Structural and evolutionary insights into ribosomal RNA methylation.

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ABSTRACT

Methylation of nucleotides in ribosomal RNAs (rRNAs) is a ubiquitous feature that occurs in all living organisms. Identification of all enzymes responsible for rRNA-methylation, as well as mapping of all modified rRNA residues, is now complete for a number of model species, such as *Escherichia coli* and *Saccharomyces cerevisiae*. Recent high-resolution structures of bacterial ribosomes provided the first direct visualization of methylated nucleotides. The structures of ribosomes from various organisms and organelles also became available lately and allowed for comparative structure-based analysis of rRNA methylation sites in various taxonomic groups. In addition to the conserved core of modified residues in ribosomes from the majority of studied organisms, structural analysis points to the functional roles of some of the rRNA methylations, which are discussed in this review in an evolutionary context.

KEYWORDS

Ribosomal RNA, rRNA, methylation, modification, ribosome, 70S, 80S, 55S.

MAIN TEXT

The ribosome is the central component of the protein synthesis apparatus. It is one of the most conserved and the most sophisticated molecular machines of the cell. The ribosome is composed of two unequal subunits, small (SSU, 30S in bacteria) and large (LSU, 50S in bacteria), which join together to form complete ribosome particle (70S in bacteria; **Figure 1**). While each ribosomal subunit contains a number of ribosomal proteins, it is the ribosomal RNA (rRNA) that plays the most critical functional role defining ribosome as a ribozyme. The small subunit decodes genetic information delivered by messenger RNA (mRNA), whereas the large subunit covalently links amino acids into a nascent protein, which is then threaded through the tunnel that spans the body of the large subunit (**Figure 1**). The ribosome provides a platform for binding the mRNA and transfer RNAs (tRNAs), which have two functional ends, one carrying the amino acid (CCA-end) and the other end containing the anticodon that recognizes the mRNA codon. tRNAs bind to the ribosome in three places: aminoacyl (A), peptidyl (P) and exit (E) sites. The A site binds the incoming aminoacyl-tRNA (aa-tRNA), the P site binds the peptidyl-tRNA carrying the nascent polypeptide chain, and the E site binds deacyl-tRNA before it dissociates from the ribosome. The tRNA molecules are indispensable adaptors that bridge the two main functional centers of the ribosome: the decoding center (DC) in the small subunit and the catalytic peptidyl transferase center (PTC) at the heart of the large subunit (**Figure 1**). The DC monitors the accuracy of base pairing between the codon of the mRNA and the anticodon of the aa-tRNA, ensuring that the correct tRNAs are selected. PTC catalyzes the chemical reaction of peptide bond formation between the amino acid moiety of the aa-tRNA and the nascent polypeptide chain attached to the P-site tRNA.

Post-transcriptionally modified nucleotides are commonly found in most types of RNA molecules[1] in all organisms from the simplest prokaryote *Nanoarchaeum equitans*[2] to human[3]. Currently, there are 144 known types of RNA-modifications in nature[1]. They are most diverse in tRNA molecules, while rRNAs contain only a few dozen of different types (predominantly methylated nucleotides)[1]. Studies of modifications of RNAs spurred much interest after the discovery of the functional role of methylation of mRNA in eukaryotes. A sophisticated enzymatic machinery capable of “writing”, “reading”, and “erasing” methylation marks on mRNAs exists in all eukaryotes. This system was uncovered in several pioneering studies that were reviewed recently[4, 5].The field of rRNA modifications reached several long-awaited

milestones. In 2012, the last unknown rRNA methyltransferase of *Escherichia coli* was discovered[6], while in 2014 the list of all rRNA-methyltransferases from yeast was completed[7]. At the same time, the rRNA-methylation machinery of bacterial species, other than *E. coli*, has also been characterized[8-11]. Several key advancements in the studies of mammalian rRNA modification machinery[12-17] make us believe that a complete inventory of human and mouse rRNA methyltransferases will become available in the near future. An in-depth comparison of the rRNA-modification enzymes from various species illustrates numerous cases of conservation, as well as examples of convergent and divergent evolution discussed below.

Recent advances in ribosome crystallography techniques allowed for the first time to directly visualize all methylated rRNA residues within the 70S ribosomes from *Thermus thermophilus*[18] and *E. coli*[19]. Crystallographic and cryo-EM studies of cytoplasmic[20-22] and mitochondrial[23, 24] ribosomes from a number of eukaryotic species also made stunning progress and provided the first near-atomic-resolution structures, allowing to model the modified nucleotides in their molecular environments. These data enable comparative structure-based analysis of the rRNA methylation sites in ribosomes from various taxonomic groups, namely *E. coli*, yeast, and human that we present here. This review also focuses on the rRNA methylation systems to highlight the evolutionary and structural significance of some of the rRNA methylations.

Methylation of ribosomal RNAs in bacteria and eukaryotes

In all studied organisms, rRNA contains modified nucleotide residues, which are represented by methylations in most cases (**Figure 2; Table 1**). Methylations of rRNA may be achieved through the action of specific enzymes, methyltransferases, each of which is usually responsible for modification of only one, rarely two, specific nucleotides[25] at a particular stage during ribosome assembly[26]. Alternatively, archaeal and eukaryotic rRNA residues, which undergo 2'-O-methylation or uridine-to-pseudouridine isomerization, are recognized *via* complementary interactions between the snoRNA component of small nucleolar ribonucleoproteins (snoRNPs) and the rRNA region flanking the modification site[27, 28]. Modification of the target nucleotide, in this case, is catalyzed by a specific protein subunit of the snoRNP. A complete list of all known 2'-O-methylation and pseudouridinylation sites in yeast rRNAs[29] has been established along with the catalogs of snoRNPs from yeast[30] and human[31]. In contrast to snoRNP-guided enzymes, all

bacterial and remaining eukaryotic rRNA-methyltransferases are enzymes that directly recognize structural features of their target sites (**Table 1**)[25].

All bacteria and eukaryotes, including well-studied *E. coli*, *S. cerevisiae,* and *Homo sapiens*, possess an overlapping, yet distinct repertoire of methylated rRNA residues (**Figure 3; Table 1**). Complete mapping of methylated rRNA residues is also available for other bacterial species, such as *T. thermophilus*[11], and archaea, such as *Haloarcula marismortui*[32]. Methylated nucleotides comprise only a small fraction of all rRNA residues and cluster mainly near the ribosome functional centers[25, 33]. Numerous studies show that the loss of rRNA methylation results in the alteration of ribosomal active sites[34], causing slower rates and lower accuracy of translation[35] as well as impaired responses to metabolites and antibiotics[36-38]. The presence of distinct, but overlapping sets of methylated nucleotides in various species (**Table 1**) points to the existence of a common ancestor, which had only a small subset of rRNA methyltransferases. The remaining enzymes, most likely, evolved independently in each taxonomic group.

In bacteria, most methylated rRNA nucleotides are exposed on the surface of the ligand-free small ribosomal subunit and should be readily accessible to the enzymes that perform modifications (**Figure 3A**). This observation is supported by the data showing that the majority of methylated nucleotides in bacterial 16S rRNA are modified either during the late steps of subunit assembly or introduced into the assembled subunits[26]. However, there is also a 5'-to-3' sequential order of methylation events in the 16S rRNA suggesting that they occur during the late steps of assembly of the individual domains (body, head, platform, etc.) of the small ribosomal subunit, which assemble simultaneously and independently from each other, rather than during the final assembly of the entire small ribosomal subunit[39]. In contrast to the small subunit, nucleotide modifications in the large subunit of the bacterial ribosome take place predominantly during the early stages of the subunit assembly[26]. This finding agrees with the higher proportion of buried modified nucleotides in the 23S rRNA that are inaccessible to solvent and, thus, to any of the modifying enzymes (**Figure 3B**)[18, 19]. While the buried methylated nucleotides likely stabilize the structure of the large ribosomal subunit, the few surface-exposed modified residues directly interact with the key ligands (*e.g.*, tRNAs and translation factors).

In eukaryotes, post-transcriptional modification of rRNA is intricately linked to the extremely complex rRNA processing and ribosome assembly[33]. Moreover, various stages of ribosome assembly in eukaryotes take place in different cellular compartments[33]. SnoRNA-guided 2'-O-methylation takes place in the nucleolus during the early stages of ribosome subunit assembly[40, 41], while the target sites in pre-rRNAs are accessible for base-pairing with snoRNA[33]. At later stages of ribosome assembly, the assistance of RNA helicase is required for snoRNA-guided modification of pre-rRNAs[42]. In contrast, rRNA methylation by snoRNA-independent enzymes, at least in some cases, occurs at the late stages of ribosome assembly in the cytoplasm[15, 43, 44]. For example, formation of the unique eukaryote-specific hyper-modified residue $m^1acp^3\Psi$ present only in the rRNA of the small ribosomal subunit (**Table 1**) begins with snoRNA-guided pseudouridinylation[45, 46] and N1-methylation in nucleolus[13, 14], followed by additional derivatization in the cytoplasm, where the 3-amino-3-carboxypropyl group is added by a Trs3 enzyme (**Figures 2B and 3C**)[15].

rRNA methylation and human disease

Methylation of rRNA has two very important implications for human health. Firstly, one of the most abundant and clinically relevant mechanisms of resistance to multiple ribosome-targeting antibiotics among pathogenic bacteria is based on the methylation of specific nucleotides in their rRNAs. For example, mono- or dimethylation of the N6 position in the A2058 residue of the 23S rRNA by Erm-type methyltransferases confers resistance to macrolides, lincosamides, and group B streptogramins (reviewed in [47]). Methylation of the C8 position of the universally conserved nucleotide A2503 in the 23S rRNA by Cfr methyltransferase confers resistance to a wide range of PTC-targeting antibiotics, including phenicols, lincosamides, oxazolidinones, pleuromutilins, group A streptogramins (reviewed in [47]). Methylation of either G1405 or A1408 nucleotides mediated by specific 16S rRNA methyltransferases confers resistance to many aminoglycosides (reviewed in [47]). Secondly, several genetic disorders in human are caused by mutations in rRNA methylation machinery. For instance, methyltransferases WBSCR22 and WBSCR20/NSUN5, which are responsible for the modification of m^7G1639 in the 18S rRNA and m^5C3782 in the 28S rRNA (**Table 1**), respectively, are hemizygously deleted together with several other genes in Williams-Beuren syndrome (reviewed in [33]). WBSCR20/NSUN5 methyltransferase is also

implicated in the lifespan control[12]. A point mutation in the EMG1 methyltransferase, which is responsible for the methylation of the N1 position of residue $m^1acp^3\Psi$1248 in the 18S rRNA (**Table 1**), leads to the loss of its nucleolar localization and development of Bowen-Conradi syndrome[13, 14, 48]. NML methyltransferase modifies position N1 of the A1322 nucleotide in the 28S rRNA and also regulates rRNA transcription and lipid metabolism in liver[49]. A particular polymorphous variant of mitochondrial methyltransferase TFB1M is associated with increased risk of type 2 diabetes[50]. Moreover, a direct link has been established recently between rRNA methylation and cancer development: NOP2 methyltransferase, which is responsible for C5 methylation of the C4447 nucleotide in the 28S rRNA (**Table 1**), is also a well-known tumor marker[51]. The fact that alterations in human rRNA modification machinery are associated with a number of pathological conditions is not surprising due to the central role of the ribosomes in gene expression.

Universally conserved rRNA methylation sites in the SSU

Comparing methylated rRNA nucleotides in model organisms allows for the identification of the universally conserved rRNA modifications. In the rRNA of the small ribosomal subunit, there are two universally conserved and modified N6-dimethylated adenine residues, m_2^6A1518 and m_2^6A1519 (*E. coli* 16S rRNA numbering) (**Figure 3A,C,E,G; Table 1**)[52]. These residues are located in helix 45, a “contact hub” of the ribosome, which is extremely close to the large ribosomal subunit, the P-site tRNA and mRNA (**Figure 4A**)[18]. Both modifications are introduced into 16S rRNA by the KsgA methyltransferase and are dispensable in bacteria. However, the absence of these modifications leads to rearrangement of the decoding center of the ribosome[34] and decreases the fidelity of translation initiation and elongation[37]. Inactivation of the *ksgA* gene, which eliminates dimethylation of the adenine residues A1518/A1519, also renders ribosomes resistant to antibiotic kasugamycin[37]. This effect is triggered by a small conformational change observed in this region when it lacks A1518/A1519-dimethylation[34]. Even mollicutes, organisms that underwent significant genome reduction, still retain a gene encoding for the helix 45 methyltransferase. Only extreme genome minimization, such as in *N. equitans*, results in a total loss of KsgA-equivalent, where it could be functionally replaced by 2’-O methyltransferase[2].

While bacteria possess only a single KsgA methyltransferase, eukaryotes have several homologous enzymes to modify rRNAs of both cytoplasmic and mitochondrial ribosomes. Dim1, a yeast homolog of KsgA, is responsible for the methylation of cytoplasmic ribosomes and, unlike the bacterial homolog, is essential for viability[53]. Mammalian mitochondria possess two paralogs of KsgA methyltransferase, TFB1M and TFB2M. While TFB1M is mainly responsible for methylation of the mitochondrial 12S rRNA[54], TFB2M primarily functions as a transcription factor, although retaining rRNA-methyltransferase activity[55].

In addition to the structural role of nucleotides m_2^6A1518 and m_2^6A1519 in the formation of the P site of the small ribosomal subunit, the KsgA methyltransferase plays an important role in the assembly of the 30S subunit. KsgA binds to a late assembly intermediate of the 30S subunit and performs "quality control check" by sequestering the intermediate until methylation occurs and the methyltransferase is released[56]. The functional importance of Dim1 methyltransferase (the yeast homolog of KsgA) acting on helix 45 during ribosome assembly is beyond the methylation itself because lethality caused by Dim1 inactivation can be suppressed by the expression of a catalytically incompetent Dim1 variant[57]. Yeast Dim1 and its mammalian homolog DIMT1L were shown to bind 90S small ribosomal subunit processome in the nucleolus[16, 57]. However, a manifestation of their methyltransferase activities is delayed until late steps of the 40S subunit assembly in the nucleus of a human cell or in the cytoplasm of a yeast cell[33]. Thus, similar to its bacterial homolog KsgA, yeast Dim1 protein was ascribed quality control function during the assembly of small ribosomal subunit[33]. Combination of the dispensable rRNA methylation function and the essential ribosome assembly function is not unique for KsgA/Dim1 proteins. Yeast methyltransferase Bud23, which is responsible for N7-methylation of the G1575 of the 18S rRNA (**Table 1**), is also required for the export of the small ribosomal subunit from the nucleus[58]. Separation of these two functions is also observed in human homolog of Bud23, WBSCR22/MERM1 (**Table 1**)[16, 59]. Another example of rRNA methyltransferase that also acts as a ribosome assembly factor in yeast is Nep1/Emg1, which adds a methyl group to the N1 position of the Ψ1191 in the 18S rRNA[14].

Universally conserved rRNA methylation sites in the LSU

The large ribosomal subunit carries two universally conserved and modified nucleotides, Gm2251 and Um2552 (*E. coli* 23S-rRNA numbering), that are located in the P-loop (Helix 80) and A-loop (Helix 92) of the 23S rRNA, respectively (**Figure 3B,D,F,H; Table 1**). Watson-Crick base-pairing between nucleotides of the A- and P-loops and the CCA-ends of the A- and P-site bound tRNAs play functionally significant roles in accommodation of aa-tRNA (**Figure 4B**) and in proper positioning of the peptidyl-tRNA (**Figure 4C**). It is likely that methylations of G2251 and U2552 are required to maintain the active conformation of the nucleotides involved in base-pairing with P- and A-site tRNAs, respectively. While lack of Gm2251 modification in *E. coli* has no phenotype[60], a deficiency in the Um2552 modification, although not lethal, leads to one of the most severe growth defects among all knockouts of rRNA methyltransferase genes[61]. Lack of the Um2552 methylation leads to significant accumulation of assembly intermediates of the large ribosomal subunit, which could be suppressed by overexpression of the specific ribosome assembly factors[62]. This observation points to the direct involvement of U2552 methylation in the assembly of the large ribosomal subunit. Interestingly, whereas nucleotide G2553 (also located in the A-loop) in bacterial 23S rRNA is unmodified, the equivalent position in both cytoplasmic and mitochondrial ribosomes in eukaryotes is 2'-O-methylated (**Table 1**).

In bacteria, 2'-O-methylation of Gm2251 and Um2552 is catalyzed by RlmB[60] and RlmE[61] enzymes, respectively. The equivalent positions in eukaryotic cytoplasmic rRNAs are methylated *via* a snoRNP-guided mechanism (**Table 1**), representing an elegant example of convergent evolution when the same biomodification is achieved through the action of conceptually different and evolutionarily unrelated enzymes. Surprisingly, 2'-O-methylation of nucleotide Gm2922 of the 25S rRNA in yeast (equivalent to G2553 in bacteria that is not modified) occurs through the action of enzyme Spb1[43], and not *via* the snoRNP-guided pathway typical for all 2'-O-methylations in eukaryotes. Mitochondria, being evolutionarily related to bacteria, use snoRNP-independent 2'-O-methyltransferases to modify nucleotides Gm1145 and Um1369 (human mitochondrial 16S rRNA numbering) that are equivalent to Gm2251 and Um2552 in bacteria, respectively (**Table 1**). As expected, RlmE, Spb1 and Mrm2 methyltransferases, which are responsible for the 2'-O-methylation of bacterial Um2552, yeast cytoplasmic Gm2922 and yeast mitochondrial Um2791 nucleotides in the rRNA of their large ribosomal subunits, respectively, display significant sequence similarity pointing to their common evolutionary origin[63].

Multiple modifications of single nucleotides

Several positions in rRNAs are differentially modified depending on the taxonomic group of the organism (**Table 1**). For example, helix 31 of the 16S rRNA in bacteria carries a tandem of methylated nucleotides, m^2G966 and m^5C967 (**Figure 5A; Table 1**)[64, 65]. These nucleotides are involved in the recruitment of the initiator tRNA and directly interact with the anticodon stem-loop of the P-site bound tRNA (**Figure 5A**)[18], whose nucleotide composition and repertoire of modifications are especially diverse among various organisms[1]. The methyl group of m^2G966 residue is adjacent to the mRNA-tRNA duplex and contacts the wobble base-pair in the P site (**Figure 5A**)[18]. These contacts rationalize why the loss of m^2G966 methylation adversely affects translation initiation, a stage when interactions between the P-site tRNA and the small ribosomal subunit are particularly important[66, 67].

Various other modifications in helix 31 are found even among different bacteria. For example, 16S rRNA of the thermophilic bacterium *T. thermophilus* (*Tth*) contains N2-dimethylated m_2^2G966 (*E. coli* numbering) instead of the monomethylated residue as in mesophilic *E. coli*[11]. The presence of the second methyl group on G966 correlates with the methylation of C1400 that is also observed in 16S rRNA of *Tth*, but not in *E. coli*. Both of these *Tth*-specific methyl groups form a direct hydrophobic contact with each other and with the mRNA-tRNA duplex, which likely reflects adaptation of *Tth* ribosomes to high temperature *via* the extended stacking between m^5C1400 base and the mRNA-tRNA wobble-pair in the P site (**Figure 5A**)[18]. The eukaryotic equivalent of bacterial m^2G966 is also modified, however, in a much more sophisticated way (**Table 1**). The same position in yeast and human 18S rRNA is occupied by uridine, which is modified in a three-step reaction to yield hypermodified $m^1acp^3\psi$1191 in yeast and $m^1acp^3\psi$1248 in human (**Figures 2B, 3C, and 3E; Table 1**)[13-15].

Another modified nucleotide, m^4Cm1402 of the bacterial 16S rRNA, is also located within the P site of the small ribosomal subunit and directly contacts the mRNA codon (**Figures 2D, 5A, and 5B**). In bacteria, this nucleotide carries two modifications simultaneously: one methyl group is attached to the amino group of the nucleobase and the other to the 2'-hydroxyl of the ribose. Curiously, in eukaryotes, both methylations are also present, however, they are segregated between the cytoplasmic 80S ribosomes, carrying only the 2'-O-methylation, and the mitochondrial 70S-like ribosomes, carrying only the N4-methylation of the nucleobase, at

positions equivalent to the bacterial C1402 (**Table 1**). Base methylation of the C1402 in bacteria is catalyzed by the RsmH methyltransferase and appears to be important for preventing erroneous initiation of translation at AUU codons[68]. The 2'-O-methylation of the same nucleotide in bacteria is performed by the RsmI methyltransferase and contributes to the maintenance of the reading frame and correct recognition of stop codons[68]. The roles of the equivalent modifications in eukaryotes are unknown.

Another region of the ribosome enriched in modified nucleotides is the peptidyl transferase center located at the heart of the large ribosomal subunit (**Figures 1 and 3**). An exclusive modification of the bacterial 23S rRNA is m^2A2503 (**Figure 5C; Table 1**), which is catalyzed by RlmN – a radical S-adenosyl-L-methionine-dependent (SAM) methyltransferase[69]. Interestingly, none of the nucleotides in eukaryotic rRNAs is methylated by the same mechanism. The C2 methyl group of m^2A2503 extends the stacking between nucleotides A2059 and A2503, which supports the fold of the two single-stranded rRNA segments of the peptidyl transferase loop that forms the wall of the peptide exit tunnel (**Figure 5C**)[18, 19]. Another methyltransferase, Cfr, also targets nucleotide A2503 of the 23S rRNA and is present only in a number of clinical isolates of pathogenic bacteria[70]. This enzyme methylates nucleotide A2503 at the position C8 of the nucleobase, resulting in the formation of dimethylated m^2m^8A2503 residue that confers resistance to many A-site binding antibiotics[70, 71].

Nucleotide ho^{5}C2501 in the bacterial 23S rRNA is adjacent to m^2A2503 in the wall of the ribosomal tunnel and is sub-stoichiometrically hydroxylated by a recently identified enzyme RlhA (**Figure 5C**)[72]. The equivalent nucleotide in cytoplasmic ribosomes of eukaryotes, m^5C4447 (human 28S rRNA numbering), is methylated (**Table 1**)[73]. The enzymology and chemical properties of these modifications are different. However, attachment of an additional group to a flat aromatic heterocycle of a nucleotide can extend its stacking potential for added stability, regardless of the chemical nature of the attached group (**Figure 5C**).

Domain IV of the rRNA in the large ribosomal subunit forms a central system of functionally important bridges between the large and the small subunits and is highly enriched in modified nucleotides[18, 25]. For example, one of the most important intersubunit bridges – bridge B2a – is formed between the helix 69 of the 23S rRNA and the top of the helix 44 of the 16S rRNA. This bridge contains nucleotide U1915 that undergoes dual modification in bacteria:

pseudouridinylation with subsequent methylation to yield $m^3\psi$1915 residue (**Figure 5D**). Interestingly, lack of pseudouridinylation, but not methylation, at this position is reported to cause severe growth defects or even lethality in bacteria[74]. In eukaryotes, the equivalent residue is pseudouridinylated, but not methylated (**Table 1**). Therefore, it is unclear why methylation of U1915 is needed for bacteria.

Single modification of multiple nucleotides

Another example of convergent evolution of rRNA-modification systems is taxonomy-specific reciprocal methylation of either one of the two nucleotides that interact with each other in a tri-dimensional structure (**Table 1**). This peculiar methylation arrangement can be found repeatedly in the structure of the large ribosomal subunit (**Figure 6**). Two nucleotides, U571 and m^6A2030, of the 23S rRNA in bacteria form stacking interactions with each other (**Figure 6A**). The equivalent nucleotides in archaea and eukaryotes also stack, however, they are reciprocally modified as compared to bacteria with the U571-equivalent being modified (m^1A628 in archaea; m^1A1322 in human) and A2030-equivalent being unmodified (**Figure 6B; Table 1**)[25, 32]. A pair of stacked nucleotides, G1935 and m^5C1962, participates in one of the functionally significant intersubunit bridges in *E. coli* (**Figure 6C**). The equivalent two G/C nucleotides in eukaryotes are swapped to C/G and are reciprocally methylated, m^5C3782 and G3809 (**Figure 6D; Table 1**). In this as well as in the previous examples, differentially methylated nucleobases form stacking interactions with each other. Given that modification of either of the two nucleotides enhances overall stacking, it does not matter which of the two interacting residues is actually modified. Furthermore, any of these two modifications might be required to establish a tighter interaction with the neighboring residues, located adjacent to the modified edge of the nucleotide.

In another example, nucleotides m^3U2634 and A2643 in yeast 25S rRNA form a non-Watson-Crick base pair (**Figure 6E**), while equivalent residues in the human 80S ribosome, C4211 and m^6A4220, are reciprocally methylated (**Figure 6F; Table 1**). In both arrangements, the methyl group is positioned in approximately the same spatial location relative to its environment. It is likely that in both cases methylation is used to avoid otherwise favorable Watson-Crick base-pair geometry of the interacting nucleotides.

334 ***Evolution of the rRNA methylation machinery***

335 Evolution of the enzymes involved in methylation of cellular RNAs could have proceeded
336 through convergent and/or divergent events. For convergent evolution, the same conserved
337 nucleotide can be modified by completely unrelated enzymes in different taxonomic groups,
338 whereas, for divergent evolution, closely related homologous enzymes can modify different
339 targets. Both types of evolutionary relationships could be best illustrated with the systems for
340 uridine methylation to yield m^5U (ribothymidine) residues in RNAs of various organisms.
341 Enzymes that form ribothymidine can proceed *via* two biochemical pathways – either using
342 SAM or N^5,N^{10}-methylenetetrahydrofolate (THF) as a donor of the methyl group. Bacteria use
343 both pathways to introduce m^5U residues into their RNAs. For example, *Bacillus subtilis* (and
344 likely a common ancestor) utilizes a single SAM-dependent RlmCD methyltransferase to form
345 both m^5U747 and m^5U1939 residues in the 23S rRNA (**Figure 7A**)[9]. At the same time, the
346 formation of the m^5U54 in the TψC-loops of tRNAs in this bacterium is catalyzed by the folate-
347 dependent TrmFO methyltransferase (**Figure 7A**)[75]. Gene duplications that likely occurred in the
348 ancestor of *E. coli* and other proteobacteria resulted in the three related SAM-dependent
349 enzymes, RlmC, RlmD, and TrmA, two of which are used for the modification of m^5U747 and
350 m^5U1939 in the 23S rRNA (**Figure 7A**)[76], and one to form m^5U54 in the TψC-loops of the
351 tRNAs. In contrast, *Mycoplasma capricolum* lacks m^5U nucleotides in its tRNAs, while folate-
352 dependent methyltransferase RlmFO is responsible for the formation of m^5U1939 in the 23S
353 rRNA (**Figure 7A**)[10].

354 Yet one more example of divergent evolution and acquisition of a secondary target for
355 modification is the system for m^1A formation. Bacterial $TrmI_2$ and yeast Trm61-Trm6 dimers
356 introduce an m^1A58 modification into tRNA molecules (**Figure 7B**). In addition to Trmt61A and
357 Trmt6 enzymes, which modify A58 in cytoplasmic tRNAs, vertebrates possess mitochondrial
358 homodimeric paralog, Trmt61B, which is responsible for modification of A947 in the
359 mitochondrial 16S rRNA, as well as A58 in mitochondrial tRNAs (**Figure 7B; Table 1**)[77, 78].

360

CONCLUDING REMARKS

All organisms are endowed with rRNA methylation machinery. The methylated nucleotides in rRNA cluster predominantly around the functional centers of the ribosome, which, from an evolutionary perspective, suggests that modifications of essential contact points between the ribosome and its ligands benefit protein synthesis. Structural analysis reveals three major types of contacts formed by rRNA modifications: (i) with the ribosome ligands; (ii) within the interior of the rRNA; and (iii) between the ribosomal subunits. *Universally conserved* modifications within rRNAs, as well as corresponding modifying enzymes, likely represent descendants of the most ancient RNA-methylation machinery. Multiple examples of *divergent* as well as *convergent evolution* exist within the system of RNA methylation. On the one hand, duplication of the methyltransferase genes and their subsequent specialization yields similar enzymes that modify different targets. On the other hand, different taxonomic groups may independently evolve enzymes introducing the same or similar modifications into the equivalent rRNA nucleotides. Another recurring theme in the modification of rRNA from different taxonomic groups is methylation of either one or another residue from a pair of nucleotides juxtaposed in the tertiary structure of the ribosome. Once the identification of all rRNA methyltransferases and their modification sites will be completed for all model organisms, the main challenge in the field will remain to understand the functional roles of each specific biomodification in rRNAs.

TABLES

Table 1. Methylated rRNA residues in the small and large ribosomal subunits of model organisms *E. coli, S. cerevisiae,* and *H. sapiens* (including mitochondria). Modified nucleotides are highlighted in bold. Nomenclature of the modified nucleotides is the same as in **Figure 2**. For human mitochondrial rRNAs, positions of the nucleotides relative to the start of their transcripts are shown in parentheses (+647 for the 12S rRNA and +1670 for the 23S rRNA).

Methylated rRNA residues in the small ribosomal subunit.

***Escherichia coli* 16S rRNA**		***S. cerevisiae* 18S rRNA**		***H. sapiens* 18S rRNA**		***H. sapiens* mitochondrial 12S rRNA**	
Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme
m^7G527	RsmG[38]	G574	Unmodified	G623	Unmodified	G252 (899)	Unmodified
U788	Unmodified	**ψ999**	snR31[79]	**ψ1056**	ACA8[45]	**m^5U429 (1076)**	Unknown
m^2G966	RsmD[64]	**$m^1acp^3ψ$1191**	snR35[46]; Emg1 (Nep1)[13, 14]; Tsr3[15]	**$m^1acp^3ψ$1248**	ACA13[45]; hEMG1[48]; TSR3[15]	A573 (1220)	Unmodified
m^5C967	RsmB[65]	C1192	Unmodified	C1249	Unmodified	A574 (1221)	Unmodified
m^2G1207	RsmC[80]	G1438	Unmodified	G1500	Unmodified	U690 (1337)	Unmodified
G1338	Unmodified	**m^7G1575**	Bud23/ Trm112[58]	**m^7G1639**	WBSCR22/ TRMT112[16, 59]	G775 (1422)	Unmodified
m^4Cm1402	RsmH; RsmI[68]	**Cm1639**	snR70[81]	**Cm1703**	U43[27]	**m^4C839 (1486)**	Unknown
C1404	Unmodified	C1641	Unmodified	C1705	Unmodified	**m^5C841 (1488)**	NSUN4[82]
m^5C1407	RsmF[83]	C1644	Unmodified	C1708	Unmodified	C844 (1491)	Unmodified
m^3U1498	RsmE[84]	U1761	Unmodified	U1830	Unmodified	U916 (1563)	Unmodified
A1500	Unmodified	A1763	Unmodified	**m^6A1832**	Unknown[3]	A918 (1565)	Unmodified
m^2G1516	RsmJ[85]	U1779	Unmodified	U1848	Unmodified	G934 (1581)	Unmodified
m_2^6A1518 m_2^6A1519	KsgA (RsmA)[52]	**m_2^6A1781 m_2^6A1782**	Dim1[53]	**m_2^6A1850 m_2^6A1851**	DIMT1L[16]	**m_2^6A936 (1583) m_2^6A937 (1584)**	TFB1M[54]

Methylated rRNA residues in the large ribosomal subunit.

***Escherichia coli* 23S rRNA**	***S. cerevisiae* 25S rRNA**	***H. sapiens* 28S rRNA**	***H. sapiens* mitochondrial 16S rRNA**

Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme
U571	Unmodified	**m^{1}A645**	Rrp8 (Bmt1)[86]	**m^{1}A1322**	NML[17]	C182 (1852)	Unmodified
m^{1}G745	RlmAI (RrmA, YebH)[87]; RlmAII[8]	C877	Unmodified	C1594	Unmodified	G285 (1955)	Unmodified
m^{5}U747	RlmC (RumB)[76];	U879	Unmodified	U1596	Unmodified	A287 (1957)	Unmodified
m^{6}A1618	RlmF (YbiN)[88]	A1850	Unmodified	A2798	Unmodified	A764 (2434)	Unmodified
A1784	Unmodified	**m^{1}A2142**	Bmt2[89]	A3646	Unmodified	A834 (2504)	Unmodified
m^{2}G1835	RlmG (YgjO)[90]	G2194	Unmodified	G3698	Unmodified	G876 (2546)	Unmodified
m$^3\Psi$1915	RluD (YfiI)[74]; RlmH (YbeA)[91]	**ψ2258**	snR191[92]	**ψ3762**	U19[93]	C908 (2578)	Unmodified
G1935	Unmodified	**m^{5}C2278**	Rcm1 (Bmt3)[94]	**m^{5}C3782**	NSUN5 (WBSCR20)[12]	A928 (2598)	Unmodified
m^{5}U1939	RlmD (YgcA, RumA)[76]	U2282	Unmodified	U3786	Unmodified	U932 (2602)	Unmodified
G1954	Unmodified	U2297	Unmodified	U3801	Unmodified	**m^{1}A947 (2617)**	TRMT61B[78]
m^{5}C1962	RlmI (YccW)[95]	G2305	Unmodified	G3809	Unmodified	C955 (2625)	Unmodified
m^{6}A2030	RlmJ[6]	A2372	Unmodified	A3876	Unmodified	A1023 (2693)	Unmodified
m^{7}G2069	RlmKL (YcbY)[96]	U2411	Unmodified	U3915	Unmodified	G1062 (2732)	Unmodified
Gm2251	RlmB[60]	**Gm2619**	snR67[81]	**Gm4196**	U31[27]	**Gm1145 (2815)**	MRM1[97]
U2265	Unmodified	**m^{3}U2634**	Bmt5[98]	C4211	Unmodified	C1159 (2829)	Unmodified
A2274	Unmodified	A2643	Unmodified	**m^{6}A4220**	Unknown[3]	A1168 (2838)	Unmodified
m^{2}G2445	RlmKL (YcbY)[96, 99]	G2814	Unmodified	G4391	Unmodified	G1262 (2932)	Unmodified
U2474	Unmodified	**m^{3}U2843**	Bmt6[98]	U4420	Unmodified	C1291 (2961)	Unmodified
Cm2498	RlmM (YgdE)[100]	C2867	Unmodified	C4444	Unmodified	C1315 (2985)	Unmodified
ho^{5}C2501	RlhA[72]	**m^{5}C2870**	Nop2 (Bmt4)[94]	**m^{5}C4447**	NSUN1 (NOP2, NOL1)[51, 73]	C1318 (2988)	Unmodified
m^{2}A2503	RlmN (YfgB)[69]	A2872	Unmodified	A4449	Unmodified	A1320 (2990)	Unmodified
Um2552	RlmE[61]	**Um2921**	snR52[81]	**Um4498**	Unknown	**Um1369 (3039)**	MRM2[97]
G2553	Unmodified	**Gm2922**	Spb1[43]	**Gm4499**	Unknown	**Gm1370 (3040)**	MRM3[97]
U2584	Unmodified	U2953	Unmodified	**m^{3}U4530**	Unknown[3]	U1401 (3071)	Unmodified

388

FIGURE LEGENDS

Figure 1. Functional elements of the bacterial ribosome. Overview of the 70S ribosome from *T. thermophilus*. The view is from the cytoplasm onto the A site. 30S subunit is shown in light yellow, 50S subunit is in light blue. mRNA is shown in magenta and tRNAs are displayed in green for the A site, in dark blue for the P site, and in orange for the E site. Growing polypeptide chain in the nascent peptide exit tunnel is schematically depicted with white circles. Two main functional centers of the ribosome are labeled.

Figure 2. Chemical structures of modified and unmodified rRNA nucleosides. Unmodified adenosine (**A**), uridine and pseudouridine (**B**), guanosine (**C**) and cytidine (**D**) nucleosides are shown in black on the left of each panel. Their naturally occurring derivatives found in rRNAs of various species are shown to the right. Ribose moieties are omitted. Chemical groups that are enzymatically added to the nucleosides are highlighted in red.

Figure 3. Modification sites in ribosomal RNAs. Spatial distribution of modified nucleotides in the structures of small (left) and large (right) ribosomal subunits from different taxonomic groups: (**A**,**B**) *Escherichia coli* (PDB entry 4YBB[19]), (**C**,**D**) *Saccharomyces cerevisiae* (PDB entry 4V88[20]), (**E**,**F**) *Homo sapiens* (PDB entry 4UGO[21]), and (**G**,**H**) human mitochondria (PDB entry 3J9M[23]). Nucleotide numbering is specific to each organism and corresponds to **Table 1**. Both small and large subunits are viewed from their interface sides as indicated by the insets. Methylation sites are highlighted in yellow. Universally conserved methylation sites that are the same among all taxonomic groups are shown in dark blue.

Figure 4. Universally conserved rRNA methylation sites in the small and large ribosomal subunits. (**A**) N6-dimethylated adenines, m_2^6A1518 and m_2^6A1519, in the helix 45 of the 16S rRNA (*E. coli* numbering), are located next to the codon-anticodon double helix formed by the mRNA (magenta) and the P-site tRNA (dark blue) molecules. (**B**,**C**) 2'-O-methylated nucleotides (blue), Um2552 (B) and Gm2251 (C), in the A-loop (Helix 92) and the P-loop (Helix 80) of the 23S rRNA (*E. coli* numbering), respectively. Note that nucleotides of the A- and P-

418 loops play key functional roles in accommodation of the aminoacyl-tRNA (green) and proper
419 positioning of the peptidyl-tRNA (dark blue) through Watson-Crick base-pairing. Modified
420 residues are highlighted in orange for the 16S rRNA and blue for the 23S rRNA, while
421 unmodified residues are shown in light yellow and light blue. Methylation sites are shown as red
422 spheres.

423

424 **Figure 5. Molecular contacts of modified nucleotides with the ribosome ligands and within**
425 **the ribosome structure.** (**A**) rRNA methylation sites around the P site in the small ribosomal
426 subunit. (**B**) rRNA modifications that maintain the structure of helix 44 in the mRNA channel
427 (mRNA is not shown for clarity). (**C**) Modified nucleotides, ho^{5}C2501 and m^{2}A2503, in the wall
428 of the peptide exit tunnel. Note that in both cases the added chemical groups extend the stacking
429 surface between the modified nucleotide and its neighbors. (**D**) Methylated m^{5}ψ1915 of the 23S
430 rRNA at the interface between the two ribosomal subunits. The color scheme is the same as in
431 Figure 4. Modifications are indicated by the red arrows.

432

433 **Figure 6. Reciprocally methylated nucleotide pairs found in various taxonomic groups.**
434 Pairs of interacting rRNA nucleotides that are methylated at either one or another heterocyclic
435 nucleobase depending on the taxonomic group. (**A**) Interaction of the *E. coli* m^{6}A2030 with
436 unmodified U571 of the 23S rRNA. (**B**) Nucleotides of *H. sapiens* cytoplasmic ribosome,
437 equivalent to those shown in (A). Note that the base in human ribosome that is equivalent to the
438 modified base in bacterial ribosome is unmodified and *vice versa*. (**C,D**) Example of reciprocally
439 modified bases in the rRNAs of the LSU of *E. coli* (C) and *H. sapiens* (D) similar to the one
440 shown in (A,B). (**E,F**) Pair of interacting nucleotides in the *S. cerevisiae* LSU (E) and the
441 equivalent nucleotides in the *H. sapiens* LSU (F). The species-specific differences in the
442 positions of the methyl groups are illustrated. The color scheme is the same as in Figure 4.
443 Methylation sites are shown as red spheres.

444

445 **Figure 7. Examples of convergent and divergent evolution of rRNA/tRNA methylation**
446 **systems.** (**A**) Evolution of m^{5}U methylation systems among various bacteria. SAM – S-adenosyl-

447 L-methionine, THF – N^5,N^{10}-methylenetetrahydrofolate. (**B**) Evolution of m^1A modification
448 systems in eukaryotes.

449

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest declared.

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TABLES

Table 1. Methylated rRNA residues in the small and large ribosomal subunits of model organisms *E. coli, S. cerevisiae,* and *H. sapiens* (including mitochondria). Modified nucleotides are highlighted in bold. Nomenclature of the modified nucleotides is the same as in **Figure 2**. For human mitochondrial rRNAs, positions of the nucleotides relative to the start of their transcripts are shown in parentheses (+647 for the 12S rRNA and +1670 for the 23S rRNA).

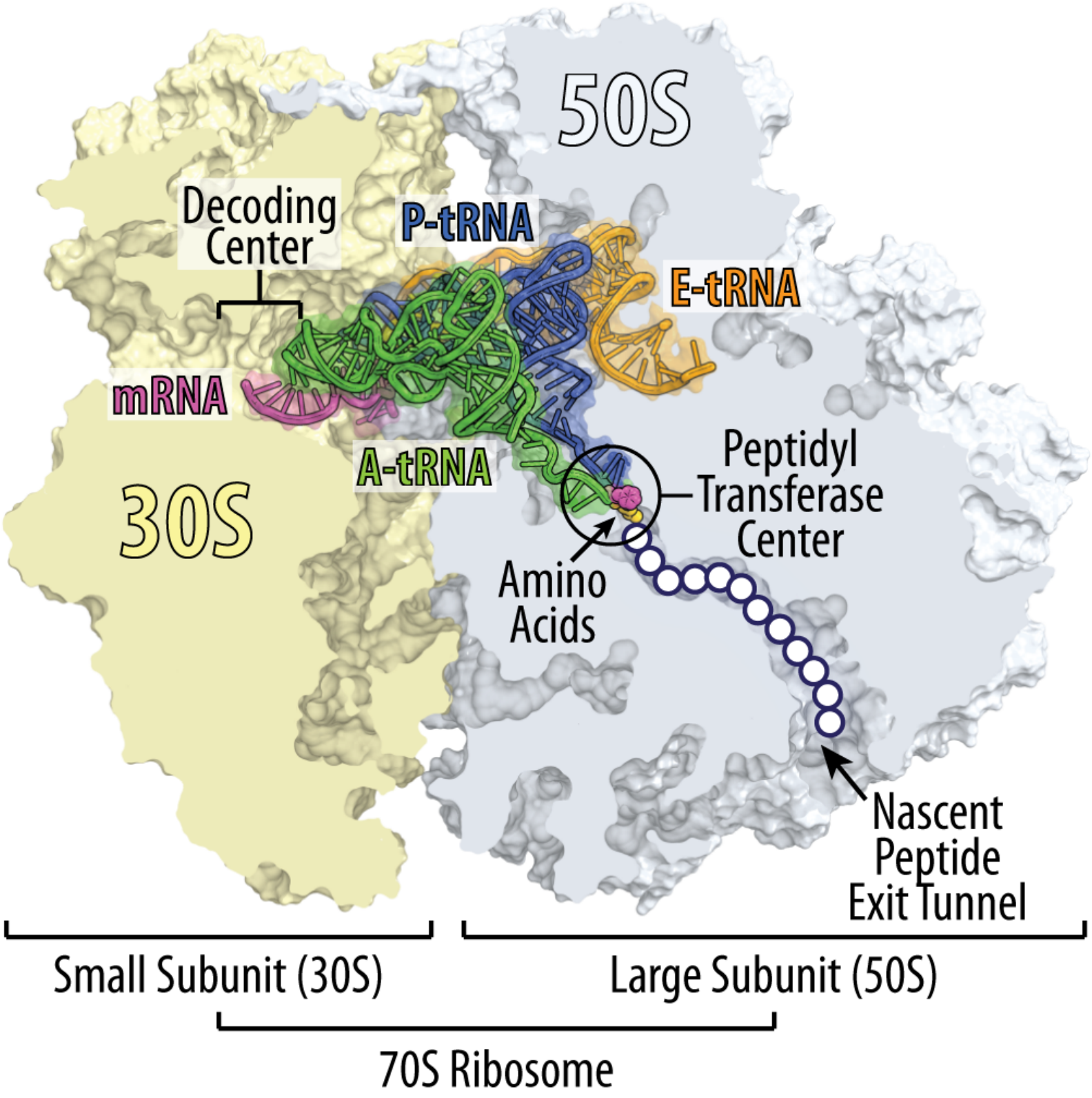
Methylated rRNA residues in the small ribosomal subunit.

***Escherichia coli* 16S rRNA**		***S. cerevisiae* 18S rRNA**		***H. sapiens* 18S rRNA**		***H. sapiens* mitochondrial 12S rRNA**	
Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme
m^7G527	RsmG[38]	G574	Unmodified	G623	Unmodified	G252 (899)	Unmodified
U788	Unmodified	**ψ999**	snR31[79]	**ψ1056**	ACA8[45]	**m^5U429 (1076)**	Unknown
m^2G966	RsmD[64]	**$m^1acp^3ψ$1191**	snR35[46]; Emg1 (Nep1)[13, 14]; Tsr3[15]	**$m^1acp^3ψ$1248**	ACA13[45]; hEMG1[48]; TSR3[15]	A573 (1220)	Unmodified
m^5C967	RsmB[65]	C1192	Unmodified	C1249	Unmodified	A574 (1221)	Unmodified
m^2G1207	RsmC[80]	G1438	Unmodified	G1500	Unmodified	U690 (1337)	Unmodified
G1338	Unmodified	**m^7G1575**	Bud23/ Trm112[58]	**m^7G1639**	WBSCR22/ TRMT112[16, 59]	G775 (1422)	Unmodified
m^4Cm1402	RsmH; RsmI[68]	**Cm1639**	snR70[81]	**Cm1703**	U43[27]	**m^4C839 (1486)**	Unknown
C1404	Unmodified	C1641	Unmodified	C1705	Unmodified	**m^5C841 (1488)**	NSUN4[82]
m^5C1407	RsmF[83]	C1644	Unmodified	C1708	Unmodified	C844 (1491)	Unmodified
m^3U1498	RsmE[84]	U1761	Unmodified	U1830	Unmodified	U916 (1563)	Unmodified
A1500	Unmodified	A1763	Unmodified	**m^6A1832**	Unknown[3]	A918 (1565)	Unmodified
m^2G1516	RsmJ[85]	U1779	Unmodified	U1848	Unmodified	G934 (1581)	Unmodified
m_2^6A1518 m_2^6A1519	KsgA (RsmA)[52]	**m_2^6A1781 m_2^6A1782**	Dim1[53]	**m_2^6A1850 m_2^6A1851**	DIMT1L[16]	**m_2^6A936 (1583) m_2^6A937 (1584)**	TFB1M[54]

Methylated rRNA residues in the large ribosomal subunit.

***Escherichia coli* 23S rRNA**	***S. cerevisiae* 25S rRNA**	***H. sapiens* 28S rRNA**	***H. sapiens* mitochondrial 16S rRNA**

Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme	Nucleotide	Enzyme
U571	Unmodified	**m^{1}A645**	Rrp8 (Bmt1)[86]	**m^{1}A1322**	NML[17]	C182 (1852)	Unmodified
m^{1}G745	RlmAI (RrmA, YebH)[87]; RlmAII[8]	C877	Unmodified	C1594	Unmodified	G285 (1955)	Unmodified
m^{5}U747	RlmC (RumB)[76];	U879	Unmodified	U1596	Unmodified	A287 (1957)	Unmodified
m^{6}A1618	RlmF (YbiN)[88]	A1850	Unmodified	A2798	Unmodified	A764 (2434)	Unmodified
A1784	Unmodified	**m^{1}A2142**	Bmt2[89]	A3646	Unmodified	A834 (2504)	Unmodified
m^{2}G1835	RlmG (YgjO)[90]	G2194	Unmodified	G3698	Unmodified	G876 (2546)	Unmodified
m$^3\Psi$1915	RluD (YfiI)[74]; RlmH (YbeA)[91]	**ψ2258**	snR191[92]	**ψ3762**	U19[93]	C908 (2578)	Unmodified
G1935	Unmodified	**m^{5}C2278**	Rcm1 (Bmt3)[94]	**m^{5}C3782**	NSUN5 (WBSCR20)[12]	A928 (2598)	Unmodified
m^{5}U1939	RlmD (YgcA, RumA)[76]	U2282	Unmodified	U3786	Unmodified	U932 (2602)	Unmodified
G1954	Unmodified	U2297	Unmodified	U3801	Unmodified	**m^{1}A947 (2617)**	TRMT61B[78]
m^{5}C1962	RlmI (YccW)[95]	G2305	Unmodified	G3809	Unmodified	C955 (2625)	Unmodified
m^{6}A2030	RlmJ[6]	A2372	Unmodified	A3876	Unmodified	A1023 (2693)	Unmodified
m^{7}G2069	RlmKL (YcbY)[96]	U2411	Unmodified	U3915	Unmodified	G1062 (2732)	Unmodified
Gm2251	RlmB[60]	**Gm2619**	snR67[81]	**Gm4196**	U31[27]	**Gm1145 (2815)**	MRM1[97]
U2265	Unmodified	**m^{3}U2634**	Bmt5[98]	C4211	Unmodified	C1159 (2829)	Unmodified
A2274	Unmodified	A2643	Unmodified	**m^{6}A4220**	Unknown[3]	A1168 (2838)	Unmodified
m^{2}G2445	RlmKL (YcbY)[96, 99]	G2814	Unmodified	G4391	Unmodified	G1262 (2932)	Unmodified
U2474	Unmodified	**m^{3}U2843**	Bmt6[98]	U4420	Unmodified	C1291 (2961)	Unmodified
Cm2498	RlmM (YgdE)[100]	C2867	Unmodified	C4444	Unmodified	C1315 (2985)	Unmodified
ho^{5}C2501	RlhA[72]	**m^{5}C2870**	Nop2 (Bmt4)[94]	**m^{5}C4447**	NSUN1 (NOP2, NOL1)[51, 73]	C1318 (2988)	Unmodified
m^{2}A2503	RlmN (YfgB)[69]	A2872	Unmodified	A4449	Unmodified	A1320 (2990)	Unmodified
Um2552	RlmE[61]	**Um2921**	snR52[81]	**Um4498**	Unknown	**Um1369 (3039)**	MRM2[97]
G2553	Unmodified	**Gm2922**	Spb1[43]	**Gm4499**	Unknown	**Gm1370 (3040)**	MRM3[97]
U2584	Unmodified	U2953	Unmodified	**m^{3}U4530**	Unknown[3]	U1401 (3071)	Unmodified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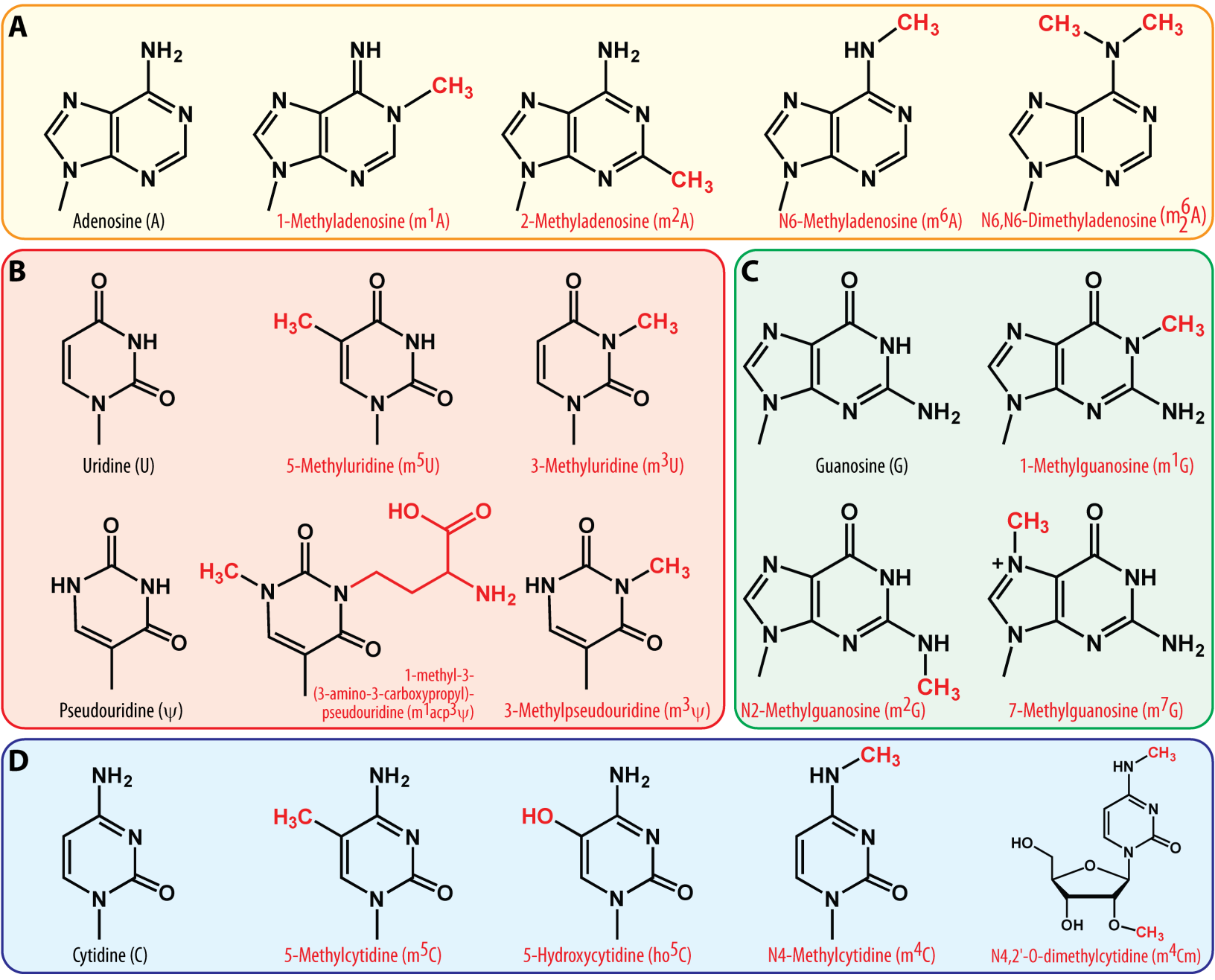
Decoding Center
P-tRNA
E-tRNA
mRNA
A-tRNA
30S
Peptidyl Transferase Center
Amino Acids
Nascent Peptide Exit Tunnel
Small Subunit (30S)
Large Subunit (50S)
70S Ribosome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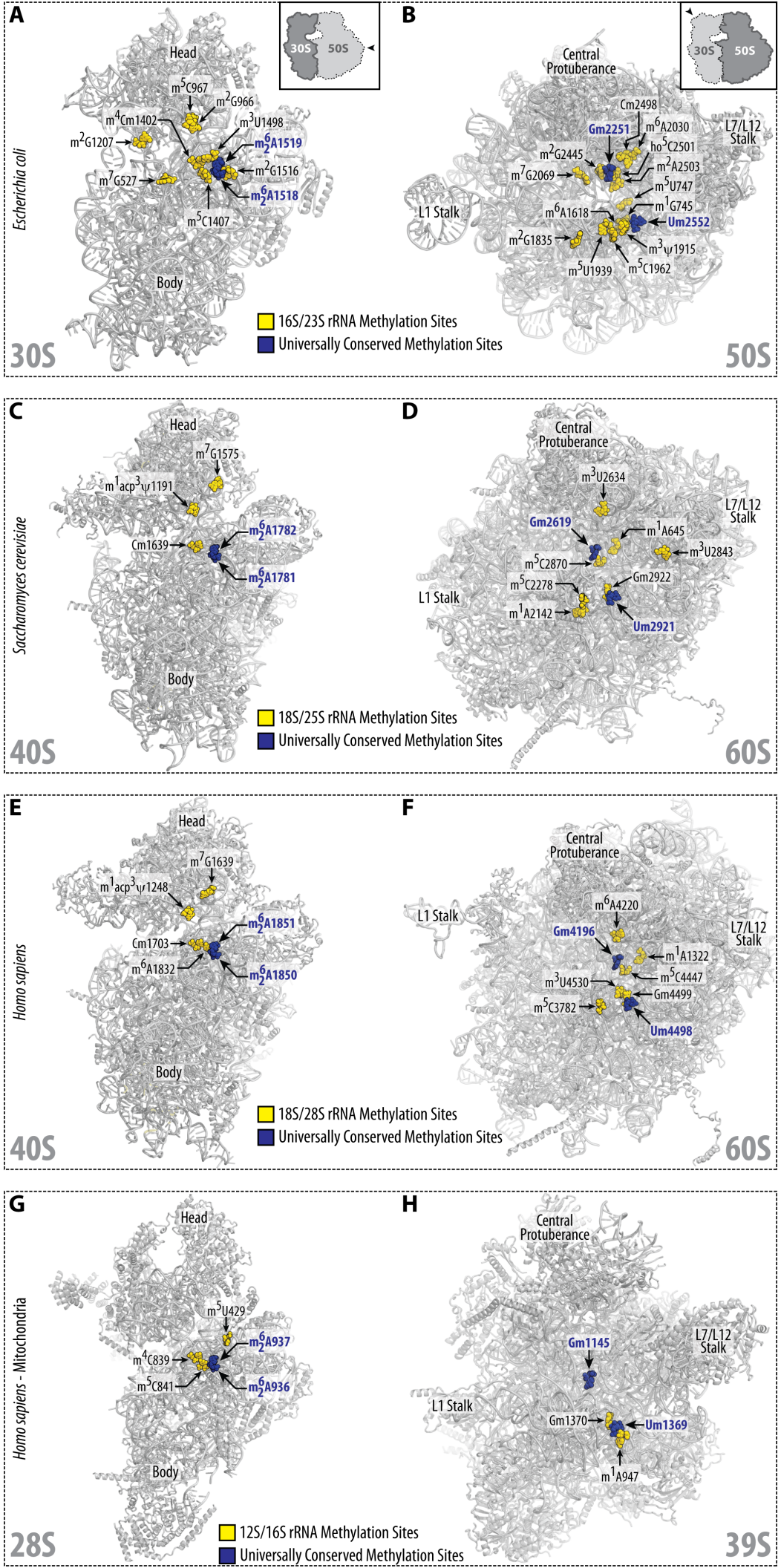
1-Methyladenosine (m1A)
2-Methyladenosine (m2A)
N6-Methyladenosine (m6A)
N6,N6-Dimethyladenosine (m62A)
B
Uridine (U)
5-Methyluridine (m5U)
3-Methyluridine (m3U)
Pseudouridine (ψ)
1-methyl-3-(3-amino-3-carboxypropyl)-pseudouridine (m1acp3ψ)
3-Methylpseudouridine (m3ψ)
C
Guanosine (G)
1-Methylguanosine (m1G)
N2-Methylguanosine (m2G)
7-Methylguanosine (m7G)
D
Cytidine (C)
5-Methylcytidine (m5C)
5-Hydroxycytidine (ho5C)
N4-Methylcytidine (m4C)
N4,2'-O-dimethylcytidine (m4Cm)


A
B
C
D
E
F
G
H
Escherichia coli
Saccharomyces cerevisiae
Homo sapiens
Homo sapiens - Mitochondria
30S
50S
40S
60S
28S
39S
Head
Body
Central Protuberance
L1 Stalk
L7/L12 Stalk
m5C967
m2G966
m4Cm1402
m3U1498
m2G1207
m62A1519
m2G1516
m7G527
m62A1518
m5C1407
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m5C1962
16S/23S rRNA Methylation Sites
Universally Conserved Methylation Sites
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m1acp3ψ1191
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Cm1639
m62A1781
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Gm2619
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m3U2843
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Gm2922
m5C2278
m1A2142
Um2921
18S/25S rRNA Methylation Sites
Universally Conserved Methylation Sites
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m1acp3ψ1248
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Cm1703
m6A1832
m62A1850
m6A4220
Gm4196
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m5C4447
Gm4499
m5C3782
Um4498
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Universally Conserved Methylation Sites
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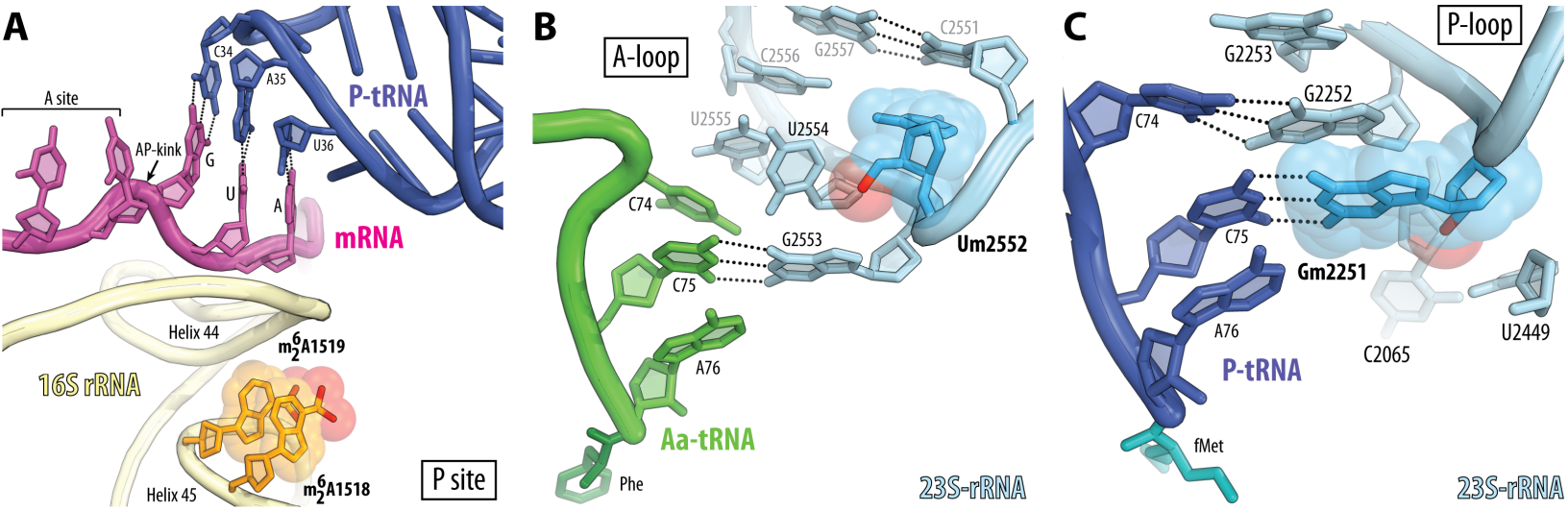
12S/16S rRNA Methylation Sites
Universally Conserved Methylation Sites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A35
P-tRNA
AP-kink
G
U
U36
A
mRNA
Helix 44
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16S rRNA
Helix 45
m26A1518
P site
B
A-loop
C2551
C2556
G2557
U2555
U2554
C74
G2553
Um2552
C75
A76
Aa-tRNA
Phe
23S-rRNA
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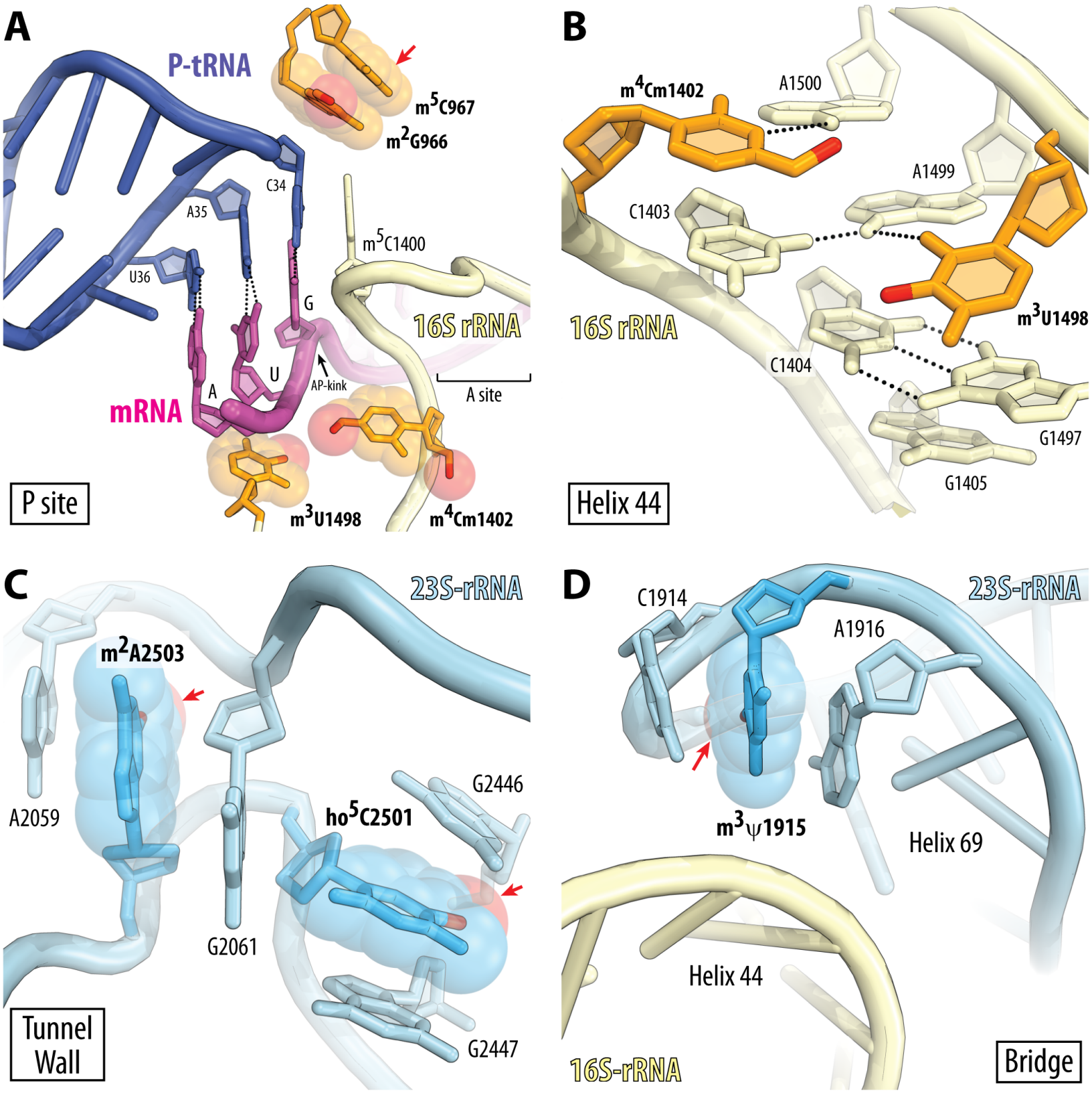
23S-rRNA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mRNA
P site
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B
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A1499
C1403
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G2446
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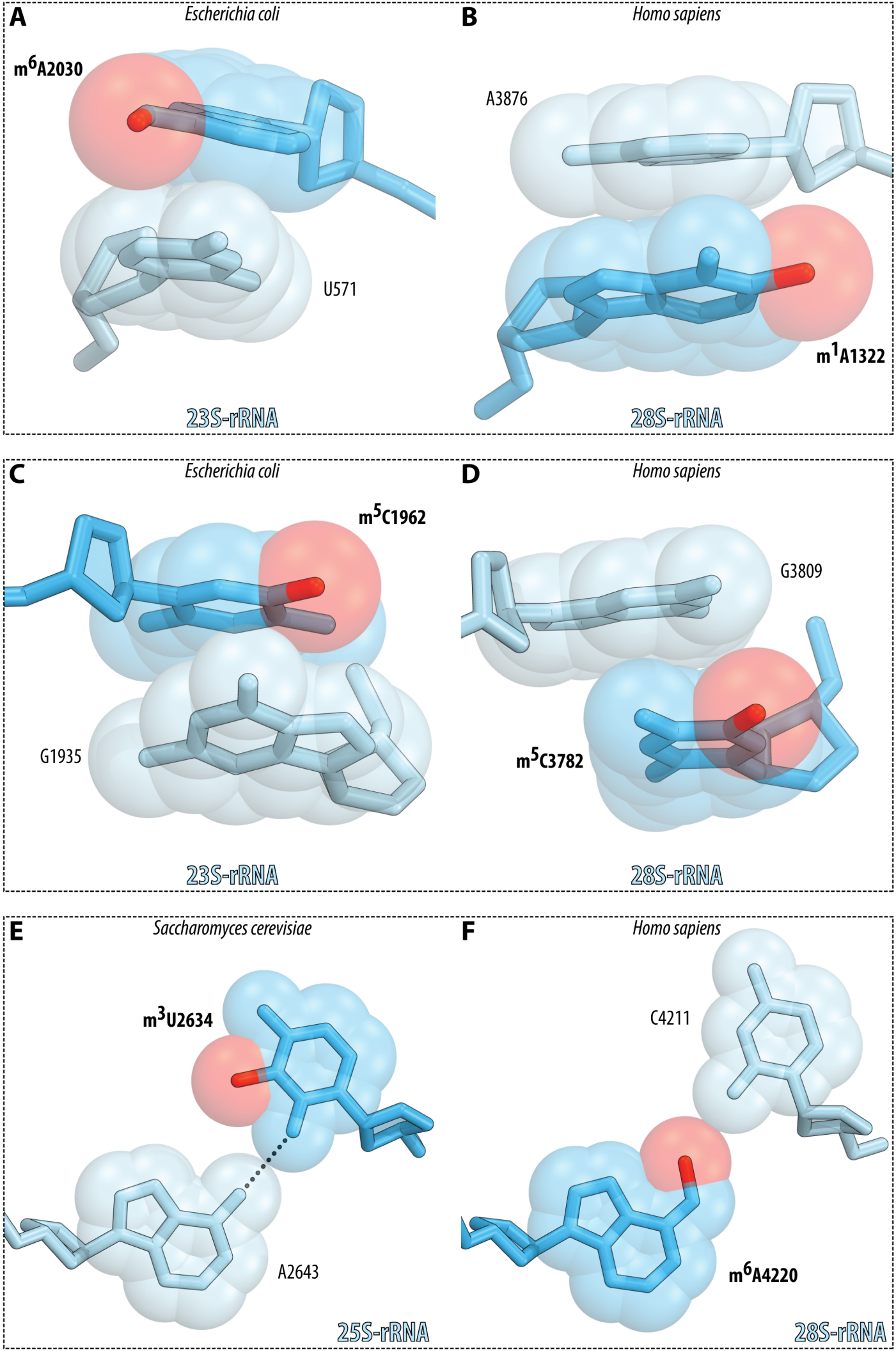
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U571
23S-rRNA
B
Homo sapiens
A3876
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C
Escherichia coli
m5C1962
G1935
23S-rRNA
D
Homo sapiens
G3809
m5C3782
28S-rRNA
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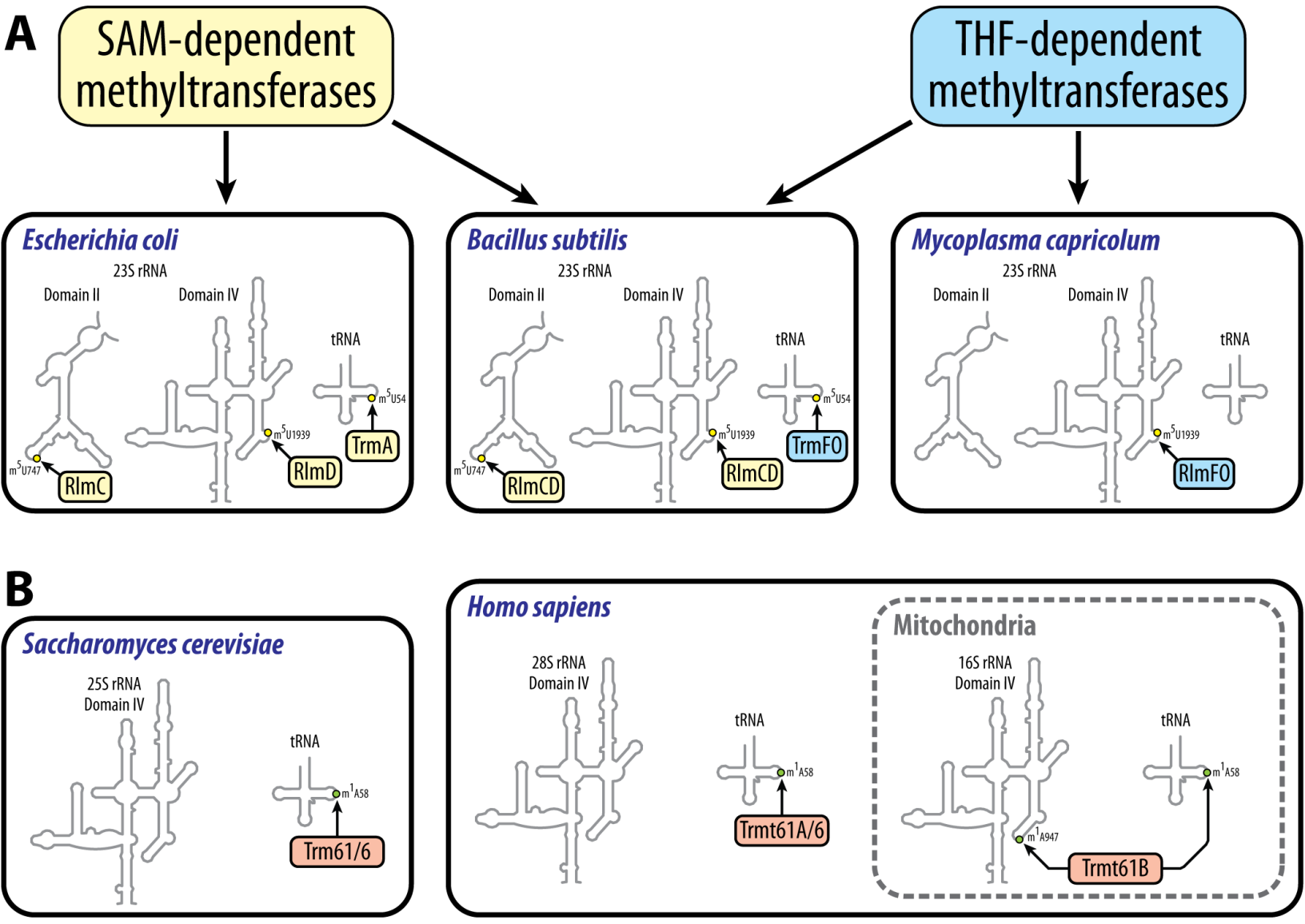
A
SAM-dependent methyltransferases
THF-dependent methyltransferases
Escherichia coli
23S rRNA
Domain II
Domain IV
tRNA
m5U54
m5U1939
m5U747
TrmA
RlmD
RlmC
Bacillus subtilis
23S rRNA
Domain II
Domain IV
tRNA
m5U54
m5U1939
m5U747
TrmFO
RlmCD
RlmCD
Mycoplasma capricolum
23S rRNA
Domain II
Domain IV
tRNA
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RlmFO
B
Saccharomyces cerevisiae
25S rRNA
Domain IV
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m1A58
Trm61/6
Homo sapiens
28S rRNA
Domain IV
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Mitochondria
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