Review TRENDS in Biochemical Sciences Vol.32 No.3



# *S*-adenosylmethionine as an oxidant: the radical SAM superfamily

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**A recently discovered superfamily of enzymes function using chemically novel mechanisms, in which *S*-adenosylmethionine (SAM) serves as an oxidizing agent in DNA repair and the biosynthesis of vitamins, coenzymes and antibiotics. Members of this superfamily, the radical SAM enzymes, are related by the cysteine motif CxxxCxxC, which nucleates the [4Fe–4S] cluster found in each. A common thread in the novel chemistry of these proteins is the use of a strong reducing agent – a low-potential $[4Fe–4S]^{1+}$ cluster – to generate a powerful oxidizing agent, the 5′-deoxyadenosyl radical, from SAM. Recent results are beginning to determine the unique biochemistry for some of the radical SAM enzymes, for example, lysine 2,3 aminomutase, pyruvate formate lyase activase and biotin synthase.**

## *S*-adenosylmethionine and controlled cleavage of C–H bonds

One of the most chemically difficult reactions in enzymology is the controlled cleavage of unreactive C–H bonds in alkyl groups. Such reactions invariably require specialized coenzymes such as hemes, di-iron clusters or vitamin $B_{12}$ coenzymes. A class of enzymes has emerged that use *S*-adenosylmethionine (SAM) and specialized [4Fe–4S] clusters to potentiate the cleavage of unreactive C–H bonds in metabolism, biosynthesis, and nucleic-acid repair and modification. The reactions of the enzymes in this class are chemically diverse but the mechanisms share a common theme: the reductive cleavage of SAM to the 5′-deoxyadenosyl radical.

## SAM and adenosylcobalamin as radical initiators

In 1970, Horace A. Barker and his associates described lysine 2,3-aminomutase (LAM) and the interconversion of L-lysine and L-β-lysine [1] (Figure 1a). LAM was found to have a role in the metabolism of L-lysine by *Clostridium subterminale* strain SB4 and in the production of β-lysine in antibiotic biosynthesis. LAM proved to be active only under anaerobic conditions and in the presence of a strong reductant, dithionite. The purified enzyme contained pyridoxal-5′-phosphate (PLP) and iron, and the addition of ferrous iron and PLP increased the activity. SAM and dithionite were absolutely required for activity.

The PLP requirement was not surprising for an amino-acid-metabolizing enzyme, but the requirement for SAM was extraordinary. The 1,2-cross migration of a functional group and hydrogen atom (Figure 1a) was known at the time to be facilitated by adenosylcobalamin in isomerization reactions, but no vitamin $B_{12}$ coenzyme was required by LAM [1]. SAM was known primarily as a biological methylating agent, not as a stand-in for adenosylcobalamin. For these reasons, LAM was regarded as a biochemical curiosity. Thirty-one years later, it was recognized as the first of a large family of SAM-dependent enzymes known as the radical SAM superfamily [2].

Adenosylcobalamin functions as a coenzyme by binding to a cognate enzyme and undergoing reversible cleavage of the Co–C5′ bond to generate cob(II)alamin and the 5′-deoxyadenosyl radical at the active site. The 5′-deoxyadenosyl radical mediates hydrogen transfer in the enzymatic transformation to the product. The adenosyl moiety of SAM similarly mediates hydrogen transfer in the reactions of LAM and other members of the radical SAM superfamily [3,4]. Thus, SAM functions as the source of the 5′-deoxyadenosyl radical.

The reaction of pyruvate formate-lyase (PFL) (pyruvate + CoA → acetyl-CoA + formate) proved to be stimulated by SAM. In 1984, the role of SAM became known [5]. PFL was the first glycyl radical enzyme to be discovered [6], and SAM proved to be the substrate for an activating enzyme, the PFL activase, which generates the glycyl radical in PFL by a process in which SAM suffers reduction to 5′-deoxyadenosine and methionine. The postulate was put forward that the cleavage of SAM by PFL activase leads to the transient formation of the 5′-deoxyadenosyl radical, which, in turn, abstracts a hydrogen atom from Gly734 of PFL to generate 5′-deoxyadenosine and the active, glycyl-radical form of PFL (Figure 1c). PFL activase was the second enzyme of what was recognized 17 years later as the radical SAM superfamily [2].

Other SAM-dependent enzymes discovered and characterized in the 1990s also involve cleavage of unreactive C–H bonds. *Escherichia coli* grown aerobically harbor a ribonucleoside diphosphate reductase that is a tyrosyl radical enzyme. Conversely, *E. coli* grown anaerobically harbor a ribonucleoside triphosphate reductase (ARR), which, in its active form, is a glycyl radical enzyme [3,4]. ARR has catalytic and activating subunits, and the activase subunit generates the Gly681 radical in the catalytic subunit by the same SAM-dependent mechanism as the activation of PFL by PFL activase (Figure 1d).

The biosyntheses of biotin and lipoic acid each conclude with the enzymatic insertion of sulfur atoms into unactivated C–H bonds. In the last step of biotin synthesis, a sulfur atom is inserted into two C–H bonds to form the

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Available online 8 February 2007.

 0968-0004/$ – see front matter  doi:10.1016/j.tibs.2007.01.002

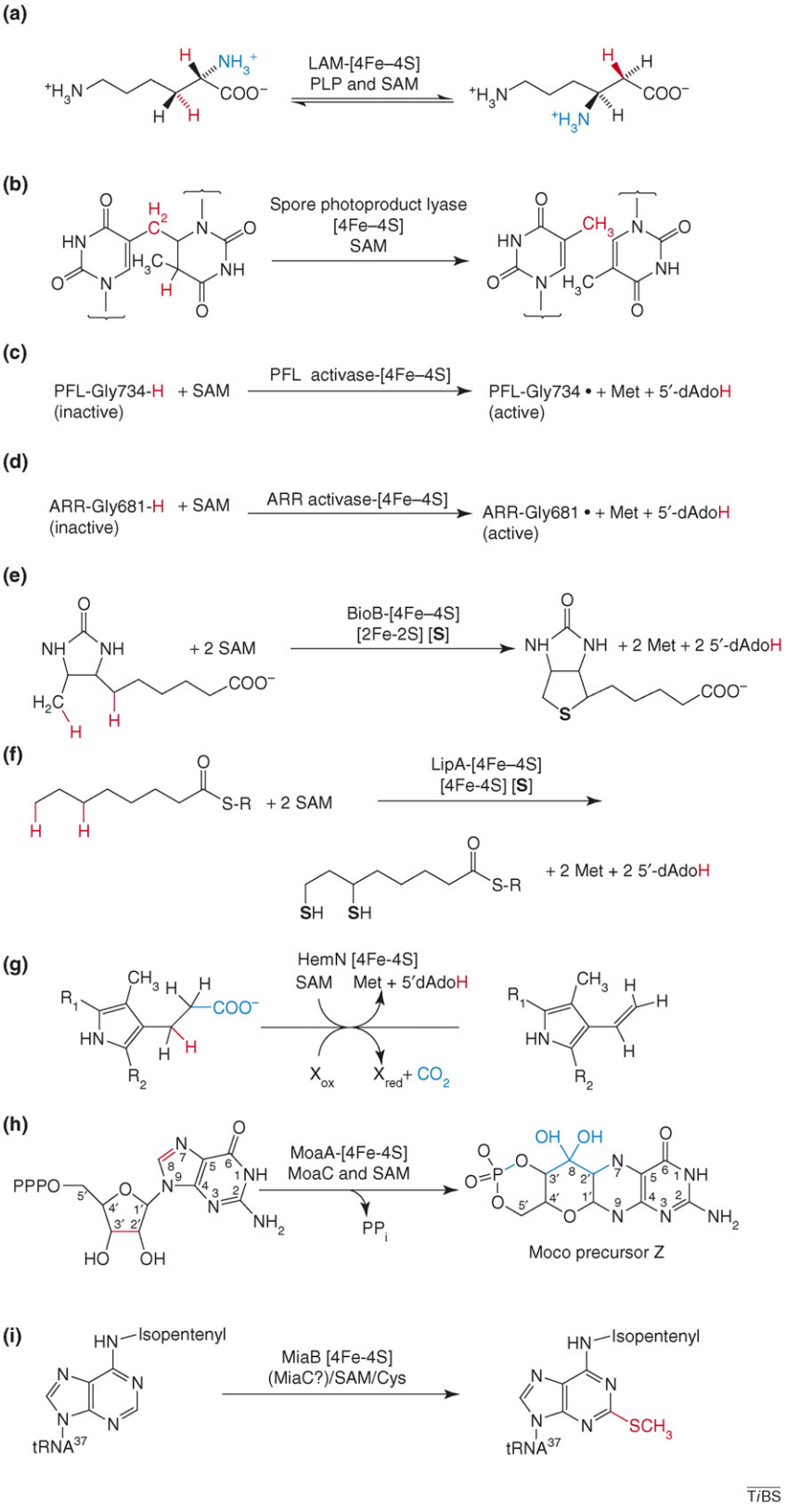


**Figure 1**. Reactions catalyzed by the most extensively studied radical SAM enzymes. In reactions of LAM **(a)** and spore photoproduct lyase **(b)**, SAM functions catalytically as a temporary oxidant and is regenerated at the end of each catalytic cycle. In reactions of PFL activase **(c)** and ARR activase **(d)**, SAM is a true substrate that is consumed as an oxidant to generate a protein-glcyl radical. In reactions of BioB **(e)** and LipA **(f)**, SAM is a true substrate that is consumed as an oxidant in the insertion of sulfur into unreactive C–H bonds. In the reaction of HemN **(g)**, SAM is a substrate consumed as an oxidant in the oxidative decarboxylation of propionate side chains of coproporphyrinogen to vinyl groups. The exact roles of SAM in the reactions of MoaA **(h)** and MiaB **(i)** are not known. Note the diversity of reaction types: isomerizations (a,b), generation of glycyl radical sites in glycyl radical enzymes (c,d), insertion of sulfur atoms into unactivated C–H bonds (e,f), oxidative decarboxylation (g), rearrangement and fusion of heterocyclic rings (h) and methylthiolation of an adenine ring (i). Abbreviations: Ado, 9-β-D-erythrofuranosidoadenine; 5′-dAdo, 5′-deoxyadenosine; Cys, cysteine; Met, methionine.

thiophane ring of biotin (Figure 1e). This process is brought about by the action of biotin synthase (BioB) in a SAM-dependent reaction, and leads to the production of methionine and 5′-deoxyadenosine together with sulfur insertion. Lipoic acid is biosynthesized from octanoic acid in a process in which octanoate is first activated and then incorporated as an octanoyl-N(ε)-lysyl moiety of a potential lipoyl protein. Lipoyl synthase (LipA) catalyzes the insertion of two sulfur atoms into the C6–H and C8–H bonds of the octanoyl group to form the dihydrolipoyl moiety. The reaction is SAM-dependent and, presumably, proceeds by a mechanism similar to that for BioB [3,4] (Figure 1f). The mechanism by which sulfur is inserted into unreactive C–H bonds by BioB and LipA is not known in detail; however, as a rule, the cleavages of unactivated alkyl C–H bonds in enzymology proceed by radical mechanisms that require the participation of redox-active transition metals. The 5′-deoxyadenosyl radical from SAM is thought to mediate the insertion of sulfur into biotin and lipoic acid by radical mechanisms.

## Iron–sulfur centers in SAM-dependent enzymes

The iron in LAM is a [4Fe–4S] cluster [4]. PFL activase, BioB, ARR activase and LipA contain similar iron–sulfur centers [3,4,7–10]. The [4Fe–4S] clusters in this group of enzymes are of a unique type among four-iron clusters in that they are formed with three cysteinyl ligands to iron and the ligands to the fourth iron originate with SAM and not the protein [11–19]. In this way, they are analogous to the [4Fe–4S] cluster in aconitase, in which ligands to the fourth iron are donated by the substrate itself [20].

The amino acid sequences of the enzymes discussed here (Figure 1) include the cysteine motif CxxxCxxC, and no other conserved cysteine is present as a potential fourth iron ligand. The cysteine motif is unlike those in other iron–sulfur proteins and is the dominant identifying characteristic of this superfamily, which also include glycine-rich segments involved in binding SAM [2]. The cysteine motif relates this group to >640 other proteins in the radical SAM superfamily. The superfamily members are involved in highly diverse biochemical processes in animals, plants and microorganisms. These enzymes catalyze steps in metabolism, DNA repair, the biosynthesis of vitamins and coenzymes, and the biosynthesis of many antibiotics. Representative examples of radical SAM enzymes and their biological functions are listed in Table 1. Although the list is incomplete, it exemplifies the great diversity of functions in the radical SAM superfamily. Although the radical SAM enzymes so-far investigated use SAM as a source of the 5′-deoxyadenosyl radical and, in this sense, are mechanistically related to the adenosylcobalamin-dependent family, the radical SAM enzymes are much more numerous and diverse in function. Most radical SAM enzymes have not yet been purified and characterized, and only ten of those listed in Table 1 have been characterized at some level.

## Roles of SAM

SAM serves as a reversible source of the 5′-deoxyadenosyl radical in the reactions of LAM and spore photoproduct lyase (Figure 1a,b). That is, the 5′-deoxyadenosyl radical mediates hydrogen transfer in the mechanism, is regenerated at some point and reverts to SAM at the end of each catalytic cycle. The probable catalytic cycles are shown in Figure 2. Thus, in these reactions, SAM serves catalytically as a coenzyme and is not consumed.

SAM does not function catalytically in the reactions of PFL and ARR activase (Figure 1c,d); instead, it is a substrate that undergoes reductive cleavage to methionine and 5′-deoxyadenosine and generates a stable radical at Gly734 (Figure 1c) or Gly681 (Figure 1d). In PFL and ARR activation, reductive cleavage of SAM leads to the 5′-deoxyadenosyl radical at the active sites of the respective activating enzymes, and the radical abstracts a hydrogen atom from C2 of Gly734 in the active site of PFL or Gly681 in ARR to activate the enzymes. Methionine and 5′-deoxyadenosine are released as by-products. SAM is the oxidant in generating the glycyl radical. Other members of this class include the activases for benzylsuccinate synthase and the adenosylcobalamin-independent glycerol dehydratase (Table 1).

BioB and LipA also use SAM as an oxidizing substrate and source of the 5′-deoxyadenosyl radical rather than for catalysis. However, unlike PFL and ARR activases, BioB and LipA do not generate stable radicals; instead, they use the oxidizing power of the 5′-deoxyadenosyl radical to catalyze the insertion of sulfur into chemically unreactive C–H bonds of dethiobiotin (in biotin synthesis) or the octanoyl group (in lipoyl synthesis), respectively. The reactions of both BioB and LipA require the cleavage of, and sulfur-insertion into, two unreactive C–H bonds, and both reactions proceed with cleavage of two mols of SAM into

**Table 1. A partial list of radical SAM enzymes and the biochemical functions**

| Enzyme[a] | Status[b] | Biochemical function | Refs |
|---|---|---|---|
| LAM | PRMS | β-Lysine antibiotics, lysine metabolism | [1] |
| SplB | PR | DNA repair | [4,39] |
| PFL activase | PRM | Glycyl radicalization in pyruvate metabolism | [3,4] |
| ARR activase | PRM | Glycyl radicalization in dNTP synthesis | [3,4] |
| BioB | PR*S | Biotin biosynthesis | [3,4] |
| LipA | PR* | Lipoic acid biosynthesis | [3,4] |
| MoaA | PS | Molybdopterin biosynthesis | [26,27] |
| HemN | PRS | Heme biosynthesis | [17,25] |
| MiaB | PR* | Thiomethylation of isopentenyl adenosine in tRNA | [28,40] |
| BssD | – | Glycyl radicalization in toluene metabolism | [41,42] |
| GD activase | PR | Glycyl radicalization of glycerol dehydratase | [43] |
| ThiH | P | Thiamine biosynthesis | [44] |
| PqqE | – | Pyrroloquinoline quinone biosynthesis | [45,46] |
| DesII | – | Desosamine biosynthesis | [47] |
| Fom3 | – | Fosfomycin biosynthesis | [48] |
| Fms7 | – | Fortimicin biosynthesis | [49] |
| BcpD | – | Bialaphos biosynthesis | [50] |
| CloN6 | – | Clorobiocin biosynthesis | [51] |

[a]Abbreviations: BcpD, gene product D in bialaphos biosynthetic pathway; GD, glycerol dehydratase; BssD, gene product D in benzylsuccinate biosynthetic pathway; CloN, gene product N in clorobiocin biosynthetic pathway; DesII, gene product II in desosamine biosynthetic pathway; Fms7, gene product 7 in fortimicin biosynthetic pathway; Fom3, gene product 3 in fosfomycin biosynthetic pathway; PqqE, gene product E in pyrroloquinoline quinone biosynthetic pathway; SplB, spore photoproduct lyase; ThiH, gene product H in thiamine biosynthetic pathway.
[b]M, mechanism established; P, purified; R, reaction defined; R*, reaction partially defined; S, structure determined.

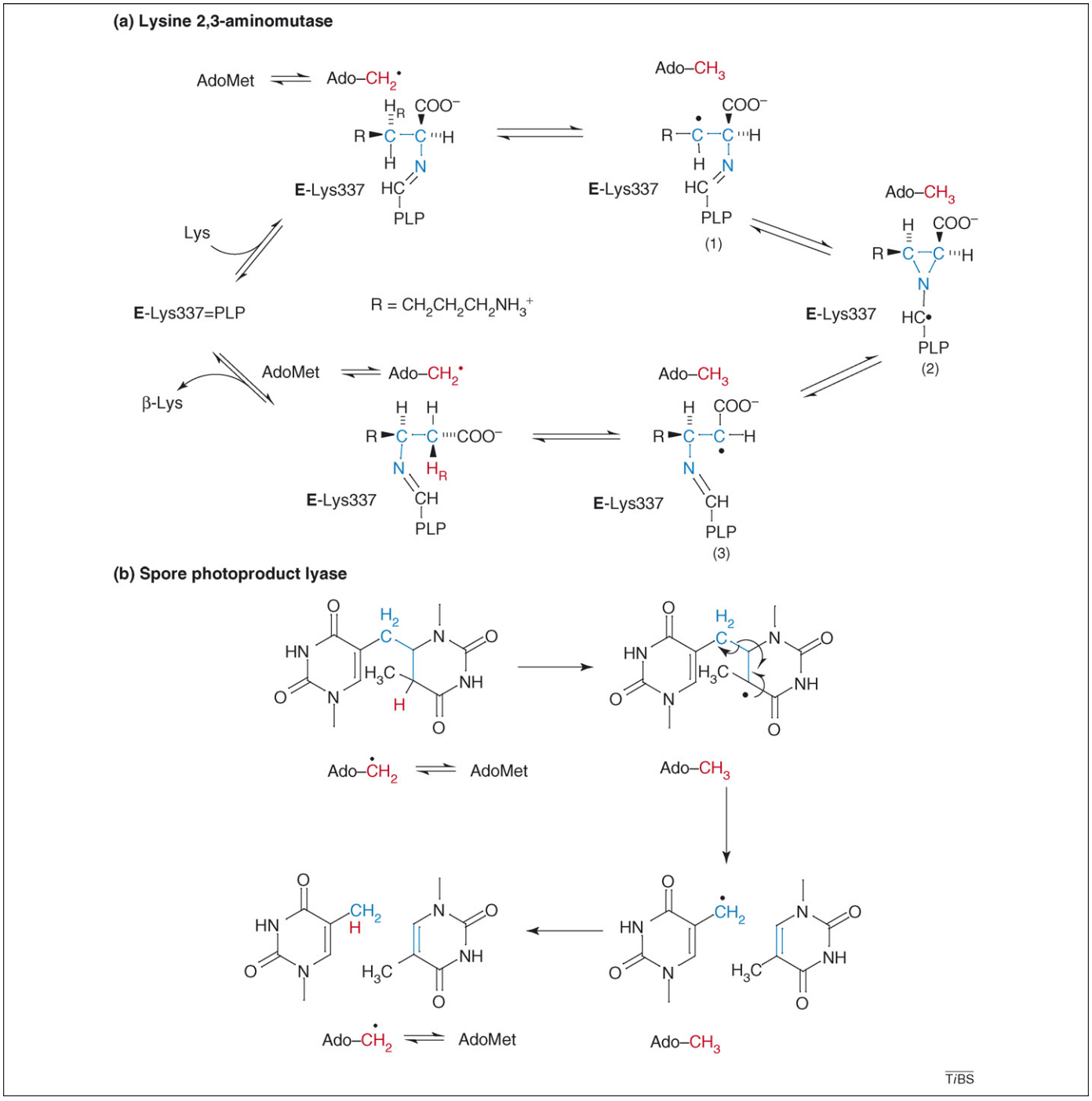


**Figure 2**. Chemical mechanisms of two reactions catalyzed by radical SAM enzymes. In the reactions of LAM (a) and spore photoproduct lyase (b), SAM functions catalytically to serve as a source of the 5′-deoxyadenosyl radical. The 5′-deoxyadenosyl radical initiates the radical mechanism by abstracting a hydrogen atom from the substrate to form 5′-deoxyadenosine and a substrate radical, which, in turn, rearranges to a product radical. At the conclusion of each reaction, the 5′-deoxyadenosine donates a hydrogen atom to the product-related radical, thereby regenerating the 5′-deoxyadenosyl radical. Mobilized hydrogens are red and the rearranging groups are blue. **(a)** The reversibly generated 5′-deoxyadenosyl radical abstracts the 3-pro-R hydrogen from the side chain of the lysine-PLP external aldimine to generate the substrate-related radical 1, which undergoes a two-step rearrangement through radical 2 to the product-related radical 3. Hydrogen-atom abstraction from 5′-deoxyadenosine by radical 3 regenerates the 5′-deoxyadenosyl radical and produces the β-lysyl-PLP external aldimine. Transaldimination with Lys337 releases β-lysine and regenerates the internal Lys337-PLP aldimine for another cycle. **(b)** The reversibly generated 5′-deoxyadenosyl radical abstracts the hydrogen atom from C5 of a thymine dimer to generate 5′-deoxyadenosine and a substrate-related radical. This radical undergoes fragmentation to thymine and a thymine carbinyl radical, the product-related radical, which abstracts a hydrogen atom from 5′-deoxyadenosine to produce the second thymine and regenerate the 5′-deoxyadenosyl radical.

two mols of methionine and 5′-deoxyadenosine for each mol of sulfur-insertion product [4,21].

The class of sulfur-insertion enzymes is likely to grow in the future as more radical SAM enzymes are discovered because sulfur insertions into C–H bonds are oxidative to the substrate and proceed with SAM as the oxidant in place of a conventional oxidizing agent. Such reactions are potentially of value in anaerobic metabolism.

A major ongoing problem in research on BioB and LipA is the source of sulfur for insertion into the C–H bonds after cleavage by the 5′-deoxyadenosyl radical [3,4]. Proximal sulfur sources are the second iron–sulfur clusters in each

enzyme. BioB has a [2Fe–2S] cluster in addition to the [4Fe–4S] cluster nucleated by the CxxxCxxC motif. The two-iron cluster donates sulfur for insertion into biotin [3,22]. LipA has a second [4Fe–4S] cluster that is distinct from the four-iron radical SAM cluster nucleated by the CxxxCxxC motif, and the second cluster is the proximal source of sulfur [23]. Both sulfur atoms in a given molecule of lipoic acid are derived from the same [4Fe–4S] cluster of LipA [23], and are incorporated by stepwise insertion [24]. The ultimate biological sources of sulfur for BioB and LipA are controversial. However, the only sulfur donors identified to date are the auxiliary iron–sulfur clusters in these enzymes.

## Other categories of radical SAM enzymes

The functional categories discussed here do not exhaust the reaction types catalyzed by radical SAM enzymes.

HemN is the coproporphyrinogen oxidase in anaerobic bacteria [17,25], and it catalyzes the oxidative decarboxylation of coproporphyrinogen (Figure 1g). The reaction decarboxylates the propionyl side chains on rings A and C and oxidizes them to vinyl groups. In aerobes, HemF catalyzes the reaction and the oxidizing agent is molecular oxygen, hence the name oxidase. In anaerobes, the radical SAM enzyme HemN uses SAM as an oxidizing agent in addition to another one-electron oxidant, $X_{ox}$ (Figure 1g). The biological electron acceptor $X_{ox}$ is not yet known. HemN is monomeric and contains a [4Fe–4S] cluster that is typical of radical SAM enzymes.

In the biosynthesis of molybdopterin, the molybdenum cofactor (Moco), MoaA and MoaC function together to catalyze the first step, which is the reaction of GTP to produce the first intermediate, precursor Z [18,26,27] (Figure 1h). Precursor Z is transformed into Moco in subsequent enzymatic steps. Little is known about the chemistry of the transformation of GTP into precursor Z apart from the fact that all of the guanosine atoms are retained and C8 of the guanine ring is inserted between C2′ and C3′ of the ribosyl ring. MoaA contains two [4Fe–4S] clusters, one typical of radical SAM enzymes and a second that also has only three cysteine ligands. The second iron–sulfur cluster binds the guanine ring of GTP [26]. The transformation of GTP into Moco requires both MoaC and MoaA, and the specific functions of the two proteins are not known.

MiaB in *E. coli* and in *Thermotoga maritima* is required in the methylthiolation of $N^6$-isopentenyladenosine at position 37 in tRNAs [28,29] (Figure 1i). MiaB from *T. maritima* contains a [4Fe–4S] cluster that is spectroscopically typical of radical SAM enzymes [28]; the protein seems to be monomeric in solution. This methylthiolation reaction (Figure 1i) can be observed *in vitro*, and SAM is a source of the methyl group in the methylthiolated-product. MiaB itself contributes sulfur slowly to complete part of a single

**Box 1. Two mechanisms for electron transfer and decarboxylation by HemN**

The radical identified in the reaction of HemN (Figure I) as arising from H-atom abstraction by the 5′-deoxyadenosyl radical is postulated to be a catalytic intermediate, and its oxidation by an external electron acceptor and decarboxylation to the vinylic product is described as occurring in a single step [52]. Concertedness in the chemical sense seems unlikely given that the rate of short-range electron transfer is much faster than C–C bond vibration frequencies. A stepwise chemical mechanism is likely. Two possible stepwise mechanisms are shown here: (i) electron transfer to the electron acceptor precedes decarboxylation and generates a resonance delocalized carbocation, which undergoes polar decarboxylation (blue arrows); (ii) the radical undergoes fragmentation to the vinylic product and the formyl radical, which is then oxidized to $CO_2$ by the electron acceptor (red arrows).

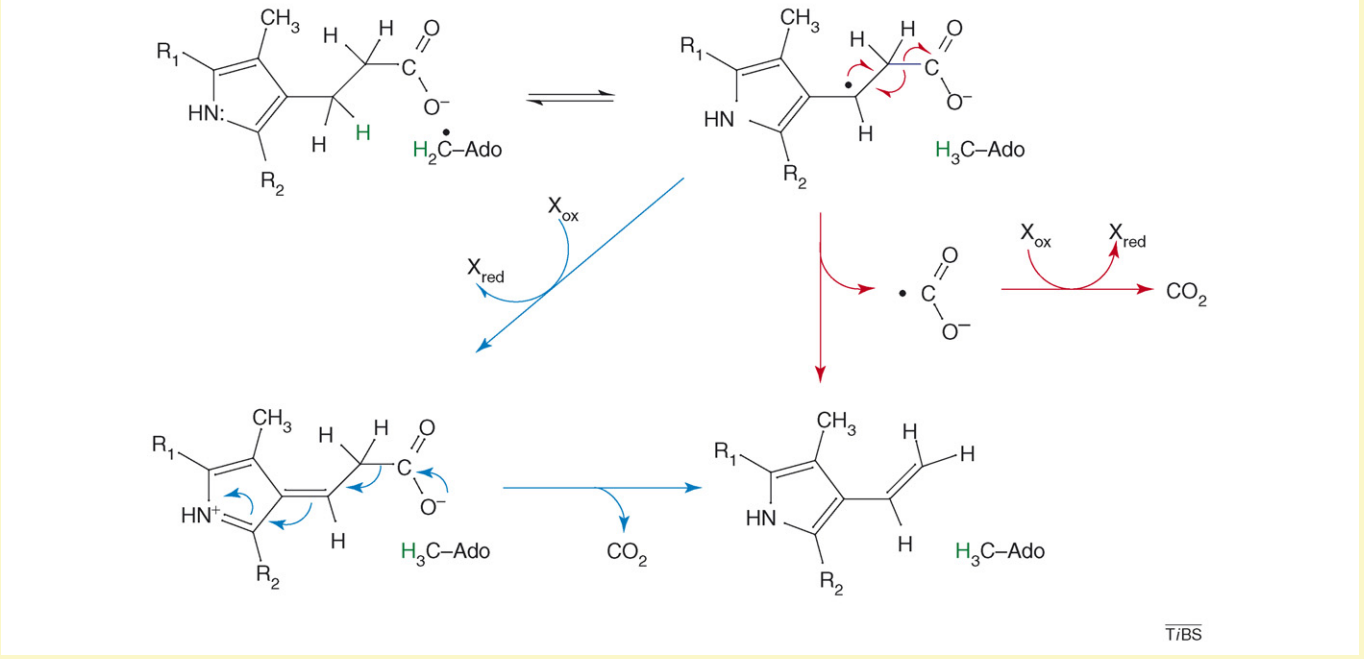


**Figure I**. Possible mechanisms for the action of HemN. The radical in the upper right results from abstraction of a hydrogen atom (green) from the substrate by the 5′-deoxyadenosyl radical derived from SAM. The two mechanisms shown (distinguished by red and blue arrows) are possible if the radical is a true intermediate. The radical shown is the only observable radical in the steady state [34], and it is reasonable to regard it as an intermediate; however, it has not been shown to be kinetically competent.

turnover (<20%). It is possible that MiaC, or another protein or cofactor, is also required but this is not known from either genetic or biochemical experiments. Cysteine is the ultimate source of sulfur *in vivo*, but the proximal source of sulfur is not known.

## Reaction mechanisms in the radical SAM superfamily

The chemical mechanism in the reaction of LAM has been well determined by the spectroscopic and kinetic characterization of the radical intermediates [4] (Figure 2a), and the mechanism is supported by the structure at the active site [19]. The 5′-deoxyadenosyl radical from the reductive cleavage of SAM initiates the catalytic cycle. Of the four radical intermediates (Figure 2a), three have been characterized by electron paramagnetic resonance (EPR) spectroscopy and rapid mix-quench EPR [4]. An allylic analog of the 5′-deoxyadenosyl radical, the 3′,4′-anhydro-5′-deoxyadenosyl radical, has been characterized as an intermediate, with 3′,4′-anhydro-*S*-adenosylmethonine as the coenzyme [4]. Electron double nuclear resonance (ENDOR) experiments in freeze-quenched samples show that radical intermediates are in van der Waals contact with the methyl group of 5-deoxyadenosine [30]. Enforced van der Waals contact is a good mechanism for preventing undesired side reactions, which is regarded as necessary for radical mechanisms in enzymatic catalysis [31].

The chemical mechanisms in the reactions of PFL and ARR activases also proceed from the 5′-deoxyadenosyl radical, which abstracts hydrogen atoms from glycyl residues at the active sites of glycyl radical enzymes [3,4]. Evidence from tritium-tracer experiments supports the role of SAM in the action of spore photoproduct lyase [32,33] (Figure 2b). The presumed radical intermediates have not been characterized or observed, neither has the enzyme structure been determined. The chemical mechanisms of sulfur insertion in the actions of BioB and LipA proceed from initial hydrogen abstraction by the 5′-deoxyadenosyl radical, but the sulfur insertion mechanism is not known.

A substrate-derived radical is observed spectroscopically in the reaction of HemN, which is a coproporphyrinogen oxidase that catalyzes the formation of vinyl side chains in heme by oxidative decarboxylation of the propionate side-chain precursors [34]. The 5′-deoxyadenosyl radical derived from SAM initiates the formation of the radical, and a one-electron acceptor is required. The mechanism of further oxidation and decarboxylation remains to be established (Box 1).

Little is known about the reaction mechanisms of other radical SAM enzymes. For example, although MoaA is necessary for the production of precursor Z in Moco biosynthesis (Figure 1h), it is not sufficient, and the role of MoaC remains undetermined. A feature of the structure of MoaA is the presence of a second [4Fe–4S] cluster with three cysteine ligands in addition to the canonical radical SAM cluster [18]. A structure of the complex of MoaA with GTP indicates that the unique iron in this second cluster binds the guanine ring of GTP [26]. GTP is tightly bound by arginine residues, which also seem to interact with the

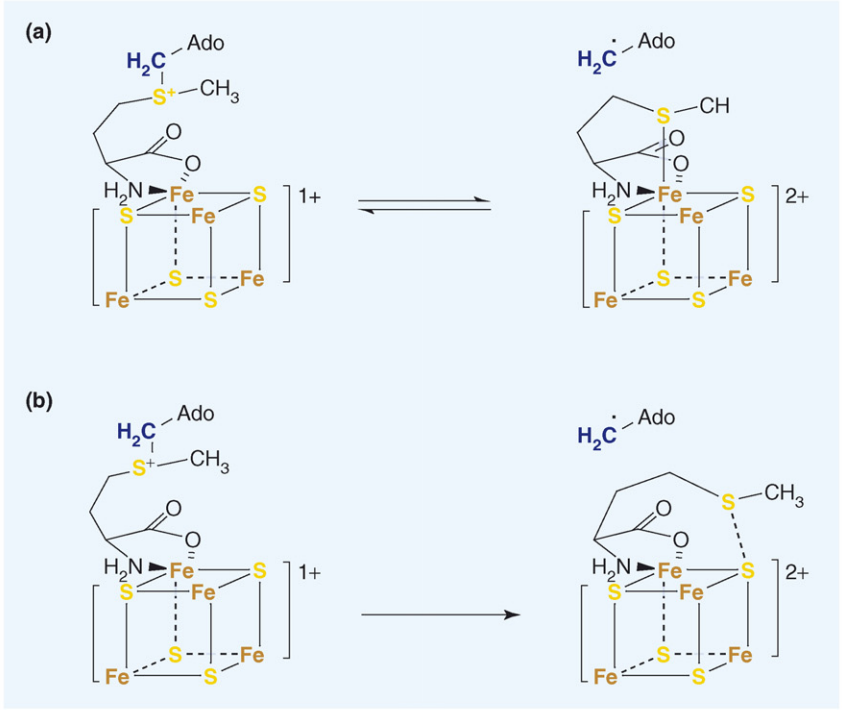


**Figure 3**. Two mechanisms for the reductive cleavage of SAM. In each mechanism, SAM is ligated through the amino and carboxylate groups to the unique iron in the [4Fe–4S]$^{1+}$ cluster. Upon inner-sphere electron transfer from the iron–sulfur cluster, the C5′–S bond breaks homolytically to generate the 5′-deoxyadenosyl radical. **(a)** The reversible cleavage with ligation of sulfur in methionine to the unique iron. This mechanism is strongly implicated by ENDOR and XAS spectroscopy and X-ray structure analysis of LAM [4,19]. **(b)** Irreversible cleavage with interaction of sulfur in methionine with a sulfide in the [4Fe–4S] cluster has been postulated for the irreversible glycyl radicalization of PFL [37].

guanine ring. The reaction mechanism and roles of MoaA and MoaC are not known, apart from the expectation that MoaA is likely to engage in hydrogen abstraction from an undetermined site in the substrate.

MiaB and SAM are necessary but not sufficient to catalyze the methylthiolation of adenosyl-37 in tRNA [28,29] (Figure 1i). The origin of the sulfur in the thiomethyl substituent of the product is not known, and the protein itself can accomplish only a partial turnover by scavenging sulfur from itself. Whether another protein or cofactor is required is not known. Constitution of methylthiolation activity *in vitro* might enable biochemical experiments to probe the mechanism.

## Reductive cleavage of SAM: the mechanism

As the source of the 5′-deoxyadenosyl radical, SAM must be reduced by one electron and this is supplied by the $[4Fe–4S]^{1+}$ cluster in radical SAM enzymes. The addition of an electron to the sulfonium group of SAM presents a mechanistic problem, in addition to a large energetic barrier because of the ideal gas electronic configuration of the sulfur. The reduction potential of a generic sulfonium ion in water is approximately −1.6 V, whereas that of a $[4Fe–4S]^{2+}$ cluster in a radical SAM enzyme ranges from −430 to −500 mV [28,35,36]. The difference of 1.1 V, corresponding to a barrier of 25 kcal $mol^{-1}$, must somehow be narrowed. The mechanism of electron transfer and reductive cleavage lowers the barrier.

Two mechanisms for reductive cleavage have been proposed [15,37]. Both depend on the direct ligation of SAM to the iron–sulfur cluster through the carboxylate and α-amino groups of SAM, and both depend on inner-sphere electron transfer from the $[4Fe–4S]^{1+}$ cluster to the sulfonium group concomitant with homolytic cleavage of the adenosyl-C5′–S bond (Figure 3). $^{15}N$- and $^{17}O$-ENDOR spectroscopy has proven direct ligation of SAM to the iron–sulfur cluster in PFL activase, BioB, and LAM [12,15]. Selenium XAS, employing *Se*-adenosylselenomethionine as the coenzyme for LAM, has shown that selenium is ligated to the unique iron in the [4Fe–4S] cluster upon reductive cleavage [3,4]. These results suggest that electron transfer cleaves the C5′–S to generate the 5′-deoxyadenosyl radical, with the sulfur of methionine becoming a ligand to iron (Figure 3a). This mechanism is further supported by the active-site structures of HemN, LAM, BioB and MoaA [16,17,19]. Another mechanism, with ligation of the methionyl sulfur to a sulfide of the [4Fe–4S] cluster upon reductive cleavage, has been proposed for PFL activase and BioB [37] (Figure 3b). Structural evidence that might support the latter mechanism is currently not available for a glycyl radical enzyme activase.

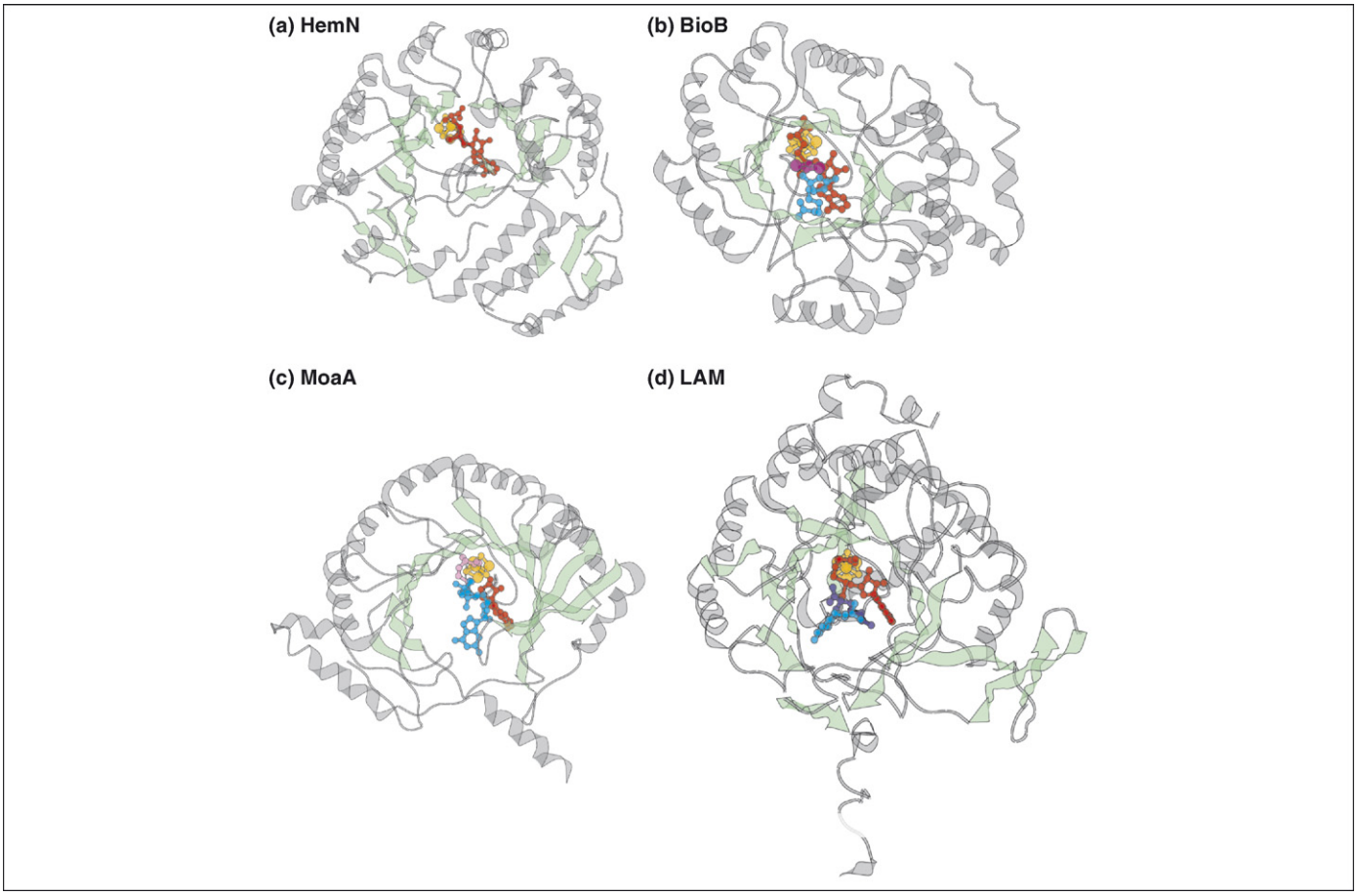


**Figure 4.** The protein folds of the radical SAM enzymes **(a)** HemN (SAM, red; [4Fe–4S], yellow), **(b)** BioB (SAM, red; [4Fe–4S], yellow; [2Fe–2S], purple; dethiobiotin, blue), **(c)** MoaA (5′deoxyadenosine, red; [4Fe–4S], yellow; methionine, pink; GTP, blue) and **(d)** LAM (SAM, red; [4Fe–4S], yellow; PLP, dark blue; lysine, blue). The β crescent, or incomplete β-barrel folds, of HemN, MoaA, and LAM are analogous. By contrast, BioB has a complete β barrel. In the structure of MoaA, the GTP-binding site is shown to include the binding of the guanine moiety to the second, non-radical SAM [4Fe–4S] cluster. The images are derived from the PDB files 1R3O, 1OLT, 2FB3 and 2A5F.

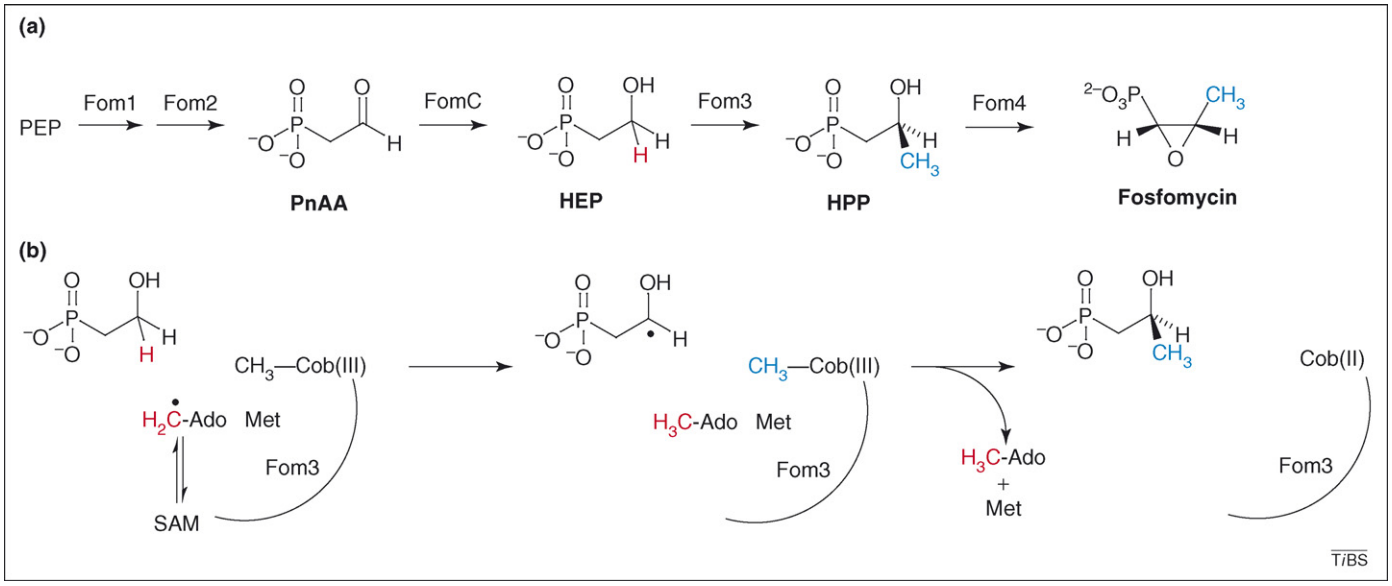


**Figure 5**. Reactions and hypothetical mechanisms in fosfomycin biosynthesis. **(a)** In the biosynthesis of fosfomycin, Fom1 and Fom2 convert phosphoenolpyruvate (PEP) into phosphonoacetaldehyde (PnAA), which is postulated to be transformed into fosfomycin in three steps [38]. Reduction to HEP is catalyzed by FomC, methylation to HPP is catalyzed by the radical SAM enzyme Fom3, and epoxidation to fosfomycin is catalyzed by Fom4. **(b)** The mechanism of action of the radical SAM enzyme Fom3 is not known; however, a reasonable chemical sequence is shown [38]. Hydrogen abstraction from HEP by the radical SAM domain of Fom3 would generate a free radical intermediate, which could be alkylated by methylcobalamin in the cobalamin-binding domain of Fom3. Groups acted upon by the radical SAM domain of Fom3 are shown in red, and those acted upon by the cobalamin domain are shown in blue.

## Structures of radical SAM enzymes

Four structures of radical SAM enzymes are available (Figure 4). The structure of BioB includes a β-barrel fold, with SAM ligated to the [4Fe–4S] cluster and dethiobiotin bound between the adenosyl group and the [2Fe–2S] cluster [16]. The other known structures are of HemN, MoaA and LAM [17–19], all of which display an open β-barrel or β-crescent fold. In all of the structures, the [4Fe–4S] cluster and the substrate-binding sites reside inside the β crescent, or β barrel in the case of BioB. In those structures with SAM, the unique iron in the [4Fe–4S] cluster is ligated to the carboxylate and α-amino groups of SAM.

There are similarities in the folds of the radical SAM enzymes (Figure 4; detailed comparisons of BioB, HemN and MoaA can be found in a recent review [25]). One feature of the structure of the MoaA complex with GTP, which is potentially functionally unique to MoaA, is that the guanine ring of GTP is bound to the second [4Fe–4S] cluster. The functional significance of this should become clear once the chemical mechanism of the action of this enzyme is discovered.

## Methylation by radical SAM enzymes

Several putative radical SAM enzymes catalyze methylation reactions that seem to be unlike those catalyzed by traditional SAM-dependent methyltransferases. Much of what is known about these reactions is based on the results of feeding experiments in crude cell extracts. Thus, the true substrates and products of these reactions are unknown, although hypotheses have been formulated. In methyl transfer by conventional DNA methylases, catechol methyltransferases and others, the methyl-accepting groups of the substrates are nucleophilic and undergo classic alkylation by the methyl group of SAM. Substrates for the radical SAM methyltransferases are not nucleophilic: they are either electrophilic or are sites normally considered to be unreactive. These reactions pose an interesting chemical problem.

An example of methyl transfer to an 'unreactive' site is found in the biosynthesis of fosfomycin (Figure 5a). Recent genetic experiments indicate that the methylation substrate is hydroxyethyl phosphonate (HEP) [38]. In addition, genetic information indicates that the enzyme Fom3 catalyzes the methylation of HEP and sequence analysis indicates that Fom3 includes a radical SAM domain and a cobalamin-binding domain. Methylation of HEP occurs on the carbon of the hydroxymethyl group to produce hydroxypropyl phosphonate (HPP), which, in turn, is converted to fosfomycin by the action of the epoxidase Fom4.

Methylation of HEP by Fom3 requires the removal of an unreactive hydrogen: a hypothetical mechanism is shown in Figure 5b [38]. Abstraction of a hydrogen atom by the 5′-deoxyadenosyl radical generated by the radical SAM domain generates a carbon-centered free radical, which is then methylated by methylcobalamin in the cobalamin-binding domain. This mechanism remains to be tested biochemically with the purified Fom3.

## Concluding remarks and future perspectives

Progress in the radical SAM field is currently limited by a lack of biochemical information (Table 1). Genetic, bioinformatic and structural information cannot be fully understood until the chemical transformations catalyzed by these enzymes become known. This information is available only for the aminomutase, spore photoproduct lyase and glycyl radical activases. Key information is lacking for all of the other reactions studied, including the proximal sources of sulfur and mechanisms of sulfur insertion in several reactions, the electron acceptor and its function in the action of HemN, and the role of MoaC and the action of MoaA in the transformation of GTP for the biosynthesis of

Moco. Other radical SAM enzymes that have important functions in animals, plants and bacteria have neither been characterized nor their biochemical mechanisms established. Therefore, the field is ripe for further research into this recently discovered superfamily and, as biochemical information becomes available, the beauty of the chemistry underlying the actions of radical SAM enzymes will be revealed.

**Acknowledgements**
We thank Adrian D. Hegeman for the preparation of Figure 4.

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