

2008

An Embarrassment of Riches: The Enzymology of RNA Modification

Dirk Iwata-Reuyl
Portland State University

Let us know how access to this document benefits you.

Follow this and additional works at: https://pdxscholar.library.pdx.edu/chem_fac

 Part of the [Biology Commons](#), and the [Chemistry Commons](#)

Citation Details

Iwata-Reuyl, Dirk, "An Embarrassment of Riches: The Enzymology of RNA Modification" (2008). *Chemistry Faculty Publications and Presentations*. 89.

https://pdxscholar.library.pdx.edu/chem_fac/89

This Post-Print is brought to you for free and open access. It has been accepted for inclusion in Chemistry Faculty Publications and Presentations by an authorized administrator of PDXScholar. For more information, please contact pdxscholar@pdx.edu.

Published in final edited form as:

Curr Opin Chem Biol. 2008 April ; 12(2): 126–133.

An Embarrassment of Riches: The Enzymology of RNA Modification

Dirk Iwata-Reuyl

Department of Chemistry, Portland State University, Portland, OR, iwatareyld@pdx.edu

Summary of Recent Advances

The maturation of transfer RNA (tRNA) involves extensive chemical modification of the constituent nucleosides, resulting in the formation of structurally diverse nucleosides. Many of the pathways to these modified nucleosides are characterized by chemically complex transformations, some of which are unprecedented in other areas of biology. To illustrate the scope of the field, recent progress in understanding the enzymology leading to the formation of 2 distinct classes of modified nucleosides are reviewed, the thiouridines and queuosine, a 7-deazaguanosine. In particular, recent data validating the involvement of several proposed intermediates in the formation of thiouridines are discussed, including 2 key enzyme intermediates and the activated tRNA intermediate. The discovery and mechanistic characterization of a new enzyme activity in the queuosine pathway is discussed.

Modification of RNA

The post-transcriptional processing of RNA involves a number of events essential for tRNA maturation [1]. From an enzymological perspective, the phenomenon of nucleoside modification is perhaps the most remarkable of these events, and results in a wealth of structural changes to the canonical nucleosides (Figure 1). While a number of RNA species are subject to nucleoside modification, it is only in transfer RNA (tRNA) that a rich structural diversity is realized. Over 100 modified nucleosides have been structurally characterized [2,3*], many of which exhibit extremely deep evolutionary conservation. The chemistry leading to nucleoside modification varies from simple methylation of the base or ribose ring to extensive "hypermodification" of the canonical bases, the latter of which can result in radical structural changes and involve multiple enzymatic steps to complete. Nucleoside modification is known to play a variety of roles necessary for proper physiological functioning of tRNA [1,4], including tertiary structural stabilization, introduction of recognition determinants, environmental sensing, and modulation of translational efficiency and fidelity.

The Enzymes of RNA Modification

While many RNA modifying enzymes had been discovered and characterized to various extents prior to the mid-1990's, in general modified nucleoside biosynthesis had not undergone comprehensive investigation due to the resistance of these enzymes to traditional biochemical and genetic characterization. For example, the design of assays for simply observing a desired enzymatic transformation was complicated by the difficulty of obtaining the appropriate tRNA substrates, the presence of endogenous RNases in cell-free extracts that degrade the RNA substrates and products, and the typically low abundance of the enzymes involved. Likewise, genetic approaches to enzyme discovery were hindered by the lack of specific phenotypes in

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

many mutants and the fact that unambiguous identification of a gene involved in RNA modification is ultimately dependent on determining the presence or absence of the specific modified nucleoside in tRNA, a laborious and technically challenging process, especially when working with large libraries of mutants. Consequently, until recently it was estimated that over 50% of the genes encoding tRNA modification enzymes still remained unknown [5]. However, the development of *in vitro* transcription in the late 1980's [6] and more efficient methods for the isolation of *in vivo* synthesized tRNA set the stage for researchers to exploit the flood of information unleashed by the genome sequencing projects for the discovery of new tRNA modification enzymes [7*]. Indeed, since 2000 the application of various genome-mining techniques has resulted in the discovery of at least 50 new genes encoding tRNA modifying enzymes [7*].

Due to the diversity of chemistries inherent in nucleoside modification the enzymology is especially rich, and coupled with the extraordinary progress that has occurred in recent years the choice of topics to cover in a short review is particularly difficult. Two quite different systems are the focus of this review; the sulfur transfer associated with the formation of thiouridines, and the novel nitrile reductase recently discovered in the pathway to the 7-deazaguanosine modified nucleoside queuosine. These systems are chosen to illustrate both the breadth of the field and the disparate problems currently being pursued. Nevertheless, they are also united by the important role that active-site cysteine residues play in catalysis, the central role of redox processes, and the potential involvement of covalent enzyme-substrate intermediates in the catalytic mechanisms.

The incorporation of sulfur to form thiouridines

Sulfur appears in tRNA nucleosides at C2 and C4 of uridine, C2 of cytidine, and C2 of adenosine. These collectively form the basis for 17 unique sulfur containing modified nucleosides present in various tRNA [2,3*]. Sulfur insertion into nucleosides occurs through 2 distinct mechanisms, an oxidative pathway in which the sulfur donor is a protein bound iron-sulfur cluster [8,9], and a non-oxidative pathway in which sulfur is incorporated at the expense of an oxygen atom [10,11]. The former, leading to the thiomethyl derivatives of adenosine, is also observed in other systems in which sulfur is inserted into unactivated C-H bonds, such as biotin and lipoic acid synthases.

The requirements for the synthesis of 2- and 4-thiouridine (s^2U and s^4U) are similar [11], and include ATP to activate the C-2 or C-4 oxygen atom, the cysteine desulfurase IscS as the source of the sulfur atom, and an enzyme to orchestrate the activation of the nucleoside and insertion of sulfur. The relevant enzymes are MnmA [12] and ThiI [13,14], which are responsible for s^2U and s^4U formation, respectively. While ThiI, IscS, tRNA, and ATP are sufficient for robust s^4U synthesis, this isn't the case for s^2U [12], which has recently been shown to require 5 additional proteins, TusA-E [15**]. These proteins participate in a complex sulfur relay in which the activated sulfur of IscS, presumably as the persulfide, is first transferred to TusA, followed by transfer to TusD, which is part of a heterohexameric complex with TusB and TusC ($\alpha_2\beta_2\gamma_2$), then to TusE, which finally transfers the sulfur to MnmA for incorporation into the tRNA. The rationale for such a complex sulfur transfer pathway is unknown, however the enhanced ability to regulate the delivery of sulfur to multiple pathways is one obvious explanation [11,15**], although no evidence is available yet to support that hypothesis.

A molecular mechanism for sulfur transfer from IscS to tRNA was first proposed by Mueller and colleagues in 2000 [14] when they demonstrated that, consistent with the presence of a "rhodanese" domain in ThiI, ThiI was capable of supporting sulfur transfer from thiosulfate to cyanide to form thiocyanide, suggesting that the catalytic mechanism for thiouridine formation might involve a protein persulfide. A persulfide intermediate was also implicated in a

simultaneous report by Lauhon and colleagues [13] in which they demonstrated sulfur transfer from IscS to ThiI. The proposed catalytic mechanism, as originally formulated and elaborated on shortly thereafter [16], is also relevant for the formation of 2-thiouridine, and is shown in figure 2. The first step is sulfur transfer from IscS to give a protein persulfide intermediate (**I**, localized on Cys⁴⁵⁶ of *E. coli* ThiI), followed by binding of the tRNA and activation of the uridine oxygen by ATP to form an adenylated intermediate. Activation as the adenylate was proposed based on the presence in ThiI of a “P-loop” motif [17], a characteristic motif found in enzymes that activate carbonyl oxygens for nucleophilic substitution by adenylation with ATP [18,19]. The critical sulfur transfer to the tRNA was envisaged to occur through either of 2 potential routes, direct attack on the adenylated uridine by the terminal sulfur of the persulfide or by free bisulfide generated after attack by another active-site sulfur (Cys³⁴⁴ of *E. coli* ThiI) on the internal sulfur of the persulfide. Subsequent decomposition of either tetrahedral intermediate then liberates AMP and thiouridine, with ThiI in the oxidized disulfide form (**II**). Reduction of the disulfide, either with added reductant *in vitro* or with an endogenous redox system *in vivo*, regenerates the dithiol form of the enzyme, ready to undergo another trans-persulfidation reaction with IscS. While indirect evidence for the overall elements of the reaction has accumulated over the last decade, it wasn't until recently that definitive evidence was reported for the existence of several key intermediates in the reaction, specifically the persulfide and disulfide forms of ThiI, and the adenylated uridine moiety.

Although the nature of the activated sulfur on ThiI and MnmA was proposed to be a persulfide, IscS was shown to readily form trisulfides and higher order polysulfides on another protein partner, the iron-sulfur cluster “scaffold protein” IscU [20], complicating the interpretation of the ThiI and MnmA data. Working with a mutant ThiI in which 4 of the 5 Cys residues had been changed to Ala, leaving Cys⁴⁵⁶, the putative site of persulfide formation, unchanged, Mueller and colleagues showed [21**] that after the sulfur transfer step from IscS the mutant ThiI could be labeled with *I*-AEDANS, a fluorescent derivative of iodoacetamide, and that treatment of the labeled protein with a reductant such as DTT liberated the fluorescent label. These data demonstrated the nucleophilic nature of the activated sulfur on ThiI, and that the label and protein were linked by an S-S bond. To identify unambiguously the chemical structure of the linkage the protein-label adduct and the reduced protein were separately subjected to tryptic digests and MALDI-MS analysis. While the mass of the peptide containing Cys⁴⁵⁶ from the reduced protein corresponded as expected to that with a free thiol, the mass of the peptide bearing the label was consistent only with the presence of a disulfide linkage, clearly demonstrating that a persulfide was the relevant form of activated sulfur on ThiI during catalysis.

In the absence of exogenous reductants such as DTT ThiI is only able to carry out a single turnover [16,22], and fewer free thiols are detected after turnover, phenomena consistent with the formation of an enzyme disulfide bond during catalysis as depicted in figure 2. Working with another mutant ThiI in which all the Cys residues except Cys⁴⁵⁶ and Cys³⁴⁴ were mutated to Ala [21**], Mueller and colleagues carried out single turnover assays in the absence of DTT, followed as above by tryptic digests and MALDI-MS analysis. In this case an intramolecular disulfide-linked peptide containing Cys³⁴⁴ and Cys⁴⁵⁶ was easily detected. Furthermore, this peptide disappeared after treatment with DTT and iodoacetamide, and was replaced by 2 separate peptides, each containing one of the alkylated Cys residues (Cys³⁴⁴ or Cys⁴⁵⁶). These data firmly establish the enzyme disulfide as a key intermediate in the catalytic mechanism.

Finally, in a beautiful display of crystallographic prowess, Nureki and colleagues reported 3 crystal structures of the MnmA/tRNA^{Glu} complex [23**] that they dubbed “snapshots” of the sulfuration reaction, interpreting the structures as an initial binding state (form I), a prereaction state (form II), and the adenylated intermediate state (form III). Notably, in the last structure

they were able to capture the activated uridine in an adenylyated form, confirming the presence of yet another putative intermediate on the reaction path.

Although previous *in vitro* sulfur transfer experiments failed to detect ^{35}S -labeled MnmA [15**], leaving open the question of whether sulfur transfer to tRNA occurred from MnmA (as with *E. coli* ThiI) or from TusE, the crystal structures clearly implicate Cys¹⁹⁹ of MnmA as the site of persulfide formation, and Cys¹⁰² as the second Cys residue required for transfer and liberation of the sulfur atom via disulfide bond formation with Cys¹⁹⁹. Furthermore, the authors also provided supporting biochemical data demonstrating ^{35}S -labeling of MnmA in *in vitro* assays. The authors note that in going from the initial binding state (form I) to the prereaction (form II) and adenylyated states (form III) the active-site of MnmA forms a closed conformation in which the activated uridine is protected from solvent. This occurs as a result of the reorganization of a helix and a preceding disordered region in form I to a loop, a β -hairpin, and a short helix in forms II and III. Assuming that the active-site architecture of MnmA and ThiI are similar, the formation of a solvent excluded chamber could explain the exceedingly high concentrations of bisulfide (50 mM) necessary to support efficient ThiI catalysis of s⁴U formation in the absence of IscS [24].

While the crystallographic data would appear to support the operation of the free bisulfide mechanism since the formation of a closed active-site is considered essential to prevent the easily diffusible bisulfide from escaping the active-site, both mechanisms benefit from a closed active-site as this better protects the reactive adenylyated uridine. Thus, none of the recent data reported for MnmA or ThiI allows a definitive conclusion regarding which of the 2 putative mechanisms for sulfur delivery is occurring, leaving this part of the puzzle still unresolved. Nonetheless, our understanding of the complicated process of tRNA sulfuration is now significantly deeper than just a few years ago, and if the recent pace of progress in this area is an indicator for the future, this issue will be resolved soon.

QueF, a biological nitrile reductase

The enzyme QueF (Figure 3) occurs in the biosynthesis of the hypermodified nucleoside queuosine [25*,26**], a pathway rich in new chemistries. Queuosine and the related modified nucleoside archaeosine share a 7-deazaquanine core structure, but differ in the substituent at the 7-position [27]. The biosynthesis of these nucleosides is unique in the realm of RNA modification in that a substantial portion of the pathway occurs at the level of the free base [27], outside the context of the RNA. Thus, the early portion of the pathway leads to a modified precursor base that is subsequently inserted into the tRNA in a transglycosylation reaction [27] with the concomitant elimination of the genetically encoded base (a guanine in both cases).

Although annotated in bacterial genomes as a type I-like GTP cyclohydrolase (GCYH-I), and initially hypothesized to be the first enzyme in the queuosine pathway [26**], the QueF enzyme was shown instead to function as a nitrile reductase [25*], converting the advanced intermediate 7-cyano-7-deazaguanine (preQ₀) to 7-aminomethyl-7-deazaguanine (preQ₁). Notably, this is the only known example of an enzyme-catalyzed reduction of a nitrile metabolite to the corresponding amine. Despite the misannotation as a cyclohydrolase-like enzyme, sequence and structural analyses were consistent with QueF belonging to the tunneling-fold (T-told) protein superfamily [28], the same structural superfamily to which GCYH-I belongs. This led to the construction of a homology model for QueF [29*] based on the GCYH I structure from *E. coli* [30], setting the stage for subsequent detailed mechanistic studies.

The reduction of a nitrile to an amine is a 4-electron process, and the electron donor in the QueF reaction is NADPH, which as a 2-electron reductant is required in a 2:1 stoichiometry to the substrate preQ₀. Because NADPH is the only cofactor required in the conversion and the protein does not possess any inherent redox capacity, the reaction must occur in a stepwise

fashion and necessarily involves the intermediacy of an imine, the 2-electron reduced product. This presents a chemical challenge in that the imine should be especially reactive towards nucleophilic attack by water, potentially leading to hydrolytic loss of the nitrogen atom. This could conceivably occur in the enzyme active-site itself after departure of the product NADP^+ generated in the first reductive cycle, or free in solution if the intermediate escaped the active-site prior to binding of the second NADPH.

So how does the enzyme catalyze the overall chemical transformation, and in particular, what strategy is employed to prevent the unproductive loss of the nitrogen atom through hydrolysis? The answer appears to be through a clever form of “protecting group” chemistry, in which the imine is temporarily masked as a covalent thiohemiaminal adduct. This adduct arises as an obligate intermediate in the catalytic mechanism due to the initial formation of a covalent thioimide adduct, which serves as the actual substrate for the first reduction cycle.

Insight into the catalytic mechanism of QueF began by considering the sequence conservation within the QueF family and the homology model mentioned above [29*]. Of particular interest was the presence of a universally conserved cysteine residue (Cys^{55} in the *B. subtilis* enzyme) that aligned with a conserved cysteine residue in the sequences of GCYH-I enzymes. Importantly, in GCYH-I this cysteine is located in the active-site where it serves as a ligand to the essential Zn^{2+} ion, which in turn coordinates the water molecule that is responsible for hydrolytic ring opening at C-8 of GTP in the first step of the cyclohydrolase reaction. The homology model placed Cys^{55} in close proximity to the nitrile group of preQ_0 , suggesting a direct role in catalysis.

Experiments designed to probe the role of Cys^{55} confirmed an essential role in catalysis [31*]; chemical labeling with iodoacetamide inactivated the enzyme, while labeling in the presence of preQ_0 protected the enzyme from inactivation, and conversion of Cys^{55} to serine or alanine via site-directed mutagenesis produced inactive enzyme. However, the key experiment in elucidating the role of Cys^{55} came from spectroscopic analysis of the enzyme in the presence of preQ_0 [31*]; titration of the wild-type enzyme, but not the Cys^{55} mutants, with preQ_0 was accompanied by the appearance of a new absorption band at 376 nm, consistent with the formation of an α,β -unsaturated thioimide and suggesting the covalent adduct **III** (Figure 3). The absorption reached a plateau when preQ_0 became stoichiometric with enzyme, and remained after extended dialysis. When excess NADPH was added to the dialyzed enzyme only 2 equivalents (based on enzyme concentration) were consumed, and reaction with NADPH abolished the absorption at 376 nm. The presence of a covalent adduct also nicely explained the crystallographic behavior of QueF [29*]; in the absence of preQ_0 only poorly diffracting crystals formed ($>8 \text{ \AA}$), but the addition of preQ_0 was accompanied by a new crystal form that diffracted to 2.25 \AA .

The minimal mechanism consistent with the experimental data is outlined in figure 3, and involves initial binding of preQ_0 to the enzyme followed by nucleophilic attack of the thiol of Cys^{55} on the nitrile group of preQ_0 to form a thioimide intermediate (**III**). The first NADPH molecule then binds to the enzyme and reduces the thioimide intermediate, giving a new covalent adduct, the thiohemiaminal (**IV**). Release of the oxidized cofactor is followed by binding of the second molecule of NADPH, collapse of the thiohemiaminal to the imine (**V**), and reduction to give preQ_1 .

With the general features of the QueF mechanism now in place, the elucidation of the molecular details of the reaction, and the potential to reengineer the enzyme for applications in the reduction of other nitrile containing compounds, are now on the horizon.

Conclusions and future projects

Nucleoside modification engenders a wealth of structurally differentiated nucleosides in tRNA, and the elucidation of the enzymology of these processes is occurring at an unprecedented rate. The systems described above, while providing some measure of the scope of the field, are by no means unusual in exhibiting important and compelling chemistries. MiaB, for example, the enzyme responsible for the thiomethylation [32] of isopentenyl adenosine (Figure 4), belongs to the newly described radical SAM superfamily [33], which utilize a [4Fe-4S] cluster and S-adenosylmethionine (SAM) to initiate radical chemistry. MiaB, along with biotin and lipoic acid synthases [8], contains 2 [Fe-S] clusters [34**], one apparently for radical initiation and the second as a source of sulfur for sulfur insertion. Radical SAM enzymes have also been discovered recently in queuosine [26**] and wyosine [35**,36*] biosynthesis, and the enzyme in the latter pathway (Tyw1) is responsible for the cryptic construction of the tricyclic ring system of wyosine. The Tyw1 enzyme has recently been crystallized [37,38], and interestingly, it also contains 2 [4Fe-4S] clusters. An *in vitro* activity has not yet been reported, nor is the identity of the donor molecule responsible for the 2 additional carbons known. While pyruvate and acetyl-CoA emerge as potential candidates for this latter role, regardless of the metabolite ultimately implicated this enzyme seems sure to add a significant new twist to the chemistry of the radical SAM family. These are but a few of the systems that are on the cusp of significant new insights into their mechanism of action. Much of the future work in this area will continue to be informed by bioinformatics analysis, both for the discovery of the remaining enzymes involved in modification pathways as well as for insights into the mechanisms, structure, and evolution of these fascinating enzymes.

Acknowledgement

I thank the present and past members of my research group who contributed to the work on the QueF enzyme, and gratefully acknowledge the NSF (MCB-9733746) and NIH (GM070641-01A3) for supporting our work on modified nucleoside biosynthesis.

References and recommended reading

Papers of particular interest, published within the annual period of review, has been highlighted as:

* of special interest

** of outstanding interest

1. Hopper AK, Phizicky EM. tRNA transfers to the limelight. *Genes Dev* 2003;17:162–180. [PubMed: 12533506]
2. Rozenski J, Crain PF, McCloskey JA. The RNA Modification Database: 1999 update. *Nucleic Acids Res* 1999;27:196–197. [PubMed: 9847178]
3. * Dunin-Horkawicz S, Czerwoniec A, Gajda MJ, Feder M, Grosjean H, Bujnicki JM. MODOMICS: a database of RNA modification pathways. *Nucleic Acids Res* 2006;34:D145–D149. [PubMed: 16381833] An excellent database that brings together the structures, biosynthetic pathways, and enzymes of RNA modification, as well as RNA sequences.
4. Bjork, GR. Biosynthesis and Function of Modified Nucleosides. In: Soll, D.; RajBhandary, UL., editors. *tRNA: Structure, Biosynthesis, and Function*. ASM Press; 1995. p. 165-206.
5. de Crécy-Lagard, V. Bioinformatics leads the path to the identification of missing tRNA modification genes. In: Bujnicki, JM., editor. *Practical Bioinformatics*. vol 15. Springer-Verlag; 2004. p. 169-190.
6. Milligan JF, Groebe DR, Witherell GW, Uhlenbeck OC. Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic Acids Research* 1987;15:8783–8798. [PubMed: 3684574]

7. * de Crécy-Lagard V. Identification of genes encoding tRNA modification enzymes by comparative genomics. *Methods Enzymol* 2007;425:153–183. [PubMed: 17673083] An excellent overview of the application of bioinformatic analyses to the field of tRNA modification.
8. Booker SJ, Cicchillo RM, Grove TL. Self-sacrifice in radical S-adenosylmethionine proteins. *Curr Opin Chem Biol* 2007;11:543–552. [PubMed: 17936058]
9. Fontecave M, Ollagnier-de-Choudens S, Mulliez E. Biological radical sulfur insertion reactions. *Chem Rev* 2003;103:2149–2166. [PubMed: 12797827]
10. Lauhon CT. Orchestrating sulfur incorporation into RNA. *Nat Chem Biol* 2006;2:182–183. [PubMed: 16547479]
11. Mueller EG. Trafficking in persulfides: delivering sulfur in biosynthetic pathways. *Nat Chem Biol* 2006;2:185–194. [PubMed: 16547481]
12. Kambampati R, Lauhon CT. MnmA and IscS are required for in vitro 2-thiouridine biosynthesis in *Escherichia coli*. *Biochemistry* 2003;42:1109–1117. [PubMed: 12549933]
13. Kambampati R, Lauhon CT. Evidence for the transfer of sulfane sulfur from IscS to ThiI during the in vitro biosynthesis of 4-thiouridine in *Escherichia coli* tRNA. *J Biol Chem* 2000;275:10727–10730. [PubMed: 10753862]
14. Palenchar PM, Buck CJ, Cheng H, Larson TJ, Mueller EG. Evidence that ThiI, an enzyme shared between thiamin and 4-thiouridine biosynthesis, may be a sulfurtransferase that proceeds through a persulfide intermediate. *J Biol Chem* 2000;275:8283–8286. [PubMed: 10722656]
15. ** Ikeuchi Y, Shigi N, Kato J, Nishimura A, Suzuki T. Mechanistic insights into sulfur relay by multiple sulfur mediators involved in thiouridine biosynthesis at tRNA wobble positions. *Mol Cell* 2006;21:97–108. [PubMed: 16387657] A powerful example of the use of "ribonucleome analysis" for gene discovery in RNA modification pathways. The authors report the discovery of 5 genes essential in s^2U biosynthesis. These genes are shown to encode proteins involved in a complex sulfur relay from the cysteine desulfurase IscS to tRNA.
16. Mueller EG, Palenchar PM, Buck CJ. The role of the cysteine residues of ThiI in the generation of 4-thiouridine in tRNA. *J Biol Chem* 2001;276:33588–33595. [PubMed: 11443125]
17. Mueller EG, Palenchar PM. Using genomic information to investigate the function of ThiI, an enzyme shared between thiamin and 4-thiouridine biosynthesis. *Protein Sci* 1999;8:2424–2427. [PubMed: 10595545]
18. Bork P, Koonin EV. A P-loop-like motif in a widespread ATP pyrophosphatase domain: implications for the evolution of sequence motifs and enzyme activity. *Proteins* 1994;20:347–355. [PubMed: 7731953]
19. Tesmer JJ, Klem TJ, Deras ML, Davisson VJ, Smith JL. The crystal structure of GMP synthetase reveals a novel catalytic triad and is a structural paradigm for two enzyme families. *Nat Struct Biol* 1996;3:74–86. [PubMed: 8548458]
20. Smith AD, Frazzon J, Dean DR, Johnson MK. Role of conserved cysteines in mediating sulfur transfer from IscS to IscU. *FEBS Lett* 2005;579:5236–5240. [PubMed: 16165131]
21. ** Wright CM, Christman GD, Snellinger AM, Johnston MV, Mueller EG. Direct evidence for enzyme persulfide and disulfide intermediates during 4-thiouridine biosynthesis. *Chem Commun (Camb)* 2006:3104–3106. [PubMed: 16855700] An elegant example of the application of mass spectrometry to characterize protein intermediates along a reaction pathway. The authors provide definitive data on the presence of persulfide and disulfide forms of ThiI, the enzyme catalyzing the formation of s^4U .
22. Wright CM, Palenchar PM, Mueller EG. A paradigm for biological sulfur transfers via persulfide groups: a persulfide-disulfide-thiol cycle in 4-thiouridine biosynthesis. *Chem Commun (Camb)* 2002:2708–2709. [PubMed: 12510310]
23. ** Numata T, Ikeuchi Y, Fukai S, Suzuki T, Nureki O. Snapshots of tRNA sulphuration via an adenylated intermediate. *Nature* 2006;442:419–424. [PubMed: 16871210] Beautiful crystallographic studies of the MnmA catalyzed formation of s^2U . The authors succeed in capturing several enzyme forms along the reaction pathway to s^2U , including the proposed adenylated form of the substrate uridine residue.
24. Lauhon CT, Erwin WM, Ton GN. Substrate specificity for 4-thiouridine modification in *Escherichia coli*. *J Biol Chem* 2004;279:23022–23029. [PubMed: 15037613]

25. * van Lanen SG, Reader JS, Swairjo MA, de Crecy-Lagard V, Lee B, Iwata-Reuyl D. From cyclohydrolase to oxido-reductase: discovery of nitrile reductase activity in a common fold. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:4264–4269. [PubMed: 15767583] Demonstration of nitrile reductase activity of the QueF enzyme in the queuosine pathway. and structural analysis establishing it as member of the T-fold superfamily.
26. ** Reader JS, Metzgar D, Schimmel P, de Crecy-Lagard V. Identification of four genes necessary for biosynthesis of the modified nucleoside queuosine. *J Biol Chem* 2004;279:6280–6285. [PubMed: 14660578] A powerful example of the power of comparative genomics for the discovery of genes in poorly characterized metabolic pathways. The authors identify 4 genes encoding putative enzymes in the queuosine biosynthetic pathway, and confirm their predictions through the generation of genetic knockouts.
27. Iwata-Reuyl D. Biosynthesis of the 7-deazaguanosine hypermodified nucleosides of transfer RNA. *Bioorg Chem* 2003;31:24–43. [PubMed: 12697167]
28. Colloch N, Poupon A, Mornon JP. Sequence and structural features of the T-fold, an original tunnelling building unit. *Proteins* 2000;39:142–154. [PubMed: 10737935]
29. * Swairjo MA, Reddy RR, Lee B, Van Lanen SG, Brown S, de Crécy-Lagard V, Iwata-Reuyl D, Schimmel P. Crystallization and preliminary X-ray characterization of the nitrile reductase QueF - a queuosine biosynthesis enzyme. *Acta Cryst* 2005;F61:945–948. Preliminary crystallization and structural analysis of the QueF enzyme. The authors construct a homology model based on the structure of GCYH-I, and use this to formulate structural and mechanistic hypothesis for QueF.
30. Nar H, Huber R, Meining W, Schmid C, Weinkauff S, Bacher A. Atomic structure of GTP cyclohydrolase I. *Structure* 1995;3:459–466. [PubMed: 7663943]
31. * Lee BW, Lanen SG, Iwata-Reuyl D. Mechanistic Studies of *Bacillus subtilis* QueF, the Nitrile Oxidoreductase Involved in Queuosine Biosynthesis. *Biochemistry* 2007;46:12844–12854. [PubMed: 17929836] Elucidation of key elements of the mechanism of nitrile reduction by the enzyme QueF. Through site-directed mutagenesis and spectroscopic studies the enzyme is shown to utilize covalent catalysis for nitrile reduction in the conversion of preQ₀ to preQ₁.
32. Pierrel F, Douki T, Fontecave M, Atta M. MiaB protein is a bifunctional radical-S-adenosylmethionine enzyme involved in thiolation and methylation of tRNA. *J Biol Chem* 2004;279:47555–47563. [PubMed: 15339930]
33. Sofia HJ, Chen G, Hetzler BG, Reyes-Spindola JF, Miller NE. 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* 2001;29:1097–1106. [PubMed: 11222759]
34. ** Hernandez HL, Pierrel F, Elleingand E, Garcia-Serres R, Huynh BH, Johnson MK, Fontecave M, Atta M. MiaB, a bifunctional radical-S-adenosylmethionine enzyme involved in the thiolation and methylation of tRNA, contains two essential [4Fe-4S] clusters. *Biochemistry* 2007;46:5140–5147. [PubMed: 17407324] Utilizing a variety of spectroscopic and biochemical techniques the authors report the presence of 2 [4Fe-4S] clusters in MiaB.
35. ** Noma A, Kirino Y, Ikeuchi Y, Suzuki T. Biosynthesis of wybutosine, a hyper-modified nucleoside in eukaryotic phenylalanine tRNA. *EMBO J* 2006;25:2142–2154. [PubMed: 16642040] Another example of the application of "ribonucleome analysis" to the discovery of genes encoding tRNA modification enzymes. Here the authors report the discovery of 4 genes encoding enzymes in the biosynthesis of wybutosine, and reconstitute a portion of the pathway *in vitro*.
36. * Waas WF, de Crecy-Lagard V, Schimmel P. Discovery of a gene family critical to wyosine base formation in a subset of phenylalanine-specific transfer RNAs. *J Biol Chem* 2005;280:37616–37622. [PubMed: 16162496] Another good example of comparative genomics and gene discovery. Using comparative genomics the authors identify a putative gene in wyosine biosynthesis. After construction of a gene knockout and tRNA analysis they are able to assign the encoded protein to the conversion of m¹G to imG-14.
37. Goto-Ito S, Ishii R, Ito T, Shibata R, Fusatomi E, Sekine SI, Bessho Y, Yokoyama S. Structure of an archaeal TYW1, the enzyme catalyzing the second step of wye-base biosynthesis. *Acta Crystallogr D Biol Crystallogr* 2007;63:1059–1068. [PubMed: 17881823]

38. Suzuki Y, Noma A, Suzuki T, Senda M, Senda T, Ishitani R, Nureki O. Crystal Structure of the Radical SAM Enzyme Catalyzing Tricyclic Modified Base Formation in tRNA. *J Mol Biol* 2007;372:1204–1214. [PubMed: 17727881]

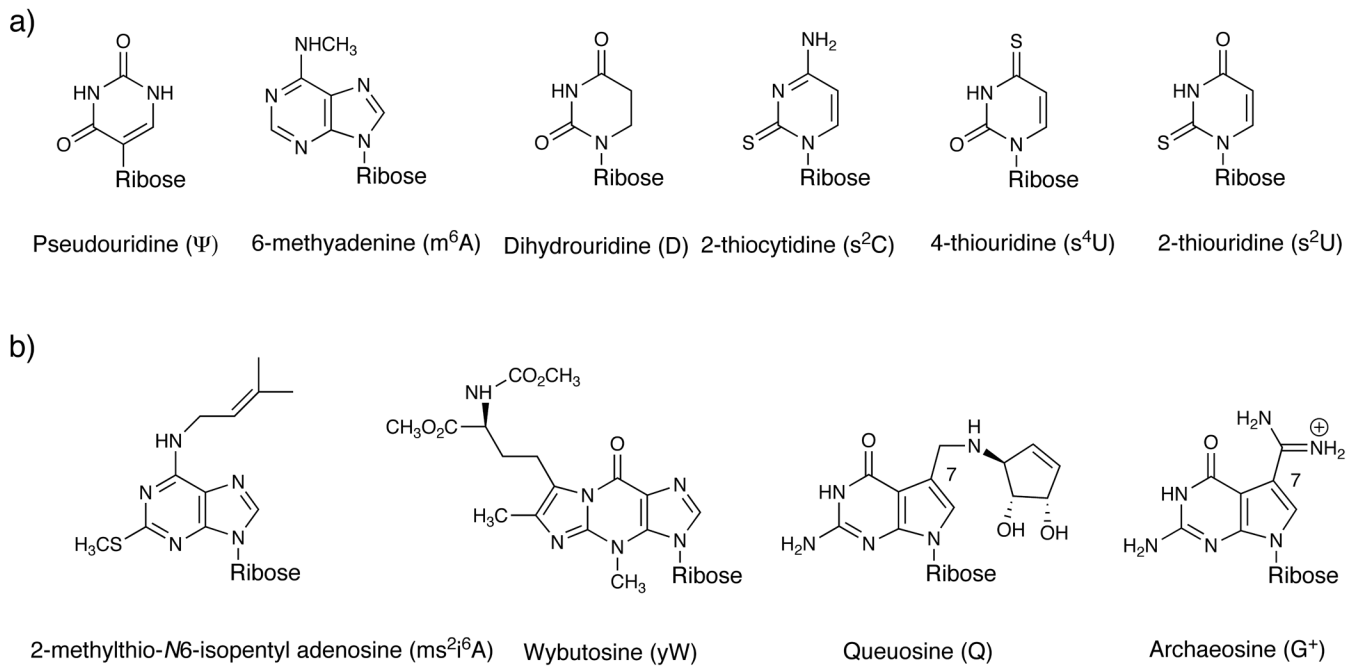


Figure 1.

Examples of modified nucleosides found in tRNA. a) Simple modifications that involve relatively small structural changes to the parent nucleoside. b) Complex or hypermodifications that involve significant structural changes to the parent nucleoside.

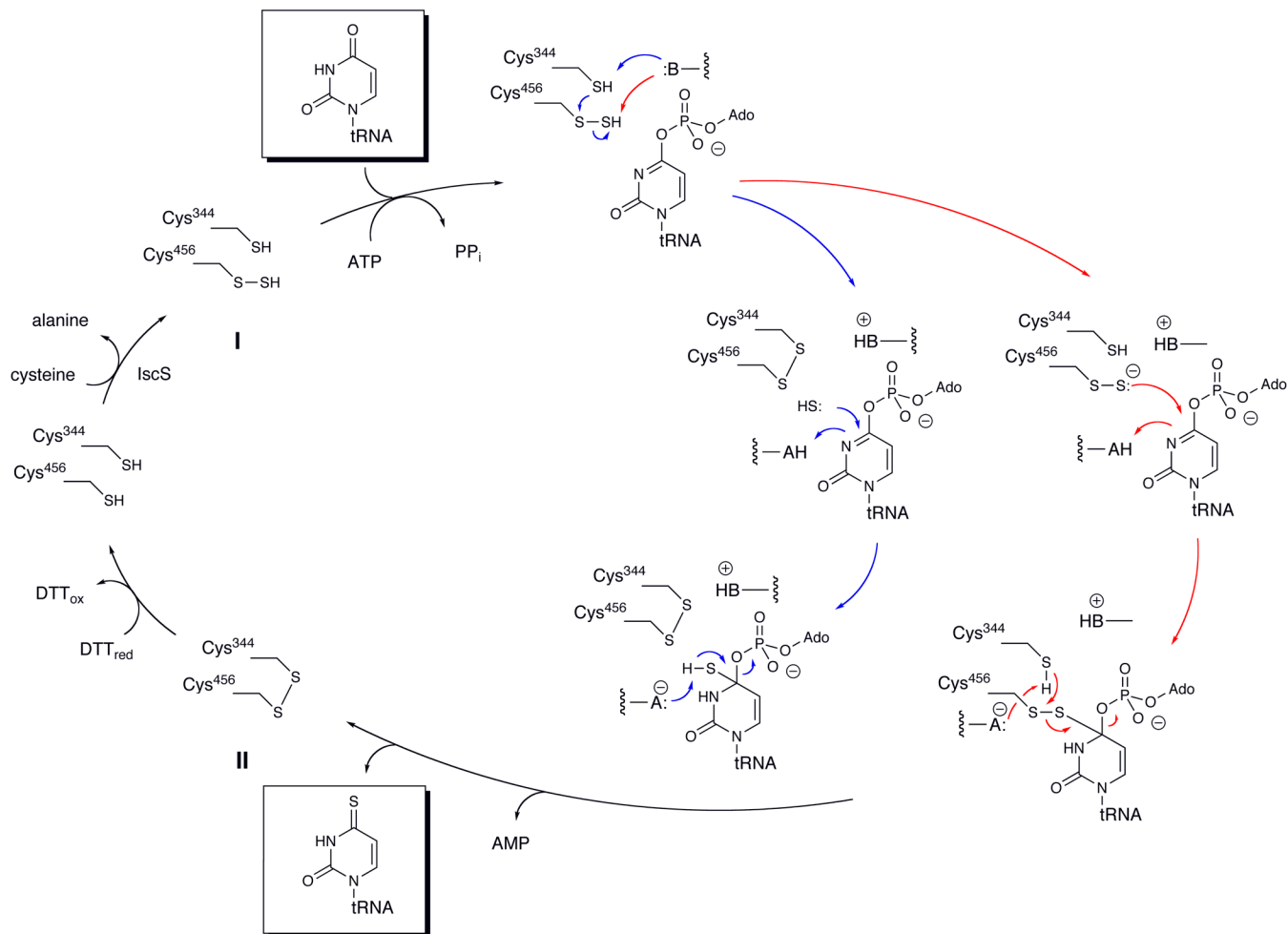


Figure 2.

Proposed mechanism for the formation of thiouridine. The mechanism is explicit for *E. coli* ThiI catalyzed formation of s⁴U, but the overall chemistry is relevant for MnmA catalyzed formation of s²U. Two potential routes to sulfur insertion have been proposed and are compatible with all the experimental data. In a direct sulfur transfer the nucleophilic, terminal sulfur of the enzyme persulfide (shown on Cys⁴⁵⁶) attacks the activated adenylylated uridine (red path) to form a covalent, disulfide linked protein-tRNA adduct. Subsequent nucleophilic attack of a second active-site Cys residue (Cys³⁴⁴) on the Cys-sulfur of the disulfide completes sulfur transfer to the tRNA. In a free bisulfide mechanism to sulfur transfer (blue path) the second active-site Cys residue attacks the enzyme persulfide at the internal sulfur, releasing free bisulfide, which subsequently attacks the activated adenylylated uridine. In both routes the breakdown of the tetrahedral intermediates releases AMP and leaves the enzyme in the oxidized disulfide form. Reduction of the disulfide prepares the enzyme for another round of trans-persulfidation from IscS. Although persulfide formation is shown prior to tRNA and ATP binding, formation of the adenylylated uridine has been shown to occur with MnmA without sulfur transfer from IscS.

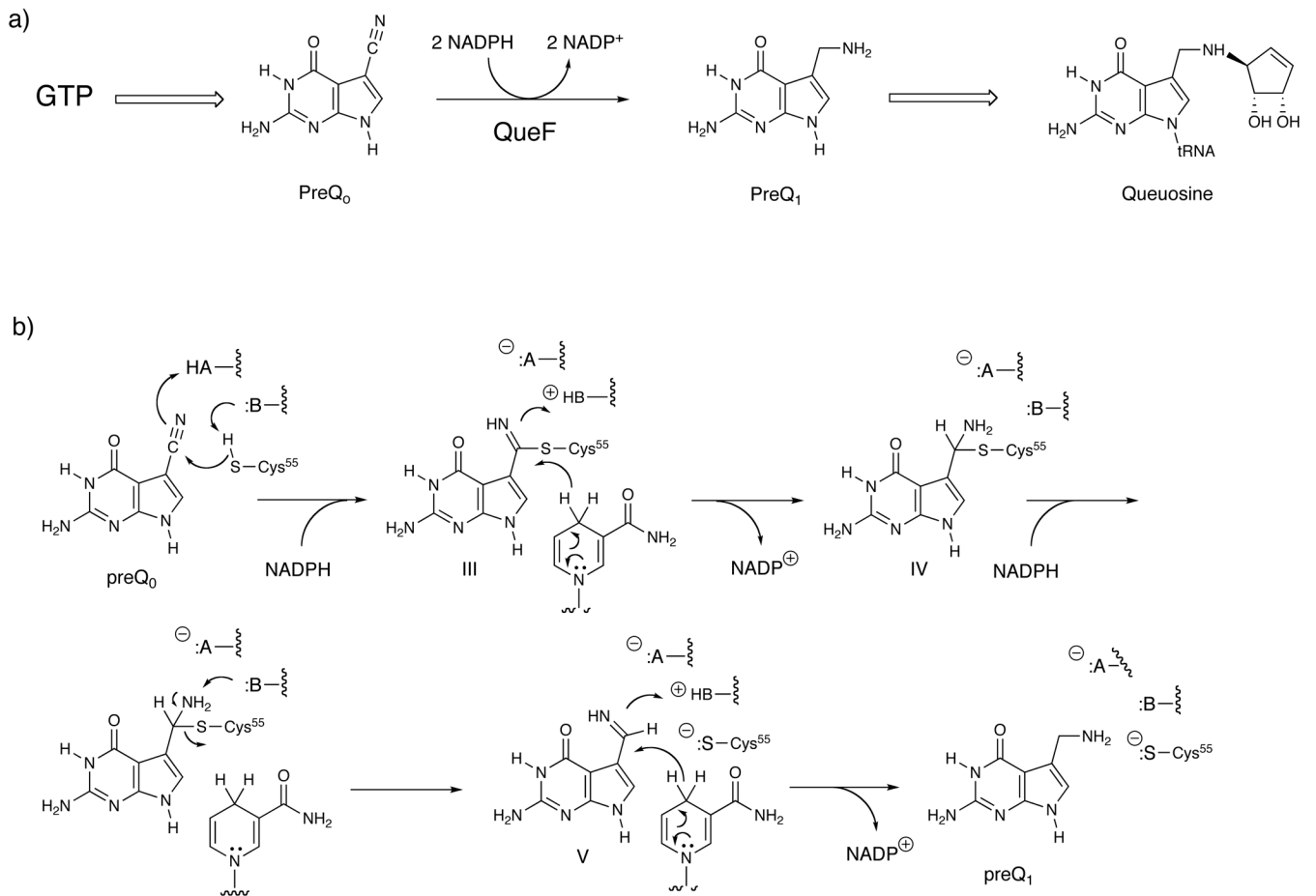


Figure 3.

a) The reaction catalyzed by the QueF enzyme and its role in the biosynthesis of queuosine.
 b) The proposed mechanism for reduction of the nitrile group of preQ₀ to the aminomethyl group of preQ₁. After binding of preQ₀ the active-site Cys residue (Cys⁵⁵ in *Bacillus subtilis* QueF) attacks the nitrile group to form a thioimide (III). Binding of the first equivalent of NADPH and hydride transfer gives the thiohemiaminal IV followed by the dissociation of NADP⁺. Binding of the second equivalent of NADPH is followed by breakdown of IV to give the imine V. The second hydride is then transferred to generate preQ₁, followed by dissociation of NADP⁺ and preQ₁ from the enzyme. By keeping the intermediate thiohemiaminal form until after the binding of the second NADPH hydrolysis of the intermediate imine is avoided.

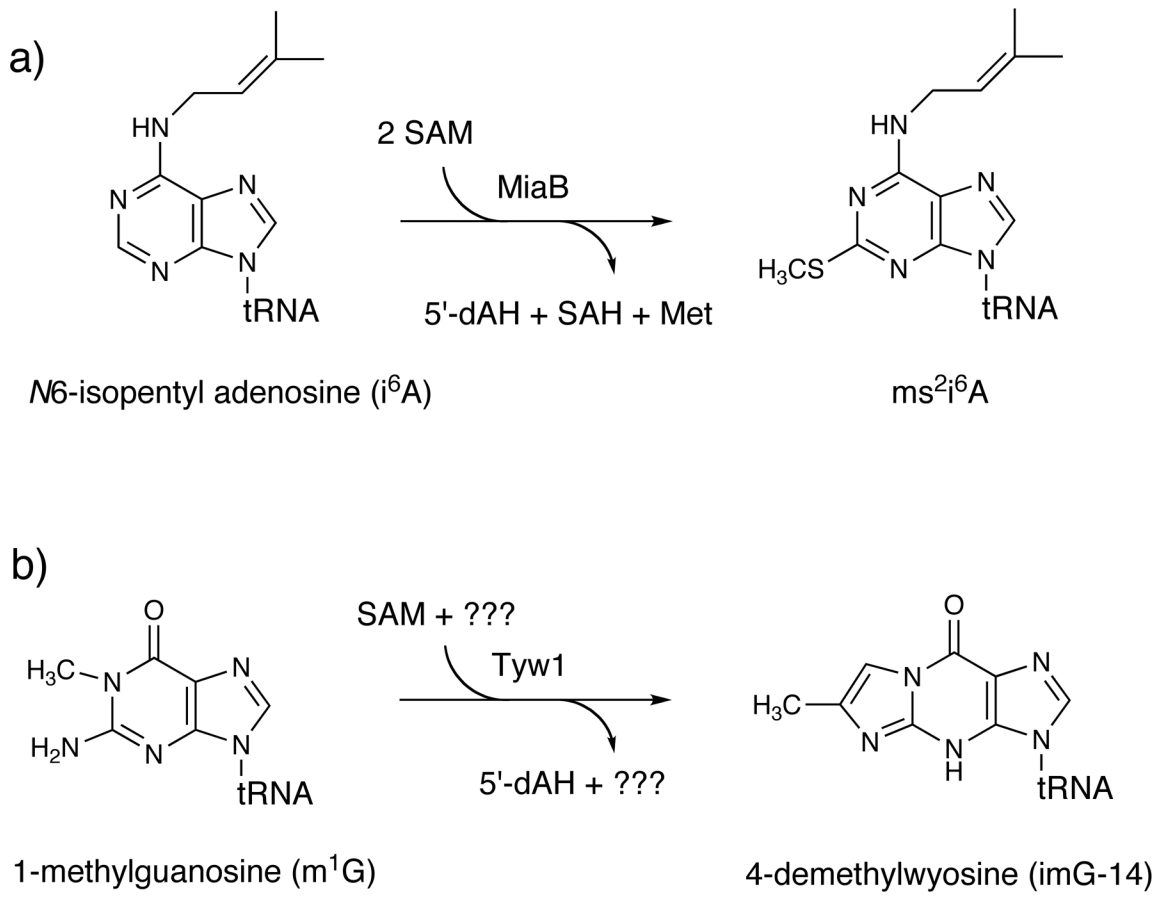


Figure 4.

a) The MiaB catalyzed thiomethylation of $i^6\text{A}$ to give ms^2i^6A . b) The reaction catalyzed by the Tyw1 enzyme in the wyosine pathway. The co-substrate(s) in the Tyw1 reaction has not yet been identified.