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Advances in the Structural and Functional Understanding of m^{1}A RNA Modification

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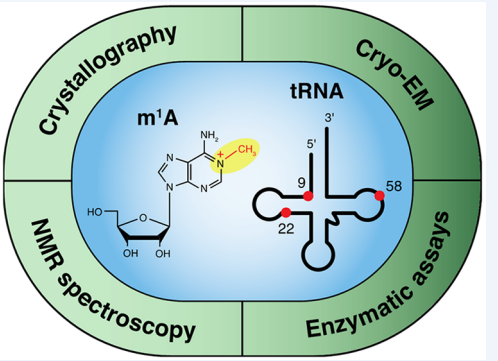
CONSPECTUS: RNA modification is a co- or post-transcriptional process by which specific nucleotides are chemically altered by enzymes after their initial incorporation into the RNA chain, expanding the chemical and functional diversity of RNAs. Our understanding of RNA modifications has changed dramatically in recent years. In the past decade, RNA methyltransferases (MTases) have been highlighted in numerous clinical studies and disease models, modifications have been found to be dynamically regulated by demodification enzymes, and significant technological advances have been made in the fields of RNA sequencing, mass spectrometry, and structural biology. Among RNAs, transfer RNAs (tRNAs) exhibit the greatest diversity and density of post-transcriptional modifications, which allow for potential cross-talks and regulation during their incorporation. N^1-methyladenosine (m^{1}A) modification is found in tRNAs at positions 9, 14, 16, 22, 57, and 58, depending on the tRNA and organism.



Our laboratory has used and developed a large panel of tools to decipher the different mechanisms used by m^{1}A tRNA MTases to recognize and methylate tRNA. We have solved the structures of TrmI from *Thermus thermophilus* (m^{1}A58), TrmK from *Bacillus subtilis* (m^{1}A22), and human TRMT10C (m^{1}A9). These MTases do not share the same structure or organization to recognize tRNAs, but they all modify an adenosine, forming a non-Watson−Crick (WC) interaction. For TrmK, nuclear magnetic resonance (NMR) chemical shift mapping of the binding interface between TrmK and tRNA^{Ser} was invaluable to build a TrmK/tRNA model, where both domains of TrmK participate in the binding of a full-length L-shaped tRNA and where the non-WC purine 13-A22 base pair positions the A22 N1-atom close to the methyl of the S-adenosyl-L-methionine (SAM) TrmK cofactor. For TRMT10C, cryoEM structures showed the MTase poised to N^1-methylate A9 or G9 in tRNA and revealed different steps of tRNA maturation, where TRMT10C acts as a tRNA binding platform for sequential docking of each maturation enzyme. This work confers a role for TRMT10C in tRNA quality control and provides a framework to understand the link between mitochondrial tRNA maturation dysfunction and diseases.

Methods to directly detect the incorporation of modifications during tRNA biosynthesis are rare and do not provide easy access to the temporality of their introduction. To this end, we have introduced time-resolved NMR to monitor tRNA maturation in the cellular environment. Combined with genetic and biochemical approaches involving the synthesis of specifically modified tRNAs, our methodology revealed that some modifications are incorporated in a defined sequential order, controlled by cross-talks between modification events. In particular, a strong modification circuit, namely Ψ55 → m^{5}U54 → m^{1}A58, controls the modification process in the T-arm of yeast elongator tRNAs. Conversely, we showed that m^{1}A58 is efficiently introduced on unmodified initiator tRNA$_i^{Met}$ without the need of any prior modification. Two distinct pathways are therefore followed for m^{1}A58 incorporation in elongator and initiator tRNAs.

We are undoubtedly entering an exciting period for the elucidation of the functions of RNA modifications and the intricate mechanisms by which modification enzymes identify and alter their RNA substrates. These are promising directions for the field of epitranscriptomics.

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- Dégut, C.; Roovers, M.; Barraud, P.; Brachet, F.; Feller, A.; Larue, V.; Al Refaii, A.; Caillet, J.; Droogmans, L.; Tisné, C. Structural Characterization of *B. subtilis* m^{1}A22 tRNA Methyltransferase TrmK: Insights into tRNA

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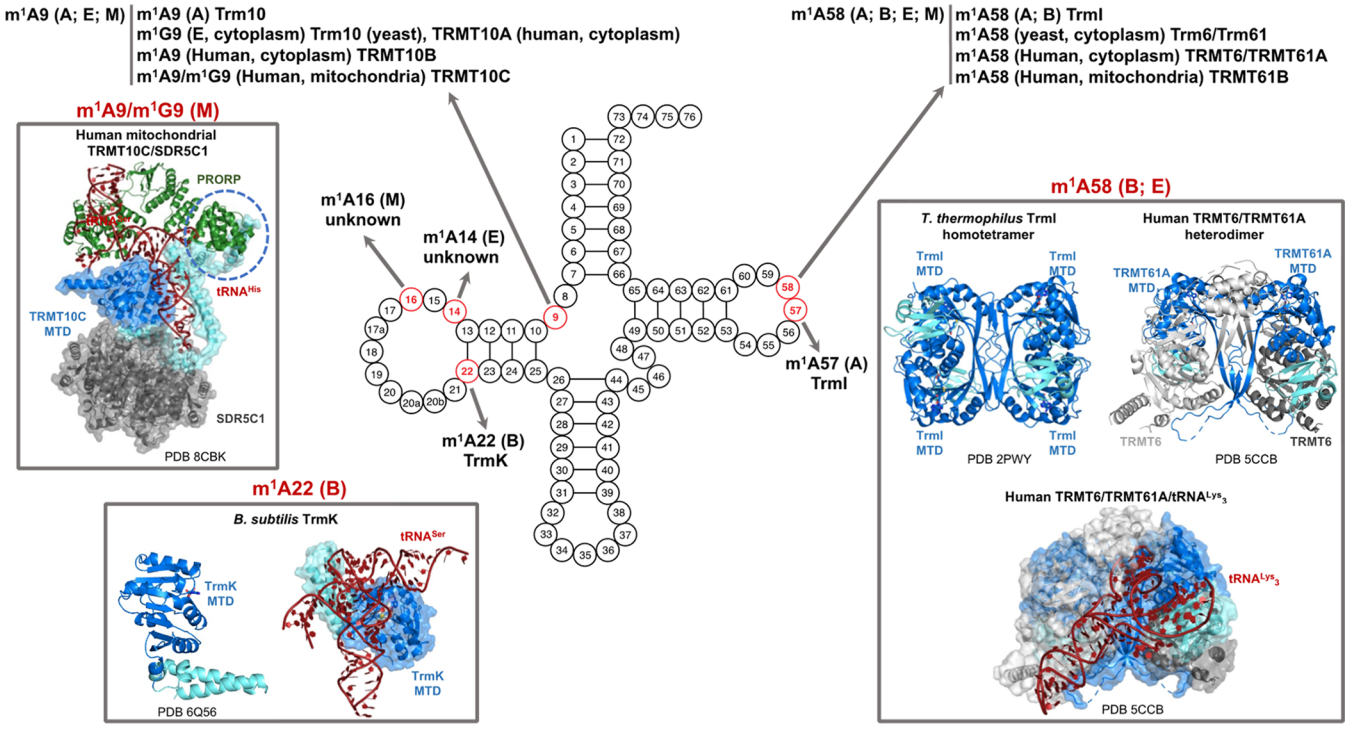

Figure 1. m^{1}A modifications in tRNAs and summary of available structural data. Schematic of a tRNA, showing the sites where m^{1}A modification can occur in red. The domains in which the modification has been identified are indicated with A (Archaea), B (Bacteria), and E (Eukarya). Some sites are specific to mitochondrial tRNAs, which are indicated with M. Structural data of m^{1}A9/m^{1}G9, m^{1}A22, and m^{1}A58 tRNA MTases are presented in the boxes with the methyltransferase domain (MTD) of the MTase in marine blue and their additional domain in cyan; places where a partner required for MTase activity are in gray. The endonuclease PRORP is in green, and the dashed blue circle indicates the interaction between the pentatricopeptide repeat (PPR) domain of PRORP and TRMT10C, which is required for RNase P activity of PRORP.

Recognition. *Nucleic Acids Res.* 2019, *47*, 4736−4750.[1] This pioneer work reported a structural and functional study of *B. subtilis* TrmK, the m^{1}A22 transfer RNA (tRNA) methyltransferase. The combined use of X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy allowed us to propose a model of the TrmK-SAM cofactor/tRNA complex, which reveals how TrmK recognizes and modifies tRNAs.

- Meynier, V.; Hardwick, S. W.; Catala, M.; Roske, J.; Oerum, S.; Chirgadze, D.Y.; Barraud, P.; Yu, W.; Luisi, B. F.; Tisné, C. Structural Basis for Human Mitochondrial tRNA Maturation. *bioRxiv* 2023.[2] We used cryo-electron microscopy (cryoEM) to solve the structures of four sequential steps of human mitochondrial precursor-tRNA maturation. In particular, these structures visualize tRNA purine-9 N^1-methylation by TRMT10C.
- Barraud, P.; Gato, A.; Heiss, M.; Catala, M.; Kellner, S.; Tisné, C. Time-Resolved NMR Monitoring of RNA Maturation. *Nat. Commun.* 2019, *10*, 3373−3387.[3] This study introduced a novel methodology using NMR spectroscopy to monitor the deposition of nucleotide modifications on tRNA in cell extracts. Our findings revealed that some modifications occur in a precise sequential order, regulated by intricate cross-talks between different modification events.
- Yared, M.-J.; Yoluç, Y.; Catala, C.; Tisné, C.; Kaiser, S.; Barraud, P. Different Modification Pathways for m^{1}A58 Incorporation in Yeast Elongator and Initiator tRNAs. *Nucleic Acids Research* **2023**, *51*, 10653−10667.[4] We implemented a generic approach enabling the preparation of tRNAs containing specific modifications. Using these specifically modified tRNAs, we revealed distinct mechanisms governing the incorporation of the m^{1}A58 modification in elongator tRNAs compared to initiator tRNAs in yeast.

INTRODUCTION

RNAs are highly co- or post-transcriptionally chemically modified at nucleobases or at the ribose 2′−OH by modification enzymes. Long regarded as molecular decoration, RNA modifications have become the focus of intense research, grouped under the heading of epitranscriptomics. This field of research aims at determining RNA modification profiles and investigating the molecular processes that establish and regulate the information carried by RNAs beyond the simple canonical nucleotides. The detection, localization, and quantification of these RNA modifications were the first steps for understanding their elusive function. The emergence of epitranscriptomics was thus accompanied by the development of new tools dedicated to RNA modification landscape determination, such as high-throughput sequencing and mass spectrometry (reviewed in ref 5). The recently introduced nanopore RNA sequencing has also shown promise as a method for discriminating and identifying different RNA modifications in native RNA.[6−9] In particular, new mapping methods have drawn considerable attention to the modification content of eukaryotic messenger RNAs (mRNAs) and its potential for the regulation of gene expression. The initial transcriptome-wide mapping of thousands of internally located N^6-methyladenosine (m^{6}A) on mRNAs identified m^{6}A as a common and prevalent base modification in mRNA[10,11] and

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has boosted this field of research. The prevalence and location of m^1A in the transcriptome have been a matter of debate and discrepancy in the literature. It is now clear that m^1A is infrequent in mRNA[12] and that the prevalence of this modification was substantially overestimated in previous studies[13,14] due to cross-reactivity of the antibody used to detect m^1A, which recognizes the m^7G modification of the mRNA cap, or samples contaminated with tRNA and ribosomal RNAs (rRNAs) in mass spectrometry analyses. Unlike mRNAs, tRNAs are commonly modified with the m^1A modification, which is found in a large subset of tRNAs from bacteria to humans at positions 9, 14, 16, 22, 57, and 58 (Figure 1). The N^1-methylation of adenosine excludes the formation of base pairing via its Watson−Crick (WC) face, and nucleosides at positions 9, 14, and 58 form non-WC base pairs via their Hoogsteen face. These noncanonical base pairs are important to establish the tRNA three-dimensional structure and to maintain the D- and the T-loops in close contact. The N^1-methylation of adenosine also drastically increases the pK_a of the nucleobase ($\Delta pK_a \simeq 5.0$) and confers a positive charge under physiological conditions.[15] m^1A is thus considered to contribute to the functions of RNA by affecting local and overall RNA topology and by altering interactions with proteins. Different sets of enzymes are responsible for m^1A incorporation in tRNAs (Figure 1). The m^1A9 in mitochondrial (mt) and archaeal tRNAs is formed by Trm10 family enzymes (TRMT10C in human mitochondria), which belong to the SPOUT family of MTases.[16] Remarkably, yeast Trm10 forms 1-methylguanosine at position 9 (m^1G9), and TRMT10C and the Trm10 enzymes from several archaea display dual specificity, forming both m^1A9 and m^1G9. So far, TRMT10C is the only member of the Trm10 family that is active in complex with another protein, namely SDR5C1.[16−18] Two additional enzymes from the Trm10 family, TRMT10A and TRMT10B, are expressed in humans. TRMT10A, the direct homologue of yeast Trm10, and TRMT10B are both nuclear/cytosolic. Each human Trm10 enzyme N^1-methylates a unique subset of tRNAs, modifying only G9 of some cytosolic tRNAs (TRMT10A),[19−21] only A9 of cytosolic $tRNA^{Asp}$ (TRMT10B),[19−21] or exhibiting dual specificity to modify either A9 or G9 of mt-tRNAs (TMRT10C).[22] TRMT10C also catalyzes a single m^1A modification in the ND5 mt-mRNA, with methylation levels that are highly tissue-specific and tightly developmentally controlled.[20] The presence of m^1A14 has only been reported in a limited number of mammalian cytoplasmic $tRNA^{Phe}$,[23] and the gene encoding the corresponding MTase is still unknown.[24] m^1A modification is also found at positions 16 and 58 in human mt-tRNAs. TRMT61B, which introduces m^1A58 in mt-tRNAs[25] and m^1A496[26] in mt-rRNA, has been proposed to be responsible for the newly discovered m^1A16 modification in mt-$tRNA^{Arg}$.[27] The m^1A22 modification is catalyzed by TrmK, an enzyme that is widely found in Gram-positive bacteria and in some Gram-negative bacteria, without any equivalent found in Eukarya or Archaea.[28] Finally, m^1A58 modification is found in tRNAs of organisms from all domains of life. The genes and corresponding enzymes responsible for m^1A58 (Trm6/Trm61 in Eukarya and TrmI in Bacteria and Archaea) have been identified and extensively structurally characterized (previously reviewed in ref 22). All of these enzymes catalyze the same chemical reaction but use different organizations and strategies to recognize their tRNA targets and access the adenosine to modify (Figure 1). Besides the MTase domain, all have additional domains of different structures (in cyan, Figure 1), which are required for tRNA binding. Moreover, TrmI is active as a tetramer (heterodimer for Trm6/Trm61 proteins), and TrmK is active as a monomer; TRMT10C is only active in the presence of SDR5C1. This Account focuses on recent advances in our understanding of the incorporation of m^1A9, m^1A22, and m^1A58 in tRNAs, their function, and their implications in human health.

M^{1}A9 IN HUMAN MITOCHONDRIAL TRNAS

mt-tRNAs have comparatively low structural stability because they have a low GC content and their D-, T- and variable loops, which support the folds of cytosolic tRNAs, are either absent or differ in length compared to cytosolic tRNAs.[6] In this context, nucleotide modifications are crucial to ensure the functional folding and stability of mt-tRNAs. Of the 18 different nucleotide modifications found in human mt-tRNAs, the N^1-methylation at position 9 of tRNA is the most prevalent modification.[27] Indeed, 19 of the 22 mt-tRNAs have a N^1-methylated purine at position 9, with 14 occurring on an adenine (m^1A) and 5 on a guanine (m^1G).[27] N^1-methylation at position 9 has been proposed to stabilize the mature tRNA fold of some of these tRNAs.[29] For instance, at least two alternative conformations of unmodified human mt-$tRNA^{Lys}$ coexist in equilibrium. The incorporation of the m^1A9 modification shifts this equilibrium toward the functional L-shaped conformation, making it available for further maturation.[29]

The TRMT10C/SDR5C1 complex has been proposed to serve as a platform for the multistep tRNA maturation process in human mitochondria, i.e., TRMT10C/SDR5C1 forms a stable complex with mt-tRNAs, enabling ordered cleavages by first PRORP (RNase P) and then ELAC2 (RNase Z), followed by 3′-CCA addition by TRNT1.[30] We have solved the cryoEM structures of each of these maturation steps at high resolution (2.7−3.1 Å).[2] The organization of the TRMT10C/SDR5C1/tRNA complex is globally the same in all structures; in other words, TRMT10C straddles a tetramer of SDR5C1 while wrapping around the L-shaped pre-tRNA (Figure 1). Both domains of TRM10C interact with the tRNA that is stabilized in an L-shaped conformation, where the T- and D-loops do not interact and where all the nucleotides of the anticodon loop interact with the N-terminal domain of TRMT10C.[2,31] This leaves one side of the pre-tRNA exposed for interaction with maturation enzymes (Figure 1). We visualize the SAH, the SAM cofactor product, in the active site of TRMT10C, allowing us to visualize the precatalytic states for m^1A9 and m^1G9 reactions.[2] The purine-9 is flipped in the active site of TRMT10C, stacked against the conserved V313, and further stabilized by interaction with the same set of conserved residues, i.e., N222, Q226, D314, and N350. D314 is poised to form a hydrogen bond with the NH_2 group of A9, supporting a SN_2 mechanism for the methyl transfer from the SAM to the base.[2]

We have also identified direct interactions between the pentatricopeptide repeat (PPR) domain of PRORP and TRMT10C (Figure 1) and have shown that these interactions are required for the RNase P activity of PRORP.[2] Binding the mt-pre-tRNA in the TRMT10C/SDR5C1 complex for mt-pre-tRNA maturation offers an alternative to compensate for the structural variability of the mt-tRNA substrates and an additional attachment point for the maturation enzymes on the pre-tRNA. The TRMT10C/SDR5C1 complex recognizes the entire mt-tRNA structure, and misfolded tRNAs will

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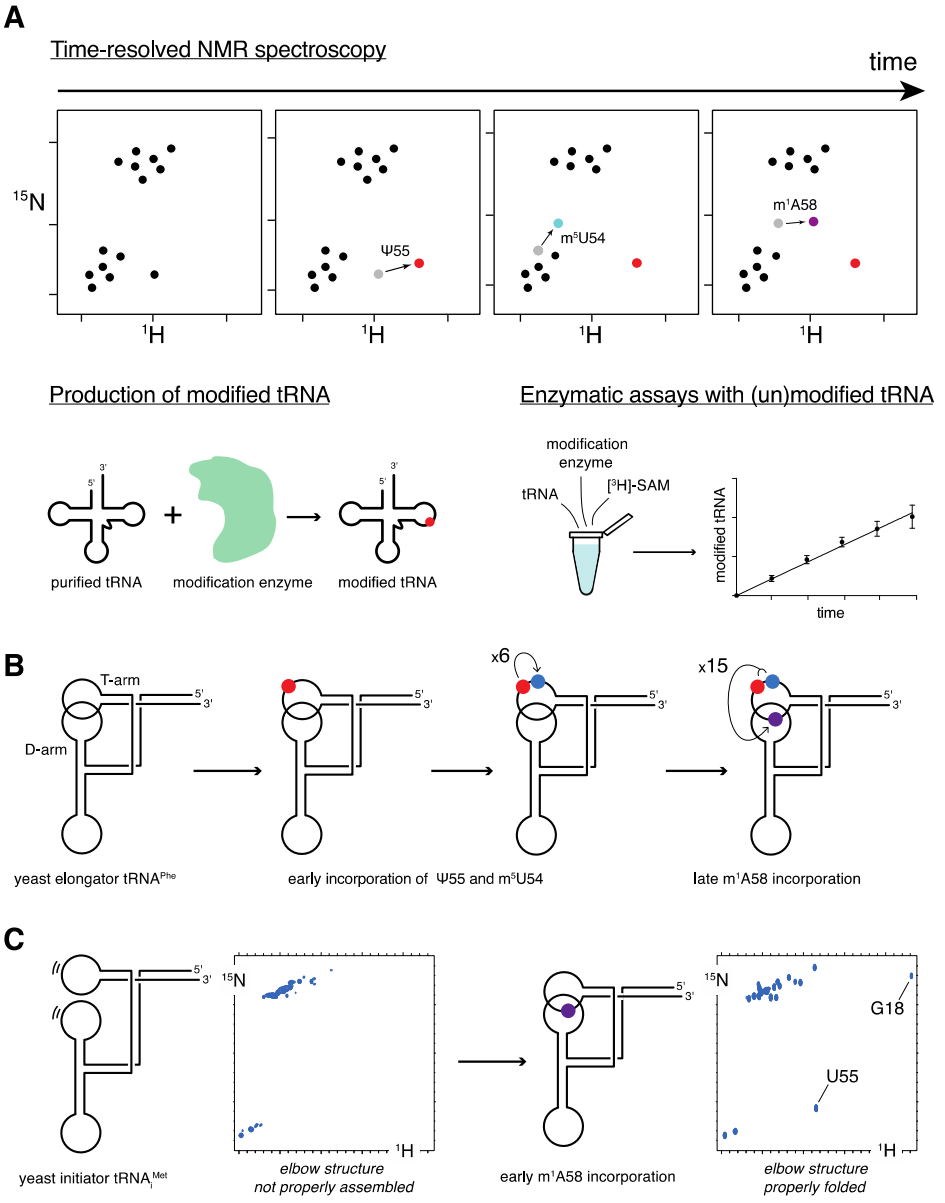

Figure 2. Temporality and circuit of modifications in pathways for m^{1}A58 introduction in yeast elongator and initiator tRNAs. (A) Overview of the methodological approaches used in combination for characterizing the temporality of modification incorporation and the potential modification circuits controlling their introduction. (B) Pathway for late m^{1}A58 incorporation in elongator tRNAs. The introduction of m^{1}A58 highly depends on the prior presence of Ψ55 and m^{5}U54. Circles in red, blue, and purple represent modifications Ψ55, m^{5}U54, and m^{1}A58, respectively. (C) Pathway for early m^{1}A58 incorporation in initiator tRNA$_i^{Met}$. NMR imino (^{1}H,^{15}N) correlation spectra are shown next to unmodified- and m^{1}A58-tRNA$_i^{Met}$ schematic drawings. In m^{1}A58-tRNA$_i^{Met}$, the U55 and G18 imino signals indicate that the elbow structure is properly folded.

therefore either not bind the TRMT10C/SDR5C1 complex or bind incorrectly and fail being processed. This confers a role of tRNA quality control to TRMT10C/SDR5C1 to ensure that pre-tRNAs entering the maturation pathways are well-folded and can be taken over for processing.

■ M^{1}A22 IN BACTERIAL TRNAS

TrmK from *B. subtilis* methylates, in vitro, seven out of the eight tRNA species that have an A at position 22 in this bacteria (tRNA^{Ser}, tRNA^{His}, tRNA^{Tyr}, tRNA^{Cys}, tRNA^{Gln}, tRNA^{Glu}, and tRNA^{Leu}).[1] So far, all of these tRNA substrates have been confirmed in vivo, except for tRNA^{His}.[32] For tRNA^{Gly}, which is not a substrate of TrmK, we have shown that the WC base pairing between U13 and A22 causes a loss of the methyl group acceptance activity in this tRNA. Indeed, U13G mutation is sufficient to transform tRNA^{Gly} into a TrmK substrate.[1] TrmK thus requires a purine at position 13 of the tRNA to catalyze m^{1}A22 formation. We have also shown that an intact three-dimensional L-shaped structure of tRNA is required and that the variable arm does not participate in the binding to TrmK. The same determinants for tRNA substrates of *Geobacillus stearothermophilus* TrmK have been recently described.[33]

The TrmK structure consists of an N-terminal Class I MTase domain linked to a C-terminal coiled-coil domain (Figure 1). Both domains are required for MTase activity.[1] We and others have failed to obtain a crystal structure of TrmK bound to a tRNA substrate. Crystallization of these RNA modification complexes remains a highly challenging task

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because of the flexibility of the tRNAs, which often limits crystallization, the low binding affinity of the partners, and the salt concentration needed in many crystallization assays, which weakens the electrostatic enzyme−RNA interactions. In the absence of diffracting crystals, we were pioneers in using NMR to obtain information on the binding interface between TrmK and a full-length tRNA substrate.[1] The strategy relies on isotopically labeling either TrmK or $tRNA^{Ser}$ and observing the binding of the unlabeled partner, invisible in NMR, to the NMR signals of the labeled species. This NMR chemical shift mapping between TrmK and $tRNA^{Ser}$ showed that TrmK binds an L-shaped tRNA and that most interactions are located around the SAM cofactor binding site, even if the C-terminal domain is also required for binding. The measured NMR data were subsequently used to guide the docking of $tRNA^{Ser}$ into the enzyme and build a model of the TrmK/SAM/$tRNA^{Ser}$ complex (Figure 1). This model was validated by measuring the decreased MTase activity of TrmK variants with single-point mutations located at the proposed protein−RNA interaction surface. TrmK recognizes the overall L-shaped structure of tRNA via extensive interactions with the phosphodiester backbone of nucleotides in the D-loop and interactions with the phosphodiester backbone of the T-loop and anticodon stem. Both domains of TrmK interact with $tRNA^{Ser}$, but the catalytic domain of TrmK shares, to a large extent, a larger interaction surface with the tRNA. The coiled-coil motif of the C-terminal domain of TrmK interacts at the level of the D- and T-loops of $tRNA^{Ser}$, which helps maintain A22 near the catalytic pocket, explaining why removal of these helices abolishes TrmK MTase activity. The base pairing of A22 with G13 also allows for positioning the N1-atom of A22 in close proximity to the methyl group of SAM, and a pyrimidine at this position would form hydrogen bonds to the WC face of A22, which would render the N1-atom nonaccessible. Recent structural and mutational mapping data obtained for *G. stearothermophilus* TrmK[33] and *Staphylococcus aureus* TrmK[34] suggest that their tRNA recognition mechanism is likely similar to that of *B. subtilis* TrmK.

Lastly, we showed that the formation of m^1A22 by *B. subtilis* TrmK does not require a general base catalyst. This result adds to the growing lines of evidence that m^1A formation in tRNA does not require a base catalyst and agrees with the previously proposed m^1A methylation mechanism for the bacterial m^1A58 tRNA MTase TrmI[22,35,36] and with the data published on the dual-specific m^1G9/m^1A9 tRNA MTase Trm10 from *Thermococcus kodakarensis*.[37,38]

■ M^1A58 IN YEAST TRNAS

The structural organization and interaction between the enzymes introducing the m^1A58 modification and target tRNAs have been previously reviewed.[22] Here, building on key references 3 and 4, we focus on the temporality and cross-talks of modification events. In these studies, we investigated some molecular aspects controlling the m^1A58 modification pathways in yeast elongator and initiator tRNAs. Their characterization was only possible by combining several methodological approaches, namely NMR spectroscopy, the enzymatic production of modified tRNAs, and enzymatic assays with purified tRNA substrates (Figure 2A).[3,39−41,4] Post-transcriptional modifications are typically introduced in tRNAs independently of each other, but in some cases, the incorporation of certain modifications was shown to be influenced by pre-existing ones. These modification cross-talks, referred to as "modification circuits", influence the sequential order of modification incorporation.[42−44] Conversely, monitoring tRNA maturation in a time-resolved fashion represents an interesting approach for the characterization of modification circuits. Toward that objective, we have developed several tools (Figure 2A), including an NMR spectroscopy-based methodology enabling the monitoring of tRNA maturation in a time-resolved fashion. The strategy relies on the introduction of ^{15}N isotope-labeled tRNAs into unlabeled cell extracts containing the cellular enzymatic activities and on the use of regular ^{15}N-edited NMR experiments to only detect the ^{15}N-labeled tRNA within the cell extract. This approach enables the detection of successive modification events occurring during the maturation of the target tRNA molecule.[3,41] In our initial application of this method to the maturation of yeast $tRNA^{Phe}$ in yeast extracts, we uncovered a sequential order in the introduction of modifications. Notably, m^1A58 emerges as a late modification, introduced after more initial modifications such as Ψ55 and m^5U54.[3]

Using cell extracts derived from specific deletion strains, we actually uncovered two modification circuits connecting these three modifications. Indeed, in a *pus4*Δ strain lacking Ψ55, we observed a severe slow-down in the introduction of both m^5U54 and m^1A58, and in a *trm2*Δ strain lacking m^5U54, we observed a slow-down in the introduction of m^1A58, thus revealing the Ψ55 → T54 → m^1A58 long-branch circuit and the Ψ55 → m^1A58 direct circuit.[3] In addition, we showed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) that levels of m^1A58 and m^5U54 are impacted in the *pus4*Δ and *trm2*Δ strains in both yeast $tRNA^{Phe}$ and total yeast tRNAs in a manner consistent with the cross-talks identified with NMR spectroscopy. This demonstrated that these modification circuits in the T-loop are found in $tRNA^{Phe}$ and other yeast tRNAs, which was later corroborated by nanopore sequencing of the entire *pus4*Δ strain tRNA population.[9]

We then characterized the molecular mechanisms related to the modification circuits involving Ψ55, m^5U54, and m^1A58. For this, we prepared tRNAs containing specific modifications via an enzymatic method (Figure 2A), and we measured the initial velocity of m^1A58 incorporation on several tRNAs each exhibiting a unique modification profile. Our data demonstrated that, compared to an unmodified $tRNA^{Phe}$, m^1A58 introduction by Trm6/Trm61 is approximately three times more efficient in the presence of m^5U54, seven times more efficient in the presence of Ψ55, and 15 times more efficient if both Ψ55 and m^5U54 are present together. Overall, the combined use of time-resolved NMR spectroscopy and the specific production of modified tRNAs for their use in downstream enzymatic assays (Figure 2A) allowed us to identify and characterize the T-loop modification circuits present in yeast elongator tRNAs (Figure 2B).[4]

Our finding that m^1A58 is introduced as a late modification after Ψ55 and m^5U54, particularly in yeast elongator $tRNA^{Phe}$, appears to contradict previous reports suggesting that m^1A58 is introduced early in the tRNA modification process, particularly on initial pre-$tRNA_i^{Met}$ transcripts (reviewed in ref 45). Notably, yeast initiator pre-$tRNA_i^{Met}$ lacking m^1A58 is targeted and degraded by the nuclear surveillance and rapid tRNA decay (RTD) pathways,[46−49] resulting in cell growth defects, particularly at elevated temperatures.[50] To understand the apparent inconsistency regarding the temporality of m^1A58

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incorporation, we conducted enzymatic assays with the initiator $tRNA_i^{Met}$. We showed that m^1A58 is efficiently incorporated on the unmodified initiator $tRNA_i^{Met}$ with an efficiency that is comparable to that observed for an optimally modified $tRNA^{Phe}$ carrying both Ψ55 and m^5U54. Clearly, Trm6/Trm61 follows two distinct modification pathways depending on whether it is acting on yeast elongator or initiator tRNAs (Figure 2B,C).

To explain the molecular origin of these different modification pathways, it is key to bear in mind that in yeast $tRNA_i^{Met}$, m^1A58 takes part in a unique tRNA elbow substructure, which is assembled via a network of interactions involving nucleotides A20, A54, m^1A58, and A60 in the D- and T-loops.[51] In our study, a comparison of the NMR spectra of unmodified- and m^1A58-$tRNA_i^{Met}$ revealed considerable changes in the structural properties of $tRNA_i^{Met}$ upon m^1A58 modification (Figure 2C).[4] In contrast to unmodified-$tRNA_i^{Met}$, m^1A58-$tRNA_i^{Met}$ displays the characteristic of a stable and homogeneously folded tRNA with a well-assembled elbow structure, where interactions between the D- and T-loops are properly established, as evidenced by the fact that the imino signals of U55 and G18 are visible (Figure 2C).[4] Given that tRNA degradation pathways primarily monitor the structural integrity of tRNAs,[52,53] the fact that the single m^1A58 modification has major structural effects on yeast $tRNA_i^{Met}$ likely explains why hypomodified $tRNA_i^{Met}$ lacking m^1A58 is targeted by degradation pathways. Altogether, these findings lead to the model that m^1A58 is introduced on pre-$tRNA_i^{Met}$ transcripts without the need of any prior modification, which allows for the proper assembly of the $tRNA_i^{Met}$ elbow region, thereby preventing its degradation by RNA decay pathways.

TRNA M^1A MODIFICATIONS IN HUMAN HEALTH

Advances in m^1A methyltransferase research and the exploration of biological mechanisms and functions related to m^1A modifications have deepened our understanding of their association with human diseases.

m^1A9-tRNA Modification and Mitochondrial Diseases

Mitochondrial diseases linked to mutations in mt-tRNA sequences are common. They affect binding and m^1R9 methylation by TRMT10C and TRMT10C-mediated tRNA-processing by MRPP3.[54] Impaired processing of mt-tRNAs has a detrimental effect on mt gene expression and the assembly of mt ribosomes.[55,56] As a consequence, mutations that disturb mt-tRNA structures or any step of mt-tRNA maturation are linked to severe human diseases.[57−59] Most of these mutations cause defects in mt-tRNA maturation and respiratory chain deficiency in patients.[60,61,54,62−66] The disease-associated mutations of TRMT10C, R181 and L182, are involved in the stabilization of the distinctive anticodon loop conformation observed in all structures of tRNA bound to the TRMT10C/SDR5C1 complex.[2] Hypo m^1A modification in tRNAs has also been proposed to play a role in Alzheimer's disease pathogenesis.[67]

m^1A22-tRNA Modification in Pathogenic Bacteria

TrmK is well-conserved among pathogenic bacteria, such as *Vibrio cholerae*, *Listeria monocytogenes*, *S. aureus*, and *Streptococcus pneumoniae*, and displays high sequence identity across the family. TrmK is essential for the survival and growth of *S. aureus*[68,69] and *S. pneumoniae*[70] and has no homologue in mammals,[28] making TrmK a promising target for the development of novel antibiotics. Indeed, disruption of the TrmK-encoding gene prevents *S. aureus* survival during infection of the bloodstream, vitreous fluid, and cutaneous abscesses in mice.[68] Moreover, the TrmK-encoding gene is expressed almost constitutively in cell models of infection,[71] during acute and chronic osteomyelitis,[72] and in the highly infectious and multidrug-resistant USA300 strain of *S. aureus* during infection.[73] In silico screening of compounds has identified a cryptic binding pocket in the vicinity of the SAM-binding site,[34] and SAM-adenine bisubstrate analogues have been proposed to serve as scaffolds to target TrmK,[74] laying foundations for future structure-based drug discovery. The challenge lies in specifically targeting TrmK, whose catalytic domain structure is shared by all class I MTases. Such specific inhibition by small molecules has been demonstrated both in vitro and in vivo for METTL3.[75]

m^1A58-tRNA Modification in Humans

TRMT6 and TRMT61A are highly expressed in advanced hepatocellular carcinoma and are negatively correlated with survival. TRMT6/TRMT61A increases the level of m^1A methylation of a subset of tRNAs, which promotes PPARδ protein translation. This triggers cholesterol biogenesis to activate Hedgehog signaling, leading to self-renewal of carcinoma stem cells and tumor development.[76] Drugs that specifically decrease the levels of m^1A58 in tRNAs by blocking the interaction between TRMT6 and TRMT61A have been shown to markedly inhibit tumor growth. Their combination with PPARδ antagonists has a synergetic effect on the inhibition of liver cancer development and tumor growth.[76] In urothelial carcinoma of the bladder, a higher expression of TRMT6/TRMT61A leads to a higher level of m^1A modification in tRNA-derived fragments involved in the regulation of the unfolded protein response. m^1A attenuates the gene-silencing normally promoted by these fragments. This reveals a mechanism of regulation of gene expression by changing the tRNA-derived fragment base modification.[77] Lastly, to protect the host from pathogens, $CD4^+$ T cells must be able to respond and reprogram themselves rapidly. This critical process is achieved through the timely synthesis of a large number of functional proteins. Upon T cell activation, TRMT6/TRMT61A levels increase rapidly, concomitant with enhanced expression of a subset of tRNAs modified with m^1A58. This is required to ensure the efficient translation of a group of key proteins for T cell proliferation. This cellular control of global translation might also be used by other proliferating cells, such as cancer cells.[78]

CONCLUSIONS AND FUTURE OUTLOOK

A general trend that emerges is that the m^1A modification is clustered in the core of tRNA on the D- or T-arms and that m^1A tRNA MTases modify adenosine involved in non-WC base pairing. Different sets of MTases are used to modify different positions, and the tRNA recognition by the MTase is completely different. In the case of m^1A9 modification, the MTase TRMT10C is used as a trap for the tRNA to present it for successive maturation steps with the first step, the processing by MRPP3, which requires the TRMT10C/SDR5C1/tRNA complex as a substrate. The progress made in solving RNA/protein complexes with cryo-electron microscopy in combination with AlphaFold[79,80] for the prediction of enzyme structures should provide high-resolution structures of RNA maturation complexes.[2,81,82] We anticipate

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that many enzymes involved in RNA modification that have been crystallized alone or with their cofactor SAM but have so far remained reluctant to let us glimpse how they recognize and modify their RNA substrate will reveal their secrets in the coming years. We believe that structures of m^1A MTases in complex with their tRNA substrates and accompanying thorough studies on the m^1A mechanism would greatly benefit the field going forward.

The question of the temporality of modification events in tRNAs is a long-standing question that has been examined directly or indirectly in a few studies (see, for instance, refs 83−85). The interdependence of certain tRNA modification events (reviewed in refs 42−44) strongly indicates a step-by-step nature of at least some parts of the tRNA maturation process. However, the direct detection of RNA modification events during tRNA biosynthesis is a technical challenge. As a consequence, a precise description or even a global picture of the stepwise modification pathways remains difficult to assemble. Importantly, modifications characterized as early ones in certain tRNAs may occur at a later time-point in others, as described in this Account with the case of m^1A58 in yeast elongator and initiator tRNAs.[4] Methods and investigations such as those described above on yeast $tRNA^{Phe}$[3] but performed on other systems and/or combined with different methodologies are thus essential to obtain a more detailed description of the modification steps and cross-talks that occur during tRNA maturation.

tRNA maturation not only concerns the incorporation of chemical modification but also includes the processing of pre-tRNA extremities, the splicing of introns, if any, the addition of the 3′CCA sequence, and aminoacylation. To get a full picture of tRNA maturation, future investigations should aim to link all of the different maturation events in order to understand how chemical modifications and/or modifying enzymes influence other maturation processes. Demethylation of m^1A tRNA modification has been shown for m^1A9[86] and m^1A58,[87] and these events must also be taken into account in a global understanding of modification implication in cellular processes.

More and more studies point to tRNA MTases as potential targets for diagnosis and therapy. The challenge in the future will be to be able to specifically target one tRNA MTase without affecting the methylation of other positions in tRNA.

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Notes

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