol dehydratase and others (Selmer *et al.*, 2005). The role of SAM as an oxidant is made clear in Eq. 2, showing the net reduction of SAM to methionine and 5′-deoxyadenosine concomitant with radicalization of Gly734 in PFL. Hydrogen abstraction from C2 of a glycyl residue by the 5′-deoxyadenosyl radical is a one-electron oxidation, but the reduction of SAM to methionine and 5-deoxyadenosine is a two-electron reduction. The second electron in the reduction of SAM comes from the iron-sulfur cluster. Figure 7 shows how the two-electron reduction of SAM is mechanistically coupled to the one-electron oxidation of Gly734 in PFL, one electron is derived from the $[4Fe–4S]^{1+}$ cluster upon reductive cleavage of SAM to the 5′-deoxyadenosyl radical, and the second is derived from Gly734 in the hydrogen abstraction. The mechanistic sequence for PFL activase is regarded as general for glycyl radical enzyme activases.

Figure 7 also shows the stereochemistry of hydrogen abstraction from Gly734 in the action of PFL activase (Frey *et al.*, 1994). A heptapeptide with the sequence surrounding Gly734 in PFL is a substrate for the activase. Two lines of evidence implicate C2(H_S) of Gly734 as the hydrogen abstracted by the 5′-deoxyadenosyl radical. Reaction of the heptapeptide labeled with 2*S*-[2-$^2H_1$]Gly leads to 5′-[$^2H_1$]deoxyadenosine. And the chemically mutated heptapeptide with D-Ala in place of Gly is a substrate, whereas the heptapeptide with L-Ala is not a substrate. Both results implicate the hydrogen in the 2-pro-*S* position of Gly734 as mobilized in the activation process.

The octapeptide Succinyl-Arg-Val-Pro-*dehydro*Ala-Tyr-Ala-Val-Arg-NH_2 inactivates PFL activase in a SAM-dependent reaction, in which SAM is cleaved and the adenosyl moiety becomes covalently linked to the peptide (Wagner *et al.*, 1999). The dehydroalanyl residue

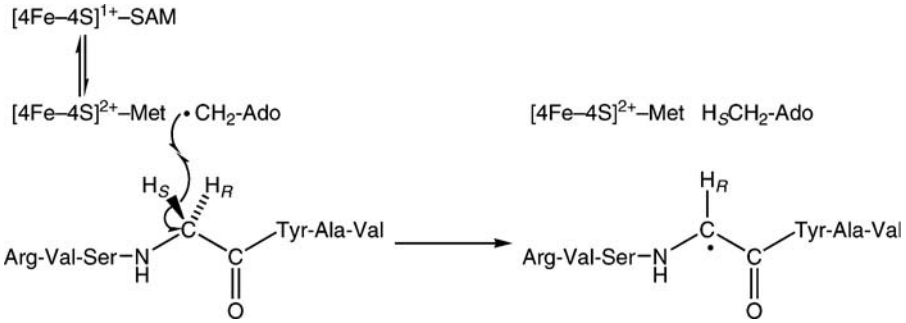

FIGURE 7 **Chemistry and stereochemistry in the action of PFL activase.**

of the peptide replaces the glycyl residue that would become radicalized in a substrate, and the methylidene group ($H_2C{=}C_\alpha$) could react by radical addition with the 5′-deoxyadenosyl radical. The results are consistent with and support the intermediate formation of the 5′-deoxyadenosyl radical in the action of PFL activase.

All glycyl radical enzymes studied to date use the glycyl radical as a haven for an unpaired electron. The captodative effects of the glycyl-carbonyl and glycyl-α-N stabilize the unpaired electron and attenuate its reactivity. The glycyl radical does not itself react directly with a substrate. Instead, as illustrated in Figure 8, each glycyl radical enzyme incorporates at least one cysteine residue in the active site that chemically communicates with the glycyl radical, and several of them have two cysteine residues in the site. Hydrogen transfer from the cysteine to the glycyl radical generates a cysteine-thiyl radical and temporarily quenches the glycyl radical. The thiyl radical in each enzyme then initiates one of the diverse radical mechanisms. The radical mechanisms of PFL, ARR, B12-independent glycerol dehydratase, and benzylsuccinate synthase are outlined without details in Figure 8. PFL converts pyruvate to acetyl-CoA in bacteria grown anaerobically, ARR generates 2-deoxyribonucleoside triphosphates for DNA biosynthesis in bacteria grown anaerobically, and B12-independent glycerol dehydratase acts in place of the adenosylcobalamin-dependent enzyme in bacteria lacking B12 (O'Brien *et al.*, 2004). Outlines of the chemical mechanisms are shown in Figure 8A, B, and C.

Alkane metabolism encounters a chemical barrier because of the absence of functional groups. Aerobic bacteria overcome this by carrying out hydroxylation (monooxygenation) on alkanes to introduce alcoholic

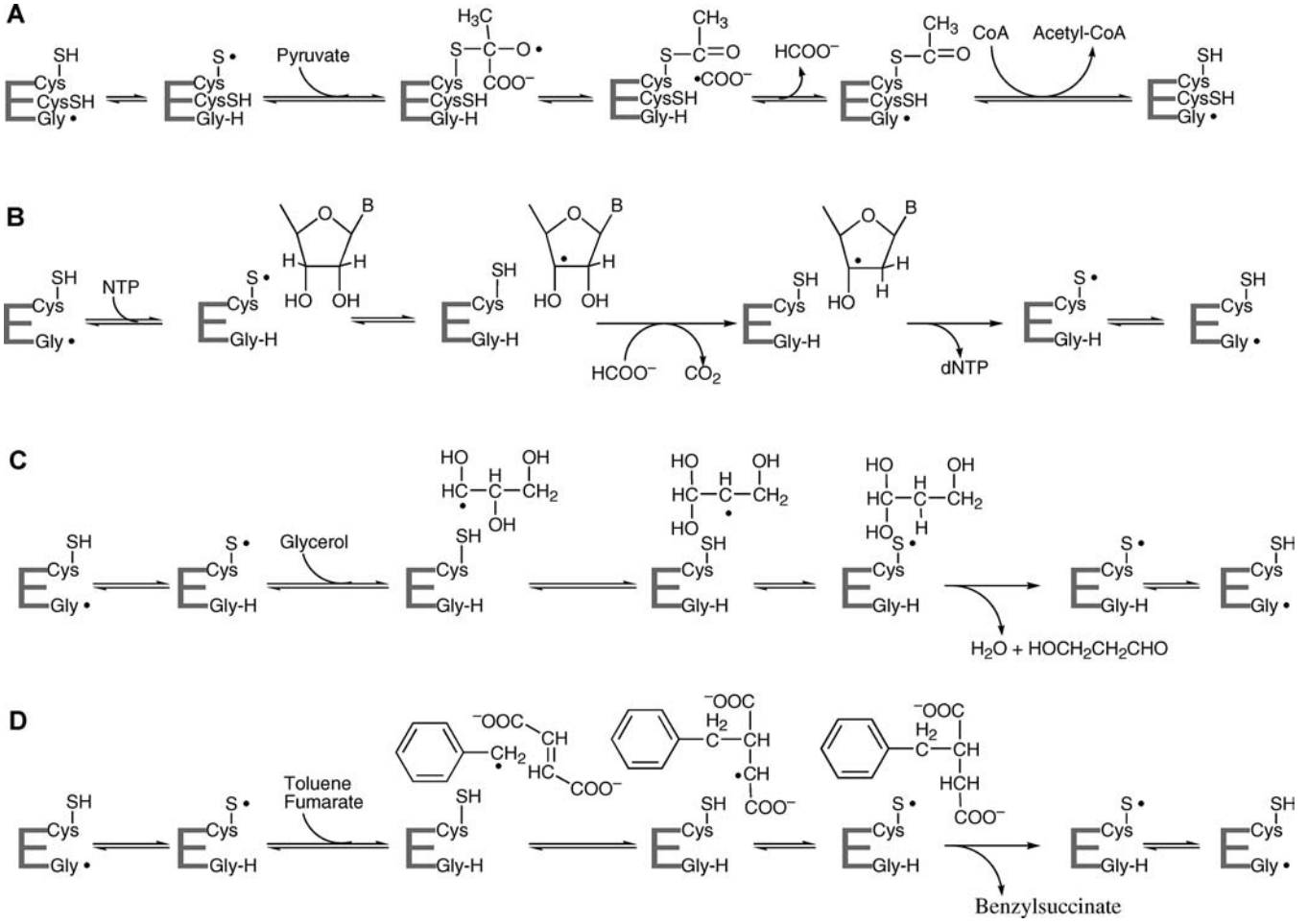

FIGURE 8 **Representative reactions catalyzed by glycyl radical enzymes. Shown in outline are diverse mechanistic sequences initiated by glycyl radical in four enzymes. The exact mechanisms are not known definitively, and the steps shown here are representative of the mechanisms under consideration. The figure is composed to illustrate the mechanistic diversity in this class of enzymes. A. PFL; B. ARR; C, B12-independent glycerol dehydratase; D. benzylsuccinate synthase.**

groups as leverage for further metabolism (van Beilin & Funhoff, 2007). Anaerobic bacteria, which cannot use oxygen, chemically functionalize alkanes through the actions of glycyl radical enzymes (Spormann & Widdel, 2000; Krieger *et al.*, 2001). Figure 8D shows how the methyl group of toluene is chemically functionalized by attachment to fumarate through the action of the glycyl radical in benzylsuccinate synthase (Krieger *et al.*, 2001; Qiao & Marsh, 2005). The functionalized product can be metabolized in more conventional enzymatic processes. The glycyl radical in benzylsuccinate synthase originates through the action of the SAM-dependent activase, BssD in Table 1, a radical SAM enzyme.

A wealth of data from ENDOR and Mossbäuer spectroscopy implicate complexation between SAM and the [4Fe–4S] cluster in PFL activase (Walsby *et al.*, 2002a,b; Krebs *et al.*, 2002). A mechanism for the reductive cleavage of SAM in this enzyme inspired by this research is shown in Figure 9. This mechanism includes ligation of SAM to the unique iron of the [4Fe–4S] cluster and inner sphere electron transfer concomitant with the cleavage of SAM, in common with the mechanism in Figure 4 in the action of LAM. Unlike the latter mechanism, reductive cleavage in Figure 9 does not lead to ligation of the methionyl-sulfur to the unique iron in the [4Fe–4S] cluster. Instead, the methionyl sulfur is shown associated with a sulfide ion in the [4Fe–4S] cluster. Unlike the reaction of LAM, reductive cleavage of *Se*SAM by PFL activase does not permit the observation of an interaction between the [4Fe–4S] cluster and *seleno*-L-methionine, which dissociates from the enzyme (Cosper *et al.*, 2003). This result has been interpreted to imply the existence of two mechanisms for the generation of the 5′-deoxyadenosyl radical, the mechanism in Figure 4 by enzymes that use SAM catalytically and the mechanism in Figure 9 by enzymes that use SAM irreversibly as a substrate and release methionine with each catalytic turnover. Sulfur in both methionine and sulfide have inert gas electronic configurations, so that it is not clear how strong their interaction would be and whether it would potentiate electron transfer and cleavage of the C5′–S bond in SAM. A structure of PFL activase with SAM bound to the cluster could clarify the issue. Such a structure is available for HemN, which cleaves SAM irreversibly, and the conformation of SAM ligated to the [4Fe–4S] cluster is similar to that in LAM; *i.e.*, the sulfur is in position to become ligated to the unique iron (Figure 1).

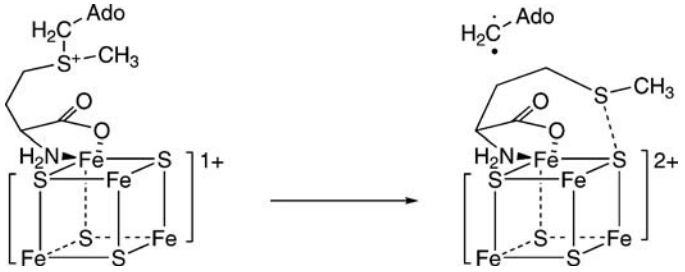

FIGURE 9 **A mechanism for reductive cleavage of SAM by PFL activase.**

Sulfur Inserting Enzymes

Biotin synthase (BioB) and lipoyl synthase (LipA) promote the insertion of sulfur atoms into unreactive C–H bonds of dethiobiotin and the octanoyl moiety, respectively, as illustrated in Figure 10. This is a difficult process, and no detailed chemical mechanism is available for either enzyme. However, considerable information about these enzymes place them in the radical SAM superfamily and show that the reactions are initiated by the 5′-deoxyadenosyl radical derived from SAM (Cosper *et al.*, 2002; 2004; Jarrett, 2005; Lotierzo *et al.*, 2005).

A chemical barrier in the reaction of biotin synthase is the necessity to cleave two unreactive C–H bonds, between which one sulfur atom is inserted. Two 5′-deoxyadenosyl radicals can surmount these barriers by abstraction of hydrogen atoms from the two alkyl-type C–H groups. Careful stoichiometric analysis indicates that at least two moles of SAM are cleaved per mole of d-biotin formed (Shaw *et al.*, 1998; Guianvarc'h *et al.*, 1997), allowing the reaction in Figure 10A to be written for the reaction of biotin synthase. In practice, more than two moles of SAM are cleaved, and this excess SAM consumption is regarded as arising from a degree of uncoupling between SAM cleavage and biotin synthesized (Lotierzo *et al.*, 2005).

A source of sulfur in biotin formation was found to be a second iron-sulfur center, a [2Fe–2S] cluster, in biotin synthase (Ugulava *et al.*, 2001a,b; Ugulava *et al.*, 2002; Tse Sum Bui *et al.*, 2003; Jameson *et al.*, 2004). The structure with both SAM and dethiobiotin bound to the active site is shown as BioB in Figure 1. The ball-and-stick diagram shows the spatial relationships in the active site. SAM ligated to the [4Fe–4S] cluster lies on one side of dethiobiotin, with the [2Fe–2S] cluster nearby on the other side. The structure is compatible with the

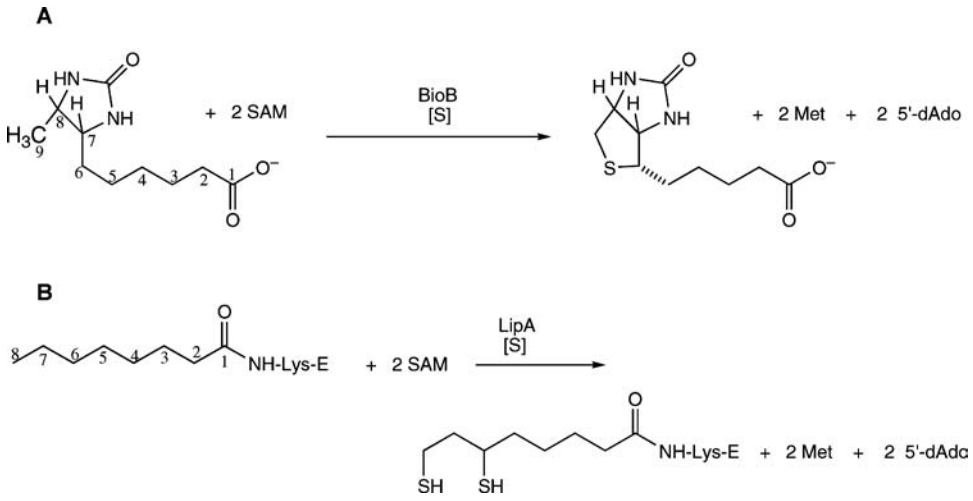

FIGURE 10 **Reactions of biotin synthase (BioB) and lipoyl synthase (LipA). *A*. Biotin synthase catalyzes the SAM-dependent insertion of a sulfur atom between C6 and C9 of dethiobiotin. This process requires the abstraction of hydrogen atoms C6–H and C9–H by 5′-deoxyadenosyl radicals derived from two molecules of SAM. *B*. Lipoyl synthase catalyzes the insertion of two sulfur atoms into two C—H bonds of octanoyl-ACP, C6–H and C8–H, to form lipoyl-ACP. Two molecules of SAM are required for this process, presumably because one 5′-deoxyadenosyl radical is required to abstract a hydrogen atom from each of the two carbon atoms.**

[2Fe–2S] cluster serving as the immediate source of sulfur for biotin synthase. The ultimate sulfur source is likely to be cysteine, the source of sulfide for the biosynthesis of iron-sulfur clusters. A mechanism by which the [2Fe–2S] cluster is regenerated with sulfur after a cycle of biotin synthesis is not currently available and is a subject of continuing research.

An unusual feature of the [2Fe–2S] cluster in biotin synthase is the presence of arginine-260 as one ligand in place of cysteine. Mutation of this residue to a variety of other residues does not abolish or seriously impair biotin synthesis, leading to the conclusion that arginine-260 does not play an important role in the function of this enzyme (Broach & Jarrett, 2006).

The reaction mechanism for sulfur insertion is not known definitively, although there is general agreement that the 5′-deoxyadenosyl radical derived from SAM abstracts hydrogen atoms from C9 and C6 to generate radical intermediate species. Unresolved is the mechanism of sulfur insertion, although 9-mercapto-dethiobiotin might be an intermediate (Tse Sum Bui *et al.*, 2004). It seems likely that the dethiobiotin-radical intermediates capture, or are quenched by, sulfide ions in the [2Fe–2S] cluster. Recent reviews probe this subject more deeply (Lotierzo *et al.*, 2005; Jarrett, 2005).

Several of the mechanistic barriers in the reaction of lipoyl synthase are similar to those in the reaction of biotin synthase. Two unreactive C–H bonds, the octanoyl-C6–H and C9–H bonds in Figure 10B, must be broken in lipoyl synthesis: As in biotin synthase this requirement is overcome by the reduction of two moles of SAM per mole of lipoyl groups formed (Cicchillo *et al.*, 2004a). A source of sulfur for insertion into the cleaved C–H bonds is needed for lipoyl synthase, and as in biotin synthase there are two iron-sulfur clusters in lipoyl synthase, one of which is the SAM-binding cluster typical of radical SAM enzymes. The second cluster is a [4Fe–4S] cluster, unlike the [2Fe–2S] center in biotin synthase (Cicchillo *et al.*, 2004b).

Several aspects of lipoyl formation are fundamentally different from biotin biosynthesis. Free octanoic acid is not a substrate for lipoyl synthase, which functions on the octanoyl-dihydrolipoyl transacetylase component of the pyruvate dehydrogenase complex (Miller *et al.*, 2000). Lipoyl synthase inserts two sulfur atoms to transform the octanoyl into the lipoyl group, and both sulfur atoms are derived from the same polypeptide chain in a given turnover (Cicchillo & Booker, 2005). Insertion of the two sulfur atoms takes place in distinct steps (Douglas *et al.*, 2006).

To date, immediate sources of sulfur for biotin and lipoyl synthases are the auxiliary iron-sulfur clusters in these proteins. If these are the true biological sulfur donors, there must be a mechanism for reconstituting the auxiliary iron-sulfur centers if these enzymes are to undergo multiple turnovers. Biotin synthase functions catalytically *in vivo* (Choi-Rhee & Cronan, 2005), so that replenishment of sulfur in the [2Fe–2S] center appears likely. The subject is under intensive investigation (Fontecave *et al.*, 2003; Marquet, 2001).

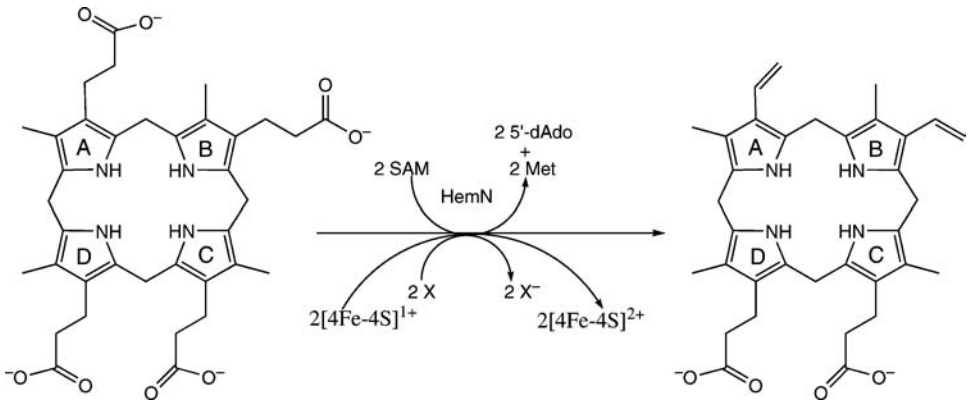

FIGURE 11 **Reaction of coproporphyrinogen oxidase III (HemN). HemN catalyzes the anaerobic oxidative decarboxylation of the propionate side chains on both rings A and B to vinyl groups, concommitant with reductive cleavage of two moles of SAM into methionine and 5-deoxyadenosine. Two oxidants are required, SAM and another one-electron acceptor X that has not been identified. The reductive cleavage of two molecules of SAM requires four electrons, two from the iron-sulfur clusters and two from the propionate side chains. Two more electrons must be removed by the electron acceptor X.**

Coproporphyrinogen Oxidase (HemN)

Heme contains vinyl groups on rings A and B, and they are derived from propionate side chains in the biosynthetic intermediate coproporphyrinogen, shown in Figure 11. In aerobic organisms, oxidative decarboxylation of the propionate side chains is catalyzed by coproporphyrinogen oxidase, an enzyme that uses molecular oxygen as an electron acceptor (Brekau *et al.*, 2003). Heme is also biosynthesized in anaerobes, but molecular oxygen is not available as an electron acceptor. In anaerobes, the reaction takes place as shown in Figure 11 and is catalyzed by HemN, a radical SAM enzyme (Layer *et al.*, 2003; 2005; 2006). HemN uses SAM as an oxidizing agent and radical initiator. As an oxidizing agent, SAM accepts one electron from the substrate in one catalytic turnover, and an electron acceptor X, not yet identified, accepts another electron. *In vitro*, the latter electron acceptor function can be carried out by *o*-phenanthroline/Fe^{3+}. Electron donation by the Radical SAM-[4Fe–4S]$^{1+}$ in HemN is required to generate the oxidizing 5′-deoxyadenosyl radical from SAM. Thus, in the overall reaction SAM is reduced by two electrons, one from the [4Fe–4S]$^{1+}$ cluster and one from the substrate. Overall, SAM is a one-electron oxidant in the oxidative decarboxylation, and the electron acceptor X is the other one-electron oxidant.

Hypothetical oxidation and decarboxylation steps in the mechanism are outlined in Figure 12. The exact sequence of steps is not known. Two steps are reasonably well understood; the reductive cleavage of SAM into methionine and the 5′-deoxyadenosyl radical by the [4Fe–4S]$^{1+}$ cluster, and the abstraction by the 5′-deoxyadenosyl radical of a hydrogen atom from the β-carbon of the propionate side chain. The structure of the SAM-[4Fe–4S] complex, shown in a ball-and-stick diagram in Figure 1, is compatible with the SAM-cleavage mechanism in Figure 4. The β-propionate-radical is known to be an intermediate through its observation and characterization by EPR spectroscopy (Layer *et al.*, 2006).

Two mechanisms, A and B in Figure 12, may be considered for the oxidative decarboxylation of the β-propionate-radical. In mechanism A, the radical is oxidized by the one-electron acceptor, designated X in Figure 12, to the cationic intermediate, which readily undergoes decarboxylation to form the vinyl group. In mechanism B, the β-propionate-radical undergoes fragmentation to the vinyl product and formate radical, and the latter is oxidized to CO_2 by the electron acceptor X. A decision between these mechanisms awaits further research.

Also to be resolved is the question of how HemN carries out oxidative decarboxylations in both ring A and ring B in Figure 11. Outstanding questions are the following: Which ring is processed first? Is the enzyme processive; that is, are rings A and B of a substrate molecule processed together in a single turnover of the enzyme? If catalysis is processive, how is the substrate reoriented to allow the second ring to react with the SAM-[4Fe–4S] center? The structure suggests the possibility of processivity by showing the presence of two molecules of SAM in adjacent locations in the unit cell. In a processive

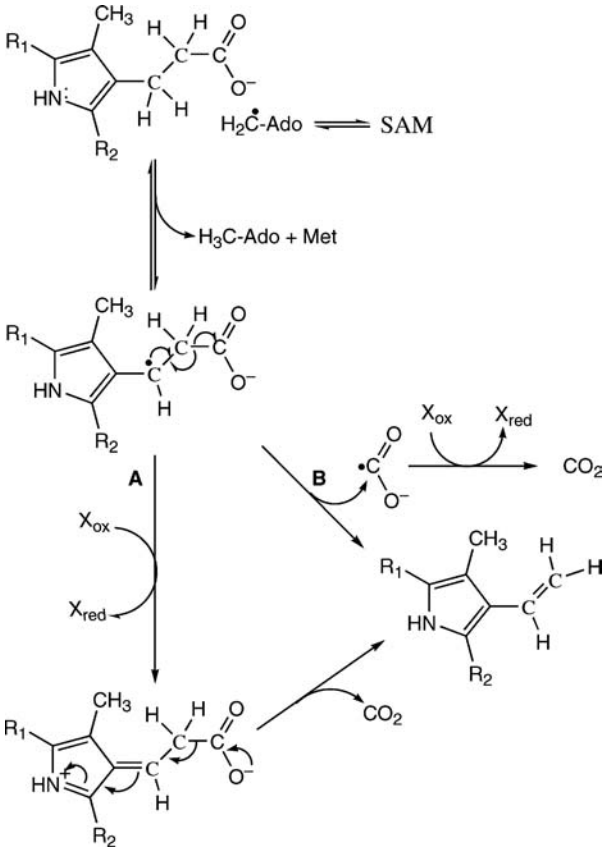

FIGURE 12 **Two mechanisms for oxidative decarboxylation of propionate groups by HemN. Oxidative decarboxylation of propionate side chains in heme biosynthesis is carried out by coproporphyrinogen oxidase (HemF) in aerobes, which employ dioxygen as the electron acceptor. In anaerobes, the reaction is catalyzed by the Radical SAM enzyme HemN, and the electron acceptors are SAM and an unidentified biological acceptor designated X in the figure. Two mechanisms are shown, both of which begin with the reductive cleavage of SAM to the 5-deoxyadenosyl radical, which abstracts a β-hydrogen from the propioate moiety to form the corresponding radical intermediate. In mechanism A, the radical intermediate is oxidized by X to a cationic intermediate, which undergoes decarboxylation to the vinyl product. In mechanism B, the radical intermediate undergoes fragmentation to the vinyl product and the formyl radical. The electron acceptor X oxidizes the formyl radical to CO_2.**

mechanism, the molecule of SAM in complex with the [4Fe–4S] cluster would react with one ring of the substrate, methionine and 5-deoxyadenosine would dissociate, and the second molecule of SAM would then bind to the [4Fe–4S] cluster for the second cycle of reaction with the other ring of the re-oriented substrate. These questions remain for further research.

MoaA and Molybdopterin Biosynthesis

GTP is the biosynthetic precursor of molybdopterin (Moco), which is essential for life and is produced in a complex and poorly understood series of enzymatic reactions. The radical SAM enzyme MoaA from *E. coli* acts together with another protein MoaC to catalyze the first step of the process. The overall transformation of GTP leads to the production of Moco precursor Z, shown in Figure 13A (Santamaria-Araujo *et al.*, 2004), and is accompanied by the cleavage of SAM into methionine and 5′-deoxyadenosine. In this reaction of GTP, C8 of the guanine ring is inserted between C2′ and C3′ of the ribosyl ring, N7 of the guanine ring becomes bonded to C2′ of the ribosyl ring, and the 3′-OH displaces PP_i to form the 3′,5′-cyclic phosphodiester. The mechanism of this process is not known.

In humans, molybdopterin biosynthesis requires the protein MOCS1A, a radical SAM enzyme with two [4Fe–4S] clusters (Hänzelmann *et al.*, 2004). Molybdopterin deficiency in humans is a pleiotropic genetic disorder resulting in loss of the activities of sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Moco precursor Z rescues Z-deficient knock-out mice displaying the human phenotype (Schwarz *et al.*, 2004).

A structure of MoaA is shown in Figure 1 with GTP bound. MoaA contains two [4Fe–4S] clusters, both nucleated by three-cysteine motifs, one of which is typical of the radical SAM superfamily and binds SAM (Hänzelmann & Schindelin, 2004). The second [4Fe–4S] cluster, like the Radical SAM cluster, has three cysteine ligands and a unique iron. The structure in Figure 1 shows the guanine ring of GTP ligated to the unique iron in the second cluster, which is located across the barrel from the radical SAM-type cluster (Hänzelmann and Schindelin, 2006).

MiaB and Methylthiolation of tRNA

The maturation of tRNA requires the chemical modification of adenine-37. The first step in modification is isopentenylation of N6 by isopentenyl pyrophosphate. MiaB catalyzes the SAM-dependent second step, methylthiolation of isopentenyladenine-37, as illustrated in Figure 13B (Pierrel *et al.*, 2002; 2003; 2004; Hernandez *et al.*, 2007). The enzyme contains two [4Fe–4S] clusters, one of which is typical of radical SAM enzymes. SAM serves as the source of the methyl group in thiomethylation, and it is also cleaved to 5′-deoxyadenosine. Experiments with selenium-reconstituted MiaB containing Fe-selenium clusters showed the incorporation of selenium into the product, presumably as the methylseleno group. The

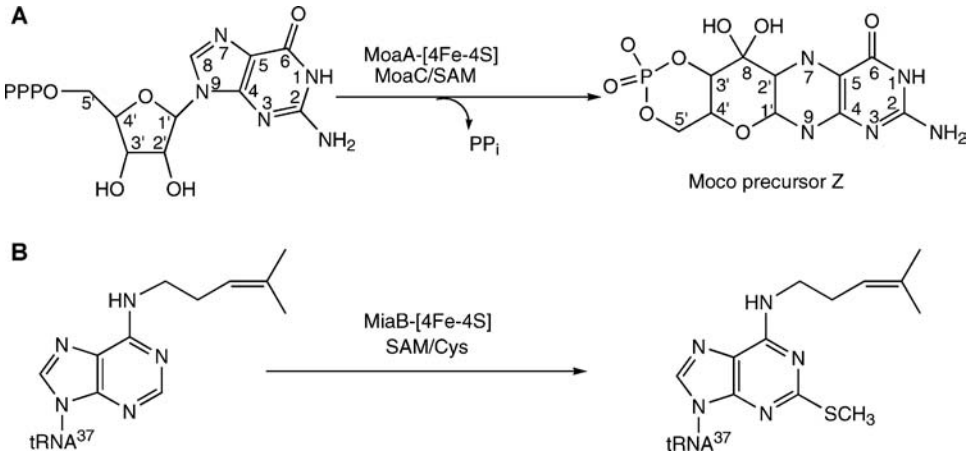

FIGURE 13 **Reactions of MoaA and MiaB. *A*. MoaA is required for the SAM-dependent transformation of GTP into the Moco precursor Z. One of the two [4Fe–4S] clusters in MoaA participates in the cleavage of SAM into 5′-deoxyadenosine and methionine in the course of the reaction. The other [4Fe–4S] cluster binds the guanine ring of GTP (Figure 1). B. MiaB facilitates the thiomethylation of isopentenyladenine at position 37 in tRNA in a SAM-dependent reaction. One of the two [4Fe–4S] clusters in MiaB participates in the cleavage of SAM into methionine and 5′-deoxyadenosine. The other cluster might serve as the proximal source of sulfur for the methylthio group.**

experiments ruled out SAM as the source of sulfur in methylthiolation and implicated the MiaB protein itself as the source of sulfur. It is possible that, in analogy with biotin and lipoyl synthases, the second iron-sulfur cluster in MiaB can serve as the source of sulfur. The mechanism of methylthiolation is not known, and its revelation is certain to be a novel and interesting chapter in biochemistry.

Wybusine in Eukaryotic tRNA^{Phe}

Hypermodification of guanine at position 37 of eukaryotic tRNA^{Phe} produces the tricyclic base wybusine, shown at the bottom of Figure 14 (Noma *et al.*, 2006). Wybusine biosynthesis requires at least five enzymes, all of which require SAM. The first step in the proposed biosynthetic sequence shown in Figure 14 is methylation of guanine-37 by SAM, catalyzed by the methyltransferase TRS5. A gene deletion search coupled with mass spectrometric analysis of tRNA^{Phe} in *Saccharomyces cerevisiae* led to the identification of four genes required to convert N^8-methylguanine-37 into wybusine (Noma *et al.*, 2006). The proposed functions of gene products TYW1 – 4 are shown in Figure 14. Although the structures of the intermediates in Figure 14 have not been rigorously assigned, they are consistent with the exact masses of intermediates produced in gene deletion strains.

TYW1 incorporates a Radical SAM domain and a flavodoxin domain, and it requires SAM, and presumably flavin mononucleotide (FMN) for electron transfer. TYW1 incorporates a two-carbon fragment into N^8-methylguanine between the N^8-methyl group and 5-amino group of guanine. The biosynthetic origin of the two carbons is not known but might be an acetyl compound such as acetyl-CoA. Elucidation of chemistry in this process awaits the identification of the two-carbon donor. TYW2 catalyzes the alkylation of the TYW1-product by SAM, in which the alkyl group transferred is the α-amino-α-carboxypropyl group of the methionyl moiety in SAM. This alkylation is analogous to methyltransfer or adenosyltransfer by SAM (Frey and Hegeman, 2007b) but with transfer of the α-amino-α-carboxypropyl group. TYW3 catalyzes SAM-dependent methylation at N4 of the guanine ring. The actions of TYW4 complete the wybutosine structure by SAM-dependent methylation of the carboxylate group and methyloxycarbonylation of the amino group. Obvious precedents for the chemistries of the radical SAM and methoxycarbonylation steps are not available, and the explication of these processes will enhance biochemical knowledge.

MEMBERS WITH CHEMICALLY UNCHARACTERIZED FUNCTIONS

Most of the enzymes in Table 1 have not been characterized, and the chemical mechanisms by which they act are not known. Hypotheses have been advanced for some of them, and more could be put forward for others. Here we consider a few of the uncharacterized systems and the current ideas regarding how they might function.

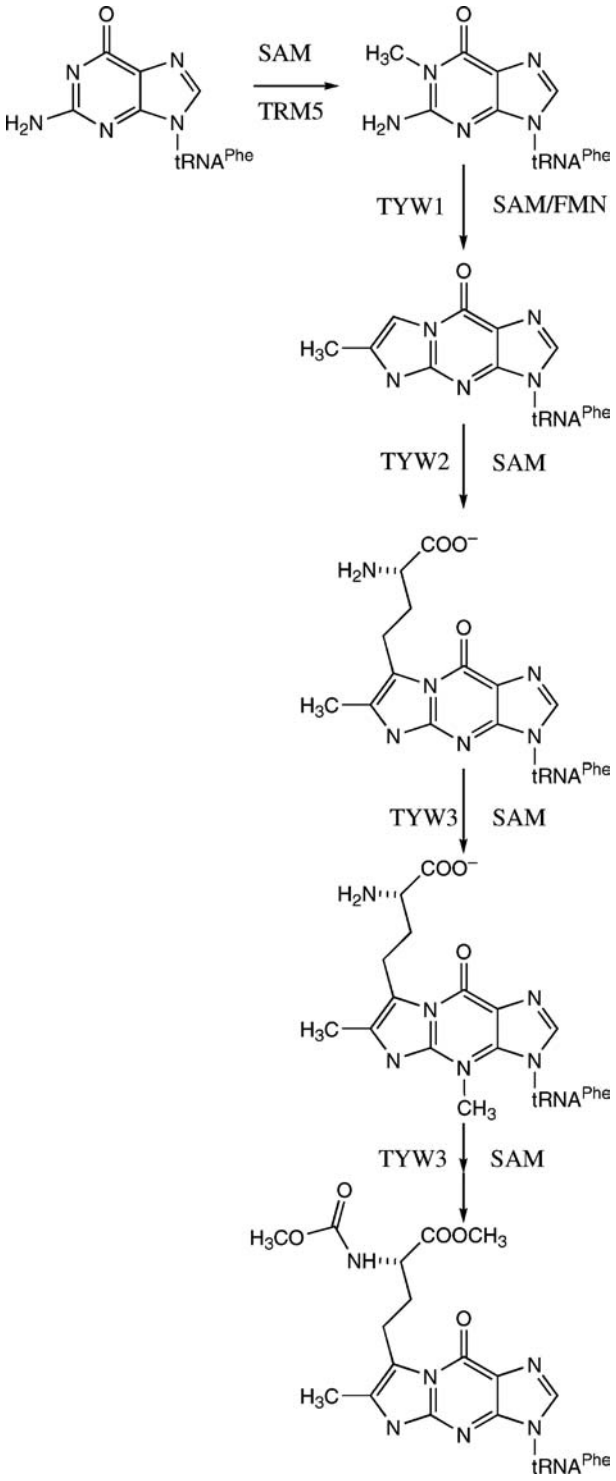

FIGURE 14 **Proposed assembly of wybusine in eukaryotic tRNA^{Phe}. Wybusine at position 37 of eukaryotic tRNAPhe arises from the hypermodification of guanine by five enzymes in *Saccharomyces cerevisiae*. SAM plays a key role in each step, beginning with the methylation of guanine-37 to N^8-methylguanine by TRS5, a SAM-dependent methyltransferase. N^8-Methylguanine undergoes a multi-step transformation into wybusine in a process requiring four gene products designated TWY1 – 4 (Noma *et al.*, 2006). TWY1 is a radical SAM enzyme with a flavodoxin domain, and so probably also requires FMN to function in electron transfer. The diverse roles of SAM in each of the steps is discussed in the text.**

SAM as a Catalytic Coenzyme

Currently, the 2,3-aminomutases and spore photoproduct lyase are the only radical SAM enzymes that use SAM as a reversible source of the 5′-deoxyadenosyl radical as an oxidant and radical initiator. More are likely to be found. DesII in the desosamine biosynthetic pathway is thought to function as an aminomutase (Szu *et al.*, 2005; Zhao *et al.*, 2001). The plant enzyme littorine mutase catalyzes a rearrangement in cell extracts similar to that catalyzed by methylmalonyl-CoA mutase, an adenosylcobalamin-dependent enzyme (Figure 15). Littorine mutase might use the SAM–[4Fe–4S] system in place of adenosylcobalamin, which is not found in plants, as the reversible source of the 5′-deoxyadenosyl radical (Ollagnier *et al.*, 1998; Layer *et al.*, 2005). Alternatively, littorine mutase might be a glycyl radical enzyme that is activated in cell extracts by a radical SAM activating enzyme. The latter is the role of SAM in the function of adenosylcobalamin-independent glycerol dehydratase (O'Brien *et al.*, 2004). The question of the biochemical role of SAM in the function of littorine mutase remains to be determined through biochemical characterization of the enzyme and the role of SAM in activating it.

Methylation by Radical SAM Enzymes

Experiments on the biogenesis of antibiotics indicate that certain methylation reactions do not take place by the nucleophilic displacement mechanism typical of standard SAM-dependent methyltransferases, such as catechol methyltransferase and DNA methylases. Genomic information implicates radical SAM enzymes in several instances where the methylated atom is not intrinsically nucleophilic.

Structural formulas for three antibiotics in which Radical SAM proteins appear to participate in

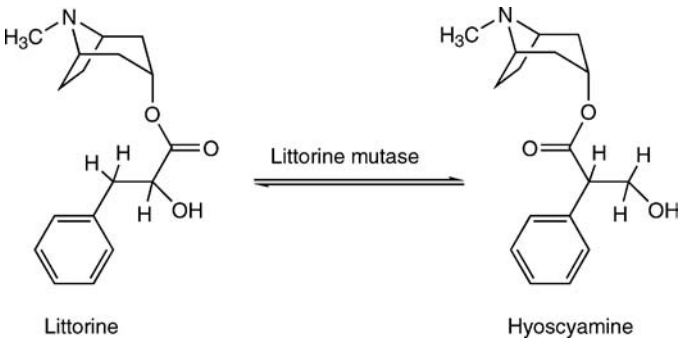

FIGURE 15 **The isomerization catalyzed by littorine mutase.**

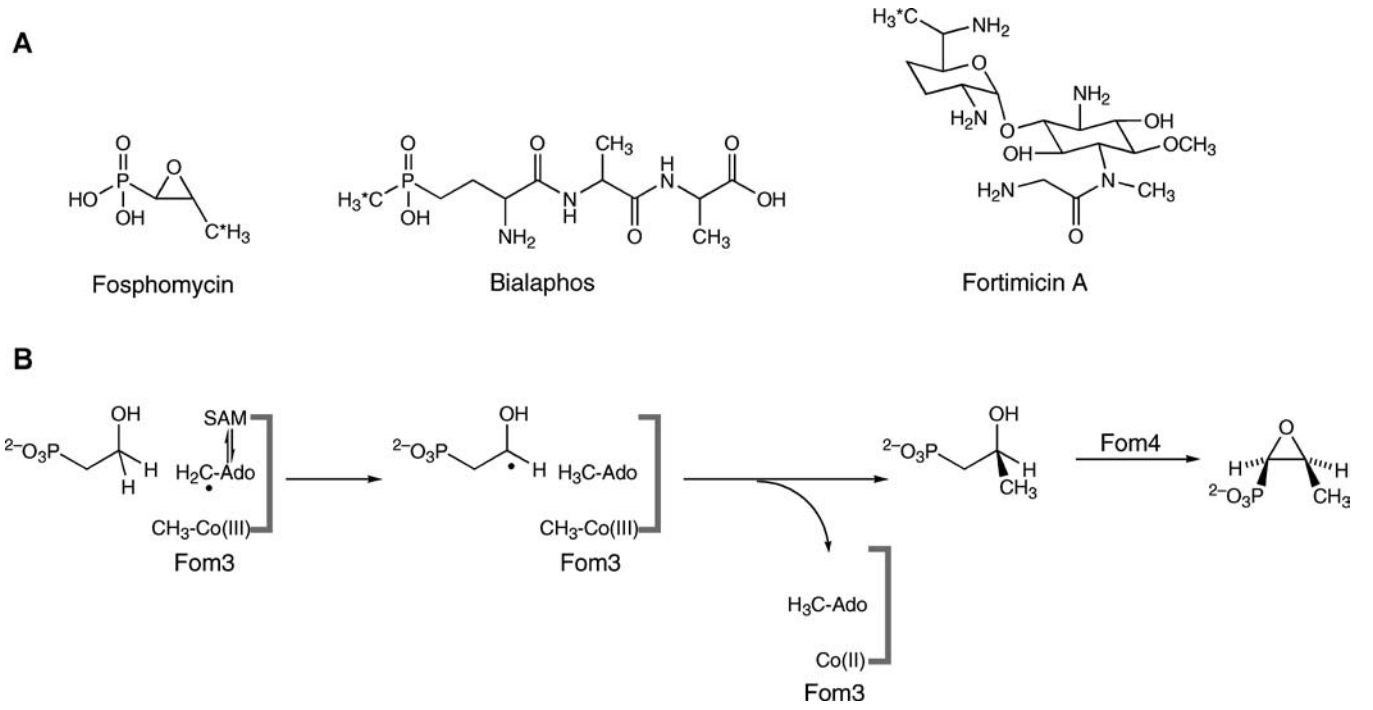

FIGURE 16 **Radical SAM enzymes in methylation of antibiotics. *A.* Structures of three antibiotics methylated on non-nucleophilic atoms. *B.* A mechanism that rationalizes the role of the radical SAM enzyme Fom3 in the methylation step of fosfomycin biosynthesis. The enzyme is thought to comprise radical SAM and cobalamin binding domains.**

methylation are shown in Figure 16A, with the methyl groups in question marked by asterisks. Fom3 appears to catalyze methyl transfer to carbon in the biosynthesis of fosfomycin (Kuzuyama *et al.*, 1992). Bialaphos is an herbicide (Thompson & Seto, 1995; Hoerlein, 1994), and BcpD in the biosynthetic pathway appears to be a Radical SAM enzyme. BcpD is thought to methylate a phosphinate group, producing the only known biological C–P–C linkage. Fms7 appears to be a Radical SAM enzyme required in the biosynthesis of the aminoglycoside fortimicin, where the methyl group is bonded to carbon (Kuzuyama *et al.*, 1995). While Fom3, BcpD, and Fms7 do not share high degrees of similarity in amino acid sequences, all have the CxxxCxxC motif in one domain, and they all have hypothetical cobalamin-binding sites, analogous to methionine synthase, in another domain.

BcpD, Fom3, and Fms7 appear to require methylcobalamin, which serves as the source of methyl groups in the antibiotics (Kuzuyama *et al.*, 1992; Seto *et al.*, 1982; Okumura, 1981). These proteins might act by mechanisms that draw from the rescue property of methylcobalamin-dependent methionine synthase, MetH (Matthews, 2001). Met H catalyzes methyl transfer from methyltetrahydrofolate to cob(I)alamin, producing tetrahydrofolate and methylcob(III)alamin. Subsequent transfer of the methyl group to homocysteine regenerates cob(I)alamin and produces methionine. Separate protein domains bind cobalamin, methyltetrahydrofolate, and homocysteine. The intermediate cob(I)alamin is highly reactive with molecular oxygen and is occasionally oxidized to cob(II)alamin, inactivating the enzyme. This event calls into play a fourth domain, which binds SAM. Reductive methylation of cob(II)alamin by SAM regenerates methylcobalamin and reactivates the enzyme (Matthews, 2001; Bandarian *et al.*, 2001).

The differences between the radical SAM methyltransferases and MetH indicate that they are unlikely to share a mechanism. Although MetH is dependent on both cobalamin and SAM, it is not a radical SAM enzyme. Feeding experiments show that methyltetrahydrofolate is not used as a methyl group donor by either BcpD or Fom3 (Kuzuyama *et al.*, 1992; Seto, H. *et al.*, 1982; Okumura, 1981). It remains unclear whether radical SAM methyltransferases generate methylcobalamin as an intermediate or whether methylcobalamin arises from an outside source.

Although methylcobalamin is a methyl donor for Fom3, BclD, and Fms7, the putative substrates are unreactive carbon atoms or electrophilic atoms, making it unlikely that they will engage in nucleophilic attack.

Recent results have led to the suggestion of a suitable mechanism for carbon methylation by Fom3 (Woodyer *et al.*, 2007). The experiments show that the substrate for Fom3 is hydroxyethyl phosphonate, shown in Figure 16B. Abstraction of a hydrogen atom from the alcoholic group by the 5′-deoxyadenosyl radical in the radical SAM domain would generate a hydroxyethyl phosphonate radical, which could be methylated by methycobalamin in the cobalamin binding domain to form hydroxypropyl phosphonate (Figure 16B). Fom4 could then catalyze epoxidation of hydroxypropyl phosphonate to produce fosfomycin. Biochemical evidence bearing on the mechanism of methylation in the biosynthesis of fosfomycin would test the hypothetical mechanism in Figure 16B.

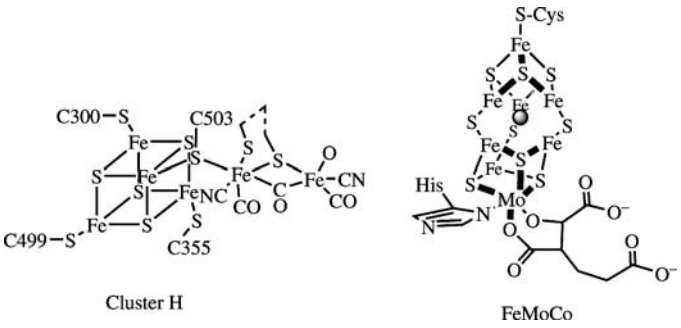

FIGURE 17 **Structures of complex iron clusters in hydrogenase and nitrogenase. The structures of cluster H in FeFe hydrogenase and of FeMoCo in nitrogenase are illustrated here as determined by x-ray crystallography. The dithiolate ligand in cluster H has not been chemically characterized. The interstitial atom in FeMoCo has not been chemically identified. Two radical SAM enzymes, HydE and HydG participate in the biosynthesis of cluster H, and NifB participates in the biosynthesis of FeMoCo.**

SAM in Cofactor Biosynthesis

Studies of other radical SAM enzymes are in early stages. For example, ThiH is under intensive biochemical and genetic analysis of its participation in thiamine biosynthesis in *E. coli* (Kriek *et al.*, 2007; Martinez-Gomez *et al.*, 2004). Tyrosine, cysteine, and 1-deoxyxylulose-5-phosphate are required to assemble the thiazole ring, and current information suggests that the 5′-deoxyadenosyl radical generated by ThiH may initate the fragmentation of tyrosine into a glycyl radical for incorporation into the ring (Leonardi & Roach, 2004; Park *et al.*, 2003). Future research should elucidate the beautiful chemistry of thiazole biosynthesis in *E. coli*. Radical SAM enzymes are implicated in bacteriochlorophyll and pyrroloquinoline quinone biosynthesis as well. The explicit chemistry of the steps in which the radical SAM enzymes participate remain to be elucidated.

Radical SAM enzymes participate in the assembly of the complex metallocofactors cluster H in FeFe hydrogenase (Leach & Zamble, 2007; Peters *et al.*, 2006) and the iron-molybdenum cofactor FeMoCo in nitrogenase (Curatti *et al.*, 2006) structures of which are shown in Figure 17. Both clusters incorporate unusual features. The H-cluster includes cyanide, carbon monoxide, and a chemically uncharacterized dithiol as ligands to iron. It has been postulated that HydE and HydG catalyze the biosyntheses of these ligands in the assembly of the H-cluster (Peters *et al.*, 2006). NifB is a radical SAM protein that catalyzes the assembly of the FeMoCo cluster in nitrogenase (Curatti *et al.*, 2006). Among the unusual features of this cluster, in addition to the presence of molybdenum and homocysteine, is the presence of an unidentified interstitial atom, similar in electron density to C, N, or O. SAM stimulates the assembly process in the presence of NifB, Fe^{2+}, sulfide, and homocitrate. Assembly is inhibited by *S*-adenosylhomocysteine (SAH), and SAH-inhibition is overcome by high concentrations of SAM. The explicit function of SAM is under investigation.

CONCLUSION

Available information about radical SAM enzymes indicates that they are of ancient origin, and were among the earliest biological catalysts to function by radical mechanisms. While the biochemical functions of radical SAM enzymes are highly diverse, those that have been investigated to date have in common the one-electron reductive cleavage of SAM into methionine and the 5′-deoxyadenosyl radical, which initiates a radical mechanism by abstraction of a hydrogen atom from a substrate. The subsequent radical chemistry is well understood for aminomutases, glycyl radicalization of glycyl radical enzymes, and spore photoproduct lyase. The initial SAM-dependent hydrogen abstraction steps in the reactions of biotin synthase, lipoyl synthase, and HemN are established, while basic questions remain about subsequent steps in the overall transformations. Little is known about the chemistry of the remaining reactions of radical SAM enzymes. Future research is likely to reveal a rich universe of radical mechanisms in the actions of radical SAM enzymes.

REFERENCES

Aberhart, D.J., Gould, S.J., Lin, H.-J., Thiruvengadam, T.K., and Weiller, B.H. 1983. Stereochemistry of Lysine 2,3-Aminomutase Isolated from *Clostridium subterminale* Strain SB4. *J Am Chem Soc* 105:5461–5470.

Akhtar, M. 1994. The modification of acetate and propionate side chains during the biosynthesis of haem and chlorophills: mechanistic and stereochemical studies. *Ciba Found Symp* 180:131–155.

Allen, R.M., Chatterjee, R., Ludden, P.W., and Shah, V.K. 1995. Incorporation of iron and sulfur from NitB cofactor into the iron-molybdenum cofactor of nitrogenase *J Biol Chem* 270:26890–26896.

Baker, J.J., van der Drift, C., and Stadtman, T.C. 1973. Purification and properties of β-lysine mutase, a pyridoxal phosphate and B12 coenzyme dependent enzyme. *Biochemistry* 12:1054–1063.

Ballinger, M.D., Reed, G.H., and Frey, P.A. 1992a. An organic radical in the lysine 2,3-aminomutase reaction. *Biochemistry* 31:949–953.

Ballinger, M.D., Frey, P.A., and Reed, G.H. 1992b. Structure of a substrate radical intermediate in the reaction of lysine 2,3-aminomutase. *Biochemistry* 31:10782–10789.

Bandarian, V., Pattridge, K.A., Lennon, B.W., Huddler, D.P., Matthews, R.G., and Ludwig, M.L. 2001. Domain alternation switches B12-dependent methionine synthase to the activation conformation. *Nat Struct Biol* 9:53 – 56.

Baraniak, J., Moss, M.L., and Frey, P.A. 1989. Lysine 2,3-aminomutase. Support for a mechanism of hydrogen transfer involving S-adenosylmethionine. *J Biol Chem* 264:1357–1360.

Becker, A., Küster, H., Niehaus, K., and Pühler, A. 1995. Extension of the *Rhizobium meliloti* succinoglycan biosynthesis gene cluster: identification of the *exsA* gene encoding an ABC transporter protein, and the *exsB* gene which probably codes for a regulator of succinoglycan biosynthesis. *Mol Gen Genet* 249:487–497.

Begley, T.P., Xi, J., Kinsland, C., Taylor, S., and McLafferty, F. 1999. The enzymology of sulfur activation during thiamin and biotin biosynthesis. *Curr Opin Chem Biol* 3:623–629.

Berkovitch, F., Nicolet, Y., Wan, J.T., Jarrett, J.T., and Drennan, C.L. 2004. Crystal structure of biotin synthase, an *S*-adenosylmethionine-dependent radical enzyme. *Science* 303:767–769.

Boll R., Hofmann, C., Heitmann, B., Hauser, G., Glaser, S., Koslowski, T., Friedrech, T., and Bechthold, A. 2006. The active conformation of Avilamycin A is conferred by AviX12, a radical AdoMet enzyme. *J Biol Chem* 281:14756–14763.

Brede D.A., Faye, T., Johnsborg, O., Odegard, I., Nes, I.F., and Holo, H. 2004. Molecular and genetic characterization of propionicin F, a bacteriocin from *Propionibacterium freudenreicii*. *Appl Environ Microbiol* 70:7303–7310.

Brekau, D., Mahlitz, E., Sauerwald, A., Layer, G., and Jahn, D. 2003. Oxygen-dependent coproporphyrinogen oxidase (HemF) from *Escherichia coli* is stimulated by manganese. *J Biol Chem* 278:46625–46631.

Broach, R.B. and Jarrett, J.T. 2006. Role of the [2Fe-2S]2+ cluster in biotin synthase: mutagenesis of the atypical metal ligand arginine 260. *Biochemistry* 45:14166–14174.

Broderick, J.B., Duderstadt, R.E., Fernandez, D.C., Wojtuszewski, K., Henshaw, T.F., and Johnson, M.K. 1997. Pyruvate formate-lyase activating enzyme is an iron-sulfur protein. *J Am Chem Soc* 119:7396–7397.

Broderick, J.B., Henshaw, T.F., Cheek, J., Wojtuszewski, K., Smith, S.R., Trojan, M.R., McGhan, R.M., Kopf, A., Kibbey, M., and Broderick, W.E. 2000. Pyruvate formate-lyase-activating enzyme: strictly anaerobic isolation yields active enzyme containing a [3Fe-4S](+) cluster. *Biochem Biophys Res Commun* 269:451–456.

Buis, J.M.. Cheek, J., Kalliri, E., and Broderick, J.B. 2006. Characterization of an active spore photoproduct lyase, a DNA repair enzyme in the radical S-adenosylmethinonine superfamily. *J Biol Chem* 281:25994–26003.

Busby, W.B., Schelvis, J.P.M., Yu, D.S., Babcock, G.T., and Marletta, M.A. 1999. Lipoic acid biosynthesis: LipA is an iron-sulfur protein. *J Am Chem Soc* 121:4706–4707.

Chandor, A., Berteau, O., Douki, T., Gasparutto, D., Sanakis, Y., Ollagnier-de-Choudens, S., Atta, M., and Fontecave, M. 2006. Dinucleotide spore photoproduct, a minimal substrate of the DNA repair spore photoproduct lyase enzyme from Bacillus subtilis. *J Biol Chem* 281:26922–32691.

Chang, C.H., Ballinger, M.D., Reed, G.H., and Frey, P.A. 1996. Lysine 2,3-aminomutase: Rapid mix-freeze-quench electron paramagnetic resonance studies establishing the kinetic competence of a substrate-based radical intermediate. *Biochemistry* 35:11081–11084.

Cheek, J. and Broderick, J.B. 2002. Direct H atom abstraction from spore photoproduct C-6 initiates DNA repair in the reaction catalyzed by spore photoproduct lyase: evidence for a reversibly generated adenosyl radical intermediate. *J Am Chem Soc* 124:2860–2861.

Chen, D., Frey, P.A., Lepore, B.W., Ringe, D., and Ruzicka, F.J. 2006. Identification of structureal and catalytic classes of highly conserved amino acid residures in lysine 2,3-aminomutase. *Biochemistry* 45:12647–12653.

Chen, D., and Frey, P.A. 2001. Identification of lysine 346 as a functionally important residue for pyridoxal 5′-phosphate binding and catalysis in lysine 2,3-aminomutase from *Bacillus subtilis Biochemistry* 40:596–602.

Chen, D., Walsby, C., Hoffman, B.M., and Frey, P.A. 2003. Coordination and mechanism of reversible cleavage of *S*-adenosylmethionine by the [4Fe-4S] center in lysine 2,3-aminomutase. *J Am Chem Soc* 125:11788–11789.

Chen, H.P., Wu, S.H., Lin, Y.L., Chen, C.M., and Tsay, S.S. 2001. Cloning, sequencing, heterologous expression, purification, and characterization of adenosylcobalamin-dependent D-ornithine aminomutase from *Clostridium sticklandii*. *J Biol Chem* 276:44744–44750.

Ching, Y.P., Qi, Z., and Wang, J.H. 2000. Cloning of three neuronal Cdk5 activator binding proteins. *Gene* 242:285–294.

Chirpich, T.P., Zappia, V., Costilow, R.N., and Barker, H.A. 1970. Lysine 2,3-aminomutase. Purification and properties of a pyridoxal phosphate and *S*-adenosylmethionine-activated enzyme. *J Biol Chem* 245:1778–1789.

Choi-Rhee, E., Cronan, J.E. 2005. Biotin synthase is catalytic *in vivo*, but catalysis engenders destruction of the protein. *Chem Biol* 12:461–468.

Christenson, S.D., Liu, W., Toney, M.D., and Shen, B. 2003. A novel 4-methylideneimidazole-5-one-containing tyrosine aminomutase in enediyne antitumor antibiotic C-1027 biosynthesis. *J Am Chem Soc* 125:6062–6063.

Cicchillo, R.M. and Booker, S.J. 2005. Mechanistic investigations of lipoic acid biosynthesis in *Escherichia coli*: both sulfur atoms in lipoic acid are contributed by the same lipoyl synthase polypeptide. *J Am Chem Soc* 127:2860–2861.

Cicchillo, R.M., Iwig, D.F., Jones, A.D., Nesbitt, N.M., Baleanu-Gogonea, C., Souder, M.G., Tu, L., Booker, S.J. 2004a. Lipoyl synthase requires two equivalents of *S*-adenosyl-L-methionine to synthesize one equivalent of lipoic acid. *Biochemistry 43*:6378–6386.

Cicchillo, R.M., Lee, K.H., Baleanu-Gogonea, C., Nesbitt, N.M., Krebs, C. and Booker, S.J. 2004b. *Escherichia coli* lipoyl synthase binds two distinct [4Fe-4S] clusters per polypeptide. *Biochemistry* 43:11770–11781.

Colichman, E.L. and Love, D.L. 1953. Polarography of sulfonium salts. *J Org Chem* 18:40–46.

Cone, M.C., Yin,X., Grochowski, L.L., Parker, M.R., and Zabriskie T.M. 2003. The Blasticidin S biosynthesis gene cluster from *Streptomyces griseochromogenes*: Sequence analysis, organization, and initial characterization. *Chem Bio Chem* 4:821–828.

Cosper, N.J., Booker, S.J., Ruzicka, F., Frey, P.A., and Scott, R.A. 2000. Direct FeS cluster involvement in generation of a radical in lysine 2,3-aminomutase. *Biochemistry* 39:15668–15673.

Cosper, M.M., Cosper, N.J., Hong, W., Shokes, J.E., Broderick, W.E., Broderick, J.B., Johnson, M.K., and Scott, R.A. 2003. Structural

studies of the interaction of *S*-adenosylmethionine with the [4Fe-4S] clusters in biotin synthase and pyruvate formate-lyase activating enzyme. *Protein Sci* 12:1573–1577.
Cosper, M.M., Jameson, G.N., Davydov, R., Eidsness, M.K., Hoffman, B.M., Huynh, B.H., and Johnson, M.K. 2002. The $[4Fe\text{-}4S]^{2+}$ cluster in reconstituted biotin synthase binds *S*-adenosyl-L-methionine. *J Am Chem Soc* 124:14006–14007.
Cosper M.M., Jameson, G.N., Hernandez, H.L., Krebs, C., and Booker, S.J. 2004. Characterization of the cofactor composition of *Escherichia coli* biotin synthase. *Biochemistry* 43:2007–2021.
Curatti, L., Ludden, P.W., and Rubio, L.M. 2006. NifB-dependent *in vitro* synthesis of the iron-molybdenum cofactor of nitrogenase. *Proc Natl Acad Sci U.S.A* 103:5297–5301.
DeMong, D.E. and Williams, R.M. 2003. Asymmetric synthesis of (2*S*, 3*R*)-capreomycidine and the total synthesis of capreomycin IB. *J Am Chem Soc* 125:8561–8565.
Douglas, P., Kriek, M., Bryant, P., and Roach, P.L. 2006. Lipoyl synthase inserts sulfur atoms into an octanoyl substrate in a stepwise manner. *Angew Chem Int Ed Engl* 45:5197–5199.
Duin, E.C., Lafferty, M.E., Crouse, B.R., Allen, R.M., Sanyal, I., Flint, D.H., and Johnson, M.K. 1997. [2Fe-2S] to [4Fe-4S] cluster conversion in *Escherichia coli* biotin synthase. *Biochemistry* 36:11811–11820.
Eliasson, R., Fontecave, M., Jornvall, H., Krook, M., Pontis, E., and Reichard, P. 1990. The anaerobic ribonucleoside triphosphate reductase from *Escherichia coli* requires *S*-adenosylmethionine as a cofactor. *Proc Natl Acad Sci U S A* 87:3314–3318.
Eliasson, R., Pontis, E., Fontecave, M., Gerez, C., Harder, J., Jornvall, H., Krook, M., and Reichard, P. 1992. Characterization of components of the anaerobic ribonucleotide reductase system from *Escherichia coli*. *J Biol Chem* 267:25541–25547.
Eliot, A.C. and Kirsch, J.F. 2004. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. *Annu Rev Biochem* 73:383–415.
Esberg, B., Leung, H.-C.E.. Tsui, H.-C.T., Björk, G.R., and Winkler, M.E. 1999. Identification of the *miaB* gene, involved in methylthiolation of isopentenylated A37 derivatives in the tRNA of *Salmonella typhymurium* and *Escherichia coli J Bacteriol* 181:7256–7265.
Fang, Q., Peng, J. and Dierks, T. 2004. Post-translational formylglycine modification of bacterial sulfatases by the radical *S*-adenosylmethionine protein AtsB. *J Biol Chem* 279:14570–14578.
Florentin, D., Bui, M.T., Marquet, A., Ohshiro, T., and Izumi, Y. 1994. On the mechanism of biotin synthase of Bacillus sphaericus. *C. R. Acad Sci III* 317:485–488.
Fontecave, M. Mulliez, E. and Ollagnier-de-Choudens, S. 2001. Adenosylmethionine as a source of 5′-deoxyadenosyl radicals. *Curr Opin Chem Biol* 5:506–511.
Fontecave, M., Ollagnier-de-Choudens, S., and Mulliez, E. 2003. Biological radical sulfur insertion reactions. *Chem Rev* 103:2149–2166.
Frey, M. Rothe, M., Wagner, A.F., and Knappe, J. 1994. Adenosylmethionine-dependent synthesis of the glycyl radical in pyruvate formate-lyase by abstraction of the glycine C-2 pro-*S* hydrogen atom. Studies of [^{2}H]glycine-substituted enzyme and peptides homologous to the glycine 734 site. *J Biol Chem* 269:12432–12437.
Frey, P.A. 1993. Lysine 2,3-aminomutase: is adenosylmethionine a poor man's adenosylcobalamin? *FASEB J* 7:412–470.
Frey, P.A. and Hegeman, A.D. 2007a. *Enzymatic Reaction Mechanisms*, New York: Oxford University Press, pp. 129–188.
Frey, P.A. and Hegeman, A.D. 2007b. *Enzymatic Reaction Mechanisms*, New York: Oxford University Press, pp. 655–678.
Frey, P.A. and Magnusson, O.Th. 2003. *S*-Adenosylmethionine: a wolf in sheep's clothing, or a rich man's adenosylcobalamin? *Chem Rev* 103:2129–2148.
Frey, P.A. and Moss, M.L. 1987. *S*-Adenosylmethionine and the mechanism of hydrogen transfer in the lysine 2,3-aminomutase reaction. *Cold Spring Har Symp Quant Bio* LII:571–577.
Friedel, M.G., Bereau, O., Pieck, J.C., Atta, M., Ollagnier-de-Choudens, S., Fontecave, M., and Carell, T. 2006. The spore photoproduct lyase repairs the 5S- and not the 5R-configured spore photoproduct DNA lesion. *Chem Commun (Camb)* 445–446.
Goodwin, P.M. and Anthony, C. 1998. The biochemistry, physiology, and genetics of PQQ and PQQ-containing enzymes. *Adv. Microbiol Physiol* 40:1–80.
Graham, D.E., Xu, H., and White, R.H. 2003. Identification of the 7,8-didemethyl-8-hydroxy-5-deazariboflavin synthase required for coenzyme F(420) biosynthesis. *Arch Microbiol* 180:455–464.
Grewal, T.S., Genever, P.G., Brabbs, A.C., Birch, M., and Skerry, T.M. 2000. *Best5*: a novel interferon-inducible gene expressed during bone formation. *FASEB J* 14:523–531.
Grimshaw, J. 1981. Electrochemistry of the sulphonium group, In: *(Stirling, C.J.M. and Patai, S., eds.) The Chemistry of the Sulphonium Group* New York: Wiley & Sons Ltd., pp.141–155.
Guianvarc'h, D., Florentin, D., Tse Sum Bui, B., Nunzi, F., and Marquet, A. 1997. Biotin synthase, a new member of the family of enzymes which uses *S*-adenosylmethionine as a source of deoxyadenosyl radical. *Biochem. Biophys. Res. Commun.* 236:402–406. Erratum in: (1997) *Biochem Biophys Res Commun* 240:246.
Hänzelmann, P., Hernandez, H.L., Menzel, C., Garcia-Serres, R., Huynh, B.H., Johnson, M.K., Mendel, R.R., and Schindelin, H. 2004. Characterization of MOCS1A, an oxygen-sensitive iron-sulfur protein involved in human molybdenum cofactor biosynthesis. *J Biol Chem* 279:34721–34734.
Hänzelmann, P. and Schindelin, H. 2004. Crystal structure of the *S*-adenosylmethionine-dependent enzyme MoaA and its implications for molybdenum cofactor deficiency in humans. *Proc Natl Acad Sci U.S.A* 101:12870–12875.
Hänzelmann, P. and Schindelin, H. 2006. Binding of 5-GTP to the C-terminal FeS cluster of the radical *S*-adenosylmethionine enzyme MoaA provides insights into its mechanism. *Proc Natl Acad Sci U.S.A* 103:6829–6834.
Harder, J., Eliasson, R., Pontis, E., Ballinger, M.D., and Reichard, P. 1992. Activation of the anaerobic ribonucleotide reductase from *Escherichia coli* by *S*-adenosylmethionine. *J Biol Chem* 267:25548–25552.
Hayden, M.A., Huang, I.Y., Iliopoulos, G., Orozco, M., and Ashley, B.W. 1993. Biosynthesis of lipoic acid: characterization of the lipoic acid auxotrophs Escherichia coli W1485-lip2 and JRG33-lip9. *Biochemistry* 32:3778–3782.
Heider, J., Spormann, A.M., Beller, H.R., and Widdel, F. 1999. Anaerobic bacterial metabolism of hydrocarbons. *FEMS Microbiol Rev* 22:459–473.
Hernandez, H.L., Pierrel, F., Elleingand, E., Garcia-Serres, R., Huynh, B.H., Johnson, M.K., Fontecave, M., and Atta, M. 2007. MiaB, a bifunctional radical-S-adenosylmethionine enzyme involved in the thiolation and methylation of tRNA, contains two essential [4Fe-4S] Clusters *Biochemistry* 46:5140–5147.
Hinckley, G.T. and Frey, P.A. 2006. Cofactor-dependence in reduction potentials for $[4Fe\text{-}4S]^{2+/1+}$ in lysine 2,3-aminomutase. *Biochemistry* 45:3219–3225.
Hoerlein, G. 1994. Glufosinate (phosphinothricin), a natural amino acid with unexpected herbicidal properties. *Rev. Environ Contam and Tox* 138, 73-145.
Jameson, G.N., Cosper, M.M., Hernandez, H.L., Johnson, M.K., and Huynh, B.H. 2004. Role of the [2Fe-2S] cluster in recombinant *Escherichia coli* biotin synthase. *Biochemistry* 43:2022–2031.
Jarrett, J.T. 2003. The generation of 5′-deoxyadenosyl radicals by adenosylmethionine-dependent radical enzymes. *Curr Opin Chem Biol* 7:174–182.
Jarrett, J.T. 2005. The novel structure and chemistry of iron-sulfur clusters in the adenosylmethionine-dependent radical enzyme biotin synthase. *Arch Biochem Biophys* 433:312–321.
King, D.S. and Reichard, P. 1995, Mass spectrometric determination of the radical scission site in the anaerobic ribonucleotide reductase of Escherichia coli. *Biochem Biophys Res Commun* 206:731–735.

Knappe, J. and Schmitt, T. 1976. A novel reaction of S-adenosyl-L-methionine correlated with the activation of pyruvate formate-lyase. *Biochem Biophys Res Commun* 71:1110–1117.

Knappe, J., Neugebauer, F.A., Blaschkowski, H.P., and Ganzler, M. 1984. Post-translational activation introduces a free radical into pyruvate formate-lyase. *Proc Natl Acad Sci USA* 81:1332–1335.

Krebs, C., Broderick, W.E., Henshaw, T.F., Broderick, J.B., and Huynh, B.H. 2002. Coordination of adenosylmethionine to a unique iron site of the [4Fe-4S] of pyruvate formate-lyase activating enzyme: a Mossbauer spectroscopic study. *J Am Chem Soc* 124:912–913.

Krebs, C., Henshaw, T.F., Cheek, J., Huynh, B.H., and Broderick, J.B. 2000. Conversion of 3Fe-4S to 4Fe-4S clusters in native pyruvate formate-lyase activating enzyme: Mössbauer characterization and implications for mechanism. *J Am Chem Soc* 122:12497–12506.

Krieger, C.J., Roseboom, W., Albracht, S.P., and Spormann, A.M. 2001. A stable organic free radical in anaerobic benzylsuccinate synthase of Azoarcus sp. strain T *J Biol Chem* 276:12924–12927.

Kriek, M., Martins, F., Leonardi, R., Fairhurst, S.A., Lowe, D.J., and Roach, P.L. 2007. Thiazole synthase from Escherichia coli: An investigation of the substrates and purified proteins required for activity in vitro. *J Biol Chem* 282:17413–17423.

Kulzer, R., Pils, t., Kappl, R., Huttermann, J., and Knappe, J. 1998. Reconstitution and characterization of the polynuclear iron-sulfur cluster in pyruvate formate-lyase-activating enzyme. Molecular properties of the holoenzyme form. *J Biol Chem* 273:4897–4903.

Kuzuyama, T., Hidaka, T., Kamigiri, K., Imai, S., and Seto, H. 1992. Studies on the biosynthesis of fosfomycin. 4. The biosynthetic origin of the methyl group of fosfomycin. *J Antibiot* 45:1812– 1814.

Kuzuyama, T., Seki, T., Dairi, T., Hidaka, T., and Seto, H. 1995. Nucleotide sequence of fortimicin KL1 methyltransferase gene isolated from Micromonospora olivasterospora, and comparison of its deduced amino acid sequence with those of methyltransferases involved in the biosynthesis of bialaphos and fosfomycin. *J Antibiot* 48:1191–1193.

Langer, B., Langer, M., and Rétey, J. 2001. Methylidene imidazolone (MIO) from histidine and phenylalanine ammonia-lyase. *Adv Prot Chem* 58:175–214.

Layer, G., Kervio, E., Morlock, G., Heinz, D.W., Jahn, D., Rétey, J., and Schubert, W.D. 2005. Structural and functional comparison of HemN to other Radical SAM enzymes. *Biol Chem* 386:971–980.

Layer, G., Moser, J., Heinz, D.W., Jahn, D., and Schubert, W.D. 2003. Crystal structure of coproporphyrinogen III oxidase reveals cofactor geometry of Radical SAM enzymes. *EMBO J* 22:6214–6224.

Layer, G., Pierik, A.J., Trost, M., Rigby, S.E., Leech, H.K., Grage, K., Breckdau, D., Astner, I., Jansch, L., Heathcote, P., Warren, M.J., Heinz, D.W., and Jahn, D. 2006. The substrate radical of *Escherichia coli* oxygen-independent coproporphyrinogen III oxidase HemN. *J Biol Chem* 281, 15727–15734.

Lees, N.S., Chen, D., Walsby, C.J., Behshad, E., Frey, P.A., and Hoffman, B.M. 2006. How an enzyme tames reactive intermediates: positioning of the active-site components of lysine 2,3-aminomutase during enzymatic turnover as determined by ENDOR spectroscopy. *J Am Chem Soc* 128:10145–10154.

Leonardi, R. and Roach, P.L. 2004. Thiamine biosynthesis in Escherichia coli. In vitro reconstitution of the thiazole synthase activity. *J Biol Chem* 279:17054–17062.

Lepore, B.W., Ruzicka, F.J., Frey, P.A., and Ringe, D. 2005. The x-ray crystal structure of lysine-2,3-aminomutase from Clostridium subterminale. *Proc Natl Acad Sci USA* 102:13819–13824.

Lieder, K.W., Booker, S., Ruzicka, F.J., Beinert, H., and Frey, P.A. 1998. *S*-Adenosylmethionine-dependent reduction of lysine 2,3-aminomutase and observation of the catalytically functional iron-sulfur centers by electron paramagnetic resonance. *Biochemistry* 37:2578–2585.

Lotierzo, M., Tse Sum Bui, B., Florentin, D., Escalettes, F., and Marquet, A. 2005. Biotin synthase mechanism: an overview. *Biochem Soc Trans* 33:820–823.

Liu, A. and Graslund, A. 2000. Electron paramagnetic resonance evidence for a novel interconversion of [3Fe-4S](+) and [4Fe-4S](+) clusters with endogenous iron and sulfide in anaerobic ribonucleotide reductase activase in vitro. *J, Biol Chem* 275:12367–12373.

Lyutzkanova, D., Distler, J., and Altenbuchner, J. 1997. A spectinomycin resistance determinant from the spectinomycin producer *Streptomyces flavopersicus*. *Microbiology* 143:2135–2143.

Magnusson, O.Th., Reed, G.H., and Frey, P.A. 1999. Spectroscopic evidence for the participation of an allylic analogue of the 5′-deoxyadenosyl radical in the reaction of lysine 2,3-aminomutase. *J Am Chem Soc* 121:9764–9765.

Magnusson, O.T., Reed, G.H., and Frey, P.A. 2001. Characterization of an allylic analogue of the 5′-deoxyadenosyl radical: An intermediate in the reaction of lysine 2,3-aminomutase. *Biochemistry* 40:7773–7782.

Mao, U., Varoglu, M., and Sherman, F.H. 1999. Molecular characterization and analysis of the biosynthetic gene cluster for the antitumor antibiotic mitomycin C from *Streptomyces lavendulae* NRRL2564. *Chem Biol* 6:251–263.

Marquet A. 2001. Enzymology of carbon-sulfur bond formation. *Curr Opin Chem Biol* 5:541–549.

Marsh, E.N., Patwardhan, A., and Huhta, M.S. 2004. *S*-adenosylmethionine radical enzymes. *Bioorg Chem* 32:326–440.

Martinez-Gomez, N.C., Robers, M., and Downs, D.M. 2004. Mutational analysis of ThiH, a member of the radical *S*-adenosylmethionine (AdoMet) protein superfamily *J Biol Chem* 279:40505–40510.

Matthews, R.G. 2001. Cobalamin-dependent methyltranfserases, *Acc Chem Res* 34:681–689.

Miller, J., Bandarian, V., Reed, G.H., and Frey, P.A. 2001. Inhibition of lysine 2,3-aminomutase by the alternative substrate 4-thialysine and characterization of the 4-thialysyl radical intermediate. *Arch Biochem Biophys* 387:281–288.

Miller, J.R., Busby, R.W., Jordan, S.W., Cheek, J., Henshaw, T.F., Ashley, G.W., Broderick, J.B., Cronan, J.E. Jr., and Marletta, M.A. 2000. *Escherichia coli* LipA is a lipoyl synthase: *in vitro* biosynthesis of lipoylated pyruvate dehydrogenase complex from octanoyl-acyl carrier protein. *Biochemistry* 39:15166–15178.

Möhrle, V., Roos, U., and Bormann, C. 1995. Identification of cellular proteins involved in nikkomycin production in *Streptomyces tendae* Tü901. *Mol Microbiol* 15:561–571.

Morita, M., Tomita, K., Ishizawa, M., Takagi, K., Kawamura, F., Takahashi, H., and Morino, T. 1999. Cloning of oxetanocin A biosynthetic and resistance genes that reside on a plasmid of *Bacillus megaterium* strain NK84-0128. *Biosc Biotechnol Biochem* 63:563–566.

Moss, M., and Frey, P.A. 1987. The role of *S*-adenosylmethionine in the lysine 2,3-aminomutase reaction. *J Biol Chem* 262:14859–14862.

Mulliez, E., Fontecave, M., Gaillard, J., and Reichard, P. 1993. An iron-sulfur center and a free radical in the active anaerobic ribonucleotide reductase of *Escherichia coli J Biol Chem* 268:2296–2299.

Nicolet, Y. and Drennan, C.L. 2004. AdoMet radical proteins–from structure to evolution—alignment of divergent protein sequences reveals strong secondary structure element conservation. *Nucleic Acids Res.* 32:4015–4025.

Noma, A., Kirino, Y., Icheuchi, Y., and Suzuki, T. 2006. Biosynthesis of wybutosine, a hypermodified nucleoside in eukaryotic phenylalanine tRNA. *EMBO J* 25:2142–2154.

Nordlund, P. and Reichard, P. 2006. Ribonucleotide reductases. *Annu Rev Biochem* 75:681–706.

O'Brien, J.R., Raynaud, C., Croux, C., Girbal, L., Soucaille P., and Lanzilotta, W.N. 2004. Insight into the mechanism of the B12-independent glycerol dehydratase from *Clostridium butyricum*: preliminary biochemical and structural characterization. *Biochemistry* 43:4635–4645.

Okumura, S. 1981. Biosynthetic incorporation of methyl group into fortimicins. *J Antibiot* 34:1360–1362.

Ollagnier, S., Kervio, W., and Rétey, J. 1998. The role and source of 5′-deoxyadenosyl radical in a carbon skeleton rearrangement catalyzed by a plant enzyme. *FEBS Lett* 437:309–312.

Ollagnier, S., Mulliez, E., Gaillard, J., Eliasson, R., Fontecave, M., and Reichard, P. 1996. The anaerobic *Escherichia coli* ribonucleotide reductase. Subunit structure and iron sulfur center. *J Biol Chem* 271:9410–9416.

Ollagnier, S., Mulliez, E., Schmidt, P.P., Eliasson, R., Gaillard, J., Deronzier, C., Bergman, T., Graslund, A., Reichard, P., and Fontecave, M. 1997. Activation of the anaerobic ribonucleotide reductase from *Escherichia coli*. The essential role of the iron-sulfur center for *S*-adenosylmethionine reduction. *J Biol Chem* 272:24216–24223.

Ollagnier-De Choudens, S. and Fontecave, M. 1999. The lipoate synthase from *Escherichia coli* is an iron-sulfur protein. *FEBS Lett* 453:25–28.

Ollagnier-De Choudens, S. *et al.* 2000. Iron-sulfur center of biotin synthase and lipoate synthase. *Biochemistry* 39:4165–4173.

Ono, K., Okajima, T., Tani, M., Kuroda, S., Sun, D., Davidson, V.L., and Tanizawa, K. 2006. Involvement of a putative [Fe-S]-cluster-binding protein in the biogenesis of quinohemoprotein amine dehydrogenase. *J Biol Chem* 281:13672–13684

Paraskevopoulou C, Fairhurst S.A., Lowe D.J., Brick P., and Onesti S. 2006. The Elongator subunit Elp3 contains a Fe4S4 cluster and binds *S*-adenosylmethionine. *Mol Microbiol* 59:795–806.

Park, J. H., Dorrestein, P. C., Zhai, H., Kinsland, C., McLafferty, F. W., and Begley, T. P. 2003. Biosynthesis of the thiazole moiety of thiamin pyrophosphate (vitamin B1) *Biochemistry* 42:12430–12438

Peters, J.W., Szilagyi, R.K., Naumov, A., and Douglas, T. 2006. A radical solution for the biosynthesis of the H-cluster of hydrogenase. *FEBS Lett* 580:363–367.

Petrovich, R.M.. Ruzicka, F.J., Reed, G.H. and Frey, P.A. 1991. Metal cofactors of lysine-2,3-aminomutase. *J Biol Chem* 266:7656–7660.

Petrovich, R.M., Ruzicka, F.J., Reed, G.H. and Frey, P.A. 1992. Characterization of iron-sulfur clusters in lysine 2,3-aminomutase by electron paramagnetic resonance spectroscopy. *Biochemistry* 31:10774–10781.

Pierrel, F., Bjork, G.R., Fontecave, M., and Atta, M. 2002. Enzymatic modification of tRNAs: MiaB is an iron-sulfur protein. *J Biol Chem* 277:13367–13370.

Pierrel, F. Douki, T., Fontecave, M., and Atta, M. 2004. MiaB protein is a bifunctional radical-S-adenosylmethionine enzyme involved in thiolation and methylation of tRNA. *J Biol Chem* 279:47555–47563.

Pierrel, F., Hernandez, H.L., Johnson, M.K., Fontecave, M., and Atta, M. 2003. MiaB protein from *Thermotoga maritima*. Characterization of an extremely thermophilic tRNA-methylthiotransferase. *J Biol Chem* 278:29515–29524.

Posewitz, M.C., King, P.W., Smolinski, S.L., Zhang, L., Seibert, M., and Ghirardi, M.L. 2004. Discovery of two novel radical S-adenosylmethionine proteins required for the assembly of an active [Fe] hydrogenase. *J Biol Chem* 279:25711–25720.

Qiao, C. and Marsh, E.N. 2005. Mechanism of benzylsuccinate synthase: stereochemistry of toluene addition to fumarate and maleate. *J Am Chem Soc* 127:8608–8609.

Prabhakaran, P.A., Woo, N.-T., Yorgey, P.S., and Gould, S.J. 1988, Biosynthesis of Blasticidin S from L-α-Arginine. Stereochemistry in the Arginine-2,3-aminomutase Reaction. *J Am Chem Soc* 110:5785–5791.

Rebeil, R and Nicholson, W.L. 2001. The subunit structure and catalytic mechanism of the *Bacillus subtilis* DNA repair enzyme spore photoproduct lyase. *Proc. Natl Acad Sci USA* 98:9038–9043.

Rebeil, R., Sun, Y., Chooback, L., Pedraza-Reyes, M., Kinsland, C., Begley, T.P., and Nicholson, W.L. 1998. Spore photoproduct lyase from Bacillus subtilis spores is a novel iron-sulfur DNA repair enzyme which shares fratures with proteins such as class III anaerobic ribonucleotide reductases and pyruvate-formate lyases. *J Bacteriol.* 180:4879–4885.

Reed, G.H. 2004. Radical mechanisms in adenosylcobalamin-dependent enzymes. *Curr Opin Chem Biol* 8:477–483.

Reed, K.E. and Cronan, J.E. Jr. 1993. Lipoic acid metabolism in Escherichia coli: sequencing and functional characterization of the lipA and lipB genes. *J Bacteriol* 175:1325–1336.

Rieder, C., Eisenreich, W., O'Brien, J., Richter, G., Götze, E., Boyle, P., Blanchard, S., Bacher, A., and Simon, H. 1998. Rearrangement reactions in the biosynthesis of molybdopterin: an NMR study with multiply $^{13}C/^{15}N$ labeled precursors. *Eur J Biochem* 255:24–36.

Ruzicka, F.J. and Frey, P.A. 2007. Glutamate 2,3-aminomutase: a new member of the radical SAM superfamily of enzymes. *Biochim Biophys Acta* 1774:286–296.

Ruzicka, F.J., Lieder, K.W., and Frey, P.A. 2000. Lysine 2,3-aminomutase from *Clostridium subterminale* SB4: Mass spectral characterization of cyanogens bromide-treated peptides and cloning, sequencing, and expression of the gene *kamA* in *Escherichia coli*. *J Bacteriol* 182:469–476.

Saeva, F.D. and Morgan, B.P. 1984, Mechanism of one-electron electrochemical reductive cleavage reactions of sulfonium salts. *J Am Chem Soc* 106:4121–4125.

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

Sanyal, I., Cohen, G., and Flint, D.H. 1994. Biotin synthase: purification, characterization as a [2Fe-2S]cluster protein, and *in vitro* activity of the *Escherichia coli bioB* gene product. *Biochemistry* 33:3625–3631.

Scott, A.I. and Roessner, C.A 2002. Biosynthesis of cobalamin (vitamin B(12). *Biochem Soc Trans* 30:613–620.

Schwarz, G., Santamaria-Araujo, J.A., Wolf, S., Lee, H.J., Adham, I.M., Gröne, H.J., Schwegler, H., Sass, J.O., Otte, T., Hänzelmann, P., Mendel, R.R., Engel, W., and Reiss, J. 2004. Rescue of lethal molybdenum cofactor deficiency by a biosynthetic precursor from *Escherichia coli Hum Mol Genet* 13:1249–1255.

Selmer, T., Pierik, A.J., and Heider, J. 2005. New glycyl radical enzymes catalyzing key metabolic steps in anaerobic bacteria. *Biol Chem* 386:981–988.

Seto, H. *et al.* 1982. Studies on the biosynthesis of bialaphos (SF-1293). 1. Incorporation of ^{13}C- and ^{2}H-labeled precursors into bialaphos. *J Antibiot* 35, 1719–1721.

Shaw, N.M., Birch, O.M., Tinschert, A., Venetz, V., Dietrich, R., and Savoy, L.A. 1998. Biotin synthase from *Escherichia coli*: isolation of an enzyme-generated intermediate and stoichiometry of *S*-adenosylmethionine use. *Biochem J* 330:1079–1085.

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

Song, K.B. and Frey, P.A. 1991, Molecular properties of lysine-2,3-aminomutase. *J Biol Chem* 266:7651–7655.

Spormann, A.M. and Widdel, F. 2000. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. *Biodegradation* 11:85–105.

Stadtman. T.C. 1973, *Adv Enzymol* 38:413–447.

Suzuki, J.Y., Bollivar, D.W., and Bauer, C.E. 1997. Genetic analysis of chlorophyll biosynthesis. *Annu Rev Genet* 31:61–89.

Szu, P.H., He, X., Zhao, L., and Liu, H.W. 2005. Biosynthesis of TDP-D-desosamin: Identification of a strategy for C4 deoxygenation. *Angew Chem Int Ed Engl* 44:6742–6746.

Tagusagawa, F., Fujioka, M., Spies, A., and Schowen, R.L. 1998. *S*-Adenosylmethionine (AdoMet)-dependent methyltransferases In: *(Sinnott, M. ed.) Comprehensive Biological Catalysis*, London: Academic Press: pp. 1–33.

Tamarit, J., Mulliez, E., Meier, C., Trautwein, A., and Fontecave, M. 1999. The anaerobic ribonucleotide reductase from *Escherichia coli*. The small protein is an activating enzyme containing a [4Fe–4S](2+) center. *J Biol Chem* 274:31291–31294.

Tamarit, J., Gerez, C., Meier, C. Mulliez, E., Trautwein, A., and Fontecave, M. (2000) The activating component of the anaerobic

ribonucleotide reductase from Escherichia coli. An iron-sulfur center with only three cysteines. *J Biol Chem* 275:15669–15675.

Thiruvengadam, T.K., Gould, S.J., Aberhart, D.J., and Lin, H.-J. 1983, Biosynthesis of Streptothricin F. 5. Formation of β-lysine by *Streptomyces* L-1689-23. *J Am Chem Soc* 105:5470–5476.

Thompson, C.J. and Seto, H. 1995. *Bialaphos Genetics and Biochemistry of Antibiotic Production*, 197–222.

Tse Sum Bui, B., Benda, R., Schunemann, V., Florentin, D., Trautwein, A.X., and Marquet, A. 2003. Fate of the (2Fe-2S)(2+) cluster of Escherichia coli biotin synthase during reaction: a Mössbauer characterization. *Biochemistry* 42:8791–8798.

Tse Sum Bui, B., Lotierzo, M., Escalettes, F., Florentin, D., and Marquet, A. 2004. Further investigation on the turnover of Escherichia coli biotin synthase with dethiobiotin and 9-mercaptodethiobiotin as substrates. *Biochemistry* 43:16432–16441.

Trefzer, A., Salas, J.A., and Bechtold, A. 1999. Genes and enzymes involved in deoxysugar biosynthesis in bacteria. *Nat Prod Rep* 16:283–299.

Ugulava, N.B., Frederick, K.K., and Jarrett, J.T. 2002. Evidence from Mossbauer spectroscopy for distinct [2Fe-2S](2+) and [4Fe-4S](2+) cluster binding sites in biotin synthase from *Escherichia coli J Am Chem Soc* 124:9050–9051.

Ugulava, N.B., Gibney, B.R., and Jarrett, J.T. 2001b Biotin synthase contains two distinct iron-sulfur binding sites: chemical and spectroelectrochemical analysis of iron-sulfur interconversions. *Biochemistry* 40:843–851.

Ugulava, N.B., Sacanell, G.J., and Jarrett, J.T. 2001a Spectroscopic changes during a single turnover of biotin synthase: destruction of a [2Fe-2S] cluster accompanies sulfur insertion. *Biochemistry* 40:8352–8358.

van Beilin, J.B. and Funhoff, E.G. 2007. Alkane hydroxylases involved in microbial bioremediation. *Appl Microbiol Biochechnol* 74:13–21.

Wagner, A.F., Demand, J., Schilling, G., Pils, T., and Knappe J. 1999. A dehydroalanyl residue can capture the 5′-deoxyadenosyl radical generated from *S*-adenosylmethionine by pyruvate formate-lyase-activating enzyme. *Biochem Biophys Res Commun* 254:306–310.

Wagner, A.F., Frey, M., Neugebauer, F.A., Schafer, W., and Knappe, J. 1992. The free radical in pyruvate formate-lyase is located on glycine-734. *Proc Natl Acad Sci USA* 89:996–1000.

Walker, K.D., Klettke, K., Akiyama, T., and Croteau, R. 2004. Cloning, heterologous expression, and characterization of a phenylalanine aminomutase involved in Taxol biosynthesis, *J Biol Chem* 279:53947–53954.

Walsby, C.J., Hong, W., Broderick, W.E., Cheek, J., Ortillo, D., Broderick, J.B., and Hoffman, B.M. 2002a. Electron-nuclear double resonance spectroscopic evidence that *S*-adenosylmethionine binds in contact with the catalytically active [4Fe-4S]+ cluster of pyruvate formate-lyase activating enzyme. *J Am Chem Soc* 124:3143–3151.

Walsby, C.J., Ortillo, D., Broderick, W.E., Broderick, J.B., and Hoffman, B.M. 2002b. An anchoring role for FeS clusters: Chelation of the amino acid moiety of *S*-adenosylmethionine to the unique iron site of the [4Fe-4S] cluster of pyruvate formate-lyase activating enzyme. *J Am Chem Soc* 124:11270–11271.

Wang, S.C. and Frey, P.A. 2007a. S-Adenosylmethionine as an oxidant: The Radical SAM superfamily. *Trends Biochem Sci* 32:101–110.

Wang, S.C. and Frey, P.A. 2007b. Binding energy in the one-electron reductive cleavage of S-adenosylmethionine by lysine 2,3-aminomutase, a Radical SAM enzyme. *Biochemistry* 46:12889–12895.

Westrich, L., Heide, L., and Li, S.M. 2003. CloN6, a novel methyltransferase catalysing the methylation of the pyrrole-2-carboxyl moiety of clorobiocin. *ChemBioChem* 4:768–773.

Wong, K.K., Murray, B.W., Lewisch, S.A., Baxter, M.K., Ridky, T.W., Ulissi-DeMario, L., and Kozarich, J.W. 1993. Molecular properties of pyruvate formate-lyase activating enzyme. *Biochemistry* 32:14102–14110.

Woodyer, R.D., Li, G., Zhao, H., and van der Donk, W.A. 2007. New insight into the mechanism of methyl transfer during the biosynthesis of fosfomycin. *Chem Commun* :359 – 361.

Woodyer, R.D. et al 2007. New insight into the mechanism of methyl transfer during the biosynthesis of fosfomycin. *Chem Comm (Camb)* 359–361.

Wu, W., Booker, S., Lieder, K.W., Bandarian, V., Reed, G.H., and Frey, P.A. 2000. Lysine 2,3-aminomutase and *trans*-4,5-dehydrolysine: Characterization of an allylic analogue of a substrate-based radical in the catalytic mechanism. *Biochemistry* 39:9561–9570.

Wu, W., Lieder, K.W., Reed, G.H., and Frey, P.A. 1995. Observation of a second substrate radical intermediate in the reaction of lysine 2,3-aminomutase: A radical centered on the β-carbon of the alternative substrate, 4-Thia-L-lysine. *Biochemistry* 34:10532–10537.

Yu, L., Blaser, M., Andrei, P.I., Pierik, A.J., and Selmer, T. 2006. 4-Hydroxyphenylacetate decarboxylases: properties of a novel subclass of glycyl radical enzyme systems. *Biochemistry* 45:9584–9592.

Zappia, V. and Barker, H.A. 1970. Studies on lysine-2,3-aminomutase. Subunit structure and sulfhydryl groups. *Biochim Biophys Acta* 207:505–513.

Zhang, G., Yan, L.Z., Vederas, J.C., and Zuber, P. 1999. Genes of the *sbo-alb* locus of *Bacillus subtilis* are required for production of the antilisterial bacteriocin subtilosin. *J Bacteriol* 181:7346–7355.

Zhao, L., Borisova, S., Yeung, S.M., and Liu, H.W. 2001. Study of C-4 deoxygenation in the biosynthesis of desosamine: evidence implicating a novel mechanism. *J Am Chem Soc* 123:7909–7910.

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