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Radical *S*-Adenosylmethionine (SAM) Enzymes in Cofactor Biosynthesis: A Treasure Trove of Complex Organic Radical Rearrangement Reactions*

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In this minireview, we describe the radical *S*-adenosylmethionine enzymes involved in the biosynthesis of thiamin, menaquinone, molybdopterin, coenzyme F_{420}, and heme. Our focus is on the remarkably complex organic rearrangements involved, many of which have no precedent in organic or biological chemistry.

Radical *S*-adenosylmethionine (SAM)[2] enzymes are among the most intriguing enzymes discovered over the past 15 years. In these enzymes, reduction of SAM by a $[4Fe\text{-}4S]^{1+}$ cluster generates the adenosyl radical, which then abstracts a hydrogen atom from the substrate. The resulting substrate radical usually undergoes a rearrangement or fragmentation reaction to give the product radical (1). Sequence analysis suggests that a large number of enzymes use the radical SAM catalytic motif (2). We are still at an early stage of defining the catalytic mechanisms and the structural enzymology of this fascinating enzyme family.

This minireview focuses on the organic chemistry of the radical SAM enzymes involved in cofactor biosynthesis: thiamin (**1**), F_0 (**2**), menaquinone (**3**), molybdopterin (**4**), and heme (**5**) (Fig. 1). (Biotin and lipoic biosynthesis also uses radical SAM enzymology and is covered separately in another minireview in this series (42).) In contrast to iron(IV)-oxo-derived radicals, where the dominant chemistry involves radical recombination with the iron-bound oxygen (P_{450} rebound rate of $>10^{10}$ s^{-1}) (3), radicals formed by hydrogen atom transfer to the 5′-deoxyadenosyl (5′-dA) radical are more persistent because the reverse reaction is relatively slow. This allows time for complex rearrangements to occur. Radical SAM enzymes catalyze a remarkable range of reactions due to the high intrinsic reactivity of organic radicals, which can undergo rapid hydrogen atom abstraction, double-bond addition, and fragmentation reactions as shown in Fig. 2.

Prokaryotic Thiamin Pyrimidine Synthase (ThiC)

Thiamin pyrophosphate (**1**) is an important cofactor in carbohydrate metabolism and branched chain amino acid biosynthesis, where it plays a key role in stabilization of the acyl carbanion biosynthon. The thiamin pyrimidine synthase (ThiC) catalyzes the conversion of aminoimidazole ribotide (AIR; **6**) to hydroxymethylpyrimidine phosphate (HMP-P; **8**) (Fig. 3*A*) (4, 5). The origin of all of the atoms of the product and the fate of all of the atoms of the substrate have been determined using isotope labeling studies (6–8). This rearrangement, as far as we can tell, is the most complex unsolved rearrangement in primary metabolism.

A mechanistic proposal for this reaction is shown in Fig. 3*B* (5). In this proposal, radical **7** abstracts a hydrogen atom from **6** to form **10**. A β-scission followed by *N*-glycosyl bond cleavage gives **12**. Electrophilic addition to the aminoimidazole followed by hydrogen atom transfer gives **14**. The regenerated 5′-dA radical (**7**) abstracts a hydrogen atom from **14** to form **15**. Radical addition to the imine followed by β-scission gives **17**. A second β-scission followed by a diol dehydratase-like rearrangement gives **20** and **21**. Radical addition to the pyrimidine followed by tautomerization gives **23**. Loss of formate and CO followed by electron transfer back to the $[4Fe\text{-}4S]^{2+}$ cluster completes the formation of HMP-P (**8**). This mechanism is supported by labeling studies (5–8), by the identification of CO and formate as reaction products (4, 5), and by a structure of the enzyme with desamino-AIR bound at the active site (4).

Prokaryotic Thiamin Thiazole Biosynthesis (ThiH)

Tyrosine lyase (ThiH) catalyzes a tyrosine cleavage reaction to form dehydroglycine (**28**) and *p*-cresol (**29**) (9, 10). Dehydroglycine (**28**) is a substrate for the bacterial thiazole synthase involved in the formation of **32** (11). A mechanistic proposal for the lyase reaction is shown in Fig. 4 (9). Abstraction of the amine hydrogen atom by the 5′-dA radical gives **30**. A β-scission gives dehydroglycine (**28**) and radical **31**, which is quenched by protonation and reduction. This mechanism is supported by substrate analog studies and product characterization (9, 10). Radical SAM amino acid lyases are a growing family of enzymes and, in addition to ThiH, now include NosL (involved in nosiheptide biosynthesis) (12), the maturase HydG (involved in the biosynthesis of the cyanide and carbon monoxide ligands of the [FeFe]-hydrogenase cluster) (13, 14), and CofH (involved in deazaflavin biosynthesis; see below). The recent structural studies on tryptophan lyase (NosL) provides clear support for amine hydrogen atom abstraction and suggest that radical SAM amino acid lyases are likely to proceed by amine rather than phenol hydrogen atom abstraction (15).

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[2] The abbreviations used are: SAM, *S*-adenosylmethionine; 5′-dA, 5′-deoxyadenosyl; AIR, aminoimidazole ribotide; HMP-P, hydroxymethylpyrimidine phosphate.

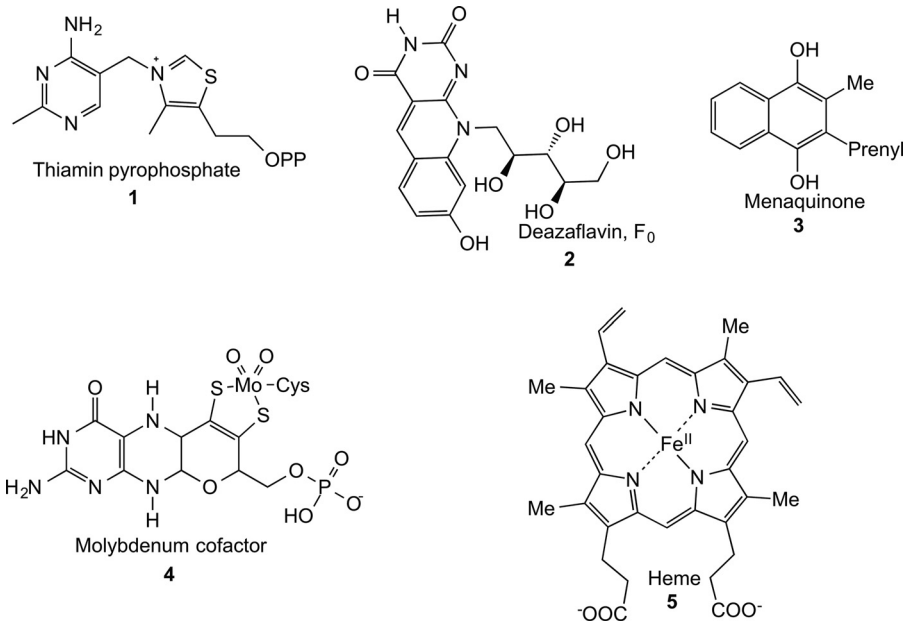

FIGURE 1. **Cofactor biosynthetic pathways make extensive use of radical SAM enzymology.** The structures of the five cofactors described in this minireview are shown.

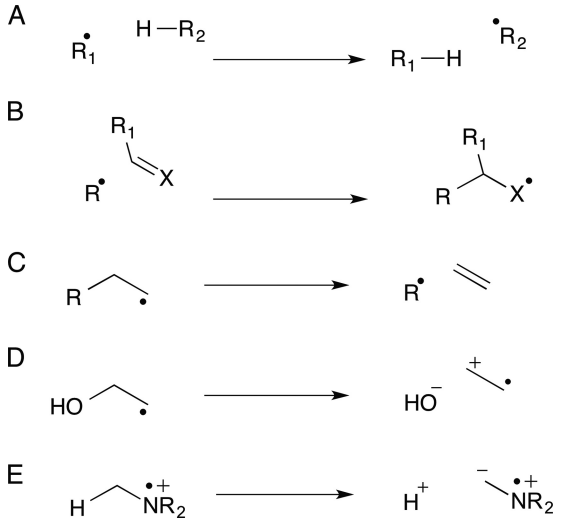

FIGURE 2. **Examples of the major radical reactions found in organic chemistry.** *A*, hydrogen atom abstraction, *e.g.* **6** to **10** and **14** to **15**. *B*, addition to double bonds, *e.g.* **15** to **16**. *C–E*, β-bond scission reactions, *e.g.* **10** to **11**, **16** to **17**, **17** to **18**+**19**, **19** to **21**, **22** to **23**, **24** to **25**, and **25** to **26**. All enzymatic examples are taken from Fig. 3.

Coenzyme F_{420} Biosynthesis (CofG/CofH or FbiC)

F_{420} is a hydride transfer cofactor found in methanogens and in some actinomyces and cyanobacteria. Its precursor, F_0 (**2**), is formed from tyrosine (**27**) and ribityldiaminouracil (**33**) (Fig. 5*A*). The F_0-synthase-catalyzed reaction has been recently reconstituted using the CofG/CofH and FbiC systems (16). The formation of F_0 is mediated by two separate radical SAM active sites, one in CofG and the other in CofH or both in separate domains of FbiC. F_0-synthase is therefore an unusual radical SAM enzyme in that it uses two separate adenosyl radicals to catalyze F_0 formation. The key mechanistic question posed by F_0-synthase is how two adenosyl radicals cooperate in the assembly of the deazaflavin cofactor. The current mechanistic proposal for this enzyme is shown in Fig. 5*B*. In this mechanism, abstraction of the tyrosine amine hydrogen atom by the CofH adenosyl radical gives **30**, which then undergoes fragmentation, leading to the formation of **28** and **31**. Addition of **31** to diaminouracil (**33**) gives **34,** which is oxidized to **35**. A second hydrogen atom abstraction by the CofG adenosyl radical gives **37**. Cyclization to **38**, oxidation, and elimination of ammonia complete the formation of the deazaflavin (**2**). This mechanism is supported by the sequence similarity of CofH and ThiH (Fig. 4). The observation of **33** bound to CofH suggests that CofH catalyzes the first step of F_0 formation.

Futalosine-dependent Menaquinone Biosynthesis (MqnC and MqnE)

Menaquinone (**3**) is a membrane-bound electron transfer cofactor that plays a key role in bacterial respiration, oxidative phosphorylation, and photosynthesis. In humans, menaquinone (vitamin K) is used for the remarkable carboxylation of glutamic acid residues in proteins involved in blood clotting and bone morphogenesis.

In the recently discovered futalosine-dependent pathway (17–20), MqnE catalyzes the conversion of **40** to aminofutalosine (**41**) (21). A mechanistic proposal is outlined in Fig. 6. In this proposal, the 5′-dA radical (**7**) adds to **40** to yield the captodative radical (**42**). Rearrangement of this radical via **43** gives **44**. Such rearrangements have previously been proposed in nonenzymatic systems, but have not yet been studied in an enzyme-mediated reaction (22–24). Decarboxylation of **44**, facilitated by the alkoxy radical, yields the ketyl radical (**45**). A final electron transfer, possibly to the $[4Fe\text{-}4S]^{2+}$ cluster, completes the formation of aminofutalosine (**41**) (21). All previously characterized rearrangements mediated by radical SAM enzymes involve radical generation by hydrogen atom abstraction from the substrate (25–28). MqnE represents a new catalytic motif involving addition of the adenosyl radical to the substrate to generate the rearranging radical.

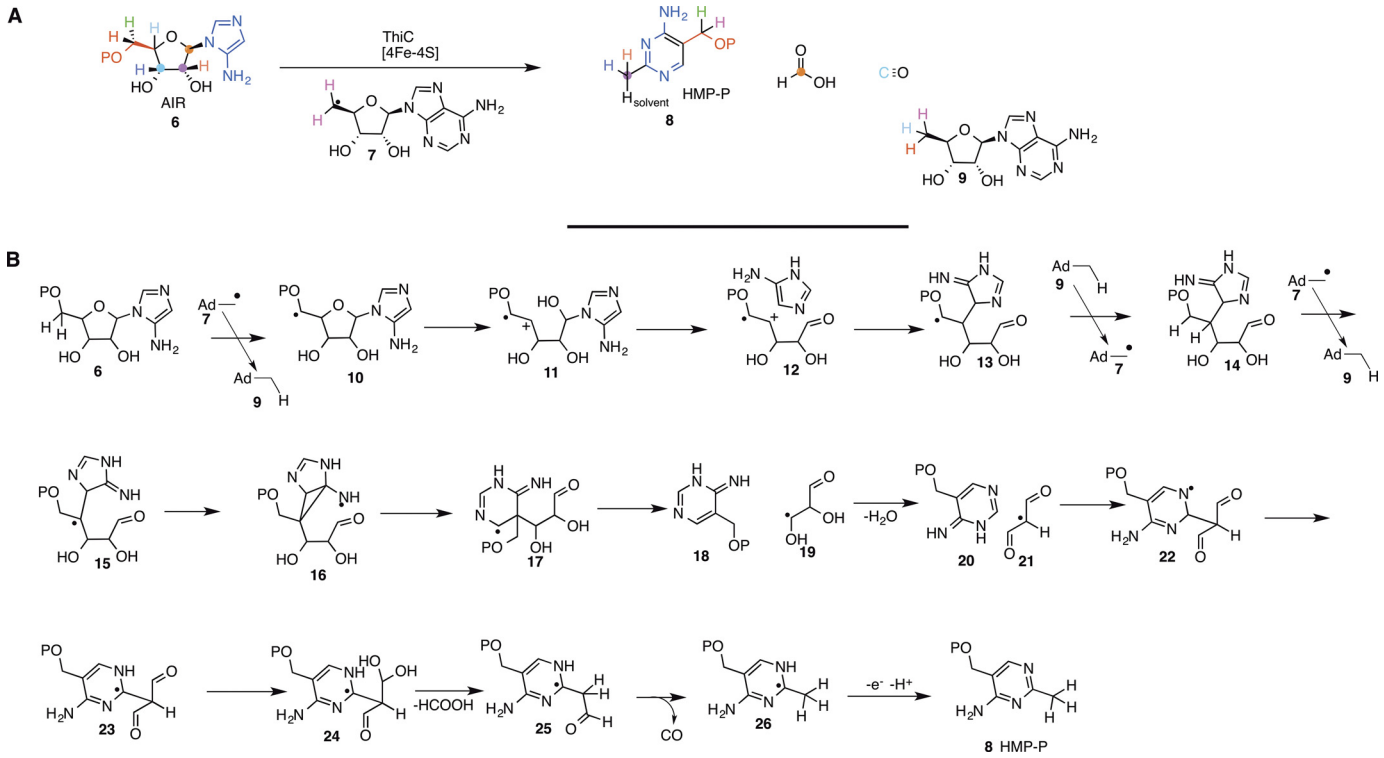

FIGURE 3. **Radical SAM enzymology in thiamin pyrimidine biosynthesis.** *A*, results of a comprehensive labeling study to determine the fate of all of the atoms of AIR (**6**) during the formation of the HMP-P (**8**). *B*, mechanistic proposal for the formation of HMP-P (**8**).

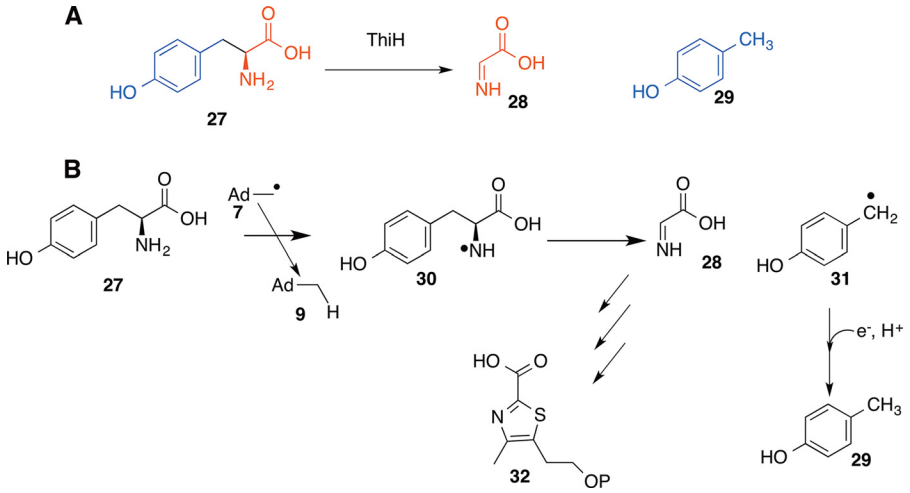

FIGURE 4. **Radical SAM enzymology in thiamin thiazole biosynthesis.** *A*, tyrosine lyase (ThiH)-catalyzed reaction. *B*, mechanistic proposal for this reaction.

MqnC catalyzes the conversion of **46** to **50** (19, 29). A mechanistic proposal is outlined in Fig. 7. In this proposal, the 5′-dA radical abstracts a hydrogen atom from C4′ of **46** to give radical **47**, which adds to the benzene ring to give radical **48**. Deprotonation followed by oxidation completes the reaction. This mechanism is supported by the identification of the site of hydrogen atom abstraction and by the product structure (29).

MqnE and MqnC both catalyze radical additions to benzene rings. Such reactions are still poorly understood biotransformations.

Molybdopterin Biosynthesis (MoaA)

Molybdopterin (**4**) is a redox cofactor used by enzymes such as xanthine oxidase, sulfite oxidase, nitrate reductase, carbon monoxide dehydrogenase, and formate dehydrogenase. The first step in the biosynthesis of this cofactor involves the MoaA-catalyzed conversion of GTP (**51**) to the pterin (**52**) (Fig. 8*A*) (30, 31). This enzyme has two [4Fe-4S] clusters at the active site. The N-terminal cluster is involved in the reductive cleavage of SAM, and the C-terminal cluster is involved in substrate binding (32, 33). A mechanistic proposal is shown in Fig. 8*B* (34, 35). In this proposal, hydrogen atom abstraction occurs from C3′ of GTP (**51**), and the resulting radical adds to C8 of the purine to form **53**. Reduction by the purine-liganded cluster gives **54**, and aminal hydrolysis yields **55**, followed by an α-ketol rearrangement to **56**. Ring opening followed by tautomerization gives **58**, which is converted to **52** by conjugate addition, water elimination, tautomerization, and ring

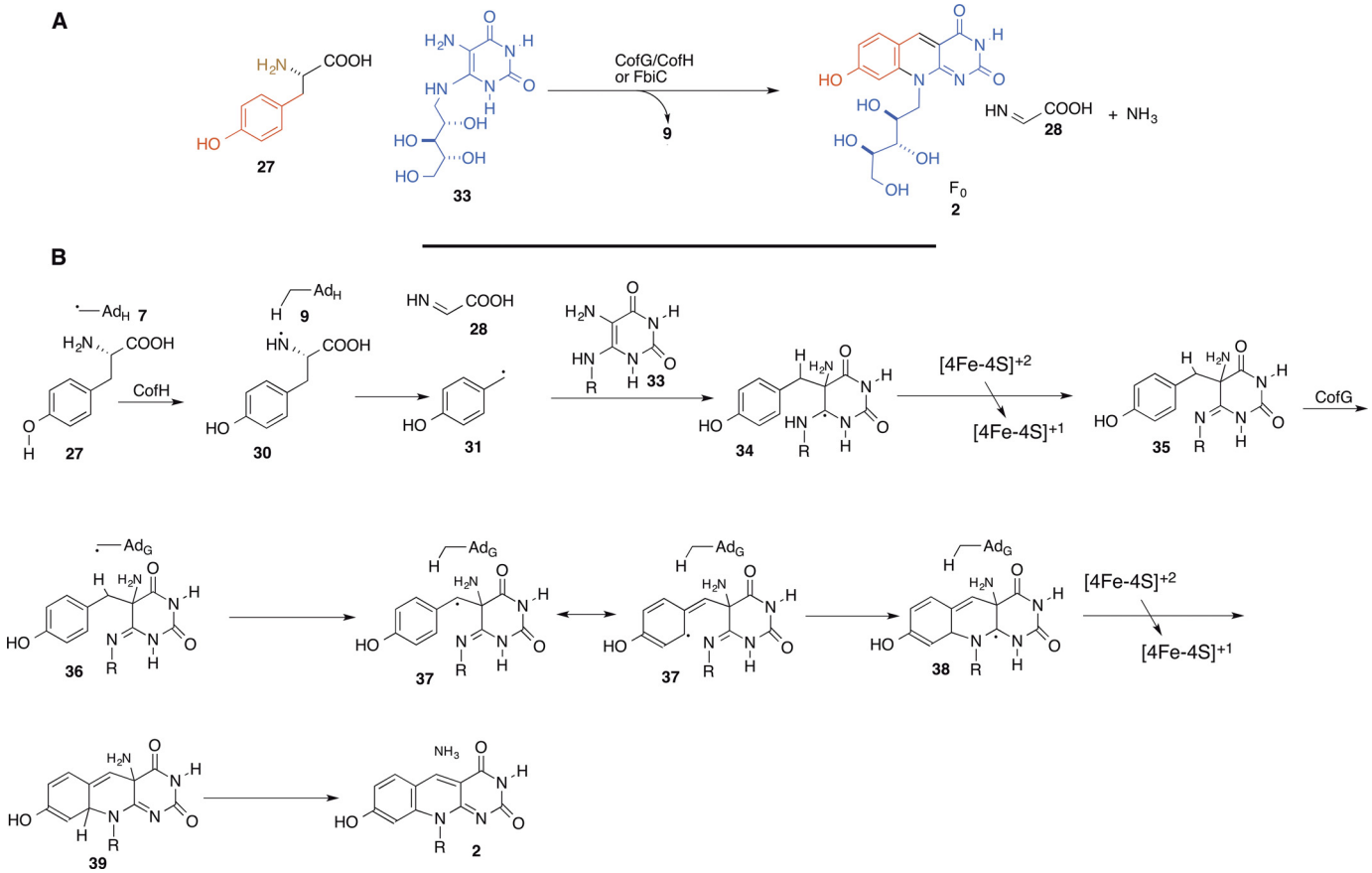

FIGURE 5. **Radical SAM enzymology in deazaflavin biosynthesis.** *A*, F_0-synthase-catalyzed reaction. *B*, mechanistic proposal for F_0-synthase.

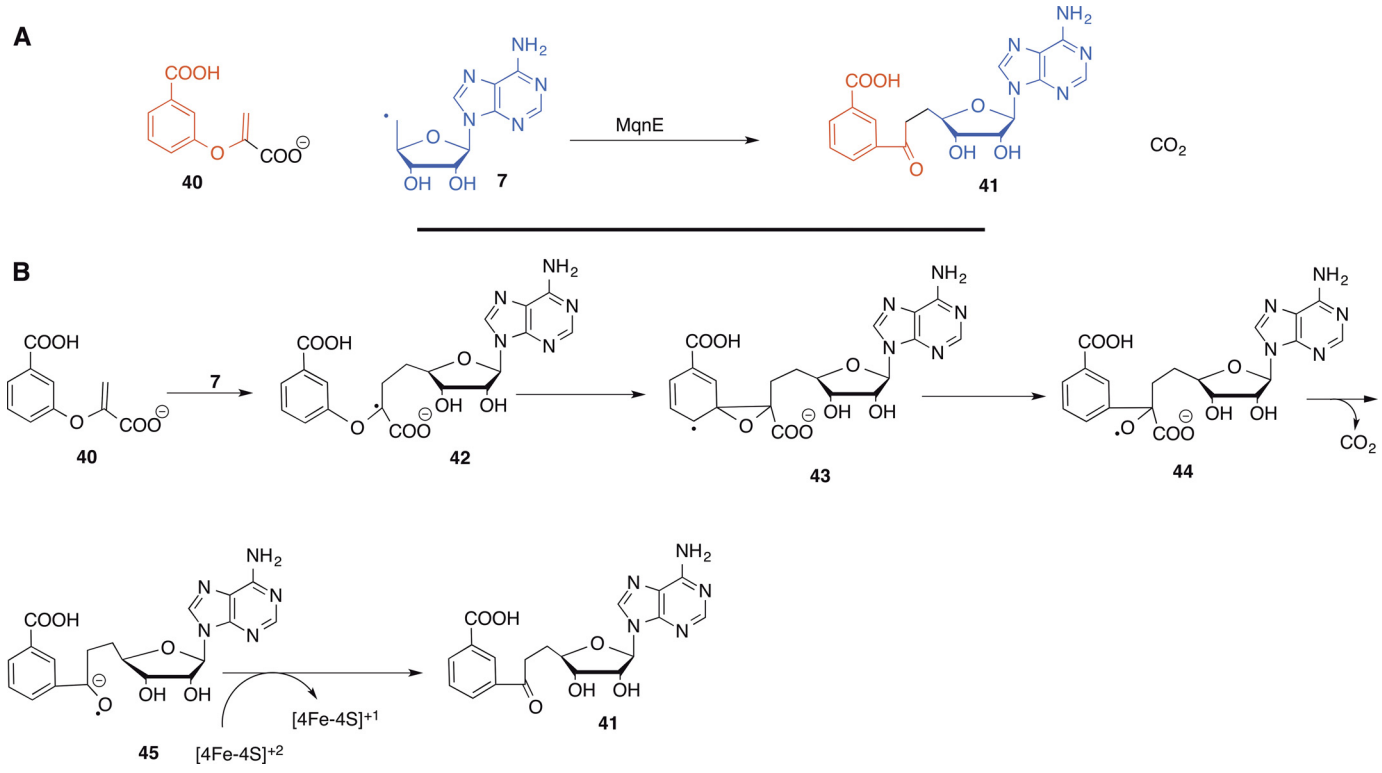

FIGURE 6. **Radical SAM enzymology in menaquinone biosynthesis.** *A*, reaction catalyzed by aminofutalosine synthase (MqnE). *B*, mechanistic proposal for the MqnE-catalyzed reaction.

closure sequence. As far as we can determine, analogous carbon insertion reactions have not been previously reported in carbohydrate chemistry.

This mechanism is supported by labeling studies demonstrating that C8 of GTP is inserted between C2′ and C3′ of the GTP ribose (36) and that the 5′-dA radical abstracts a hydrogen atom from C3′ of GTP (35). In addition, intermediate **54** has been trapped using native substrate **51** and the substrate analog 2′,3′-dideoxy-GTP (34, 37).

Oxygen-independent Coproporphyrinogen III Oxidase (HemN)

Heme (**5**) functions as an oxygen carrier, a redox cofactor, and a source of the iron-oxo intermediate in biochemical hydroxylation, epoxidation, and dehydrogenation reactions. HemN catalyzes the oxidative decarboxylation of coproporphyrinogen III (**62**) to form protoporphyrinogen IX (**63**) in heme biosynthesis. A mechanistic proposal for this reaction is shown in Fig. 9 (38). In this proposal, the 5′-dA radical abstracts a hydrogen atom from **62**, generating **64**. This then undergoes decarboxylation and oxidation to form **63**. Repetition of this chemistry generates the B-ring vinyl group. This mechanism is supported by a structure of HemN (39), by the identification of the hydrogen atoms abstracted from coproporphyrinogen III (40), and by the detection of the substrate radical by EPR (41). This decarboxylation (as well as the conversion of **44** to **45** for MqnE) nicely demonstrates how an impossible decarboxylation in a closed shell species becomes possible through radical chemistry.

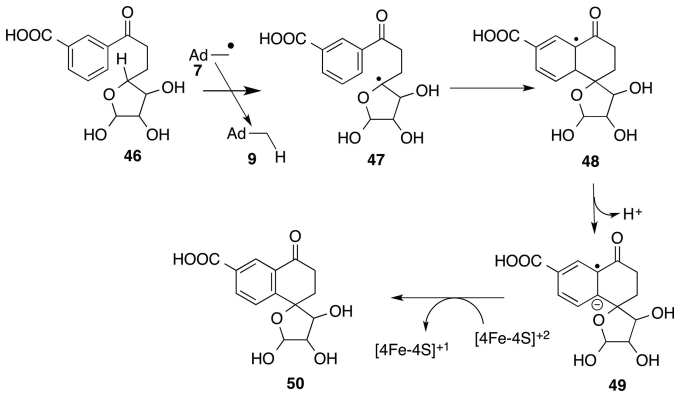

FIGURE 7. **Radical SAM enzymology in menaquinone biosynthesis.** Shown is the mechanistic proposal for the MqnC-catalyzed conversion of **46** to **50**.

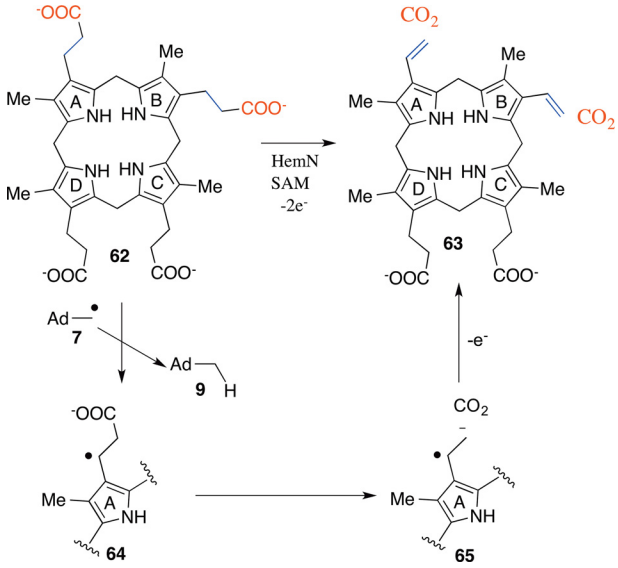

FIGURE 9. **Radical SAM enzymology in heme biosynthesis.** Shown is the mechanistic proposal for the coproporphyrinogen III oxidase (HemN)-catalyzed formation of protoporphyrinogen IX (**63**).

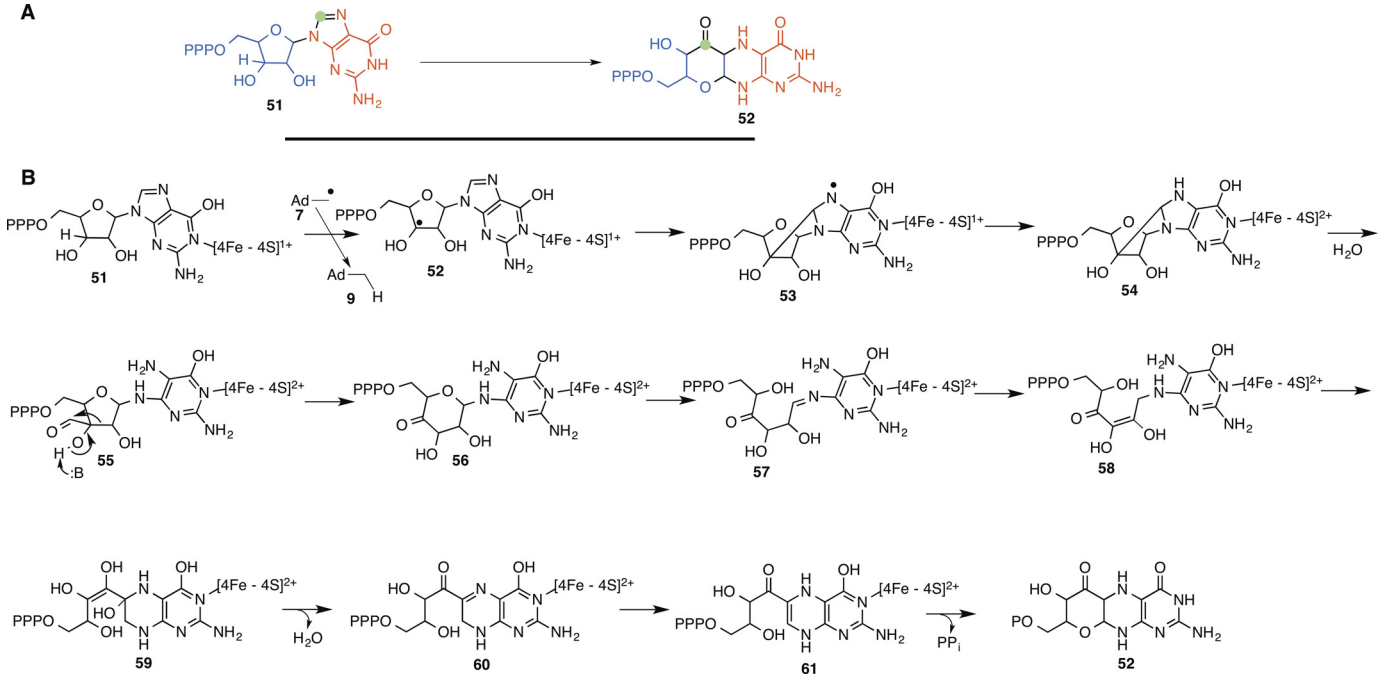

FIGURE 8. **Radical SAM enzymology in molybdopterin biosynthesis.** *A*, MoaA-catalyzed reaction. *B*, mechanistic proposal for the conversion of GTP (**51**) to the pterin (**52**).

Conclusions

Radical SAM enzymes are surprisingly prevalent in cofactor biosynthesis and play key roles in the biosynthesis of thiamin, menaquinone, molybdopterin, F_{420}, heme, biotin, and lipoic acid. The novelty of the chemistry demonstrates that, in the protected and catalytic environment of the enzyme active site, organic radicals can undergo complex rearrangements never before found in organic chemistry. As most of these enzymes have only recently become available, mechanistic analysis is still in its infancy, and much remains to be discovered in this new and exciting area of cofactor biosynthesis.

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