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The epitranscriptome of small non-coding RNAs

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ABSTRACT

Small non-coding RNAs are short RNA molecules and involved in many biological processes, including cell proliferation and differentiation, immune response, cell death, epigenetic regulation, metabolic control. A diversity of RNA modifications have been identified in these small non-coding RNAs, including transfer RNAs (tRNAs), microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small nuclear RNA (snRNA), small nucleolar RNAs (snoRNAs), and tRNA-derived small RNAs (tsRNAs). These post-transcriptional modifications are involved in the biogenesis and function of these small non-coding RNAs. In this review, we will summarize the existence of RNA modifications in the small non-coding RNAs and the emerging roles of these epitranscriptomic marks.

1. Introduction

More than 150 types of chemical modifications have been documented in RNAs, and these post-transcriptional modifications play profound roles in influencing RNA biogenesis and maintaining their biological functions [1–5]. RNA modifications are involved in many aspects of RNA metabolism including transcription, splicing, localization, stability, and translation through affecting RNA structure, base-pairing properties of RNA, and interactions with other molecules. Development of detection technologies and functional studies of those RNA modifications give rise to an emerging research field called epitranscriptome or RNA epigenetics [6–8].

Small non-coding RNAs are RNA molecules with a length of 18–200 nucleotides (nt), including transfer RNAs (tRNAs), microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small nuclear RNA (snRNA), small nucleolar RNAs (snoRNAs), and tRNA-derived small RNAs (tsRNAs) [9,10]. These small non-coding RNAs participate in a multitude of distinct biological processes including RNA transcription, processing, RNA decay, and translation. Besides, these regulatory small non-coding RNAs are also involved in various physiological and pathological processes, including tumorigenesis, neuron development and metabolic disorders [11–13]. Benefiting from the development of sensitive and robust RNA modification detection technologies, a diverse set of chemical modifications are identified in small non-coding RNAs [7,14](Fig. 1). Emerging evidence shows that these chemical modifications play pivotal roles in maintaining the proper structure and function of these small non-coding RNAs [15]. As the function of modifications in tRNAs has been well-summarized by several excellent reviews [16,17], here we will mainly focus on the epitranscriptome of miRNAs, piRNAs, snRNA, snoRNAs, and tsRNAs.

2. Chemical modifications in miRNA

MicroRNAs (miRNAs), with a length of ~22 nucleotides (nt), play important roles in regulating gene expression [18–21]. The DNA sequences are first transcribed to primary-microRNAs (pri-miRNAs) and further processed into precursor-microRNA (pre-miRNAs) by DGCR8/Drosha [20]. Then the pre-miRNAs are exported to the cytoplasm and cleaved by Dicer to release miRNA duplex. One strand of miRNA duplex associates with Argonaute (AGO) protein and forms RNA-induced silencing complexes (RISCs) to repress translation or promote mRNA decay [18].

Several post-transcriptional modifications have been identified in miRNAs or miRNA precursors and are involved in affecting their biogenesis and function [22–24]. N^6-methyladenosine (m^6A) is one of the most prevalent RNA modifications in eukaryotic cells and it influences miRNA biogenesis and function from several distinct aspects. For example, recent studies have shown that m^6A methylation on

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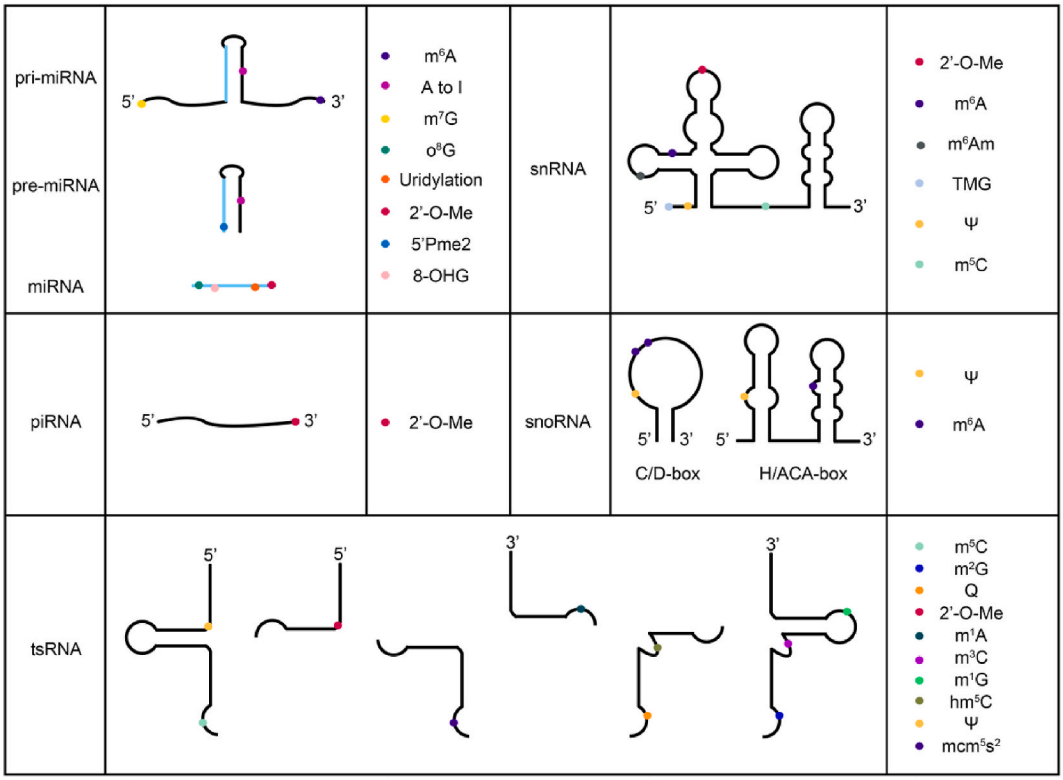

Fig. 1. Schematic depicting small non-coding RNAs and RNA modifications. Here, we show several small non-coding RNAs' secondary structure and mark the major modifications with different colored circle. Modification types are listed aside. Pri-miRNA, primary miRNA; pre-miRNA, miRNA precursor; miRNA, microRNA; piRNA, PIWI-interacting RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; tsRNA, transfer RNA-derived small RNA; m^6A, 6-methyladenosine; A to I, adenosine to inosine editing; m^7G, 7-methylguanidine; o^8G, 8-oxoguanine; Uridylation, addition of non-templated uridine(s) to the 3′ end of RNAs; 2′-O-Me, 2′-O ribose methylation; 5′Pme2, 5′ phosphodimethylation; 8-OHG, oxidatively modified 8-hydroxyguanosine; m^6Am, $N^{6,2'}$-O-dimethyladenosine; TMG, 2,2,7-trimethylguanosine; Ψ, pseudouridylation; m^5C, 5-methylcytosin; m^2G, 2-methylguanidine; Q, Queuosine; m^1A, N^1-methyladenine; m^3C, N^3-methylcytidine; m^1G, N^1-methylguanine; hm^5C, 5-hydroxymethylcytosine; mcm^5S^2, 5-methoxycarbonylmethyl-2-thiouridine.

pri-miRNAs enhances DGCR8 recognition and binding to its substrates, and thus promotes the maturation of miRNA [25–27]. Mechanistically, further study showed that m^6A-methylated pri-miRNA can recruit HNRNPA2B1, which facilitates the binding of DGCR8 to the methylated transcripts [28]. In addition, m^6A methylation in pre-miRNA affects the cleavage of Dicer and consequently promotes mature miRNAs biogenesis [29]. On the other hand, miRNAs can also regulate m^6A methylation by modulating METTL3 binding to mRNAs [30]. m^6A methylations are involved in several pathological processes through affecting the biogenesis or stability of the miRNAs that targeting oncogenes or tumor suppressors. For instance, m^6A on pre-miR-143-3p and pri-miR221/222 promotes the biogenesis of the corresponding mature miRNAs, and further promote brain metastasis and bladder carcinogenesis, respectively [27,29,31,32]. Non-templated uridine(s) addition is another important modification occurring in miRNA. Mono-uridylation and oligo-uridylation of miRNA can have both positive and negative effects on its biogenesis, respectively [33,34]. For instance, Mono-uridylation of let-7 and miR-105, catalyzed by TUT7/4/2, promotes subsequent Dicer processing and increases the mature microRNA levels [35,36]. However, TUT4-mediated 3′ RNA poly-uridylation blocks the biogenesis of let-7 miRNA [37–39]. Notably, let-7 miRNAs act as tumor suppressors by negatively regulating numerous oncogenes including c-Myc and RAS, and hence uridylation also participate in miRNA-mediated tumorigenesis [40–42]. Besides, ADARs-mediated Adenosine to Inosine (A to I) editing is also prevalent in mature miRNA and miRNA precursors [43,44]. A-to-I editing in miRNA precursors can interfere with both cleavage by Drosha/Dicer and AGO loading, and hence affects miRNA biogenesis. RNA editing events in mature miRNA alter miRNA-mRNA interaction and thus affect miRNA targeting specificity and efficiency [45]. miRNA editing plays important roles in both physiological and pathological process, including postnatal development, aging and metabolic processes [46–48]. For instance, the increased A to I editing level on miR-381 and miR-376b influences their targeting specificity, and thus affect dendritic outgrowth [47]. Besides, downregulation of the editing level on miR-589 promotes glioblastoma cancer cell migration and invasion [49].

Except these well-documented RNA modifications, several novel modifications are also identified in miRNAs, including N^7-methylguanosine (m^7G), 2′-O-methylation (2′-O-Me), 5′ phosphodimethylation (5′Pme2) and 8-oxoguanine (o^8G). Owing to the development of several m^7G sequencing technologies, m^7G methylation sites are identified in miRNA [50–52]. And the modified sites, catalyzed by METTL1, are located in a subset of miRNAs including let-7 family miRNAs. m^7G methylation destroys the secondary structure within the pri-miRNA transcript and thus regulates miRNA biogenesis and structure [51]. In plants and drosophila, 2′-O-methylation exists in the 3′-terminal of miRNAs, which is catalyzed by a methyltransferase HEN1, and this is a crucial step in miRNA biogenesis [53,54]. In addition, 2′-O-methylation has also been identified in the 3′-terminal of mammalian miRNAs and plays an important role in enhancing miRNA stability and association with AGO2 [55]. Although HENMT1, a HEN1 homologue, has been identified in mammalian cells, whether it is responsible for the 2′-O-methylation in mammalian miRNAs remains unknown. 5′Pme2 in miRNAs is catalyzed by BCDIN3D and negatively regulates miRNA maturation by antagonizing the Dicer-dependent processing [56]. 8-oxoguanine (o^8G) is identified in seed regions (positions 2–8) of selective miRNAs such as miR-1 and interferes the interaction between miRNA and targeting mRNA through o^8G•A base pairing. This

position-specific oxidation of miRNAs serves as a post-transcriptional mechanism to coordinate pathophysiological redox-mediated gene expression [57]. Excessive reactive oxygen species (ROS) can cause cell-damaging effects through oxidatively modifying guanine to 8-hydroxyguanine (8-OHG) in miRNAs. Upon oxidatively modified, miR-184 associates with the 3′ UTRs of Bcl-xL and Bcl-w, leading to the initiation of apoptosis, which is related to cancers, aging, and neurodegenerative and cardiovascular diseases [58].

Except the direct roles of RNA modifications in miRNAs, recent studies revealed that some RNA modifying enzymes can function independently of their catalytic activity. For instance, the pseudouridine synthase, PUS10, promotes miRNA biogenesis via enhance interaction between pri-miRNAs and microprocessor proteins [59]; Another pseudouridine synthase, TRUB1 functions in a similar manner and regulates the maturation of let-7 miRNA [60]. Whether there are other RNA modification related proteins function in the similar manner are still worth to be investigated.

3. Chemical modifications in piRNA

piRNAs, with 23–32 nt in length, is a kind of animal-specific small non-coding RNAs originating from single-stranded precursors [61–63]. Ranging from hydra to humans, the ancestral function of piRNAs is silencing transposons in the germ line [62,64]. Mosquitoes also use piRNAs to fight against viral infections in the soma [65].

piRNAs are methylated by an S-adenosylmethionine (SAM)-dependent methyltransferase (Hen1 in flies; HENN-1 in worms; HENMT1 in mice) [66–69], and forming 2′-O-methylation (2′-O-Me) at the 3' ends of pre-piRNA, which influences piRNA biogenesis [70–74]. In detail, it has been reported that Hili, one of the human PIWI proteins, exhibits a higher binding affinity to RNAs containing 2′-OCH_3 but not 2′-OH. And thus, RNA harboring 2′-O-Me tends to the formation of piRISC (piRNA-induced silencing complexes) [75,76]. This character is also supported by the studies of homologues of Hili in other species including Siwi (silkworm) and Miwi (mouse) [75,77]. 2'- O–Me as a defining feature of piRNA, which improves stability of piRNA by protecting piRNA from non-templated nucleotide addition and RNA decay [70,71,78]. Although the link between piRNA and cancers has been established, the role of 2'-O-Me in these pathological processes remains unclear and needs to be further explored [79].

4. Chemical modifications in snRNA

Small nuclear RNAs (snRNAs), with approximately 150 nt in length in mammals, are critical components of the spliceosome and involved in RNA splicing [80,81]. RNA-RNA and RNA-protein interactions between snRNA and other molecules are essential to correctly position the spliceosome on substrate pre-mRNAs [82].

The most abundant modifications in snRNAs are pseudouridine (Ψ) and 2′-O-Me, and majority of these modifications are catalyzed in RNA-dependent manners by box H/ACA RNP and box C/D RNP in eukaryotes [83]. Moreover, RNA-independent pseudouridine synthases, including PUS1 and PUS7, are also responsible for several Ψ sites in snRNA [84–87]. Pseudouridylation often occurs in the conserved and functional regions of snRNA, indicating that Ψ can influence snRNA function. For instance, both the mammalian cell free system and the Xenopus oocyte reconstitution system showed that Ψ in U2 snRNA are necessary for complete snRNP assembly and further pre-mRNA splicing [88–91]. Besides Ψ and 2′-O-Me, m6A and $N^{6,2\prime}$-O-dimethyladenosine (m^6Am) are also present in several snRNAs. m^6A methylation at position 43 in mammalian U6 snRNA is catalyzed by METTL16 and plays an important role in facilitating efficient recognition of the splice sites [92–97]. Also, m^6Am30 in U2 snRNA, catalyzed by METTL4, is important for proper pre-mRNA splicing and lack of m^6Am in U2 snRNA leads to over 1000 significantly altered splicing events [98,99]. Besides, several other post-transcriptional modifications have also been identified in snRNAs, including 5-methylcytosine (m^5C) [100], 5′ γ-monomethyl cap in nascent U6 transcript catalyzed by MEPCE [101], 3′ end oligouridylation catalyzed by TUTase [102], and 2,2,7-trimethylguanosine (TMG) catalyzed by TGS1. TMG cap also regulates pre-mRNA splicing and depletion of *tgs1* leads to embryo lethality in mice and Drosophila [103].

5. Chemical modifications in snoRNA

snoRNAs, with a length of 60–300 nt, are crucial small non-coding RNAs in all eukaryotic organisms and guides posttranscriptional modifications on ribosomal (rRNAs) and snRNAs [104,105].

Classified by sequences and binding proteins, there are two types of snoRNA, including box C/D snoRNA (core proteins including FBL, NOP56, NOP58, and SNU13) and box H/ACA snoRNA (core proteins including DKC1, NHP2, NOP10, and GAR1). Box C/D snoRNAs form a closed loop (boxC/D), which contained special conserved RUGAUGA and CUGA motifs, and box H/ACA snoRNAs is usually composed by two stem-loops which is linked by the H box (ANANNA motif) and an ACA sequence near the 3' end [106,107]. Six Ψ sites were identified in snoRNAs, with four within C/D box snoRNAs, and two in H/ACA box snoRNAs. All four Ψ sites in C/D box snoRNAs occurred in the 5′ terminus of the snoRNA guiding sequence, but the function of these pseudouridylations is still unknown [87]. m^6A methylations are also identified in snoRNAs, which can be demethylated by the mRNA demethylase FTO, and impacts box C/D snoRNP assembly through affecting the formation of trans Hoogsteen-sugar A·G base pairs [108–110].

6. Chemical modifications in tsRNA

tsRNAs are a class of small non-coding regulatory RNAs, derived from tRNA. Emerging studies elucidated that tsRNAs take an important part in post-transcriptional regulation [111–114]. Ranging from prokaryotes to eukaryotes, many tsRNAs have been discovered in diverse species with the advent of deep-sequencing [115–117]. Originating from mature tRNAs or precursor tRNAs, tsRNAs play distinct roles in various biological processes, including interactions with proteins or RNAs, translation inhibition, chromatin regulation, and epigenetic modifications [118,119].

As derived from the heavily modified tRNAs, tsRNAs also harbor many post-transcriptional modifications [120–122]. Among these modifications, m^5C methylation has been well studied and demonstrated to influence both tsRNAs biogenesis and biological functions. tiRNAs are generated by stress-induced tRNA cleavage mediated by endonuclease angiogenin (ANG). m^5C38 in tRNA are methylated by DNMT2 and m^5C34, m^5C48, m^5C49, or m^5C50 are catalyzed by NSUN2 [123–126]. And the biogenesis of tiRNA can be affected by m^5C methylation which inhibits ANG binding to the methylated tRNA and therefore increases tRNA stability [127]. Hence, the lack of m^5C leads to the accumulation of tiRNAs, and further reduced protein translation rate and codon mistranslation [128,129]. In addition, RNA modifications also influence tsRNA function. For instance, recent studies showed that, tsRNAs are involved in paternal epigenetic memory [130,131], and DNMT2-mediated m^5C and m^2G methylation are essential for this process [130,132]. Besides, TET2, the dioxygenase, which oxidizes m^5C to hm^5C, can also regulate distinct types of tsRNA processing [133].

Moreover, other prevalent methylations in tRNAs including N^1-methyladenine (m^1A), N^3-methylcytidine (m^3C), and N^1-methylguanine (m^1G) [134–136], also inhibit ANG-mediated tRNA cleavage and further decrease tsRNA production. Knockout of m^1A and m^3C demethylase ALKBH3 leads to hypermethylated tRNAs and decrease the abundance of 5′-tsRNAs [134]. TRMT10A deficiency leads to m^1G9 hypomethylated tRNA and further induces the accumulation of $tsRNA^{Gln}$ [135]. Another abundant tRNA modification, Ψ, is also involved in tsRNA biogenesis and function. PUS7 mediated Ψ8 promotes 18 nt 5'terminal

Table 1
The epitranscriptome of small non-coding RNAs.

SncRNAs Species	Described Chemical Modifiction	Writers	Readers
miRNA	m^6A	METTL3/ METTL14	HNRNPA2B1/ HNRNPC
	m^7G	METTL1	/
	2′-O-Me	HEN1	/
	5′Pme2	BCDIN3D	/
	Uridylation	TUT7/4/2	/
	A to I	ADARs	/
	o^8G	/	/
	8-OHG	/	/
piRNA	2′-O-Me	HEN1	
snRNA	Ψ	Box H/ACA RNP/ Pus1 and Pus7	/
	2′-O-Me	Box C/D RNP	/
	m^6A	METTL16	/
	m^6Am	METTL4	/
	TMG	TGS1	/
	m^5C	/	YPS
snoRNA	Ψ	Box H/ACA RNP	/
	m^6A	/	/
tsRNA	m^5C	DNMT2/ NSUN2	/
	m^2G	DNMT2	/
	Q	QTRT1/QTRT2	/
	2′-O-Me	TRM7/FTSJ1	/
	m^1A	TRMT6/61A	/
	m^3C	METTL2/ METTL6	/
	m^1G	TRMT10A	/
	hm^5C	TET2	/
	Ψ	PUS7	/
	mcm^5S^2	/	/

oligoguanine tRF-5s (mTOGs) biogenesis. Besides, the mTOGs harboring Ψ also represses translation initiation by displacing eIF4A/G from the m^7G cap, which further impacts hematopoiesis [137].

Besides these abundant modifications, several other chemical modifications are also involved in regulating tsRNA biogenesis. Queuosine (Q) is a hyper-modified 7-deaza-guanosine occurring at the wobble anticodon position 34 of four tRNAs ($tRNA^{His}$, $tRNA^{Asn}$, $tRNA^{Tyr}$, and $tRNA^{Asp}$). Q in tRNA is catalyzed by the heterodimeric enzyme QTRT1/QTRT2 and can protect its cognate tRNA against ANG cleavage [138]. An unanticipated role of Q34 modification is that it promotes DNMT2-mediated m^5C38 methylation in $tRNA^{Asp}$, revealing the crosstalk between distinct modifications [139,140]. SNORD97 and SCARNA97 mediated Cm34 in human elongator $tRNA^{Met}$ can also prevent site-specific cleavage of ANG and reduce tsRNA production [141]. In addition, 5-methoxycarbonylmethyl-2-thiouridine (mcm^5S^2) at the anticodon wobble position U34 plays the opposite role in tsRNA biogenesis which enhances the cleavage activity of tRNA endonuclease γ-toxin in yeast [142]. As more than 90 chemical modifications occurs in tRNA, whether there are other chemical modifications and modifying enzymes involved in regulating tsRNA biogenesis and function is still needed to be elucidated.

7. Summary

Until now, enriched knowledge of chemical modifications in small non-coding RNAs have been achieved. And for a certain chemical modification, it can occur in various RNA species, nevertheless the writer protein (modifying enzyme) and reader protein (binding protein) may be distinct, which are mainly dependent on the catalytic machinery and binding properties of the related proteins (Table 1). Therefore, the same chemical modification may play distinct roles in different RNA species.

To comprehensively understand the epitranscriptome of small non-coding RNAs, much work is still needed. Firstly, robust RNA modification detection technologies for small non-coding RNAs are urgent to be developed to identify novel types and precise positions of chemical modifications. As most existing RNA modification sequencing technologies use the polyA-enriched RNA or small RNA depleted RNA as the starting materials, detection technologies for the low abundance of small non-coding RNAs are still lack. Secondly, as several distinct chemical modifications have been identified in small non-coding RNAs, the crosstalk between those chemical modifications are needed to be elucidated. Lastly, emerging evidence has shown that the catalytic-independent functions of RNA modifying enzymes participate in the regulation of small non-coding RNA biogenesis, and cautions are needed to be taken to distinguish the non-canonical roles of modification enzymes and RNA modifications. Collectively, we summarize our current knowledge of these intriguing RNA modifications in small non-coding RNA functions, and we believe that a more comprehensive understanding of those epitranscriptomic marks in small non-coding RNA will spring up in the future.

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Declaration of competing interest

The authors declare no conflict of interest.

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