Beyond rRNA and snRNA: tRNA as a 2′-O-methylation target for nucleolar and Cajal body box C/D RNPs

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Box C/D small nucleolar RNAs (snoRNAs) and small Cajal body (CB) RNAs (scaRNAs) form ribonucleoprotein (RNP) complexes to mediate 2′-O-methylation of rRNAs and small nuclear RNAs (snRNAs), respectively. The site of methylation is determined by antisense elements in the box C/D RNAs that are complementary to sequences in target RNAs. However, numerous box C/D RNAs in mammalian cells lack antisense elements to rRNAs or snRNAs; thus, their targets remain unknown. In this issue of *Genes & Development*, Vitali and Kiss (pp. 741–746) demonstrate that "orphan" nucleolar box C/D snoRNA SNORD97 and CB box C/D scaRNA SCARNA97 contain antisense elements that target the wobble cytidine at position 34 of human elongator tRNA^{Met}(CAT) for 2′-O-methylation (C_{34}m). C_{34}m is jointly mediated by SNORD97 and SCARNA97 despite their apparently different intranuclear locations. Furthermore, the investigators demonstrate that C_{34}m prohibits site-specific cleavage of tRNA^{Met}(CAT) into tRNA fragments (tRFs) by the stress-responsive endoribonuclease angiogenin, thereby uncovering a role for SNORD97 and SCARNA97 in the biogenesis of tRFs, which modulate a diverse set of cellular functions in human health and disease.

Box C/D small nucleolar RNAs (snoRNAs) and small Cajal body (CB) RNAs (scaRNAs) target rRNAs and small nuclear RNAs (snRNAs), respectively, for 2′-O-methylation. However, many "orphan" snoRNAs and scaRNAs lack antisense elements to these RNAs, and therefore their targets remain unknown. In this issue of *Genes & Development*, Vitali and Kiss (2019) performed bioinformatic analyses of human RNAs to identify targets of two such orphan box C/D RNAs that share conserved sequence similarity: SNORD97 and SCARNA97. The analysis predicted that SNORD97 and SCARNA97 target the C_{34} wobble position of human tRNA^{Met}(CAT) for 2′-O-methylation (C_{34}m).

To investigate this prediction, Vitali and Kiss (2019) used a primer extension assay on partially alkaline hydrolyzed RNA, since Cm blocks alkaline hydrolysis. Human HAP1 cells lacking either SNORD97 or SCARNA97 displayed partial reduction of tRNA^{Met}(CAT) C_{34}m modification. Furthermore, lack of both SNORD97 and SCARNA97 elicited near-complete C_{34}m inhibition, which could be partially rescued by either SNORD97 or SCARNA97 overexpression. The findings document a coordinated role of these two box C/D RNAs in C_{34}m modification of tRNA^{Met}(CAT) (Fig. 1). Although box C/D ribonucleoproteins (RNPs) mediate 2′-O-methylation of elongator tRNA^{Met}(CAT) in archaea (Joardar et al. 2011), Vitali and Kiss (2019) provide the first evidence of a role for box C/D RNPs in tRNA modification in eukaryotes.

It has long been known that nucleoside modifications can either protect or promote tRNA cleavage. For example, the *Escherichia coli* plasmid-encoded colicin E5 acts as a tRNA-specific RNase that cleaves tRNA^{Tyr}(QUA) at the queuosine (Q)-modified wobble position (Ogawa et al. 1999). In eukaryotes, the yeast *Kluyveromyces lactis* secretes a toxin, zymocin, that cleaves tRNA$^{Glu}_{UUC}$, tRNA$^{Lys}_{UUU}$, and tRNA$^{Gln}_{UUG}$, 3′ to the wobble nucleoside when modified to 5-methoxycarbonylmethyl-2-thiouridine but not in its absence (Lu et al. 2005). Vitali and Kiss (2019) demonstrate that SNORD97- and SCARNA97-mediated 2′-O-methylation of human tRNA^{Met}(CAT) is a new example of a tRNA modification that regulates tRNA cleavage. When human HAP1 cells deficient in either SNORD97, SCARNA97, or both were treated with arsenite to induce oxidative stress and activation of the stress-responsive endoribonuclease angiogenin, tRNA^{Met}(CAT) 3′ fragments accumulated. Increase in 3′ tRNA fragment (tRF) levels was prohibited when SNORD97 and SCARNA97 levels were restored or upon

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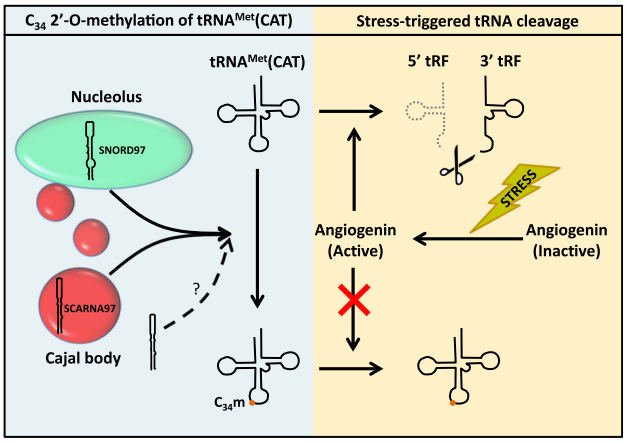

Figure 1. SNORD97 and SCARNA97 cooperatively 2′-O-methylate tRNA^{Met}(CAT), protecting it from stress-triggered angiogenin cleavage. SNORD97 and SCARNA97 localize to the nucleolus (green) and CBs (red), respectively. Upon RNP formation, SNORD97 and SCARNA97 act together to 2′-O-methylate tRNA^{Met}(CAT) at C34 (C_{34}m; orange circle). C_{34}m protects tRNA^{Met}(CAT) from cleavage into 5′ and 3′ tRNA fragments (tRFs) in response to stress-triggered activation of angiogenin.

addition of an angiogenin small molecule inhibitor. Therefore, C_{34}m modification protects tRNA^{Met}(CAT) integrity in response to stress. Thus, these studies potentially link SNORD97 and SCARNA97 to a diverse set of cellular processes such as translational regulation, ribosome biogenesis, apoptosis, the immune response, epigenetic inheritance, tumorigenesis, and neurodegeneration (Anderson and Ivanov 2014).

The joint role of these box C/D RNAs in tRNA^{Met}(CAT) C_{34}m modification is particularly interesting, since SCARNA97 localizes to CBs (Jady et al. 2012) via a novel 93-nucleotide pyrimidine-rich sequence (Vitali and Kiss 2019). SNORD97 is primarily nucleolar (Vitali et al. 2003). Although the nucleolus and CB are distinct nuclear compartments, they can physically interact (Fig. 1; Trinkle-Mulcahy and Sleeman 2017). Therefore, SNORD97 and SCARNA97 could come in close proximity for coordinated tRNA^{Met}(CAT) modification. Alternatively, SNORD97- and SCARNA97-mediated tRNA^{Met}(CAT) C_{34}m modification may occur in a tRNA^{Met} subtype-specific manner. Of the nine or more human tRNA^{Met}(CAT) genes, some transcripts may be modified at nucleoli by SNORD97, and others may be modified by SCARNA97 at CBs. However, tRNA^{Met}(CAT) was not detected at nucleoli or CBs (Vitali and Kiss 2019). Although tRNA interactions within these nuclear compartments may be too transient to detect, it is also possible that the coordinated actions of SNORD97 and SCARNA97 occur in the nucleoplasm. In support of this possibility, Deryusheva and Gall (2019) demonstrated that scaRNA localization is not limited to CBs (Fig. 1), and localization of target RNA to specific nuclear compartments is not necessary for modification. Furthermore, snRNA modification occurs in cells where CB formation is inhibited (Deryusheva et al. 2012). However, according to possible nucleoplasmic C_{34}m tRNA modification, one might expect that overexpression of SNORD97 or SCARNA97 would completely suppress the absence of either, which was not detected. Future studies are necessary to understand the mechanism of coordinated SNORD97- and SCARNA97-mediated methylation of tRNA^{Met}(CAT) as well as the involvement of nucleoli and CBs, if any.

In conclusion, the orphan SNORD97 and SCARNA97 box C/D RNAs have now been "adopted," with tRNA^{Met}(CAT) identified as their target (Vitali and Kiss 2019). Discovery of an RNA species other than rRNA or snRNA as snoRNA and scaRNA targets is likely only the tip of the iceberg. Similar future bioinformatic approaches can be used to identify targets of other orphan box C/D RNAs, which may span a wide range of RNA species. Only with the discovery of new targets will we begin to fully appreciate the likely vast regulatory role of box C/D snoRNAs and scaRNAs in cellular biology.

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