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MicroRNA assassins: factors that regulate the disappearance of miRNAs

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Abstract

MicroRNAs (miRNAs) control essential gene regulatory pathways in plants and animals. Serving as guides in silencing complexes, miRNAs direct Argonaute proteins to specific target messenger RNAs to repress protein expression. The mature, 22-nucleotide (nt) miRNA is the product of multiple processing steps, and recent studies have uncovered factors that directly control the stability of the functional RNA form. Although alteration of miRNA levels has been linked to numerous disease states, the mechanisms responsible for stabilized or reduced miRNA expression have been largely elusive. The discovery of specific *cis*-acting modifications and *trans*-acting proteins that affect miRNA half-life reveals new elements that contribute to the homeostasis of these vital regulatory molecules.

miRNAs eluded researchers for decades, stealthily participating in many of the most important biological pathways in eukaryotic cells. In recent years, our understanding of miRNAs has grown from the discovery of a single genetic oddity in worms to the recognition of an entirely new class of regulatory molecule with thousands of members[1]. The significance of miRNAs in normal development and cellular function is underscored by mounting evidence that misregulation of specific miRNA pathways is associated with complicated health afflictions, including cancer, heart disease and neurological disorders[2–4]. miRNAs are intertwined in complex regulatory pathways in plants as well[5] and represent one of the most plentiful classes of gene regulators in multicellular organisms.

Production of the functional, ~22-nt mature miRNA involves multiple processing steps[6–8] (Fig. 1). The general miRNA biogenesis pathway begins with synthesis of a primary transcript by RNA polymerase II (Pol II). Housed within the primary transcript is the hairpin precursor, which contains the sequence destined to be the mature miRNA in one arm of the stem. In animals, the Microprocessor complex, minimally composed of the Drosha RNase (RNase) and its RNA binding partner Pasha (also called DGCR8), releases the miRNA precursor from the primary transcript (Table 1). Exportin-5 delivers the precursor to the cytoplasm for final processing by the Dicer RNase and its double-stranded RNA binding cofactor TRBP (also called loquacious, Loqs) (Table 1). After loading onto Argonaute, one strand of the resulting partial duplex, designated the guide, is p referentially retained. This multistep pathway is shared in plants with a few exceptions: the Dicer-like (DCL) proteins

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catalyze both the primary and precursor processing steps in the nucleus, where the mature miRNA forms a complex with Argonaute and is transported to the cytoplasm (Table 1). miRNAs serve as guides to direct the Argonaute complex to target mRNAs through complementary base-pairing[6,9]. Typically, target recognition results in destabilization or translational repression, either of which ultimately silences gene expression.

Accumulation of a specific miRNA is dependent on the rates of transcription, processing and decay. Similar to the expression of many protein-coding genes, expression of miRNA primary transcripts is subject to regulation by specific transcription factors and chromatin marks[6–8]. Control of each processing step has also emerged as a key determinant of functional miRNA expression. The first global analysis of primary and mature miRNA levels revealed that extensive post-transcriptional regulation is involved in cellular miRNA homeostasis[10]. Several examples of proteins and mechanisms that govern processing of specific miRNAs are detailed in recent reviews[7,8]. Here, we focus on parameters that determine miRNA existence after maturation has been completed. The stability of mature miRNAs is controlled by *cis*-acting modifications, protein complex formation and exposure to nucleases. The recent discoveries of specific factors that mediate miRNA turnover offer new insights into mechanisms responsible for changes in the availability of these critical regulatory molecules.

Eluding the reapers: *cis*- and *trans*-acting stabilization elements

Unprotected 5′ or 3′ ends leave RNAs vulnerable to exonucleolytic decay pathways. To thwart degradation factors, 5′ cap structures and 3′ poly(A) tails are added to most protein-coding Pol II transcripts. Stable secondary structures help protect the ends of mature noncoding RNAs synthesized by Pol I (18S, 5.8S and 28S ribosomal RNA precursors) and Pol III (5S rRNA and tRNAs). miRNAs emerge as short, duplex RNAs with 5′ monophosphate and 3′ hydroxyl groups after processing by the sequential actions of Drosha and Dicer or DCL proteins[6–8]. Typically, one half of the hybrid, called the guide, is preferentially maintained, and the other strand, sometimes referred to as the star or passenger strand, disappears. This dichotomy has been attributed to biased Argonaute loading of the miRNA half that has weaker 5′ pairing interactions with its partner strand[11,12]. Presumably, the stably bound guide strand is protected by Argonaute while the passenger is vulnerable to degradation (Fig. 2). The profound imbalance in guide versus passenger for many miRNAs implies that an efficient decay pathway exists to clear unprotected miRNAs.

Mature miRNA abundance is sensitive to Argonaute protein levels, supporting a protective role for this core miRNA effector protein (Fig. 2). Downregulation or ectopic expression of Argonaute results in diminished or bolstered mature miRNA levels, respectively[13–16]. In some cases, depletion of Argonaute also results in impaired processing of precursor to mature miRNAs, indicating that Argonaute may function in biogenesis as well as stabilization of miRNAs[14,15]. In a screen for factors that are limiting for miRNA biogenesis in mammalian culture cells, ectopic expression of Argonaute proteins resulted in increased levels of mature miRNAs[13]. Other genes encoding proteins essential in the miRNA pathway, such as Drosha, Pasha/DGCR8 and Dicer, had no effect in this experimental system,

indicating that the availability of Argonaute proteins largely influences cellular mature miRNA levels[13].

In addition to taking refuge in protein complexes, mature miRNAs can undergo protective modifications (Table 2). In *Arabidopsis thaliana*, methyl groups are added to the 3′ ends of miRNAs by the HEN1 methyltransferase[17]. In *hen1* mutants, levels of mature miRNAs are substantially reduced and the residual species often have 1–5 uracil residues appended to their 3′ ends[18] (Table 2). Thus, 3′ methylation prevents uridylation and destabilization of miRNAs in *Arabidopsis*. In one model, unmodified miRNAs undergo uridylation, which serves as a tag to promote degradation. Alternatively, miRNAs that lack 3′ methyl groups could be exposed for direct exonucleolytic decay or U-tailing, which might instead serve as a protective modification. Interestingly, miRNAs with extra uracil residues were observed more frequently for the guide versus star strand of miR173 in *hen1* mutants[18]. Thus, either uridylation of miRNAs with unmodified 3′ ends favors the Argonaute-bound form or the unselected passenger strands with U-tails are more efficiently degraded than nonuridylated species. Although the enzyme responsible for uridylation of plant miRNAs has yet to be identified, specific nucleases that degrade mature plant miRNAs were recently determined and will be discussed below[19].

In contrast to plants, a uniform modification of animal miRNAs has not been observed. The HEN1 methyltransferase is conserved in animals, but its substrates are piRNAs (PIWI-interacting RNAs) and, in some cases, siRNAs (small interfering RNAs), instead of miRNAs[20–22]. Diverse nucleotide substitutions, additions and deletions have been detected in animal miRNAs by massive sequencing approaches to probe deeply the miRNA composition of cells and organisms[23–26]. Despite the caveat that sequencing errors can also contribute to heterogeneity in apparent miRNA composition, the extent of these types of modifications appears substantial[27]. Although a change in mature miRNA sequence has clear implications for target recognition, possible effects on miRNA half-life are less predictable.

It was recently demonstrated that 3′ adenylation can have a stabilizing effect on animal miRNAs (Table 2). Although the addition of nontemplated adenines has been detected on many different animal miRNAs[23,25,26,28], a functional consequence of this modification has so far only been established for miR-122 in liver cells[29]. The cytoplasmic poly(A) polymerase GLD-2 adds a single adenine residue to the 3′ end of mature miR-122; this modification appears to prevent shortening and to stabilize the miRNA[29]. Depletion of GLD-2 in liver cells resulted in disappearance of the 23-nt adenylated form of miR-122 with a concurrent increase in the 21-nt variant. Moreover, the total levels of mature, but not precursor, miR-122 miRNAs were substantially reduced in cells deficient in GLD-2 activity. Interestingly, the abundance of several other miRNAs expressed in liver cells was not affected by the loss of GLD-2, indicating that stabilization of miR-122 is specifically dependent on GLD-2 mediated adenylation. Because GLD-2 has other targets, including 7SL (the noncoding RNA component of the signal recognition particle) and select mRNAs, it is unclear what proportion of miRNAs is subject to adenylation by this factor. It is possible that GLD-2 nonspecifically adds adenine residues to miRNAs, but only a fraction of these miRNA species depend on the modification for stability.

Nontemplated addition of adenine residues has also been detected on plant miRNAs[18,30]. One to seven adenines were found attached to representatives of most miRNA families identified in *Populus trichocarpa* (black cottonwood)[30] (Table 2). Adenylation was observed for both full-length as well as truncated miRNAs, suggesting that mature and partially degraded miRNAs are substrates for this modification. It remains to be determined whether adenylation has a functional consequence for miRNAs *in vivo* or whether this is a promiscuous activity on unprotected miRNAs. Supporting the first possibility, replacement of the 3′ nucleotide with an adenine residue resulted in slower miRNA degradation in an *in vitro* decay assay using extracts from *P. trichocarpa*[30]. The factors responsible for adenylation of plant miRNAs and the potential effect of this modification on plant miRNA homeostasis await elucidation.

The SDN slayers: an end attack

The first factors shown to degrade mature miRNAs are the appropriately named *small RNA degrading nuclease* (*SDN*) genes in *Arabidopsis*[19] (Fig. 2). Members of this family of exonucleases catalyze 3′-to-5′ decay of single-stranded miRNAs, and depletion of *SDN* transcripts results in increased steady-state levels of mature miRNAs *in vivo*[19] (Table 1). Consistent with a protective role for the 3′ methyl group on plant miRNAs, methylated miRNAs were less efficiently degraded than unmodified miRNAs by recombinant SDN1 in *in vitro* decay assays. Notably, miRNAs with two or five uracil residues added to the 3′ end were strikingly resistant to SDN1-mediated degradation (Table 2). Thus, unmethylated miRNAs in *hen1* mutants may be subject to two opposing activities: 3′-to-5′ degradation by SDN proteins or uridylation by yet-to-be-identified factors. These findings prompt re-evaluation of the consequence of uridylation on plant miRNAs. If uridylation also impedes SDN-mediated degradation *in vivo*, then the addition of uracil residues to the 3′ ends of unmethylated miRNAs could function as a protective backup measure as opposed to a tag for destabilization.

SDN1 is related to four other predicted exonucleases in *Arabidopsis* that may have overlapping functions in regulating miRNA homeostasis[19]. Depletion of *SDN1*, *SDN2* and *SDN3* transcripts results in generally increased miRNA levels and pleiotropic developmental defects[19]. Overaccumulation of miRNAs presumably augments target downregulation, potentially reducing some targets below critical thresholds. Homologs of *SDN* genes are present in animals but roles in miRNA homeostasis or other pathways are yet to be discovered.

The XRN-2 executioners: beginning of the end

In animals, the 5′-to-3′ exonuclease XRN-2 (Rat1p in yeast) catalyzes degradation of mature miRNAs[31] (Fig. 2 and Table 1). From a panel of eight candidate nucleases, which included homologs of SDN, Chatterjee and Grosshans identified XRN-2 as a factor involved in mature miRNA accumulation in *Caenorhabditis elegans*[31]. The failure to detect an effect of the *SDN*-related genes in the worm miRNA pathway could be due to the assay and/or to the potential redundancy of the worm homologs. Thus, a conserved role for these genes in plants and animals has not been ruled out. The finding that XRN-2 degrades single- but not

double-stranded miRNAs *in vitro* indicates that after strand separation, miRNAs that fail to be incorporated into Argonaute or those that are released from the effector complex are the natural targets of XRN-2–mediated decay. Moreover, the vulnerability of a miRNA to degradation by XRN-2 *in vitro* is influenced by target availability. The evidence suggests that interaction of the miRNA–Argonaute complex with its target prevents release and subsequent destabilization of the miRNA[31]. At least *in vitro*, XRN-2 seems to both facilitate Argonaute unloading and catalyze degradation of miRNAs when target sequences are not available (Fig. 2). A relationship between miRNA homeostasis and functional utilization is an intriguing possibility and could contribute to changes in endogenous miRNA levels if this is also the case *in vivo*.

In addition to mature miRNAs, diverse RNA substrates are subject to 5′-to-3′ degradation by XRN-2. In plants, the XRN-2 related proteins XRN2 and XRN3 digest the loops resulting from miRNA precursor processing, an event that happens in the nucleus in *Arabidopsis*[32]. In mammalian cells, XRN-2 aids Pol II transcriptional termination of miRNA primary transcripts by catalyzing degradation of Drosha cleavage products downstream of the miRNA hairpin[33,34]. This role is similar to the function of XRN-2 in terminating Pol II transcription of mRNAs after cleavage by the polyadenylation machinery[35]. Additionally, XRN-2/Rat1 clears the nonfunctional products of numerous RNA processing events, including lariats from splicing, spacer regions from rRNA maturation and 5′ extensions of snoRNAs; this nuclease also targets aberrant RNAs that escape full maturation, such as hypomodified tRNAs and improperly processed mRNAs[36]. All of these functions require nuclear XRN-2 activity. Mature miRNAs, by contrast, reside primarily in cytoplasmic Argonaute complexes[9]. However, nuclear occupancy of Argonaute has been documented and is regulated by the import receptor protein Imp8 in mammalian culture cells[37]. The subcellular distribution of XRN-2 and its miRNA substrates has not yet been investigated. Nonetheless, it seems possible that cellular localization is another layer of regulation determining the turnover rate of mature miRNAs.

Outlook

In the short history of their recognized existence, miRNAs have emerged as indispensable regulators of gene expression in plants and animals. Multilevel processing steps whittle miRNAs into precise mature forms that depend on base-pairing interactions to regulate specific target genes. Transcriptional and post-transcriptional regulatory mechanisms control where, when and how much of a particular miRNA accumulates. Although miRNAs have been considered to be generally stable molecules with half-lives that are often days long[38–40], it is now clear that the absolute levels of mature miRNAs are also controlled by *cis*- and *trans*-acting factors that directly affect stability. Recent discoveries have established that specific modifications and exonucleases can profoundly influence miRNA existence. Stemming from these initial studies are three areas that warrant deeper investigation to further elucidate the causes and effects of altered miRNA homeostasis at the level of mature miRNA stability (Fig. 3).

Tagged for life or death

The 3′-methyl modification on plant miRNAs protects them against 3′-to-5′ degradation by SDN exonucleases[18,19]. This modification also appears to counter uridylation of plant miRNAs. However, it is less clear whether the addition of uracil residues marks the miRNA for destruction, or buffers against SDN activity, or instead is a spurious reaction on unmodified miRNAs that has no functional consequence. The first possibility is consistent with the observation that plants with mutations in the *HEN1* methyltransferase gene have overall reduced miRNA levels and the residual species are heterogeneous in length due to the addition of 3′-uracil residues. Uridylation of other noncoding RNAs has been shown to stimulate their degradation. For example, the nucleotidyltransferase CDE-1 (cosuppression defective) was recently demonstrated to regulate the stability of endogenous siRNAs in *C. elegans* via 3′- terminal addition of uracil residues[41]. Additionally, targeted downregulation of the precursor form of let-7 miRNA has been associated with the appendage of uracil residues by the TUT4/Zcchc11/PUP-2 terminal uridyltransferase[42–45]. Another target of uridylation mediated by Zcchc11 is mature miR-26a (ref. 46). However, in this case the addition of uracil residues to miR-26a abrogates its function without obviously affecting its expression levels. Supporting the possibility that uridylation could also have a protective effect, miRNA substrates with uracil additions were degraded less efficiently by SDN1 *in vitro*[19]. Thus, uridylation is not a definitive tag for destruction of mature miRNAs.

Addition of a limited number of adenines seems to have a stabilizing effect on miRNAs in plants and animals[29,30]. Although adenylation of eukaryotic mRNAs has long been recognized for its importance in stabilization and translation, this same modification targets several noncoding RNAs, such as tRNAs, pre-rRNAs and snRNAs, for destruction by the TRAMP (Trf4/Air1–2/Mtr4 polyadenylation) complex[47]. The addition of a single adenine to the 3′ end of miR-122 in liver cells demonstrates a new role for the cytoplasmic poly(A) polymerase GLD-2 (ref. 29). The broadly conserved GLD-2 protein regulates the poly(A) tail length of specific mRNAs, which in turn influences their translational c ompetence[47]. GLD-2 participates in diverse biological pathways, including germline development and neuronal function[47]. The discovery of miRNAs as new substrates for this poly(A) polymerase raises the possibility that the adenylation and stabilization of specific miRNAs could be important for the biological outputs of GLD-2 activity[29].

The consequence of modifications to mature miRNAs is likely to be dependent on context. Given the established examples in which adenylation can have opposite effects on RNA stability depending on the substrate and polymerase complex, a simple code that dictates miRNA half-life may not exist. The addition of an adenine residue to noncoding RNAs can prevent uridylation[29,48]. However, many mature miRNAs naturally end in adenine or uracil residues, so it is unclear whether the presence of these nucleotides *per se* or the act of modification itself elicits downstream effects on miRNA stability. Finally, an important consideration in studying the role of *cis*-acting modifications on miRNA homeostasis is the likelihood that some types of chemical changes are not apparent by current miRNA detection methods. Moreover, certain modifications would also interfere with standard miRNA cloning strategies. Thus, the extent and types of modifications as well as the

possibility of yet-to-be-discovered miRNA species that have escaped detection are unknowns that await innovative chemical and molecular investigations.

Use it or lose it

The demonstration that target availability affects the release of miRNAs from Argonaute and their subsequent vulnerability to degradation by XRN-2 *in vitro* has important implications for endogenous miRNA function as well as for therapeutic use of small RNAs[31]. The generally poor correlation between expression of miRNA primary transcripts and mature miRNA forms has been attributed to processing regulation[10]. Given the study by Chatterjee and Grosshans, mature miRNA levels might also reflect their targeting activity within a cell[31]. Another clue that activity might influence miRNA accumulation is the finding that miRNA abundance correlates with the number of potential target sites bound by Argonaute *in vivo*[49]. If target association maintains miRNAs in the Argonaute-bound state, then the mechanism of target regulation could also influence miRNA stability: miRNAs that promote mRNA degradation would lose the stabilizing effect of target association more rapidly than miRNAs that remain bound to translationally repressed targets.

The influence of target recognition on Argonaute occupancy and stabilization of miRNAs *in vivo* is not yet established. However, the evidence that Argonaute is a limiting factor for endogenous miRNA accumulation implies that there is competition among small RNAs for Argonaute protection[13–16]. Notably, miRNA regulation of endogenous targets can be perturbed by transfection of siRNAs or miRNAs into culture cells[50,51]. The upregulation of predicted miRNA targets in cells introduced to exogenous siRNAs was attributed to titration of the silencing machinery[50]. Saturation of Argonaute-binding capacity is expected to limit the function and stability of endogenous miRN As[13–16,50]. Several miRNA targets related to oncogenic pathways were found to be commonly upregulated in response to unrelated siRNA transfections[50]. Thus, potential disruption of endogenous target regulation by Argonaute titration may have unexpected but profound biological consequences.

To be or not to be

The exonucleases SDN and XRN-2 degrade unprotected mature miRNAs. In *Arabidopsis*, miRNAs devoid of 3′ methyl modifications are subject to 3′-to-5′ degradation by SDN nucleases, and in *C. elegans*, release from Argonaute exposes miRNAs to 5′-to-3′ decay by XRN-2 (refs. 19,31). These exonucleases appear to generally act on miRNAs and could potentially be responsible for efficient clearance of the unselected passenger strand after separation from its guide-strand partner. A regulatory role for SDN or XRN-2 in the clearance of specific miRNAs has not been determined. Good candidates for miRNAs subject to regulated destabilization are the brain-enriched miRNAs miR-9 and miR-183, with short half-lives of about 1 h, and miR-124, whose mature but not precursor levels rapidly drop in response to serotonin treatment in neurons from *Aplysia californica*, a marine snail[52,53]. In another example, the extreme variations in mature miRNA levels for members of a common primary-transcript cluster during embryonic stem-cell differentiation could involve targeted degradation of individual miRNAs[54]. Presumably, cofactors that recognize specific miRNA sequences would be needed to recruit exonucleases to particular

substrates. Some miRNAs show extensive sequence conservation beyond just the 5′ region important for target interaction. Maintenance of nucleotide identity may be important for recognition of certain miRNAs by sequence-specific RNA-binding proteins that regulate processing or stability. Curiously, the first *cis*-acting element shown to regulate mature miRNA fate is not broadly conserved. The 3′ terminal hexanucleotide sequence of human miR-29b promotes nuclear localization and subsequent destruction of this miRNA[55]. The *trans*-acting factors that recognize this sequence element and promote trafficking and degradation of miR-29b are yet undiscovered.

The connections between target availability, Argonaute capacity and miRNA accumulation underscore the exquisite regulation of mature miRNA expression. It is likely that some miRNAs have also evolved elements that influence Argonaute loading efficiency, recognition by modifying enzymes and vulnerability to nucleases, all of which may ultimately affect the lifespan of a miRNA. The birth and death of miRNAs have now come full circle; general features of miRNA biogenesis and degradation have been established. However, the regulatory mechanisms that govern transcription, processing and now destabilization of multitudes of different miRNAs are not yet fully defined. Determining how specific miRNAs are marked for death and identifying the assassins that do the job are vital challenges to understand the cause and consequence of dynamic changes in mature miRNA levels during development and disease.

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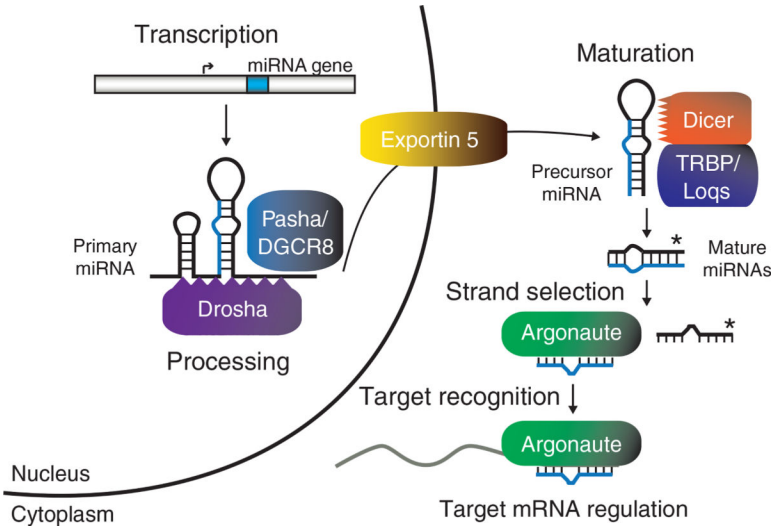

Figure 1.

A general model of miRNA biogenesis and function[6–9]. After synthesis by RNA polymerase II, miRNA primary transcripts are recognized by Pasha/DGCR8 and Drosha, which excises the hairpin precursor. Exportin 5 delivers the miRNA precursor to Dicer and its RNA binding partner, TRBP/Loqs, for final processing to the mature 22-nt miRNAs. One strand is selected for stable association with Argonaute, where it serves as a guide to target and regulate specific mRNAs.

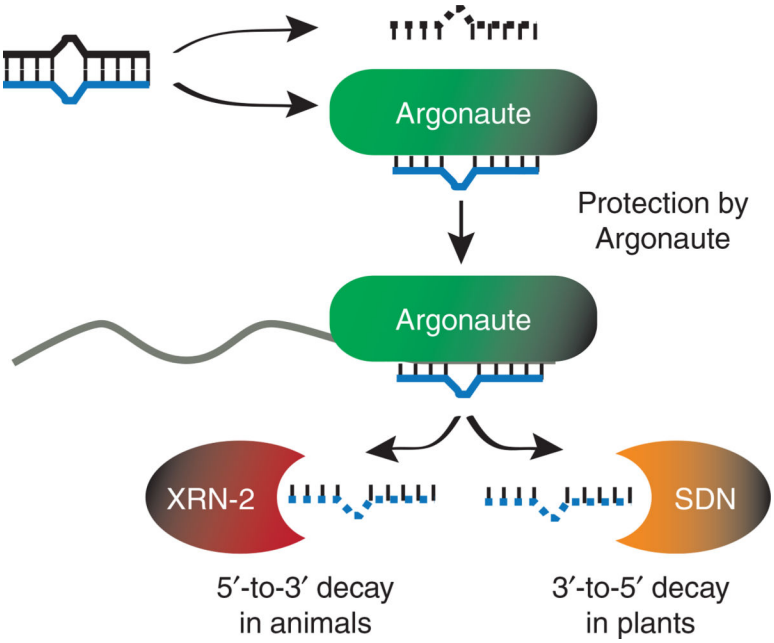

Figure 2.

Proteins that regulate miRNA stability. Incorporation into Argonaute stabilizes mature miRNAs and release from this complex leaves miRNAs vulnerable to decay by XRN-2 or SDN exonucleases[13–16,19,31]. In *C. elegans*, XRN-2 also facilitates release of miRNAs from Argonaute proteins that are not associated with targets[31].

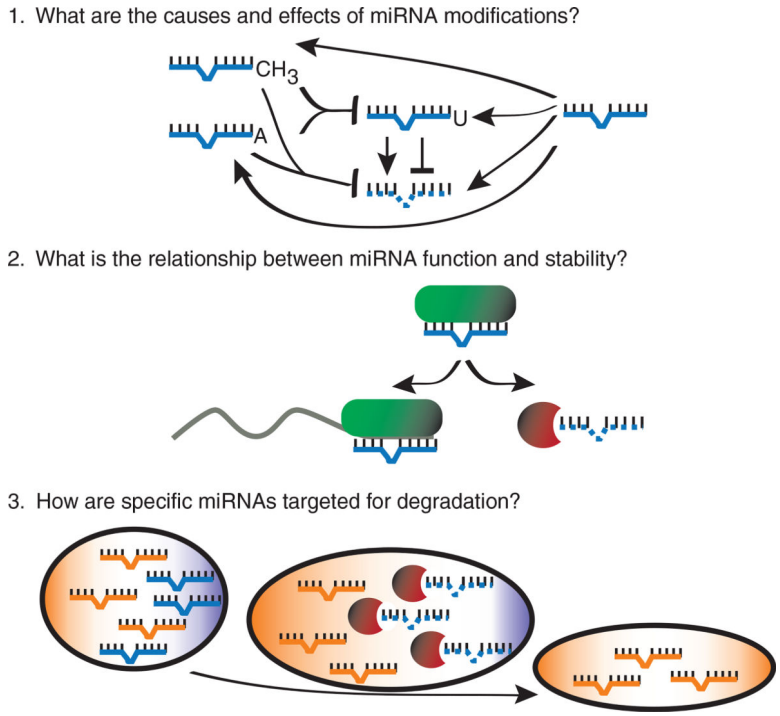

Figure 3.

Outstanding questions regarding factors that regulate miRNA stability. First, some modifications appear general, such as methylation of plant miRNAs, whereas others may be targeted to specific miRNAs, such as adenylation of miR-122 (refs. 18,29). In several cases the modifying enzyme and effect of the modification on miRNA stability are not yet known. Second, evidence is mounting that Argonaute is a limiting factor for miRNA function and stability[13–16]. Target availability has been shown to influence the association of a miRNA with Argonaute and its protection from degradation *in vitro*[31], but whether this parameter influences miRNA accumulation *in vivo* is not yet established. Third, the extent of targeted degradation of specific miRNAs as a means to transform the cellular miRNA population is unclear. Decay of select miRNAs could contribute to the dynamic changes in miRNA levels that often accompany differentiation.

Table 1

Enzymes that act on miRNAs

Name	Type	Substrate	Activity
Drosha in animals DCL in plants	RNase III endonuclease	Primary miRNAs	Generates hairpin precursor
Dicer in animals DCL in plants	RNase III endonuclease	Precursor miRNAs	Removes loop from precursor to generate mature miRNA duplex
Argonaute	PIWI-RNase H endonuclease	Precursor miRNAs	Cleaves passenger strand of some miRNA precursors
HEN1	Methyltransferase	Mature miRNAs and siRNAs in plants piRNAs and siRNAs in animals	Adds 2′-O-methyl group to the 3′ ends of small RNAs
GLD-2 in animals	Poly(A) polymerase	Mature single-stranded miRNAs	Adds adenosine to the 3′ end of miR-122 and possibly other miRNAs
TUT4/Zcchc11/ PUP-2 in animals	Uridyltransferase	Mature single-stranded and precursor miRNAs	Adds uridines to miRNA and precursor 3′ ends
SDN1 in plants	3′-to-5′ exonuclease	Mature single-stranded miRNAs	Degrades mature miRNAs
XRN-2 in animals	5′-to-3′exonuclease	Mature single-stranded miRNAs Primary miRNA cleavage products	Degrades mature miRNAs Degrades 3′ sequence after Drosha cleavage
XRN2, XRN3 in plants	5′-to-3′ exonuclease	Precursor miRNA loops	Degrades loop sequence released from miRNA precursors after Dicer cleavage

Table 2

Modifications that affect miRNA stability

Modification	Organism	Enzyme	Potential effects
2′-*O*-methylation of miRNA 3′ ends	Plants	HEN1	Stabilization Inhibition of 3′ uridylation
Uridylation of miRNA 3′ ends	Plants Animals	Unknown TUT4/Zcchc11/PUP-2	Destabilization Inhibition of 3′-to-5′ decay by SDN1 Destabilization of miR-122 Reduction of function, but not stability, of miR-26b
Adenylation of miRNA 3′ ends	Plants Animals	Unknown GLD-2	Stabilization in *P. trichocarpa* extracts Stabilization of miR-122 Inhibition of 3′ uridylation

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