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Charging the code — tRNA modification complexes

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All types of cellular RNAs are post-transcriptionally modified, constituting the so called 'epitranscriptome'. In particular, tRNAs and their anticodon stem loops represent major modification hotspots. The attachment of small chemical groups at the heart of the ribosomal decoding machinery can directly affect translational rates, reading frame maintenance, co-translational folding dynamics and overall proteome stability. The variety of tRNA modification patterns is driven by the activity of specialized tRNA modifiers and large modification complexes. Notably, the absence or dysfunction of these cellular machines is correlated with several human pathophysiologies. In this review, we aim to highlight the most recent scientific progress and summarize currently available structural information of the most prominent eukaryotic tRNA modifiers.

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Introduction

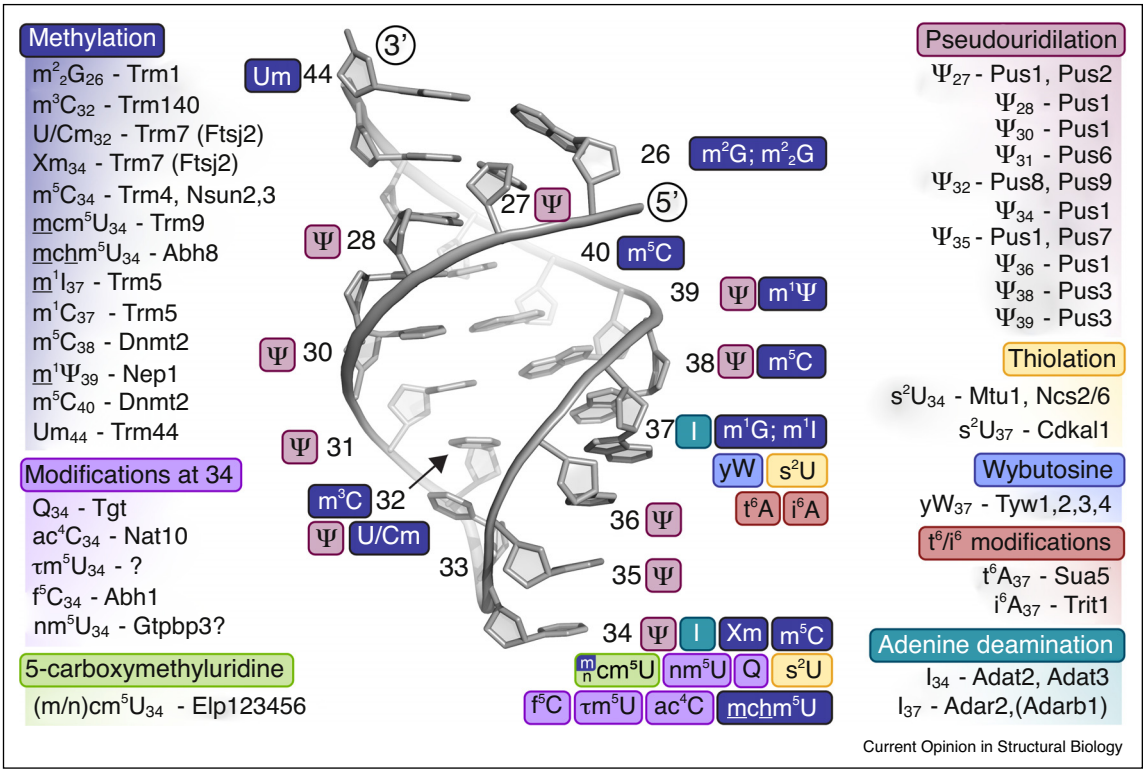
The 'Modomics' database for RNA modification [1] currently lists ~200 unique chemical modifications of RNA, with around half of them being detected in tRNAs of all known species [2–4]. Despite their strong sequence variation, all cellular tRNAs need to fold into almost identical three-dimensional structures to fit the relatively narrow tRNA binding sites of the ribosome during translation elongation. The possibility of incorporating chemical groups, which contribute additional biophysical properties to the individual RNA bases, vastly expands the range of suitable sequences that can fold into the characteristic L-shaped tRNA structure. In addition, the modification of RNA bases in and around the anticodon impacts on their intrinsic geometry and canonical Watson–Crick base pair interactions between codons and anticodons [5–7]. These alterations strongly influence the dynamics of tRNA selection at the ribosomal A-site [8] and subsequently affect the local elongation speed, co-translational folding dynamics [9], proteome stability and cell survival [10]. tRNA modifications were initially thought to be routinely and uniformly added to their respective tRNA molecules. To date, it is becoming increasingly clear that most of them are dynamically regulated in response to environmental cues [11,12] and an intense cross talk between various modifications and their pathways emerges [13]. Here, we aim to provide a comprehensive summary of the respective modification enzymes that produce this plethora of posttranscriptional modifications patterns. We summarize available structural and functional knowledge concerning the most abundant families of tRNA modification enzymes. Our focus lies on the main modification cascades and known macromolecular assemblies that target the ASL in eukaryotes (Figure 1). These partially highly complex molecular machines are not only important guardians of the proteome and regulatory factors of translational elongation, but are also clinically very important. The pathophysiological consequences and clinical implications of disease-causing mutations in tRNA modifiers are very well covered by recent expert reviews [14–17].

(t)RNA methyltransferases

Methylations affect multiple properties of tRNA molecules, including folding dynamics, thermostability, maturation as well as protection from cleavage or priming for the synthesis of subsequent modifications [18,19]. Eukaryotic tRNA methyltransferases (TRMs) typically utilize *S*-adenosyl methionine (SAM) as a methyl group donor which results in formation of a *S*-adenosyl-L-homocysteine and a methylated product [20]. In the following section, we aim to highlight structurally characterized TRMs and describe their selectivity for certain tRNA species and specific base positions within the respective ASLs (Figure 2).

In detail, Trm1, which conducts a double methylation of the exocyclic nitrogen of G26, which promotes a proper folding of multiple tRNA species by enforcing a water-mediated interaction of $m_2^2G_{26}$ with a nearby cytosine [21,22]. In yeast, Trm140 binds $tRNA^{Ser}$ and $tRNA^{Thr}$ and catalyzes m^3C_{32} in a i^6A_{37}-dependent manner [23]. In human, Trm140 functionally corresponds to the

Figure 1



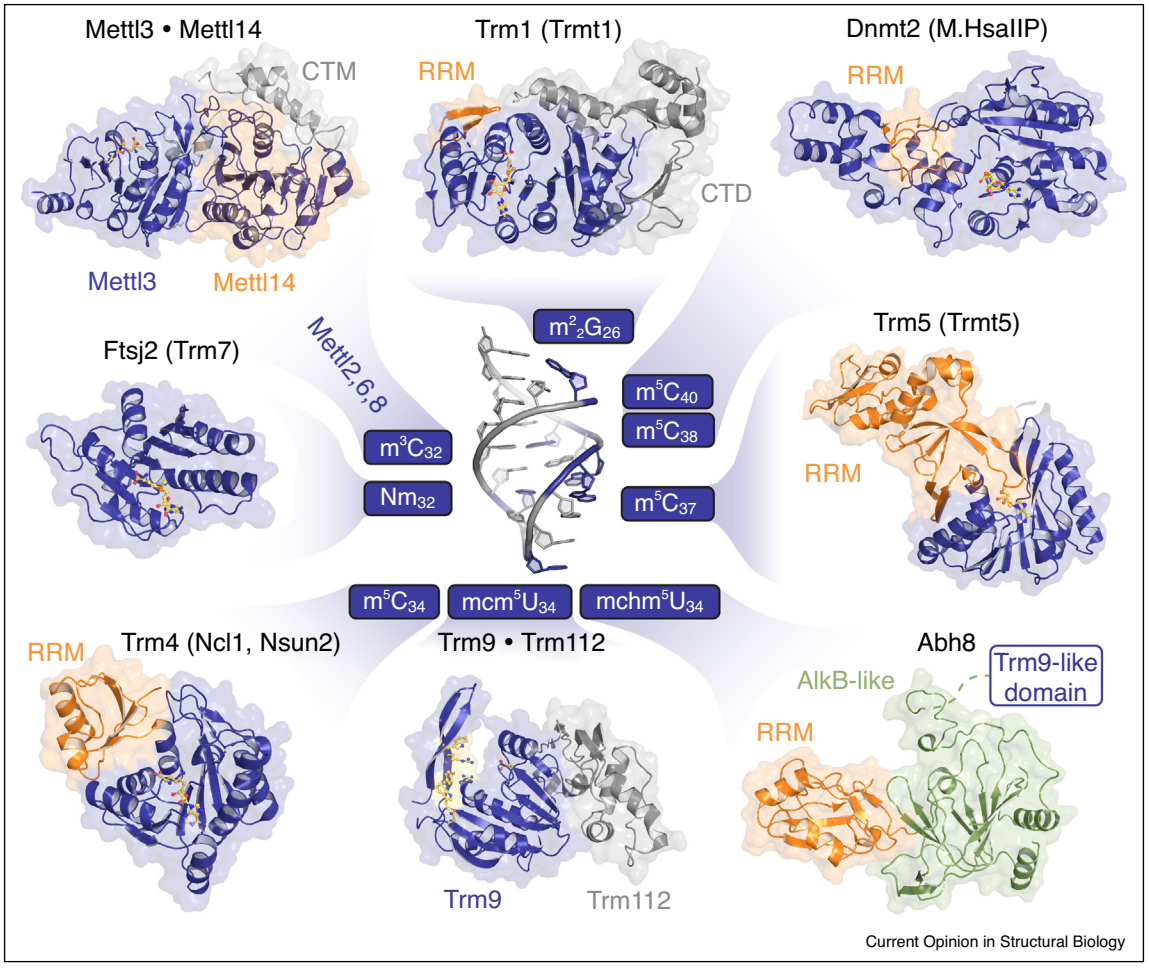
tRNA modifications occurring within the tRNA anticodon region.
Overview of the tRNA anticodon loop in cartoon representation. Individual modifications are grouped, highlighted, and labeled. Respectively from the left methylation (indigo), modification of position 34 (violet), 5-carboxymethyluridin (light green), pseudouridylation (rose), thiolation (yellow), wybutosine (yW; blue) N6-isopentenyladenosine (i^6A) and N6-threonylcarbamoyladenosine (t^6A) (red), adenine deamination (teal). Modifications occurring within anticodon region are plotted and highlighted on a model $tRNA^{Glu}$ (PDB ID 2CV2).

Methyltransferase-like (Mettl) 2, 6 and 8 proteins [24,25]. Although Mettl2/6/8 are currently not structurally characterized, structures of the Mettl3/Mettl14 complex that provides N^6-adenosine methylation [26–28] and the methyltransferase domain of Mettl16 [29] provide new insights into the METTL protein family. The known structure of human Ftsj2, a homolog of yeast Trm7, reveals a typical class I TRM fold [18]. In yeast, 2′-O ribose methylation provided by Trm7 is guided by its interactions with Trm732 and Trm734, which drive the reactions at positions 32 and 34, respectively [30]. Trm4 catalyzes modifications at positions m^5C_{48} and m^5C_{49}; however, it is also capable of generating m^5C_{34} and m^5C_{40} [31]. Archaeal Trm4 was co-crystalized in the presence of a naturally occurring inhibitor sinefungin [32]. In human, Trm4 has two functional counterparts, Nsun2 and Nsun3 catalyzing m^5 modifications in the nucleus and mitochondria, respectively. In addition to its tRNA modification activity, Nsun2 was reported to methylate miRNAs [33]. Methylation of the wobble cytosine provided by Nsun3 is required for initiation of 5-formylcytidine (f^5C_{34}) synthesis on $tRNA^{Met}$ [34]. The first known structure of Nsun family member was solved for Nsun6, which catalyzes the m^5C_{72} modification [35•]. Trm5 is another multifunctional enzyme, capable of conducting m^1 modification at G_{37} or I_{37}. Interestingly, Trm5 also plays a role in some archaeal species during wybutosine (yW_{37}) synthesis. Trm5 was co-crystalized with $tRNA^{Phe}$ and a SAM cleavage product [36••]. Available structure elucidates both the Trm5-tRNA interaction and the moonlighting activity of Trm5 in archaea [36••]. Another member of the class Dnmt2, historically considered a DNA-specific methyltransferase [37], provides a m^5C tRNA modification at C_{38} and C_{40} [38]. m^5C_{38} was demonstrated to prevent the generation of tRNA-derived fragments [39], which appear due to the tRNA cleavage under stress conditions and may act as regulatory RNAs [40]. Although available structures do not provide an explicit explanation for tRNA recognition, the enzymatic activity of a fungal Dnmt2 was recently found to be stimulated by the presence of queuosine [41•].

Pseudouridine synthases

Pseudouridylation is one of the most widely spread modification in all types of RNAs, including tRNAs, snRNAs, rRNA, ncRNAs, and mRNAs, and occurs in each domain of life [42]. This altered form of a uridine base arises

Figure 2



Structural overview of methyltransferases acting on the ASL of tRNA.
tRNA methyltransferases share a structurally similar TRM domain (dark blue) and an RNA recognition motif (RRM, orange). Ligands and relevant active site residues are highlighted in yellow. Names, alternative names or names of close homologs are shown in parentheses. Trm1 from *Pyrococcus horikoshii* co-crystalized with a SAM molecule (PDB ID 2EJT). Human Mettl3-Mettl14 complex co-crystalized with SAM (PDB ID 5IL1). Human Ftsj2, a Trm7 homolog, co-crystalized with SAM (PDB ID 2NYU). Trm4 from *Methanocaldococcus jannaschii* crystalized with an inhibitor (PDB ID 3A4T). Trm9–Trm112 complex from *Yarrowia lipolytica*. Trm9 is shown in blue, Trm112 (grey) activates the catalytic subunit (PDB ID 5CM2). N-terminal part of a human Abh8, which consists an AlkB-like domain (green) and a Trm9-like domain missing from the structure (PDB ID 3THP). Trm5 from *Pyrococcus abyssi* co-crystalized with a SAM degradation product and a $tRNA^{Phe}$ (PDB ID 5WT3). Human Dnmt2 co-crystalized with SAH (PDB ID 1G55).

through the substitution of the canonical carbon-nitrogen glycosidic bond (C1–N1) with a carbon-carbon bond (C1–C5) between the ribose and uracil. This simple isomerization reaction is conducted by stand-alone enzymes called pseudouridine synthases (PUS). Interestingly, prokaryotic and eukaryotic members of the five PUS families, namely TruA, TruB, TruD, RsuA, and RluA, display relatively low sequence identity, but show very similar folds of the catalytic domain [43,44••]. The conserved core of PUS enzymes is created by a central eight-stranded β-sheet and several surrounding helices, which properly position the catalytically active aspartate residue in proximity to the uridine base of the respective target RNA. In the ASL of various eukaryotic tRNAs, several positions, namely U_{27}, U_{28}, U_{30}, U_{31}, U_{32}, U_{34}, U_{35}, U_{36}, U_{38}, and U_{39}, are specifically converted into pseudouridine. PUS enzymes are found in the nucleus, the cytoplasm, and mitochondria and for tRNA targets can be divided into two major groups, namely modifiers of cytoplasmic or mitochondrial tRNAs.

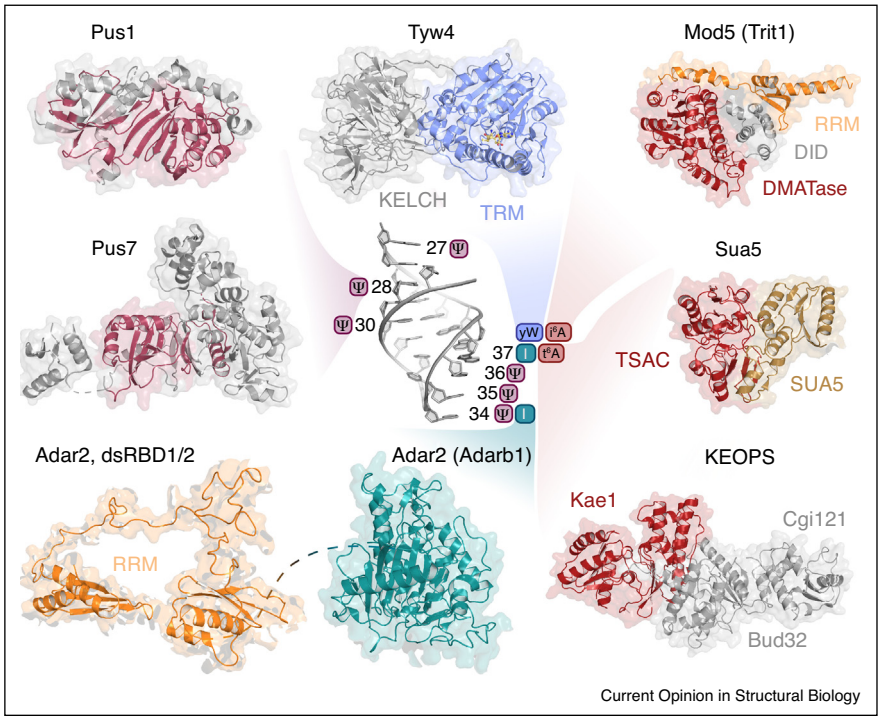
In yeast, Pus1 is the main player for cytoplasmic tRNA ASL modifications, which displays broad specificity for target uridines (U_{26}, U_{27}, U_{28}, U_{34}, U_{35}, U_{36}) [45]. Pus7 shows specific activity at U_{35} and Pus8 appears as a highly specific enzyme for U_{32} bases. Additionally, Pus3 and

Pus6 modify U_{31} and $U_{38/39}$ in both cytoplasmic and mitochondrial tRNAs. The last two pseudouridine synthases involved in anticodon modifications are Pus2 and Pus9, which are responsible for editing uridine in mitochondrial tRNAs at $U_{26/27}$ and U_{32} bases [42]. Strikingly, Pus enzymes modify the anticodon bases in cytoplasmic tRNAs, whereas they don't edit this important decoding region in mitochondrial tRNAs. In humans, TRUB2, RPUSD3, RPUSD4, and Pus1 have been predicted to target mitochondrial tRNAs, but only Pus1 has been experimentally confirmed to modify mitochondrial tRNAs, at positions 27 and 28 [46]. The other three human mitochondrial pseudouridine synthases are less well characterized [47], but have been proposed to modify positions 31 and 32 [48] or 39 in mt-tRNA^{Phe} [49]. Standalone pseudouridine synthases have broad range of activity, lack of which leads to reduced growth in yeast cells and major human diseases such as mitochondrial myopathy, sideroblastic anemia (MLASA), or intellectual disabilities [50,51].

The KEOPS complex

The bases in position 37 are highly modified in eukaryotic tRNAs. Besides previously described methylation, the formation of N^6-isopentenyladenosine (i^6A), N^6-threonylcarbamoyladenosine (t^6A), wybutosine (yW) as well as thiolation can be distinguished. The tRNA isopentenyl transferases (IPTases) are responsible for i^6A, whereas yW is catalyzed by the concerted action of Trm5 [36••] and Tyw1-4, which are able to conduct SAM-dependent methyl-transfer reactions [52,53]. t^6A is universal and present in nearly all tRNAs, which decode 'ANN' codons. In all kingdoms of life, the first step of the biosynthesis of t^6A leads to the production of threonylcarbamoyl adenylate (TC-AMP), which subsequently is used to transfer a threonylcarbamoyl group to adenosine. The enzymes Sua5 and YRDC are responsible for the formation of TC-AMP in yeast and humans. These homologs belong to the Sua5/TsaC family, function as independent monomers and are localized both in the cytoplasm and mitochondria [54,55•,56••]. In eukaryotes, the product of the first reaction step is processed by the cytoplasmic KEOPS complex, containing five subunits called OSGEP/Kae1 (catalytic core), PRPK/Bud32, TPRKB/Cgi121, LAGE3/Pcc1, and C14ORF142/Gon7 [54,57]. Despite some structural knowledge of the Sua5/TsaC family and the available structures of eukaryotic KEOPS complex components from yeast, the precise overall assembly of the complex, its mechanism of action as well as the role of particular subunits in the modification process

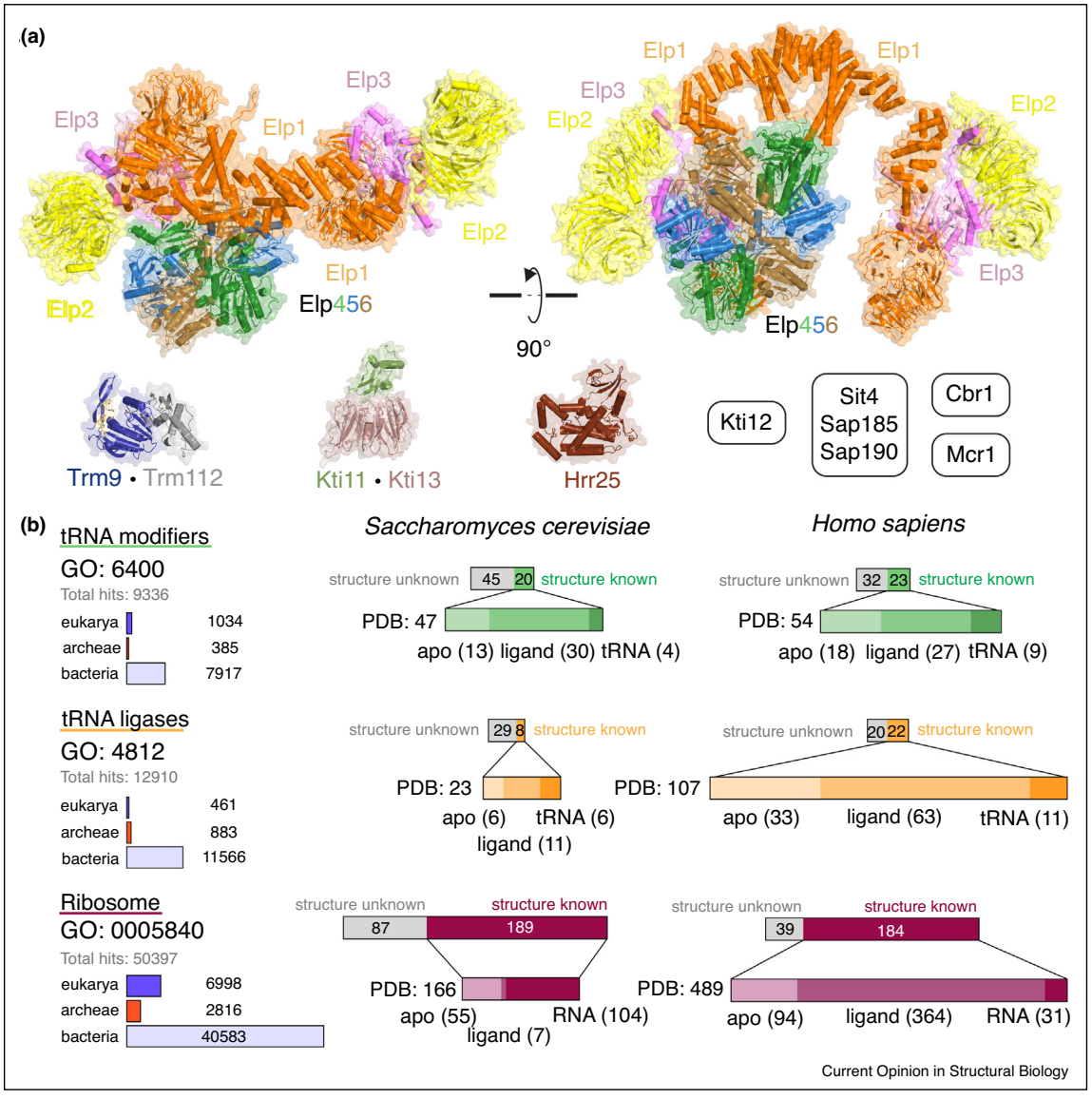
Figure 3



Structural overview for other tRNA modifiers.
Visual representation of protein structures including color illustration for tRNA binding domains (orange), enzymatic core (color corresponding to respective type of modification). Names, alternative names or names of close homologs are shown in parentheses. Pseudouridine synthases Pus1 (PDB ID 4IQM) and Pus7 (PDB ID 5KKP; both rose), wybutosine biosynthesis factor Tyw4 (PDB ID 2ZW9; blue), yeast isopentenyl transferases Mod5 (PDB ID 3EPJ; red), archaeal Sua5 (PDB ID 6F89) and Cgi121/Bud32/Kae1 KEOPS components (PDB ID 3ENH; red), and the structure of Adenine deaminases (ADARs) (PDB ID 2L3J; teal).

remain poorly understood. The hetero-dimerization of certain KEOPS subunits and the presence of a metal cluster in the active site of catalytic core formed by OSGEP/Kae1 proteins were described [55•,56••]. The functional roles of other cascade components are still unknown, although dimerization of mitochondrial Qri7 seems to be important for its functionality [56••,58]. Despite the fact that detailed aspects of t^6A biosynthesis remain elusive, recent studies showed that bicarbonate represents a rate-limiting factor for t^6A_{37} formation (Figure 3). Highlighting its importance, mutations in t^6A synthetase genes are correlated with neurodegenerative diseases, renal tubulopathy and Galloway-Mowat syndrome in humans (GAMOS) [59••,60,61].

Figure 4



The Elongator complex and structural knowledge of tRNA modifiers in general.
(a) An integrative model of the recently characterized Elongator complex and its subunits is shown from the front and the top. The complex harbors six pairs of proteins, namely Elp1(orange), Elp2 (yellow), Elp3 (catalytic subunit, pink) and Elp456 (green, blue and brown). Known accessory proteins or downstream factors are shown below. Trm9–Trm112 methyltransferase (PDB ID 5CM2) in blue and grey, Kti11–Kti13 (PDB ID 4XHL) in green and brown and Hrr25/Kti14 (PDB ID 5CYZ) in chocolate. Additional accessory proteins of unknown structure are listed (right).
(b) A comparison of available database records for tRNA modifiers, tRNA ligases and ribosomes. A SWISSPROT database was queried using GO terms (tRNA modifiers – GO:6400 excluding GO:4812, tRNA ligases – GO:4812, ribosome – GO:0005840). Distribution of records across three domains of life is shown on the left panel. Species-specific searches for *Saccharomyces cerevisiae* and *Homo sapiens* allowed to evaluate the number of annotated proteins and the number of proteins with available structural information (right panel, upper bars). Their accession numbers were used for a subsequent search in the PDB database to uncover the total number of known structures (right panel, lower bars). Every structure was categorized into ligand-free structures (lightest shade), ligand-bound structures (medium shade) and (t)RNA bound structures (darkest shade).

The Elongator complex

One of the chemically most complex modifications is catalyzed by a similarly complicated relay system. In eukaryotes, the highly conserved Elongator complex [62] carboxymethylates (cm^5) 11 out of 13 yeast tRNAs carrying a U_{34} in the wobble position [63]. The core Elongator complex harbors two copies of each of its six subunits, Elp1-6 and displays an overall molecular weight of more than 850 kDa [64]. Furthermore, the asymmetrically shaped complex [62] is regulated by various temporarily associated regulatory factors, including Kti11, Kti12, Kti13, Kti14/Hrr4, Sit4, Sap185, Sap190, Cbr1, and Mcr1 [65]. In recent years, the crystal structures of the dimeric Elp1 C-terminus [66], Elp2 [67••,68], the enzymatically active Elp3 subunit [69], the hetero-hexameric Elp456 subcomplex [64], and the temporarily associated regulatory factors Kti11, Kti13, and Kti14/Hrr25 [70,71] became available (Figure 4a). In addition, the overall architecture of the fully assembled Elongator complex has been recently determined, using negative stain electron microscopy in combination with an integrative modeling approach based on the known high resolution crystal structures and additional spatial restraints from cross linking mass spectrometry [67••,72].

The pivotal addition of cm^5 to U_{34} can subsequently leads to the synthesis of 5-methoxycarbonylmethyluridine (mcm^5U), 5-carbamoylmethyluridine (ncm^5U), 5-methoxy-carbonyl-methyl-2-thiouridine (mcm^5s^2U), 5-methoxycarbonylmethyl-2′-O-methyluridine (mcm5Um), or 5-(carboxyhydroxymethyl)uridine methyl ester ($mchm^5U$) by other enzymatic cascades. These small additional groups on the 'Elongator' modification at the fifth carbon (C5) are attached by Trm9 [73] and Abh8 [74], both of which require an obligatory activator Trm112 (Figure 1) [74,75•,76]. The identity of the protein that synthesizes ncm^5, either directly or by conversion of mcm^5, remains a mystery. Last but not least, the additional thiolation on the second carbon (C2) is catalyzed by the Urm1/Uba4 pathway [77], which in eukaryotes includes Nfs1, Tum1, Urm1, Uba4, Ncs2, and Ncs6 [78]. In summary, around 25 individual proteins are involved in wobble uridine modifications, which are highly conserved among eukaryotes and also have related counterparts in bacteria and archaea. It remains to be shown if some of these factors form dynamic intermediates with the Elongator complex and how they influence each other.

Conclusion

In summary, tRNA modifiers represent a highly dynamic network of a large number of macromolecular complexes and individual proteins. Because of space restrictions, we are not able to cover structural details regarding the enzymes that conduct adenine deamination [79], queuosine biosynthesis [80], wybutosine [53], thiolation [78], and other more unique modifications, like 5-taurinomethyluridine [81] or N4-acetylcytidine [82]. Nevertheless, we aimed to numerically evaluate the overall state of the field and compared the number of known tRNA modifiers in publicly available databases [83] to other well-established tRNA-dependent mechanisms, like ribosomes and tRNA aminoacyl transferases, also known as tRNA ligases (Figure 4b). We performed species-specific queries to more precisely identify the number of known structures of tRNA modifiers, tRNA ligases and ribosomes. While the structures of most ribosomal proteins and more than a half of all human tRNA ligases have been determined, tRNA modifiers seem to still offer many possibilities for exploratory research projects. In particular, RNA-bound or tRNA-bound structures amount to a relatively small fraction of available records in the structural databases [84,85]. Undoubtedly, structural characterization of modifiers in their tRNA-bound state most significantly improves our understanding of their specificity and catalytic mechanisms. We assume, that the ascent of cryo-EM will have a similar impact on the tRNA modification field as it had for the other fields [86].

Interestingly, expansion of tRNA gene copy numbers and iso-acceptor tRNAs correlate well with an increase in different tRNA modification enzymes [87]. This observation suggests co-evolutionary mechanisms, which expand the decoding potential of the available tRNA pool and constantly optimize translational efficiency and accuracy via novel tRNA modification pathways [87]. Last but not least, the assembly of large complexes seems advantageous for some modifications, whereas other pathways seem to rely on stand-alone enzymes. It remains to be shown, if additional molecular assemblies that spatially link different modification pathways will be discovered and characterized.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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