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Small RNAs meet their targets: When methylation defends miRNAs from uridylation

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Small RNAs are incorporated into Argonaute protein-containing complexes to guide the silencing of target RNAs in both animals and plants. The abundance of endogenous small RNAs is precisely controlled at multiple levels including transcription, processing and Argonaute loading. In addition to these processes, 3’ end modification of small RNAs, the topic of a research area that has rapidly evolved over the last several years, adds another layer of regulation of their abundance, diversity and function. Here, we review our recent understanding of small RNA 3’ end methylation and tailing.

Introduction

Small silencing RNAs (small RNAs) of 20–30 nucleotide (nt) in length are key regulators of gene expression in both animals and plants. Together with their effector protein called Argonaute (AGO), small RNAs repress gene expression at either the transcriptional or the post-transcriptional level and play important roles in various biological processes, such as cell differentiation and transgenerational inheritance. Small RNAs can be classified into microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi (a class of AGO protein)-interacting RNAs (piRNAs) based on their origin, biogenesis and/or the type of AGO they are associated with, although the boundaries of this classification become blurred and sometimes even difficult to discern. Dicer-like proteins, a class of RNAseIII-type enzymes, release miRNAs and siRNAs from their precursors as duplexes with 2 nt 3’ overhangs at each end. By contrast, piRNAs are processed via a Dicer-independent pathway. More details regarding the biogenesis and function of small RNAs have been reviewed elsewhere. After biogenesis, plant small RNAs as well as some animal small RNAs are 2’-O-methylated at their 3’ end by HUA ENHANCER1 (HEN1) in plants or its homologues in animals. 2’-O-methylation is critical for small RNA stability. Besides methylation, small RNAs are also subject to 3’ untemplated nucleotide addition (tailing), which in turn affects their function and stability. In this point of view, we summarize our recent understanding on small RNA methylation and tailing and discuss their biological relevance.

Methylation of small RNAs

Plant miRNAs and siRNAs contain a 2’-O-methyl group at their 3’ end. This modification is catalyzed by HEN1. HEN1 contains 2 double-stranded RNA binding domains (dsRBD1 and dsRBD2), a La-motif containing domain (LCD) and a methyltransferase domain (MTase). HEN1 specifically recognizes 21–24 base pair (bp) dsRNAs with 2nt overhangs and deposits a methyl group to the 2’ OH position of the 3’ end in each strand in vitro. Structural analysis reveals that HEN1 recognizes dsRNAs via its dsRBD1 and dsRBD2 domains. The substrate length of HEN1 is determined by the distance between the LCD domain and the MTase domain. Loss of 2’-O-methylation in the

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small RNA deep sequencing analyses reveal that uridylation or adenylation (usually mono- or di-nucleotide additions) at the 3’ end of miRNAs is widespread and conserved across a variety of animal species. Uridylation of small RNAs is catalyzed by the terminal uridyltransferases (TUTase) (Fig. 1). While many TUTases show overlapping activities on some miRNAs, some of them act on miRNAs in a sequence-specific manner. Uridylation often serves as an RNA decay signal whereas adenylation appears to increase RNA stability. Besides stability, 3’ modification can also regulate small RNA activities. For example, uridylation of miR-26a prevents the miRNA from repressing its mRNA target, without affecting its abundance. PAPD4/GLD-2 mediated 3’ end adenylation has little effect on miRNA abundance but reduces their AGO loading efficiency. Interestingly, miRNAs derived from the 3’ arms of their pre-miRNAs are more frequently uridylated, suggesting that at least some of the uridylation occurs to the 3’ end of pre-miRNAs before they are channeled into Dicer processing. Indeed, mono-uridylation of group II premiRNAs (with a 1nt 3’ overhang instead of the canonical 2nt 3’ overhang after Drosha cleavage) is crucial for their further maturation. In C. elegans, 22G secondary siRNAs are not methylated and are extensively oligo-uridylation by CDE-1, a germline-specific nePAP. CDE-1-mediated uridylation of CSR-1 bound 22G siRNAs prevents their over-accumulation through active degradation, which is important for both mitotic and meiotic chromosome segregation.

Lack of HEN1 function causes extensive tailing and trimming of small RNAs in higher plants. An early study demonstrated that untemplated nucleotides added to the 3’ end of small RNAs in hen1 are predominantly uridines, which is subsequently shown to be the same in all other tested plant species. Hen1 is involved in an RNA repair process. In animals, HEN1 methylates single-stranded RNAs without affecting its abundance. Interestingly, miRNAs derived from the 3’ arms of their pre-miRNAs are more frequently uridylated, suggesting that at least some of the uridylation occurs to the 3’ end of pre-miRNAs before they are channeled into Dicer processing. Indeed, monouridylation of group II premiRNAs (with a 1nt 3’ overhang instead of the canonical 2nt 3’ overhang after Drosha cleavage) is crucial for their further maturation. In C. elegans, 22G secondary siRNAs are not methylated and are extensively oligo-uridylation by CDE-1, a germline-specific nePAP. CDE-1-mediated uridylation of CSR-1 bound 22G siRNAs prevents their over-accumulation through active degradation, which is important for both mitotic and meiotic chromosome segregation.

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evolutionarily distant plant species. The Chlamydomonas MUT68 adds adenosines to the 3’ end of 5′ CP of MAA7 (the endogenous target of siRNAs generated by a transgenic inverted-repete sequence) and promotes its degradation from 3′-to-5′, likely involving the action of the exosome. In a recent study, we show that HESO1 is responsible for the uridylation of 5′ CPs in Arabidopsis. A reduction in uridylation in heso1–2 increases the accumulation of 5′ CP, demonstrating that uridylation triggers degradation of 5′ CP. The proportion of 3′ truncated 5′ CPs is increased by heso1 relative to wild-type plants (WT), suggesting that uridylation may cause 5′ CP degradation through a mechanism different from 3′-to-5′ trimming. This resembles the effect of heso1 on miRNAs in hen1. In humans, uridylation can trigger decapping of histone mRNAs followed by 5′-to-3′ degradation. This may not be the case for 5′ CPs, as the status of 5′-to-3′ truncation of 5′ CPs is not altered by heso1. 5′ CPs can also be degraded by 5′-to-3′ and 3′-to-5′ trimming since both 5′ and 3′ truncated 5′ CPs exist. In xrn4, the amount of 5′ CPs is increased compared with that in WT, revealing that XRN4 is an enzyme responsible for the degradation of 5′ CPs although it is not clear whether it acts before or after uridylation. The enzymes that trim 5′ CPs from 3′-to-5′ remain to be identified.

How does uridylation trigger the degradation of small RNAs and 5′ CPs?

Both small RNAs and 5′ CPs at least in transient associate with the AGO proteins. This suggests that a common mechanism may be used to degrade both uridylated small RNAs and 5′ CPs. Agreeing with this notion, the exosome is responsible for the degradation of both uridylated small RNAs and adenylated 5′ CPs in Chlamydomonas. Thus, it is possible that a nuclease may simultaneously target both uridylated 5′ CPs and small RNAs in higher plants. Recently, Dis3L2, which is a 3′-to-5′ RNase II nuclease encoded by a paralog of RRP44, a core component of the exosome, was shown to degrade uridylated pre-let-7 (precursor of let-7 miRNA). In yeast, the Dis3L2 homolog can trigger rapid degradation of uridylated RNAs in a tail-length dependent manner. Arabidopsis SOV (SUPPRESSOR OF VARICOSE) is the closest homologous protein of Dis3L2. It is worth testing if SOV is the enzyme responsible for the degradation of uridylated small RNAs and 5′ CPs in the near future. HESO1 can add long U-tails (up to hundreds of nt long) to its substrates in vitro. However both miRNAs and 5′ CPs only contain short U-tails (less than 15 nt) in vivo. This provides an alternative explanation for the degradation of uridylated miRNAs and 5′ CPs: long...
Figure 2. A proposed model for target cleavage mediated small RNA destabilization. In this model, small RNA mediated target cleavage (A and B) but not translational inhibition (C) may provide a signal in the recruitment both tailing and trimming enzymes to AGO for the modification and further degradation of 5’ cleavage products. (A) HEN1 mediated 2’-O-methylation protects small RNAs from both tailing and trimming activities. (B) In the absence of HEN1, small RNAs become frequently tailed and trimmed. (C) In the case of partially complementary target RNA (e.g. animal miRNAs), small RNAs predominantly suppress gene expression via translational inhibition. In this scenario, tailing and trimming enzymes will be barely recruited to AGO and small RNAs are less tailed and trimmed, regardless of methylated or not. Green oval, AGO protein; Blue oval, translational machinery.

U-tail may dissociate miRNAs and 5’ CPs from the AGO1 complex, resulting in their rapid degradation such that no long-tailed products can be detected.

HEN1 protects small RNAs from AGO-associated tailing and trimming activities that normally acts on 5’ CPs

The dual activities of HESO1 and MUT68 on both small RNAs and 5’ CPs and the fact that both small RNAs and 5’ CPs associate with AGO proteins suggest that the terminal uridyl transferase may recognize their substrates in the AGO complex (Fig. 2). Several evidences demonstrate that HESO1 indeed uridylates AGO1-associated miRNAs. First, HESO1 associates with AGO1, as revealed by colocalization and co-immunoprecipitation assays.41 Second, HESO1 is able to uridylate AGO1-bound miR165/166 in vitro.42 Third, in hen1, AGO1-bound miRNAs are tailed.11 Fourth, suppression of AGO1 function in hen1 diminishes miRNA uridylation.11,41 We envision that HESO1 also recognizes siRNAs in vivo through its physical interaction with respective AGO proteins. Further analyses show that the PAP domain rather than the unconserved C-terminal domain of HESO1 interacts with the PAZ and PIWI domain of AGO1.41 These interactions may facilitate HESO1 to recognize its substrates, as PAZ binds the 3’ end of small RNAs, which can be released by base pairing between small RNAs and their targets, and PIWI cleaves the targets. In addition, the interaction of terminal uridyl transferases with AGO may be common among different species since PAP (Fig. 1), PAZ and PIWI are evolutionarily conserved.47

Both small RNAs and 5’ CPs are also subject to 3’-to-5’ trimming activities. By analogy, it is possible that the enzymes catalyzing 3’-to-5’ trimming may also associate with AGO proteins. In fact, Nibbler has been shown to interact with AGO in Drosophila.34 The tailing and trimming activities are likely recruited to AGO to eliminate the 5’ CPs. The presence of these AGO1-associated activities confers the necessity of small RNA methylation by HEN1 to ensure their stability in the AGO complex in plants (Fig. 2). In flies, AGO2-associated siRNAs are protected by methylation.16 In contrast, AGO1-associated miRNAs are not methylated16 and display very limited tailing and trimming. This may be due to the fact that animal miRNAs are less complementary to their targets.55 Consistent with this, introduction of artificial target RNAs with high complementarity to some endogenous miRNAs triggers their tailing and trimming.39,50 Moreover, siRNAs, which often have highly complementary targets, become tailed and trimmed when misloaded into AGO1.49,50 A possible explanation for these observations is that less complementarity between miRNAs and targets inhibits target cleavage, which may signal tailing and trimming. Alternatively, the extensive complementarity between small RNA and its target may help to release its 3’ end from PAZ protection.51

Conclusions and Perspectives

In addition to transcription and processing, 3’ end modification also contributes to the accumulation of small RNAs. We are just beginning to understand the underlying biochemical pathway of this process. While it is clear that methylation plays a crucial role in stabilizing small RNAs through antagonizing uridylation and trimming activities, future studies will be required to determine whether the degree of complementarity to targets or target cleavage serves as a signal for the recruitment of tailing and trimming enzymes. There are more challenges in this field. An immediate one is the identification of all the enzymes involved in the small RNAs modification and catabolic pathway, with those responsible for trimming and degradation in particular.
outstanding questions include how these enzymes are recruited, and more importantly, whether small RNA stability control can be regulated in response to any developmental or environmental signals. We believe that addressing these questions will greatly advance our understanding of the regulation of small RNAs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
7. References

www.landesbioscience.com RNA Biology 1103
37. Orban TI, Izaurralde E. Decay of mRNAs targeted by RISC requires XRN1, the Ski complex, and the exosome. RNA 2005; 11:459-69; PMID:15703439; http://dx.doi.org/10.1261/rna.7231505

38. Souter FF, Kastenmayer JP, Green PJ. AtXRN4 degrades mRNA in Arabidopsis and its substrates include selected mRNA targets. Mol Cell 2004; 15:173-83; PMID:15260969; http://dx.doi.org/10.1016/j.molcel.2004.06.006


42. Risland OS, Norbury CJ. Decapping is preceded by 3' uridylation in a novel pathway of bulk mRNA turnover. Nat Struct Mol Biol 2009; 16:616-23; PMID:19430462; http://dx.doi.org/10.1038/nsmb.1601


44. Ustianenko D, Hrossova D, Potesil D, Chalupnikova K, Hrzadilova K, Pachernik J, Cejlikova K, Udhrjjan S, Zdrabal Z, Vlaseova S. Mammalian DIS3L2 exonuclease targets the uridylated precursors of let-7 miRNAs. RNA 2013; 19:1632-8; PMID:24141620; http://dx.doi.org/10.1021/rn4000551.113


50. Ameres SL, Hung JH, Xu J, Weng ZP, Zamore PD. Target RNA-directed tailing and trimming purifies the sorting of endo-siRNAs between the two Drosophila Argonaute proteins. Rna 2011; 17:54-63; PMID:21106652; http://dx.doi.org/10.1261/rna.2498411
