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REVIEW

Regulation of small RNA stability: methylation and beyond

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As central components of RNA silencing, small RNAs play diverse and important roles in many biological processes in eukaryotes. Aberrant reduction or elevation in the levels of small RNAs is associated with many developmental and physiological defects. The *in vivo* levels of small RNAs are precisely regulated through modulating the rates of their biogenesis and turnover. 2′-*O*-methylation on the 3′ terminal ribose is a major mechanism that increases the stability of small RNAs. The small RNA methyltransferase HUA ENHANCER1 (HEN1) and its homologs methylate microRNAs and small interfering RNAs (siRNAs) in plants, Piwi-interacting RNAs (piRNAs) in animals, and siRNAs in *Drosophila*. 3′ nucleotide addition, especially uridylation, and 3′-5′ exonucleolytic degradation are major mechanisms that turnover small RNAs. Other mechanisms impacting small RNA stability include complementary RNAs, *cis*-elements in small RNA sequences and RNA-binding proteins. Investigations are ongoing to further understand how small RNA stability impacts their accumulation *in vivo* in order to improve the utilization of RNA silencing in biotechnology and therapeutic applications.

***Keywords*:** miRNA; siRNA; piRNA; methylation; HEN1; RNA silencing

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Introduction

As sequence-specific guides in RNA silencing in all eukaryotes, small RNAs play important and diverse roles in many biological processes (reviewed in [1-5]). Small RNAs are classified into three major types, microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs) based on differences in their precursors, biogenesis and protein partners [1, 4]. miRNAs are produced from *MIR* gene transcripts that form stem-loop structures [1, 4, 6]. Mature miRNAs are usually 21-24-nt-long and are generated through the processing of the stem-loop precursors by RNase III enzymes (Drosha and Dicer in animals; DICER-LIKE (DCL) in plants) [4, 6]. miRNAs target endogenous genes for mRNA cleavage, decay and/or translational repression [7]. siRNAs are derived from double-stranded RNA (dsRNA) precursors generated through sense and antisense transcription, transcription of inverted repeat elements, viral replication, or RNA-dependent RNA polymerase (RDR) activity that converts single-stranded RNAs into dsRNAs [2, 4, 8]. siRNAs are 21-24-nt-long and their biogenesis also requires the endonuclease activity of Dicer or DCL [2, 4]. siRNAs repress the expression of transposable elements (TEs) and transgenes, and mediate the cleavage of viral mRNAs [9, 10]. piRNAs, found specifically in animals, are usually 24-32-nt-long and processed from presumably single-stranded RNA precursors in a Dicer-independent manner ([11]; reviewed in [12]). Their biogenesis is composed of a primary processing pathway and a ping-pong amplification pathway [13, 14]. piRNAs are derived from TEs, intergenic regions, and certain genes. piRNAs derived from TEs silence the TEs through the cleavage of TE-derived transcripts or DNA methylation of the genomic loci to cause transcriptional silencing [13-16]. While miRNAs and siRNAs are bound by the Argonaute (Ago) sub-clade of Ago proteins, piRNAs associate specifically with the Piwi sub-clade of Ago proteins [12].

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Small RNA stabilization by methylation

The three types of small RNAs also vary in the re-

quirement for the 2′-*O*-methyl modification on the ribose of their 3′ termini. HUA ENHANCER 1 (HEN1) was first identified in *Arabidopsis* as a small RNA methyltransferase (MTase) that methylates miRNA and siRNA duplexes [17, 18]. HEN1 homologs were found to methylate small RNAs in other plants, piRNAs in animals and Ago2-associated small RNAs in *Drosophila* [11, 19-25]. In general, 2′-*O*-methylation serves as a protective mechanism against 3′-5′ degradation and 3′ uridylation of small RNAs [17, 21, 26].

Plant miRNAs and siRNAs are methylated by HEN1

In plants, the biogenesis of small RNAs, including miRNAs and all types of siRNAs, involves 2′-*O*-methylation at their 3′ termini [17]. The requirement for methylation was uncovered through the isolation of *Arabidopsis hen1* mutants. miRNAs and siRNAs are methylated at their 3′ termini in wild-type *Arabidopsis*, but not in *hen1* mutants [17] (Figure 1A). In *hen1* mutants, miRNAs show reduction in abundance and heterogeneity in sizes [17, 26], the latter of which was reflected by a ladder of bands when total RNAs were resolved on high-resolution acrylamide gels and hybridized with a probe for specific miRNA species. Because primer extension studies demonstrated that the heterogeneous species have identical 5′ ends, the ladder of signals probably represented heterogeneity at the 3′ ends [26]. Indeed, when particular miRNAs were cloned and sequenced from wild type and a *hen1* mutant, it became clear that miRNAs tended to acquire an oligonucleotide tail enriched for U residues in the *hen1* mutant [26] (Figure 2). The process of tailing miRNAs with uridine as the preferential nucleotide will be referred to as uridylation. miRNAs also become truncated from their 3′ ends in the *hen1* mutant (Figure 2), and truncated miRNAs can also be uridylated [26]. The uridylation of the unmethylated small RNAs perhaps leads to their 3′-5′ degradation, but this still needs to be confirmed genetically or biochemically. WAVY LEAF1 (WAF1), a HEN1 ortholog in rice, is also essential for the stabilization of small RNAs. The accumulation of miRNAs and *trans*-acting siRNAs, a type of endogenous

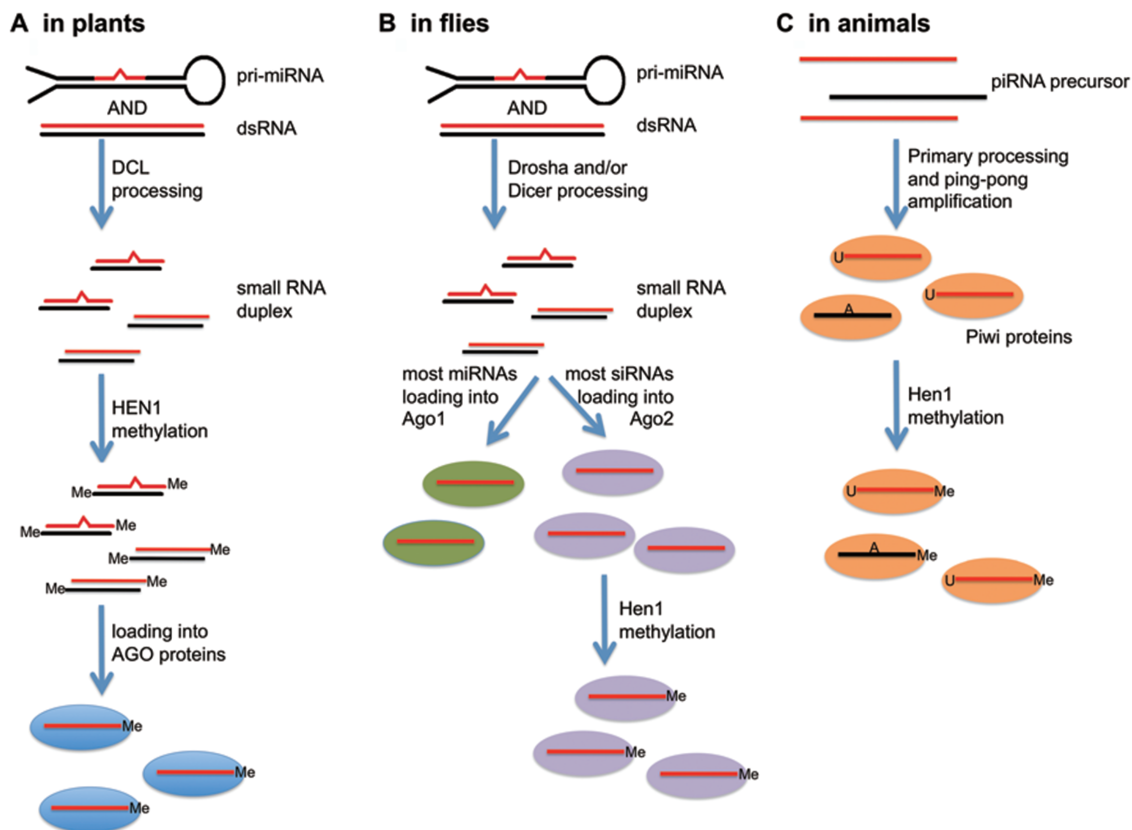


Figure 1 2′-*O*-methylation of small RNAs by HEN1. **(A)** Plant HEN1 methylates the 3′ termini of both strands of small RNA duplexes. **(B)** *Drosophila* Hen1 methylates Ago2-associated siRNAs, but not Ago1-associated miRNAs. **(C)** Animal Hen1 methylates piRNAs in germ cells. The ovals represent different Argonaute proteins.

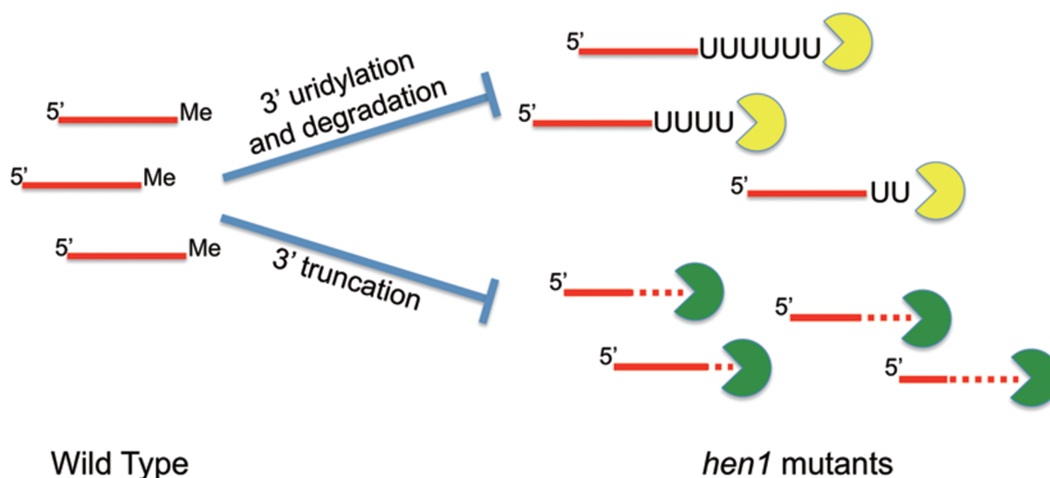


Figure 2 Methylation protects small RNAs from 3′ uridylation and truncation that lead to the degradation of the small RNAs.

siRNAs, is greatly reduced in *waf1* mutants [27].

Phylogenetic studies showed that the N-terminal three-fourth of *Arabidopsis* HEN1 is conserved only among the plant homologs, and the C-terminal domain, which constitutes approximately one-fourth of HEN1 and contains a recognizable S-adenosyl methionine (AdoMet)-binding motif [17], is conserved among many bacterial, fungal, and metazoan homologs [28] (Table 1). Bioinformatics analysis revealed that the MTase catalytic domain of HEN1 is closely related to small molecule MTases [28]. *In vitro* activity assays showed that recombinant *Arabidopsis* HEN1 acts on 21-24-nt small RNA duplexes and deposits a methyl group onto the 2′ OH of the 3′-terminal nucleotides of each strand [18]. The 2-nt overhang of the duplex, and the 2′ and 3′ OH of the 3′ nucleotide are two important features in the substrates of *Arabidopsis* HEN1 [18].

The mechanism of substrate recognition and methylation by HEN1 was revealed by the crystal structure of full-length HEN1 from *Arabidopsis* in complex with a 22-nt small RNA duplex. *Arabidopsis* HEN1 binds to the small RNA duplex substrate as a monomer [29]. The plant-specific N-terminal portion of HEN1 contains two dsRNA-binding domains (dsRBD1 and dsRBD2) and a La-motif-containing domain (LCD) [28, 29]. Both dsRBDs are involved in substrate recognition, and the substrate length specificity is determined by the distance between the MTase domain and the LCD, each interact-

Table 1 HEN1 homologs that methylate small RNAs

Name	Organism	Substrates	Mutant phenotypes
HEN1	*Arabidopsis*	Small RNA duplexes	Reduction of small RNA abundance; pleiotropic developmental defects
WAF1	Rice		Reduction of small RNA abundance; seedling lethality and pleiotropic defects
mHen1	Mouse	piRNAs	
DmHen1/Pimet	*Drosophila*	piRNAs, Ago2-assoicated small RNAs	Decrease of piRNA length and abundance; de-repression of piRNA targets; trimming and tailing of Ago2-associated siRNAs
Hen1	zebrafish	piRNAs	Defects in oocyte development; uridylation or adenylation of piRNAs; decrease of piRNA abundance; retrotransposon de-repression
Hen1p	*Tetrahymena thermophila*	scnRNAs	Reduction of scnRNA length and abundance; defects in programmed DNA elimination and production of sexual progeny

ing with one end of the small RNA duplex [29]. HEN1 methylates the 2′ OH of the 3′-terminal nucleotide in a Mg^{2+}-dependent manner [29]. Kinetic studies illustrated that HEN1 is catalytically efficient in the absence of any supplementary proteins. The enzyme modifies individual strands in succession to complete the methylation of the duplex [30]. Since the C-terminal portion of HEN1 (residues 666-942) efficiently modifies small RNA duplexes *in vitro* but exhibits weaker affinities for both small RNA duplexes and AdoMet (the methyl donor), the function of the N-terminal portion is to stabilize the catalytic complex [30].

Small RNA methylation by HEN1 in plants could be modulated *in vivo*. Several RNA-silencing suppressors from plant viruses, such as the *Beet yellows virus* 21 kDa protein (p21), the *Tomato bushy stunt virus* 19 kDa protein (p19) and the *Turnip mosaic virus*-silencing suppressor P1/HC-Pro interfere with small RNA methylation by HEN1 [31-33]. Interestingly, miRNA methylation defects in the weak *hen1-2* mutant can be partially suppressed by mutations in the endogenous 24 nt siRNA biogenesis pathway [34]. The suppression suggests that siRNAs compete with miRNAs for methylation when HEN1 function is partially compromised in *Arabidopsis* [34]. In addition, differences in phenotypic severity of the same *hen1* lesion in Col versus L*er* accessions implicate the existence of a negative modifier of HEN1 activity or small RNA activity in the Columbia background [34].

Animal piRNAs and fly siRNAs are methylated by HEN1 homologs

piRNAs are expressed in animal germlines and guide Piwi proteins to silence TEs (reviewed in [12]). piRNAs and *Drosophila* Ago2-associated siRNAs are 2′-*O*-methylated at their 3′ termini by HEN1 homologs in the animal kingdom [19, 21, 23, 25] (Figure 1B and 1C). Animal Hen1 proteins are less than half the size of the plant HEN1 proteins: they lack the plant-specific N-terminal portion [19, 21-23, 25, 35]. Most animal Hen1 proteins have a similar accumulation pattern as the Piwi proteins [21, 23, 25]. For example, mouse Hen1 is present specifically in testis as are piRNAs and Piwi proteins. Recombinant mouse Hen1 methylates single-stranded piRNAs *in vitro* [22, 23]. The zebrafish *Hen1* gene is expressed in both female and male germ lines, but is essential only for oocyte development and dispensable for testis development. The zebrafish Hen1 protein is localized to nuage, a germ cell-specific structure where Ziwi (one of the Piwi proteins in zebrafish) is localized [21]. In the testes of *hen1* mutants, retrotransposon-derived piRNAs become uridylated and reduced in abundance, and the retrotransposons are mildly de-repressed. Therefore, Hen1-mediated methylation stabilizes piRNAs for transposon silencing in ovary and testis [21].

piRNAs and Ago2-associated siRNAs in *Drosophila* are 2′-*O*-methylated at their 3′ termini [11, 19, 25]. Saito *et al.* [25] showed that loss of Pimet (piRNA MTase), the HEN1 homolog in *Drosophila*, results in the loss of 2′-*O*-methylation of piRNAs. Recombinant Pimet methylates single-stranded small RNA oligos *in vitro* and physically interacts with the Piwi protein [25]. The same HEN1 homolog in *Drosophila* was named DmHen1 by Horwich *et al* [19]. DmHen1 methylates the 3′ termini of Piwi-associated piRNAs and Ago2-associated siRNAs, but not Ago1-associated miRNAs. Without DmHen1, the length and abundance of piRNAs are decreased, and the levels of piRNA targets are elevated [19]. Horwich *et al*. [19] further showed that methylation occurs on single-stranded siRNAs in association with Ago2, which is also likely to be the case for piRNAs *in vivo*. In *Drosophila*, 2′-*O*-methylation of Ago2-associated endo-siRNAs protects the siRNAs from 3′ tailing and trimming [36]. In the absence of Hen1 activities, endo-siRNAs generally become 3′ truncated and/or acquire a 3′ tail. The most abundant nucleotide added is uridine, followed by adenine. Long tails are rare but are nearly always uridine tails [36] (Figure 2).

A HEN1 homolog is also present in the ciliated protozoan *Tetrahymena thermophila*, which contains two classes of small RNAs [35, 37]. The 28-29 nt scan (scn) RNAs are produced during sexual reproduction, and bind specifically to the Ago protein, Twi1p [38]. The dsRNA precursors of scnRNAs are generated from bidirectional transcription of non-coding DNA [39] and processed by the Dicer-like protein, Dcl1p [40, 41]. The ~23-24 nt RNAs may also be generated from dsRNA precursors and map to the genome in clusters that are antisense to predicted genes [37]. The scnRNAs are methylated by Hen1p (the *Tetrahymena* HEN1 homolog), which is co-expressed with Twi1p *in vivo* and physically interacts with Twi1p *in vitro* [35]. Loss of Hen1p causes a reduction in the abundance and length of scnRNAs, defects in programmed DNA elimination, and inefficient production of sexual progeny [35]. Recombinant Hen1p methylates single-stranded scnRNAs *in vitro*. Therefore, the stabilization of scnRNAs by Hen1p-mediated 2′-*O*-methylation ensures DNA elimination in *Tetrahymena* [35]. Intriguingly, approximately half of the ~23-24 nt sRNAs that resemble siRNAs from other organisms undergo non-templated 3′ nucleotide addition, with a single uridine as the most common addition [37]. Is this reminiscent of the 3′ tailing of unmethylated *Drosophila* endo-siRNAs? The effects of uridylation on the accumulation and activity of

these ~23-24 nt sRNAs await further investigation.

Damaged bacterial RNAs are methylated by HEN1 *in vitro*

Bacterial Hen1 is encoded by a two-gene operon that also encodes polynucleotide kinase-phosphatase (Pnkp), an RNA repair enzyme [42, 43]. Bacterial Hen1 methylates the 3′-terminal nucleotide of the 5′ fragment of a broken tRNA in a Mn^{2+}-dependent manner to protect it against further damage by a transesterifying endonuclease; then the Pnkp in the Hen1-Pnkp complex repairs the RNA substrate by end healing and sealing [43, 44]. The bacterial Hen1 MTase domain contains a core fold shared by other RNA and DNA MTases as well as motifs unique to bacterial Hen1 homologs. The interaction between bacterial Hen1 and their RNA substrates is likely similar to that of their eukaryotic counterparts [43].

Small RNA turnover mechanisms

Loss of HEN1 activity causes reduced accumulation of naturally methylated small RNAs, and studies of *hen1* mutants helped uncover previously unknown mechanisms that degrade small RNAs [17, 21, 26, 35, 36, 45]. These studies suggest that 3′ uridylation and 3′-5′ exonucleolytic degradation are two major mechanisms that turnover small RNAs when they lose methylation (Figure 2). Studies on naturally unmethylated small RNAs, such as miRNAs in animals, further support that 3′ uridylation generally marks small RNAs for degradation and reveal other 3′ tailing events that influence small RNA stability (Figure 3A). Moreover, 3′-5′ exonucleolytic degradation has been found to be a major mechanism to turnover mature small RNAs across organisms, although a 5′-3′ exonuclease was found to degrade miRNAs in *Caenorhabditis elegans*.

3′ nucleotide modification affects the stability of small RNAs

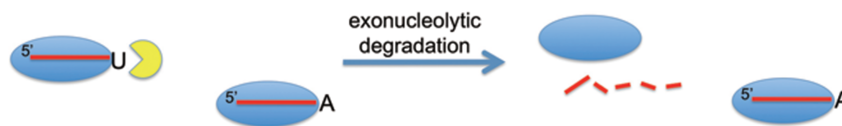
Methylation protects plant small RNAs, animal piRNAs and fly Ago2-associated siRNAs against 3′ nucleotide addition [17, 21, 26, 35, 36], which probably stimulates a 3′-5′ exonucleolytic activity to degrade the tagged small RNAs. However, for these naturally methylated small RNAs, the nucleotidyl transferases modifying the small RNAs when they lose methylation have not yet been identified in most organisms (Table 2), such that the role of uridylation in small RNA turnover has not been genetically evaluated. Once these enzymes are identified, it would be interesting to determine whether they impact small RNA metabolism under the normal condition when HEN1 function is present.

The characterization of a *Chlamydomonas reinhardtii* mutant (*Mut-68*) has shed light on the mechanisms of uridylation and degradation of miRNAs and siRNAs. In the alga *C. reinhardtii*, siRNAs and miRNAs are 2′-*O*-methylated at their 3′ termini as in angiosperms [46]. MUT68, a terminal nucleotidyl transferase, was initially shown to add adenines to the 5′ cleavage fragments from mRNAs targeted by RNA-induced silencing complexes (RISCs) to lead to their efficient decay [47]. MUT68 cooperates with the cytoplasmic exosome to degrade RISC-generated 5′ RNA cleavage products [47], and cooperates with the peripheral exosome subunit RRP6 to degrade small RNAs [48]. In a *Mut-68* mutant and an *RRP6* depletion line, the levels of a subset of miRNAs and siRNAs are elevated [48]. MUT68 plays a role in the uridylation of the 3′ ends of small RNAs *in vivo* and stimulates their degradation by RRP6 *in vitro* [48].

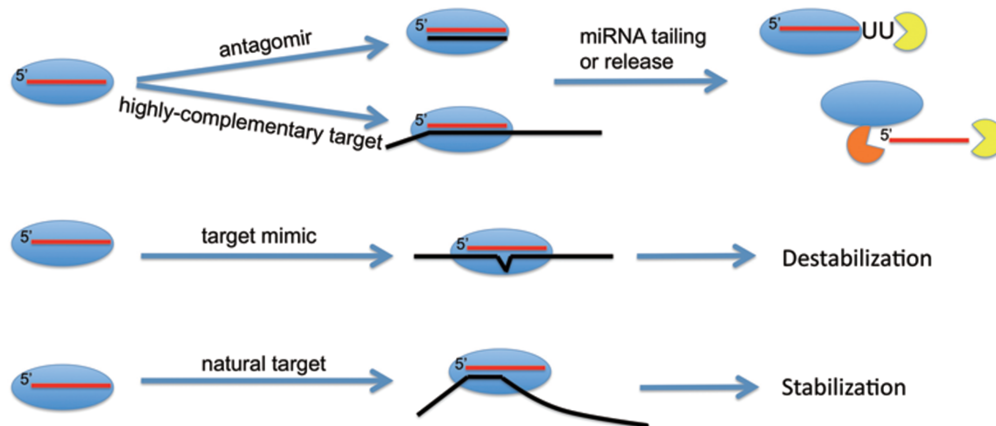
Table 2 Nucleotidyl transferases affecting mature small RNAs

Name	Organism	Substrates	Activity	Potential effects
CDE-1	*C. elegans*	siRNAs from the CSR-1 pathway	Uridylation	Destabilization
MTPAP, PAPD4, PAPD5, ZCCHC6, ZCCHC11, and TUT1	Humans	miRNAs	Uridylation or adenylation	Regulation of miRNA stability and/or activity
MUT68	*Chlamydomonas reinhardtii*	Unmethylated small RNAs	Uridylation	Destabilization
Unknown	*Populus trichocarpa*	miRNAs	Adenylation	Stabilization
Unknown	Zebrafish	Unmethylated piRNAs	Uridylation and adenylation	Regulation of piRNA stability
Unknown	*Arabidopsis*	Unmethylated small RNAs	Predominantly uridylation	Destabilization

A 3’ nucleotide addition



B complementary RNAs



C protein factors

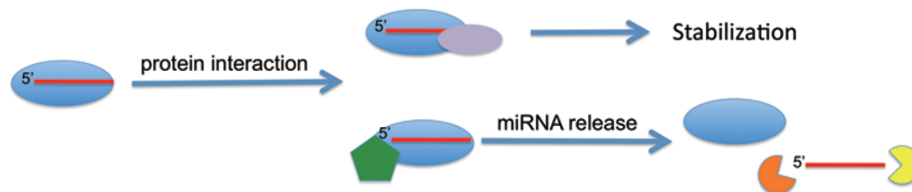


Figure 3 Mechanisms influencing small RNA stability. **(A)** 3′ nucleotide addition affects the stability of small RNAs. While U-tails probably lead to small RNA degradation, 3′ adenylation may have a protective role against degradation or have no effects on the stability of small RNAs. **(B)** Target transcripts can either enhance or suppress the degradation of the corresponding small RNAs in different situations. **(C)** Protein factors can stabilize or destabilize RISCs to influence the accessibility of nucleases to small RNAs. Yellow shapes indicate 3′-to-5′ exonucleases, orange shapes represent 5′-to-3′ exonucleases, and the purple oval and the green pentagon indicate RISC-interacting proteins.

MUT68 and RRP6 were unable to use a 2′-*O*-methylated miR912 oligonucleotide as a substrate *in vitro*, indicating that MUT68 only acts on unmethylated small RNAs [48]. However, it is not clear if MUT68 and RRP6 compose the downstream pathway, degrading unmethylated small RNAs in *hen1*, since it has not been tested yet whether *MUT68* loss of function or *RRP6* depletion suppresses the molecular defects associated with HEN1 loss of function.

A 3′-nucleotide addition of small RNAs has been observed on a global scale in high throughput sequencing studies in different model organisms [49-51]. The 3′ nucleotide addition influences small RNA stability and function differently, depending on the specific small RNAs examined and the nature of the added nucleotides (Figure 3A). A recent study showed that 3′ nucleotide addition to miRNAs is a common posttranscriptional event that exhibits selectivity for specific miRNAs ranging from *C. elegans* to humans [51]. The modifications are predominantly mono-adenylation or mono-uridylation across different tissue types, disease states, and developmental stages [51]. Multiple enzymes, including MTPAP, PAPD4, PAPD5, ZCCHC6, ZCCHC11, and TUT1, were found to be responsible for the 3′-nucleotide addition in an miRNA-specific manner [51].

Uridylation usually enhances the decay of the tailed

small RNAs (Figure 3A). For example, in *C. elegans*, uridylation of the naturally unmethylated siRNAs in the CSR-1 pathway restricts these siRNAs to CSR-1, an Ago that is not involved in regulating mRNA levels but is required for proper chromosome segregation [52-54]. Loss of CDE-1, the nucleotidyl transferase that exerts the uridylation activity, leads to an increase in the levels of CSR-1 siRNAs, defective chromosome segregation in mitosis and meiosis, and erroneous gene silencing [54]. Therefore, 3′ uridylation regulates the accumulation of the CSR-1 siRNAs by destabilizing them.

Uridylation can be triggered by the interaction with target RNAs (Figure 3B). One implication comes from the uridylation of piRNAs derived from RNA-based but not DNA-based TEs in a zebrafish *hen1* mutant [21]. In *Drosophila*, miRNAs typically associate with Ago1 to repress the translation and/or cause the decay of their target mRNAs [55]. miRNAs are complementary to their targets only at the seed sequence region, with few additional base pairs tethering the two RNAs together [56]. The abundance of the corresponding miRNAs decreases when transgenes containing one or more highly complementary sites are introduced [36]. Extensive base pairing between Ago1-associated miRNAs and their target mRNAs triggers miRNA 3′ tailing and trimming that could result from the activities of a terminal nucleotidyl transferase and a 3′-5′ exonuclease, respectively. Consistently, structural studies on the *Thermus thermophiles* Ago in complex with the guide sequence and the target strands of varying lengths show that the presence of a long, but not a short, stretch of sequence complementarity between the guide and the target induces the 3′ end of the guide to be dislodged from the Ago PAZ domain [57]. This would presumably allow a nucleotidyl transferase or an exonuclease to access the 3′ end of the small RNA. The nucleotidyl transferase and the exonuclease acting upon Ago1-associated miRNAs maybe the same as those acting upon Ago2-bound, unmethylated siRNAs in the *hen1* mutant [36]. In fact, tailing and truncation of siRNAs in the *Drosophila hen1* mutant may also be induced by the interactions with their endogenous target mRNAs, which are highly complementary to the siRNAs.

Target-dependent small RNA turnover is conserved between flies and mammals. Highly complementary targets also induce 3′ trimming and tailing of miRNAs in human cells, which is demonstrated by both synthetic target RNA analogs, “antagomirs”, and *in vitro* transcribed-target mRNAs containing sites fully complementary to miRNAs [36]. A kinetic analysis of miR-233 decay in human cells shows that the presence of target transcripts, especially those with perfect complementarity to the miRNA, increased the decay of miRNA-233 [58]. Deep sequencing unveiled that the frequency of miR-233 with 3′ mono-uridylation increased during decay, suggesting that the addition of a uridine may be a destabilizing modification [58]. The frequency of miR-233 with one or two adenines added to its 3′ end was also elevated in the cells co-expressing a target with mismatches. However, the levels of these variants did not change during decay [58].

Adenylation usually has no effect on the stability of modified small RNAs, or increase their stability (Figure 3A). For example, mature miR-122 was shown to be adenylated by the RNA nucleotidyl transferase PAPD4/GLD-2 in humans and mice, which resulted in an increase in the stability of this miRNA [59]. Considering the low *in vitro* specificity of PAPD4/GLD-2, either some specificity factors recruit PAPD4/GLD-2 to the 3′ terminus of miR-122 or a broader set of miRNAs is regulated by PAPD4/GLD-2 through adenylation *in vivo* [59]. In *Populus trichocarpa,* adenylation was observed for both full length and truncated miRNAs [60]. An *in vitro* decay assay showed that replacement of the natural 3′ nucleotide with an adenine resulted in a slower miRNA degradation in *P. trichocarpa* extracts [60].

These observations imply that 3′ nucleotide addition, predominantly uridylation and adenylation, is a widespread modification on small RNAs. Interestingly, the A- or U-tails in animals are usually only 1- or 2-nt-long, whereas plant miRNAs acquire a tail of 1-7 nt in a *hen1* mutant. Uridylation and adenylation on other RNAs, such as U6 RNA and 5S rRNA, occur competitively, and a single adenylation prevents further oligouridylation [61]. It has not been tested whether this is true for small RNAs in plants or animals.

The stability and activities of miRNAs can be regulated through active 3′ trimming during the maturation of miRNAs in RISCs [62, 63]. In *Drosophila*, many miRNAs undergo 3′ trimming by the 3′-5′ exoribonuclease Nibbler after the miRNA* strands have been removed from Ago1. Depletion of Nibbler results in reduced miRNA activities as well as the accumulation of longer miRNA isoforms. This suggests that Nibbler renders miRNAs smaller and perhaps a better fit for Ago1 to enhance their activities. Intriguingly, depletion of Nibbler in S2 cells results in miR-34 species that are longer than the lengths of Dicer products [62], suggesting that the untrimmed species are subjected to 3′ tailing and perhaps degradation. It is likely that the active 3′ trimming of miRNAs is conserved in other organisms because both *C. elegans* and humans have homologs of Nibbler, and miRNAs in humans also exhibit 3′ heterogeneity [62]. Given that Ago1-associated miRNAs are accessible to Nibbler in *Drosophila*, other exonucleases or terminal

nucleotidyl transferases may also be able to modify small RNAs when they are in association with Ago proteins.

Exonucleolytic degradation of small RNAs

The discovery of a small RNA 3′ truncation activity in *Arabidopsis hen1* mutants prompted a search for the 3′-5′ exonuclease responsible for this activity. A family of 3′-5′ exoribonucleases named *small RNA-degrading nuclease* (*SDN*) was found to turnover mature miRNAs and siRNAs in *Arabidopsis* [64]. *In vitro* assays showed that SDN1 acts specifically on single-stranded small RNA oligonucleotides that are longer than 8 nt, and is deterred but not completely inhibited by the 2′-*O*-methyl modification on the 3′ termini of small RNAs [64]. There are four closely related SDN family exoribonucleases sharing overlapping functions in *Arabidopsis*; only simultaneous knockdown of multiple *SDN* genes results in elevated miRNA levels *in vivo* and pleiotropic developmental defects [64]. Interestingly, the efficiency for SDN1 to degrade U-tailed miR173 is lower than that for miR173 *in vitro*, which suggests that SDN1 may not be the enzyme that degrades the uridylated small RNAs in the absence of HEN1 activity [64]. However, it cannot be excluded that the SDN proteins degrade U-tailed miRNAs *in vivo* with the assistance of other proteins. Whether SDNs are responsible for the 3′ truncation of small RNAs in *hen1* mutants has yet to be evaluated by knocking down multiple *SDN* genes in *hen1* mutants and observing the status of miRNA 3′ truncation in this background. Another pressing question is whether SDNs degrade small RNAs released from RISCs or small RNAs bound by Ago proteins. *SDN* homologs are universally present in eukaryotes, but whether they exert similar functions in animals is currently unknown.

XRN-2 was found to affect miRNA homeostasis in *C. elegans* by promoting the dislodging of miRNAs from RISC and degrading the free miRNAs through its 5′-3′ exoribonucleolytic activity [65] (Figure 3C). miRNAs are efficiently released from the RISC complex and degraded by XRN-2 in a *C. elegans* larval lysate [65]. The presence of wild-type target mRNAs appears to stabilize miRNAs in *C. elegans*. Both the release and the degradation of miRNAs can be blocked by adding miRNA target mRNAs. *In vivo*, the steady-state miRNA levels are increased in the presence of target mRNAs [65]. However, it is currently unknown how the dislodging of miRNAs from RISC by XRN-2 is achieved or how target mRNAs stabilize miRISCs. It is also not known whether the XRN-2-dependent turnover and target mRNA-mediated stabilization of miRNAs exist in other organisms. The plant homologs of XRN-2 are involved in miRNA biogenesis in that they degrade the non-miRNA fragments, which are by-products of pri-miRNA processing, but do not affect mature miRNA levels [66].

Both exonucleases mentioned above (SDN in *Arabidopsis* and XRN-2 in *C. elegans*) act on mature, single-stranded small RNAs. *C. elegans* Eri-1 represses RNA-mediated silencing by degrading siRNA duplexes [67]. *eri-1* encodes a conserved protein containing nucleic acid-binding and exonuclease domains [67].

Additional factors that influence small RNA stability

The life spans of small RNAs in an organism are not homogenous or constant. There are mechanisms that influence the stability of individual small RNAs. Factors that influence small RNA stability include target transcripts, *cis*-acting elements and *trans*-acting proteins.

Target RNAs affect the stability of small RNAs

In addition to the highly complementary-target-induced miRNA trimming and tailing in flies and humans, and natural-target-mediated blocking of miRNA degradation in *C. elegans*, targets with mismatches at the slicing sites promote the degradation of miRNAs in plants (Figure 3B). In *Arabidopsis*, miR399 is regulated by *IPS1* (Induced by Phosphate Starvation1), which encodes a non-coding RNA containing a short motif highly complementary to miR399 [68]. The *IPS1* sequence contains a 3-nt insertion at the cleavage site, which prevents endonucleolytic cleavage of *IPS1* transcripts by miR399 RISC, resulting in the sequestration of miR399 and reduction of miR399 activity [68]. Based on this principle, artificial target mimics have become a popular tool to downregulate miRNA activity for functional studies in *Arabidopsis* [69, 70]. Todesco *et al*. [70] generated a large set of artificial target mimics for *Arabidopsis* miRNAs. In all target mimic lines they examined, the levels of the corresponding miRNAs were decreased, suggesting that unproductive interaction of miRISC with a decoy target affects miRNA stability, even though such an effect was not observed in the interaction between *IPS1* and miR399 [70]. Does the interaction with the target mimics trigger tailing and trimming, which are the consequences of exposing *Drosophila* miRNAs to highly complementary targets? The mechanism underlying target mimic-mediated degradation has not been examined. Taken together observations in plants and animals, target mRNAs can serve as an additional layer of regulation for the activity and turnover of specific miRNAs. This can potentially be exploited for intervention of miRNA expression.

cis elements affect small RNA stability

cis elements in miRNAs have been shown to influence the stability of specific miRNAs. The identified *cis* elements vary in length, sequence and position within the miRNAs. Sequences near the 3′ end of miRNAs have been shown to affect miRNA stability [71]; this is not surprising since 3′ modification/degradation seems to be a major mode of miRNA turnover such that 3′ sequences may affect the accessibility of the decay machinery to the miRNAs. Elements in the middle of the small RNA as well as the overall percentage of AU or UA dinucleotides also serve as destabilization signals [72-74]. However, the enzymes responsible for sequence-specific decay of small RNAs have not been identified in most cases.

The 7-nt motif in miR-382 is an example of a *cis* element located at the 3′ end. miR-382, a miRNA that contributes to Human immunodeficiency virus-1 (HIV-1) latency in resting $CD4^+$ T lymphocytes, is unstable in cells [71, 75]. Bail *et al.* showed that the 3′-terminal 7 nt (GGAUUCG) were necessary for the low stability of miR-382 in a cell-free system that is able to recapitulate the differential stability of miRNAs [71]. miR-382 decay was primarily due to the exosome with a more modest contribution by Xrn1 and no detectable contribution by Xrn2 [71]. However, it was not determined whether these nucleotides were sufficient to impart instability to other miRNAs.

Central motifs that influence miRNA stability have been identified in both humans and *Drosophila*. For example, miR-200c and miR-141, differing by 5 nt and encoded by the miR-200c_141 cluster, show varying relative abundance in different human cell lines [72]. Strikingly, cell detachment induced the decay of miR-141 but not miR-200c. A sequence motif (UGUCU) in the central region was identified to be necessary for the destabilization of miR-141 by cell detachment [72]. Several other miRNAs, miR-200a, miR-429 and miR-34a, which also contain this motif, were also destabilized upon cell detachment. But the motif was not able to render let-7 unstable [72]. Zhang *et al*. [74] measured the lifespan of miRNAs in human cell lines using the pulse-chase approach. They illustrated that uridines at nucleotide positions 9-11 of miR-29b are necessary for its rapid decay in a nucleotide- and position-specific manner. In addition, a substantial subset of miRNAs with the uridine-rich sequence, such as miR-29c and fly bantam, tends to turnover faster. Different from the UGUCU motif, the destabilization effect of the uridine motif in miR-29b is conferred upon the miRNA/miRNA* duplex rather than upon mature miRNAs. Therefore, the nucleases that respond to the two motifs likely have different substrate specificities. Both studies show that the motifs are only necessary but not sufficient to confer instability [72, 74]. Therefore, these motifs may rely on the sequence context of miRNAs or additional *trans* factors that coexist with the miRNAs.

RNA editing may affect the stability of small RNAs through altering the identity of nucleotides in small RNAs. Adenosine deaminase acting on RNA (ADAR) proteins edit adenosines to inosines in dsRNAs [76, 77]. Non-coding transcripts are the most common targets of ADARs [77]. It has been demonstrated that certain miRNA precursors undergo A-I editing *in vivo*, such as miRNA-22 and miRNA-142 precursors [78, 79]. Edited pri-miR-142 is poorly processed by Drosha and degraded by Tudor-SN, a ribonuclease specific to inosine-containing dsRNAs [79]. These data illustrate that RNA editing regulates the biogenesis of miRNAs. However, the effects of RNA editing on the stability of small RNA duplexes or mature miRNAs have not been evaluated thoroughly, and the function of Tudor-SN as a component of RISC is not clear either.

In summary, some miRNAs contain sequence information to influence their own turnover, which might be a mechanism that enables adjustments of miRNA levels in response to environmental or endogenous cues. Understanding the effects of *cis* elements that influence miRNA stability will benefit the design and utilization of small RNAs for genetic manipulations.

trans factors affect small RNA stability

Components of the RISC or RISC-associated factors may influence the stability of small RNAs (Figure 3C). Most endogenous miRNAs are in RISCs, and only a very small proportion is free in cells [80]. Antagomirs that are complementary to miRNAs are able to dissociate the targeted miRNAs from RISC, causing the loss of protection of the miRNAs by RISC [80]. The antagomir-induced miRNA degradation may be similar to highly complementary-target mRNA-mediated miRNA degradation, and the two may share common mechanisms, such as the displacement of the miRNAs from RISCs or the dislodging of the 3′ end of the small RNA from the PAZ domain of Ago. In the mouse, Ago proteins promote mature miRNA accumulation posttranscriptionally, in that overexpression of *Ago* genes elevates the levels of miRNAs and *Ago2* knockout reduces the levels of miRNAs [81]. Moreover, half-lives of multiple endogenous miRNAs are correlated with the cellular Ago2 levels [82]. Similar stabilization effects of miRNAs by Ago proteins were observed in *Arabidopsis*. Loss of function of AGO1, the major miRNA effector in *Arabidopsis*, results in reduced

levels of most miRNAs [83]. *Arabidopsis* AGO2 is highly induced by the bacterial pathogen *Pseudomonas syringae*. Meanwhile, the accumulation of several miRNA star strands that are bound by AGO2 is also elevated [84]. Intriguingly, *Arabidopsis* AGO10 specifically associates with miR165/166, but the accumulation of miR165/166 is increased, rather than reduced, in *ago10* mutants, implicating that AGO10 possibly represses the level of miR165/166 instead of stabilizing this miRNA [85-87]. Therefore, different Ago proteins could affect the stability of associated small RNAs differently. Besides physical association and stabilization, the slicer activity of Ago proteins can influence the stability of associated small RNAs. For example, in the *T. thermophila* strain containing slicer defective Twi1p, the removal of the passenger strand of scnRNA is blocked [88]. The amount of scnRNAs is drastically reduced probably due to the degradation of the double-stranded scnRNAs in Twi1p by an exonuclease [88], implicating that efficient removal of passenger strands is necessary for the activity as well as the accumulation of small RNAs.

Non-Ago proteins can potentially affect small RNA stability by stabilizing or destabilizing RISCs to influence the accessibility of the small RNA-degradation machinery to small RNAs. Few such proteins have been identified. Translin, a nucleic acid-binding protein that is known to bind several mRNAs, a non-coding RNA and miR-122a in germ cells of mouse testis [89], is one example. The binding of Translin to miR-122a increases its *in vivo* stability through an unknown mechanism [89]. Translin proteins are components of C3PO that functions in RISC activation by degrading Ago-nicked passenger strands [90]. Other non-Ago proteins that may influence small RNA stability include the ADARs. Mouse ADAR1 and ADAR2 bind siRNA duplexes without editing the RNAs [91]. The binding by ADARs represses the activity of siRNAs, in that siRNAs are significantly more effective in *ADAR1* null mutant mouse cells than in wild-type cells [91]. It was proposed that the formation of ADAR-siRNA complexes interferes with the assembly of siRNA-RISCs [91]. However, it was not reported whether or how ADARs affect the turnover of siRNAs.

Conclusions and perspectives

Precise and faithful regulation of small RNA levels is critical for diverse biological processes in various organisms. Either reduction or elevation in small RNA levels results in pleiotropic developmental defects in *Arabidopsis*, as illustrated by the phenotypes of plants with *hen1* mutations or *SDN* depletion [17, 64]. In *C. elegans*, when the excess siRNAs from the CSR-1 pathway are fed into other RNAi pathways, chromosome segregation is disrupted [54]. The stability of small RNAs contributes to their steady-state levels and impacts target gene expression. Understanding small RNA turnover is becoming a new focus in the RNA-silencing field. Future investigations in this area include identification of major components of the small RNA turnover machinery, and understanding how they are recruited to specific types of small RNAs and how their activities are regulated.

Understanding the mechanisms regulating small RNA stability will benefit our utilization of RNAi. For example, the stability of fork-siRNAs (a type of siRNAs with designed mismatches in the duplexes to enhance asymmetry in Ago loading) was significantly improved by methylating the nuclease-sensitive sites. As a result, the duration of the gene-silencing activity of the modified fork-siRNAs doubled that of the unmethylated analogs [92]. Modulation of miR-382 stability could potentially impact the control of HIV-1 latency [75], which is a major barrier for the eradication of the virus in patients on suppressive highly active anti-retroviral therapy (reviewed in [93]). Moreover, novel genetic manipulations could be devised based on the knowledge of highly complementary-target-mediated miRNA instability to target miRNAs whose overexpression is associated with human disorders. The stability of individual miRNAs can be altered after we understand how the specificity of miRNA turnover or stabilization is achieved. A recent report showed that plant miRNAs can be obtained by animals through food intake [94]. This finding enlightens scientists on the production and the delivery of miRNAs into patients for therapeutic purposes.

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References

1 Ambros V, Chen X. The regulation of genes and genomes by small RNAs. *Development* 2007; **134**:1635-1641.
2 Chen X. Small RNAs and their roles in plant development. *Annu Rev Cell Dev Biol* 2009; **25**:21-44.
3 Filipowicz W. RNAi: the nuts and bolts of the RISC machine. *Cell* 2005; **122**:17-20.
4 Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 2009; **10**:126-139.
5 Sunkar R. MicroRNAs with macro-effects on plant stress responses. *Semin Cell Dev Biol* 2010; **21**:805-811.
6 Xie Z, Khanna K, Ruan S. Expression of microRNAs and its regulation in plants. *Semin Cell Dev Biol* 2010; **21**:790-797.

7 Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet* 2011; **12**:99-110.
8 Ahlquist P. RNA-dependent RNA polymerases, viruses, and RNA silencing. *Science* 2002; **296**:1270-1273.
9 Zamore PD. Ancient pathways programmed by small RNAs. *Science* 2002; **296**:1265-1269.
10 Zhang H, Zhu JK. RNA-directed DNA methylation. *Curr Opin Plant Biol* 2011; **14**:142-147.
11 Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 2006; **313**:320-324.
12 Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* 2011; **12**:246-258.
13 Brennecke J, Aravin AA, Stark A, *et al.* Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. *Cell* 2007; **128**:1089-1103.
14 Gunawardane LS, Saito K, Nishida KM, *et al.* A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in *Drosophila*. *Science* 2007; **315**:1587-1590.
15 Aravin AA, Sachidanandam R, Bourc'his D, *et al.* A piRNA pathway primed by individual transposons is linked to *de novo* DNA methylation in mice. *Mol Cell* 2008; **31**:785-799.
16 Kuramochi-Miyagawa S, Watanabe T, Gotoh K, *et al.* DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Dev* 2008; **22**:908-917.
17 Yu B, Yang Z, Li J, *et al.* Methylation as a crucial step in plant microRNA biogenesis. *Science* 2005; **307**:932-935.
18 Yang Z, Ebright YW, Yu B, Chen X. HEN1 recognizes 21-24 nt small RNA duplexes and deposits a methyl group onto the 2′ OH of the 3′-terminal nucleotide. *Nucleic Acids Res* 2006; **34**:667-675.
19 Horwich MD, Li C, Matranga C, *et al.* The *Drosophila* RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC. *Curr Biol* 2007; **17**:1265-1272.
20 Houwing S, Kamminga LM, Berezikov E, *et al.* A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. *Cell* 2007; **129**:69-82.
21 Kamminga LM, Luteijn MJ, den Broeder MJ, *et al.* Hen1 is required for oocyte development and piRNA stability in zebrafish. *EMBO J* 2010; **29**:3688-3700.
22 Kirino Y, Mourelatos Z. 2′-O-methyl modification in mouse piRNAs and its methylase. *Nucleic Acids Symp Ser (Oxf)* 2007; 417-418.
23 Kirino Y, Mourelatos Z. The mouse homolog of HEN1 is a potential methylase for Piwi-interacting RNAs. *RNA* 2007; **13**:1397-1401.
24 Kirino Y, Mourelatos Z. Mouse Piwi-interacting RNAs are 2′-O-methylated at their 3′ termini. *Nat Struct Mol Biol* 2007; **14**:347-348.
25 Saito K, Sakaguchi Y, Suzuki T, Suzuki T, Siomi H, Siomi MC. Pimet, the *Drosophila* homolog of HEN1, mediates 2′-O-methylation of Piwi- interacting RNAs at their 3′ ends. *Genes Dev* 2007; **21**:1603-1608.
26 Li J, Yang Z, Yu B, Liu J, Chen X. Methylation protects miRNAs and siRNAs from a 3′-end uridylation activity in *Arabidopsis*. *Curr Biol* 2005; **15**:1501-1507.
27 Abe M, Yoshikawa T, Nosaka M, *et al.* WAVY LEAF1, an ortholog of *Arabidopsis* HEN1, regulates shoot development by maintaining MicroRNA and trans-acting small interfering RNA accumulation in rice. *Plant Physiol* 2010; **154**:1335-1346.
28 Tkaczuk KL, Obarska A, Bujnicki JM. Molecular phylogenetics and comparative modeling of HEN1, a methyltransferase involved in plant microRNA biogenesis. *BMC Evol Biol* 2006; **6**:6.
29 Huang Y, Ji L, Huang Q, Vassylyev DG, Chen X, Ma JB. Structural insights into mechanisms of the small RNA methyltransferase HEN1. *Nature* 2009; **461**:823-827.
30 Vilkaitis G, Plotnikova A, Klimasauskas S. Kinetic and functional analysis of the small RNA methyltransferase HEN1: the catalytic domain is essential for preferential modification of duplex RNA. *RNA* 2010; **16**:1935-1942.
31 Yu B, Chapman EJ, Yang Z, Carrington JC, Chen X. Transgenically expressed viral RNA silencing suppressors interfere with microRNA methylation in *Arabidopsis*. *FEBS Lett* 2006; **580**:3117-3120.
32 Lozsa R, Csorba T, Lakatos L, Burgyan J. Inhibition of 3′ modification of small RNAs in virus-infected plants require spatial and temporal co-expression of small RNAs and viral silencing-suppressor proteins. *Nucleic Acids Res* 2008; **36**:4099-4107.
33 Jamous RM, Boonrod K, Fuellgrabe MW, Ali-Shtayeh MS, Krczal G, Wassenegger M. The helper component-proteinase of the Zucchini yellow mosaic virus inhibits the Hua Enhancer 1 methyltransferase activity *in vitro*. *J Gen Virol* 2011; **92**:2222-2226.
34 Yu B, Bi L, Zhai J, *et al.* siRNAs compete with miRNAs for methylation by HEN1 in *Arabidopsis*. *Nucleic Acids Res* 2010; **38**:5844-5850.
35 Kurth HM, Mochizuki K. 2′-O-methylation stabilizes Piwi-associated small RNAs and ensures DNA elimination in *Tetrahymena*. *RNA* 2009; **15**:675-685.
36 Ameres SL, Horwich MD, Hung JH, *et al.* Target RNA-directed trimming and tailing of small silencing RNAs. *Science* 2010; **328**:1534-1539.
37 Lee SR, Collins K. Two classes of endogenous small RNAs in *Tetrahymena thermophila*. *Genes Dev* 2006; **20**:28-33.
38 Mochizuki K, Gorovsky MA. Conjugation-specific small RNAs in *Tetrahymena* have predicted properties of scan (scn) RNAs involved in genome rearrangement. *Genes Dev* 2004; **18**:2068-2073.
39 Chalker DL, Yao MC. Nongenic, bidirectional transcription precedes and may promote developmental DNA deletion in *Tetrahymena thermophila*. *Genes Dev* 2001; **15**:1287-1298.
40 Malone CD, Anderson AM, Motl JA, Rexer CH, Chalker DL. Germ line transcripts are processed by a Dicer-like protein that is essential for developmentally programmed genome rearrangements of *Tetrahymena thermophila*. *Mol Cell Biol* 2005; **25**:9151-9164.
41 Mochizuki K, Gorovsky MA. A Dicer-like protein in Tetrahymena has distinct functions in genome rearrangement, chromosome segregation, and meiotic prophase. *Genes Dev* 2005; **19**:77-89.
42 Jain R, Shuman S. Bacterial Hen1 is a 3′ terminal RNA ribose

2′-O-methyltransferase component of a bacterial RNA repair cassette. *RNA* 2010; **16**:316-323.
43 Mui Chan C, Zhou C, Brunzelle JS, Huang RH. Structural and biochemical insights into 2′-O-methylation at the 3′-terminal nucleotide of RNA by Hen1. *Proc Natl Acad Sci USA* 2009; **106**:17699-17704.
44 Chan CM, Zhou C, Huang RH. Reconstituting bacterial RNA repair and modification *in vitro*. *Science* 2009; **326**:247.
45 Park W, Li J, Song R, Messing J, Chen X. CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. *Curr Biol* 2002; **12**:1484-1495.
46 Molnar A, Schwach F, Studholme DJ, Thuenemann EC, Baulcombe DC. miRNAs control gene expression in the single-cell alga *Chlamydomonas reinhardtii*. *Nature* 2007; **447**:1126-1129.
47 Ibrahim F, Rohr J, Jeong WJ, Hesson J, Cerutti H. Untemplated oligoadenylation promotes degradation of RISC-cleaved transcripts. *Science* 2006; **314**:1893.
48 Ibrahim F, Rymarquis LA, Kim EJ, *et al.* Uridylation of mature miRNAs and siRNAs by the MUT68 nucleotidyltransferase promotes their degradation in *Chlamydomonas*. *Proc Natl Acad Sci USA* 2010; **107**:3906-3911.
49 Ruby JG, Jan C, Player C, *et al.* Large-scale sequencing reveals 21U-RNAs and additional microRNAs and endogenous siRNAs in *C. elegans*. *Cell* 2006; **127**:1193-1207.
50 Landgraf P, Rusu M, Sheridan R, *et al.* A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 2007; **129**:1401-1414.
51 Wyman SK, Knouf EC, Parkin RK, *et al.* Post-transcriptional generation of miRNA variants by multiple nucleotidyl transferases contributes to miRNA transcriptome complexity. *Genome Res* 2011; **21**:1450-1461.
52 Claycomb JM, Batista PJ, Pang KM, *et al.* The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. *Cell* 2009; **139**:123-134.
53 Gu W, Shirayama M, Conte D Jr, *et al.* Distinct argonaute-mediated 22G-RNA pathways direct genome surveillance in the *C. elegans* germline. *Mol Cell* 2009; **36**:231-244.
54 van Wolfswinkel JC, Claycomb JM, Batista PJ, Mello CC, Berezikov E, Ketting RF. CDE-1 affects chromosome segregation through uridylation of CSR-1-bound siRNAs. *Cell* 2009; **139**:135-148.
55 Okamura K, Ishizuka A, Siomi H, Siomi MC. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. *Genes Dev* 2004; **18**:1655-1666.
56 Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. *PLoS Biol* 2005; **3**:e85.
57 Wang Y, Juranek S, Li H, *et al.* Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes. *Nature* 2009; **461**:754-761.
58 Baccarini A, Chauhan H, Gardner TJ, Jayaprakash AD, Sachidanandam R, Brown BD. Kinetic analysis reveals the fate of a microRNA following target regulation in mammalian cells. *Curr Biol* 2011; **21**:369-376.
59 Katoh T, Sakaguchi Y, Miyauchi K, *et al.* Selective stabilization of mammalian microRNAs by 3′ adenylation mediated by the cytoplasmic poly(A) polymerase GLD-2. *Genes Dev* 2009; **23**:433-438.
60 Lu S, Sun YH, Chiang VL. Adenylation of plant miRNAs. *Nucleic Acids Res* 2009; **37**:1878-1885.
61 Chen Y, Sinha K, Perumal K, Reddy R. Effect of 3′ terminal adenylic acid residue on the uridylation of human small RNAs *in vitro* and in frog oocytes. *RNA* 2000; **6**:1277-1288.
62 Han BW, Hung JH, Weng Z, Zamore PD, Ameres SL. The 3′-to-5′ exoribonuclease Nibbler shapes the 3′ ends of microRNAs bound to *Drosophila* Argonaute1. *Curr Biol* 2011; **21**:1878-1887.
63 Liu N, Abe M, Sabin LR, *et al.* The exoribonuclease Nibbler controls 3′ end processing of microRNAs in *Drosophila*. *Curr Biol* 2011; **21**:1888-1893.
64 Ramachandran V, Chen X. Degradation of microRNAs by a family of exoribonucleases in *Arabidopsis*. *Science* 2008; **321**:1490-1492.
65 Chatterjee S, Grosshans H. Active turnover modulates mature microRNA activity in *Caenorhabditis elegans*. *Nature* 2009; **461**:546-549.
66 Gy I, Gasciolli V, Lauressergues D, *et al. Arabidopsis* FIERY1, XRN2, and XRN3 are endogenous RNA silencing suppressors. *Plant Cell* 2007; **19**:3451-3461.
67 Kennedy S, Wang D, Ruvkun G. A conserved siRNA-degrading RNase negatively regulates RNA interference in *C. elegans*. *Nature* 2004; **427**:645-649.
68 Franco-Zorrilla JM, Valli A, Todesco M, *et al.* Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat Genet* 2007; **39**:1033-1037.
69 Rubio-Somoza I, Manavella PA. Mimicry technology: suppressing small RNA activity in plants. *Methods Mol Biol* 2011; **732**:131-137.
70 Todesco M, Rubio-Somoza I, Paz-Ares J, Weigel D. A collection of target mimics for comprehensive analysis of microRNA function in *Arabidopsis thaliana*. *PLoS Genet* 2010; **6**:e1001031.
71 Bail S, Swerdel M, Liu H, *et al.* Differential regulation of microRNA stability. *RNA* 2010; **16**:1032-1039.
72 Kim YK, Yeo J, Ha M, Kim B, Kim VN. Cell adhesion-dependent control of microRNA decay. *Mol Cell* 2011; **43**:1005-1014.
73 Sethi P, Lukiw WJ. Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. *Neurosci Lett* 2009; **459**:100-104.
74 Zhang Z, Zou J, Wang GK, *et al.* Uracils at nucleotide position 9-11 are required for the rapid turnover of miR-29 family. *Nucleic Acids Res* 2011; **39**:4387-4395.
75 Huang J, Wang F, Argyris E, *et al.* Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. *Nat Med* 2007; **13**:1241-1247.
76 Keegan LP, Leroy A, Sproul D, O'Connell MA. Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes. *Genome Biol* 2004; **5**:209.
77 Nishikura K. Editor meets silencer: crosstalk between RNA editing and RNA interference. *Nat Rev Mol Cell Biology* 2006; **7**:919-931.
78 Luciano DJ, Mirsky H, Vendetti NJ, Maas S. RNA editing of a miRNA precursor. *RNA* 2004; **10**:1174-1177.
79 Yang W, Chendrimada TP, Wang Q, *et al.* Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. *Nat Struct Mol Biol* 2006; **13**:13-21.

80 Tang F, Hajkova P, O'Carroll D, *et al.* MicroRNAs are tightly associated with RNA-induced gene silencing complexes *in vivo*. *Biochem Biophys Res Commun* 2008; **372**:24-29.
81 Diederichs S, Haber DA. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. *Cell* 2007; **131**:1097-1108.
82 Winter J, Diederichs S. Argonaute proteins regulate microRNA stability: Increased microRNA abundance by Argonaute proteins is due to microRNA stabilization. *RNA Biol* 2011; **8**:1149-1157.
83 Vaucheret H, Mallory AC, Bartel DP. AGO1 homeostasis entails coexpression of MIR168 and AGO1 and preferential stabilization of miR168 by AGO1. *Mol Cell* 2006; **22**:129-136.
84 Zhang X, Zhao H, Gao S, *et al. Arabidopsis* Argonaute 2 regulates innate immunity via miRNA393(*)-mediated silencing of a Golgi-localized SNARE gene, MEMB12. *Mol Cell* 2011; **42**:356-366.
85 Ji L, Liu X, Yan J, *et al*. ARGONAUTE10 and ARGONAUTE1 regulate the termination of floral stem cells through two microRNAs in *Arabidopsis*. *PLoS Genet* 2011; **7**:e1001358.
86 Liu Q, Yao X, Pi L, Wang H, Cui X, Huang H. The ARGONAUTE10 gene modulates shoot apical meristem maintenance and leaf polarity establishment by repressing miR165/166 in *Arabidopsis*. *Plant J* 2008 Nov 28. doi:10.1111/j.1365-313X.2008.03757.x
87 Zhu H, Hu F, Wang R, *et al. Arabidopsis* Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. *Cell* 2011; **145**:242-256.
88 Noto T, Kurth HM, Kataoka K, *et al*. The Tetrahymena argonaute-binding protein Giw1p directs a mature argonaute-siRNA complex to the nucleus. *Cell* 2010; **140**:692-703.
89 Yu Z, Hecht NB. The DNA/RNA-binding protein, translin, binds microRNA122a and increases its *in vivo* stability. *J Androl* 2008; **29**:572-579.
90 Liu Y, Ye X, Jiang F, *et al.* C3PO, an endoribonuclease that promotes RNAi by facilitating RISC activation. *Science* 2009; **325**:750-753.
91 Yang W, Wang Q, Howell KL, *et al.* ADAR1 RNA deaminase limits short interfering RNA efficacy in mammalian cells. *J Biol Chem* 2005; **280**:3946-3953.
92 Petrova Kruglova NS, Meschaninova MI, Venyaminova AG, Zenkova MA, Vlassov VV, Chernolovskaya EL. 2′-O-methyl-modified anti-MDR1 fork-siRNA duplexes exhibiting high nuclease resistance and prolonged silencing activity. *Oligonucleotides* 2010; **20**:297-308.
93 Zhou J, Rossi JJ. Current progress in the development of RNAi-based therapeutics for HIV-1. *Gene Ther* 2011; **18**:1134-1138.
94 Zhang L, Hou D, Chen X, *et al.* Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res* 2012; **22**:107-126.