Published in final edited form as:
Wiley Interdiscip Rev RNA. 2012 ; 3(4): 593–600. doi:10.1002/wrna.1114.

MicroRNA degradation and turnover: regulating the regulators

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Abstract

MicroRNAs (miRNAs) are endogenous, small noncoding RNAs that play important regulatory roles in gene expression. The control of miRNA biogenesis has been well characterized, but their degradation is not fully understood. Recent discoveries indicate that miRNAs have a long life span in general. However, rapid turnover dynamics of miRNAs in a variety of specific cellular contexts has been documented, as well as the requirement of sequence elements for miRNA decay. Furthermore, several ribonucleases that degrade miRNAs have been identified. Here, we discuss the cellular contexts and biochemical mechanisms of miRNA decay, together with several prominent questions in this field.

INTRODUCTION

MicroRNAs (miRNAs) have emerged as important regulators of gene expression in metazoans and plants. Their discovery has greatly enhanced our comprehension of the many layers of posttranscriptional gene regulation. The miRNAs are a type of small noncoding RNA, typically 22 nucleotides long. These small regulatory molecules are involved in a variety of biological processes, and their misregulation is often causally associated with human diseases. A miRNA originates from a hairpin structure located within a longer primary transcript. In general, two members of the RNase III family, Drosha and Dicer, sequentially cut the primary transcript, and then one strand (guide strand) is incorporated into the RNA-induced silencing complex (miRISC). While this canonical pathway of miRNA processing has been well elucidated, the regulation of miRNA biogenesis has recently attracted increased attention. As well, the turnover of mature miRNAs has become a key issue[1] in which important progress has recently been made.[2] Here, we summarize the most recent work on the degradation of mature miRNAs, with special attention given to the cellular and molecular contexts in which miRNA destabilization occurs.

miRNAs ARE GLOBALLY STABLE

A primary question about miRNA turnover is whether miRNAs are typically stable or not. Considering that mRNA is subject to active regulation and structural RNAs are often relatively long lived, we think the question concerning the stability of miRNA is of particular interest. From the viewpoint of biochemical function, miRNAs are like

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The authors declare no conflict of interest.

transcription factors that specifically regulate the expression of target genes. From the viewpoint of molecular structure, miRNAs resemble structural noncoding RNAs such as snoRNAs, which are packaged with proteins into relatively stable complexes.

Stability of miRNAs was implicated in early studies of several miRNAs. For example, miR-208, which is located within an intron of the gene encoding myosin heavy chain, persists for 3 weeks after transcription of its host gene is blocked.[3] Likewise, expression levels of mature miR-122 remains constant over 1 day in mouse liver, while its precursor levels oscillate with an approximately fivefold amplitude between daytime and night.[4] More recently, global turnover rates of miRNAs have been investigated by both exogenous and endogenous approaches. For example, in human 293T cells, blocking transcription with a chemical inhibitor had no effect upon mature miRNA expression levels even after 8 h of treatment.[5] In addition, miRNAs produced from plasmids transfected into HeLa cells persist over 12 h.[6] One could argue that miRNAs might be produced by continuous processing of primary transcripts and precursors after a transcriptional block, and exogenous miRNA might not completely recapitulate the loading of miRNA into the silencing complex. To minimize potential artifacts related to the above experiments, Gantier et al. employed an alternative approach by which Dicer in mouse embryonic fibroblasts was conditionally ablated in the presence of tamoxifen and miRNA levels were measured after a transcription block for a few days.[7] Their experiments showed that the half-lives of miRNAs ranged from 28 to 220 h, which is roughly 2- to 20-fold longer than that of typical mRNAs (about 10 h). As only residual Dicer activity has been reported after genetic ablation, these studies provide evidence that in general, the majority of miRNAs are more stable than mRNAs. Interestingly, miRNAs in serum are also long lived.

How do miRNAs resist attack by ribonucleases? The silencing complex miRISC may shield its resident miRNA from cleavage by ribonucleases. In fact, the core components of silencing complexes, Argonaute (AGO) proteins, act to enhance the abundance of miRNAs besides as acting as a silencer.[8] Structural biology data of small RNA and AGO suggest that AGO tightly binds to small RNA, in particular, both 5′ and 3′ ends of small RNA are buried within the AGO protein.[9] Thus, it would appear that little free space remains for miRNAs in miRISC. Nevertheless, it was reported that the RNA/DNA binding protein, translin, can bind to miR-122.[10] Besides the shielding effects of the silencing complex, do chemical modifications of miRNAs protect them from attack by ribonucleases? In plants, the 3′ ends of miRNAs are 2′-*O*-methylated, which is catalyzed by methyl transferase HEN1.[11] Methylated miRNAs are protected from destruction.[12] The resistance to exoribonuclease functions indirectly, that is, methylation prevents uridylation, which subsequently blocks degradation of miRNAs. In animals, methylation has not been observed so far, except for the passenger strand of miRNA in the fruitfly.[13,14]

What biological sense does it make for miRNAs to possess long half-lives? A miRNA often has a large copy number in a single cell.[15] Slow decay contributes to the high accumulation levels of mature miRNAs. Biochemical analysis indicates that turnover of miRNAs is less efficient than that of siRNAs.[16,17] Thus, the high abundance of miRNAs confers on them the ability to target multiple mRNAs.

DEGRADATION OF miRNAs

While miRNAs are globally stable, individual miRNAs display rapid decay dynamics in some specific situations. For example, while abundance of miR-29b remains low during the DNA replication phase of HeLa cells, it transiently accumulates during the mitotic phase.[18] As a second example, a set of neuron-enriched miRNAs such as miR-9, miR-125b, and miR-146a possess half-lives of 1 to 3.5 h.[19] Moreover, upon exposure to five spaced pulses

of serotonin, abundance of mature miR-124 in Aplysia neurons decreases twofold within 1 h.[20] A fascinating finding extended these observations: when mice were forced into light and dark adaptation, the abundance of retina-enriched miRNAs, including miR-183/-96/-182, miR-204, and miR-211, switched between high and low levels within 1–3 h, respectively.[21] Further analysis in cell culture showed that neurons must be mature enough to manifest neuronal activity-dependent regulation of miRNA turnover. Of note, rapid decay occurred for a few neuron-enriched miRNAs but not constitutively expressed miRNAs and for mature miRNAs but not the miRNA precursors.[21] In summary, these examples illustrate the sophisticated regulation of miRNA metabolism in several cellular contexts (Table 1).

As for the biological function of miRNA turnover, the retina-enriched miRNAs mentioned above are partially responsible for the repair of retina damaged by acute exposure to high intensity light,[29] although it is not clear whether they play a role in light/dark adaptation. Finally, decay of the miR-16 family is suggested to be involved in the cell-cycle regulation program.[27]

There are two prominent questions related to miRNA decay. One is how specific miRNAs are marked for destruction; the other is which ribonucleases catalyze the degradation reaction. With respect to the specificity of miRNA degradation, a number of factors have been identified, which are discussed below.

Target RNAs Mediate Both Protection and Degradation Effects

What happens when a miRNA binds to a target mRNA? We know that the target mRNA is subject to translational repression and/or instability, but the fate of the miRNA has not been well defined. The first clues came from a study of artificial miRNA inhibitors—AntagomiRs, which perfectly base pair with miRNAs and thereby affect miRNA abundance. This suggests that miRNA stability and target binding may be linked.[30] Recently, two studies examined the decay mechanism of miRNAs triggered by cognate target mRNAs. In both fruitfly and human cell lines, targets promoted miRNA decay.[24] The effect required extensive base pairing between miRNAs and cognate target mRNAs, as 8 nucleotide mismatches abolished miRNA decay. In situations leading to miRNA degradation in the presence of targets, miRNAs were found to be tailed with uracils at their 3′ ends, which will be discussed below. In another study, a target mRNA which is either perfectly base paired with miR-223 or had a 2 nucleotide bulge at the center was overexpressed in 293T cells and miR-223 was precisely quantified.[31] The result showed that turnover of miR-223 was significantly faster with the perfect target compared to the bulged target, and deep sequencing data revealed that the 3′ end of miR-223 had one or two uridines added.

However, both *in vitro* and *in vivo* assays with *Caenorhabditis elegans* showed that targets can also protect miRNAs from active degradation.[22,23] For example, let-7 disappeared when it was incubated in worm lysate. When target mRNAs of let-7 were supplied to the lysate, mature let-7 produced from pre-let-7 became stable and accumulated.[22] In the converse experiment, deletion of two endogenous targets of miRNA lin-4 also decreased abundance of lin-4.[23] Finally, let-7 with a point mutation in the seed sequence presented decreased expression due to the lack of cognate targets; however, supplying a target for the mutated let-7 stabilized the miRNA. Because base pairing between miRNAs and target mRNAs lays the foundation for miRNA function, this interaction plays a fundamental role in control of miRNA stability. The discrepancy in conclusions regarding target-mediated regulation of miRNA stability will be further discussed below.

Uridylation Promotes miRNA Instability

Nontemplated addition of uridines and adenosines is the predominant form of modifications at 3′ ends of mature miRNAs.[32] The causal relationship between modification and miRNA stability was first elucidated in plants. As mentioned earlier, the 3′ ends of *Arabidopsis* miRNAs are methylated, which is catalyzed by methyl-transferase HEN1. In a *hen1* mutant, miRNAs are modified by addition of one to five uridines or adenosines, and the modified miRNAs are downregulated significantly.[12] However, the authors also reported that uridylated miRNAs are resistant to degradation by 3′ → 5′ exoribonuclease SDN1, suggesting that uridylation may have a protective effect in the absence of methylation[33] (see below). Nonetheless, uridylation promotes miRNA degradation in alga. Chlamydomonas nucleotidyl transferase MUT68 uridylates mature miRNAs leading to their degradation.[34] A clear understanding of the relationship between modifications and miRNA decay in animals was not established until recently. The initial observation was that addition of a single adenosine specifically stabilized miR-122 in mice.[35] GLD-2 catalyzes polyadenylation of some noncoding RNAs and mRNAs. MiR-122 is a novel substrate of GLD-2. In mouse liver, lack of GLD-2 diminished the steady-state level of miR-122 (which normally has an added adenosine at the 3′ end), while other miRNAs remained unchanged. A genome-wide analysis showed that GLD-2 was responsible for adenosine addition to the 3′ ends of many miRNAs in the human monocytic cell line THP-1. Adenylation did not reduce stability of miRNAs in THP-1 cells but it did decrease their association with AGO2 and AGO3, thereby decreasing their effectiveness.[36] However, in fruitfly embryos, a target which perfectly base paired with miRNA *bantam* remodeled the bantam/ago1 complex, that is, bantam was tailed with adenosine and trimmed at the 3′ end, ensuring its disappearance.[24] Therefore, the fate of adenylated miRNAs largely depends on their cellular context.

As for uridylation, a variety of effects on miRNA turnover have been documented for different miRNA species. As mentioned earlier, uridylation is associated with decay of miRNAs for perfect targets.[24,31] In addition, pre-let-7 is modified by a poly(U) tail added by the nucleotidyl transferase TUTase 4, which precedes destruction of the precursor.[37] In this case, the uracil tail is more than 10 nucleotides long, and it seems to act as a platform to nucleate assembly of a ribonucleolytic machine. Nevertheless, a report noted that uridylation of miRNA does not necessarily lead to its instability but rather represses its activity. In human lung alveolar epithelial cells, miR-26a targets the mRNA encoding interleukin-6.[38] Experiments showed that TUTase 4 (also named as zcch11) modified miR-26a by adding one, and less frequently, two uridines. The addition did not decrease miR-26a abundance but significantly inhibited miR-26a activity and consequently relieved repression of interleurkin-6 synthesis.[38] Therefore, for different miRNA species and cellular contexts, uridylation may regulate miRNA stability or activity.

Viral Noncoding RNAs Direct miRNA Decay

Recently, an interesting report noted a novel factor that directs miRNA decay. *Herpesvirus saimiri* encodes several conserved noncoding RNAs, including HSUR1 and HSUR2. HSUR1 and HSUR2 can base pair with the seed sequence of miR-27a.[26] In marmoset T cells infected with Herpesvirus, the decay rate of miR-27a was much faster than others and its steady-state level was selectively reduced. Mutation analysis suggested that HSUR1 directly binds to miR-27a and regulates its degradation.

Cellular Adhesion Alters miRNA Life Span

An interesting observation is that global levels of miRNAs increase with the density of cell culture.[39] It was first attributed to alterations of miRNA biogenesis, including enhanced Drosha processing activity and enhanced miRISC formation. Alteration of miRNA stability, however, was revealed to be the novel underlying mechanism responsible for this

observation. The Kim group explored the decay of miRNAs with the logic that differences in steady-state levels of miRNAs from a common polycistronic primary transcript is likely due to posttranscriptional regulation, including turnover rates.[25] Indeed, they found that although miR-141 and miR-200c were located in an identical cluster, miR-141 abundance fluctuated dramatically with cell density, whereas miR-200c was unaffected. Loss of cellular adhesion by either trypsin digestion or addition of calcium chelator caused the level of miR-141 to drop quickly. It had been unknown whether cell–cell contact or cell–extracellular matrix adhesion plays critical roles in maintenance of miRNA stability. Nonetheless, this is not an isolated finding; a screen for miRNA pathways in *C. elegans* revealed that the cytoskeleton, particularly the microtubule, is largely required for miRNA stability.[40] The roles of the cytoskeleton for miRNA stability remain a mystery.

Intrinsic Stability

Are there sequence elements in miRNAs that determine their half-lives? Although miRNAs are as short as 22 nucleotides, several sequences that regulate miRNA decay rate have been identified. One is 7 nucleotides at the 3′ end of miR-382, specifically, GGAUUCG.[5] Mutations in this sequence enhanced stability of the miRNA in human 293T cells. An *in vitro* system that couples precursor miRNA processing and decay showed that specific decay of miR-382 (vs the stable control miR-378) required its processing *in vitro*; differential decay was lost with either single-or double-stranded versions of mature miR-382 and miR-378. Two decay elements lie within the seed sequence and 3′-end nucleotides of miR-503. Both are required for miRNA decay. A third example is three uridines at nucleotides 9–11 in miR-29b. This uridine-rich element is required for rapid decay of miR-29b, and degradation depends on both the uridine sequence and their exact position.[6] It seems that decay is also related to the unwinding or loading of the miRNA/miRNA* duplex into silencing complexes. Finally, UGUCU at nucleotides 7–11 in miR-141 determine its decay rate. Unlike the UUU sequence in miR-29b, the exact position of UGUCU in miR-141 is not important for its function. Among the four decay sequences described, it is not yet known whether the 7 nucleotide one in miR-382 is sufficient to give rise to decay in any miRNA, while the other three are not able to bring about decay in a random sequence context. It is believed that both ends of miRNAs are buried within the AGO proteins, while the middle region is relatively free.[9] With respect to miRISC structure, the decay sequences may thus be grouped into two different modes for regulation of miRNA instability, with one at the center and the other at either the 5′ or 3′ end.

EXECUTIONER OF DEGRADATION

A key to understanding miRNA decay is to identify enzymes that catalyze the breakdown of phosphodiester bonds in miRNAs. At present, four ribonucleases have been reported to act on substrate miRNAs. Among them, two exoribonucleases were identified by elegant screens. Chen and colleagues hypothesized that ribonucleases cutting miRNAs would likely be the same ones that cut structural and catalytic RNAs.[33] Following this idea, they searched for *Arabidopsis* homologs of yeast exoribonucleases that process rRNAs and tRNAs and then chose two enzymes for experimental examination. As expected, one of them, SDN1 (small RNA degrading nuclease 1), destroyed miRNAs with high efficiency.[33] The catalytic reaction proceeded in a 3′ → 5′ direction and in a sequence-independent manner. Degradation was blocked by uridylation or 2′-*O*-methyl modification at the 3′ end. In *C. elegans*, XRN2 was identified in a candidate gene screen to suppress the phenotype of a *let-7* loss-of-function mutant.[22] Molecular characterization showed that XRN2 is an exoribonuclease that cuts miRNAs in a 5′ → 3′ direction (Figure 1). Degradation also did not depend on sequence context. The authors provided further insight into the destructive mechanism: mature miRNAs are released from miRISC before degradation and XRN2 facilitates their release.[22] A subsequent study revealed that XRN1, a close relative of XRN2,

is also involved in turnover of miRNAs, for both the guide and passenger strands.[23] In human 293T cells, XRN1, but not XRN2, had a moderate effect on decay of miR-378 and miR-382.[5] PNPase PNPT1 (also known as PNPase^{old-35}) is a 3′ → 5′ exoribonuclease and an exosome subunit that catalyzes the phosphorolysis of miRNAs.[28] In human melanoma cell line HO-1, PNPT1 is induced by interferon-β; it immunoprecipitates with and degrades miR-221. In contrast to SDN1 and XRN2, which have extensive miRNA substrates, PNPT1 activity possesses a high specificity for a small subset of miRNAs, in particular miR-221. In addition to the four exoribonucleases mentioned, RRP41, which is also an exosome subunit, is required for degradation of miR-278 and miR-382.[5]

CONCLUSIONS AND FUTURE DIRECTIONS

How does a cell turn off the function of miRNAs? One way is to regulate the activity of miRNAs, the other is to modulate their abundance by turnover. Very recently, several exciting findings concerning the regulation of miRNA stability have been documented. However, there is still much unknown about this topic.

Interplay Between Targets and miRNAs

There is some discrepancy concerning target mRNA effects on miRNA fate between human and *C. elegans* cells. In human cells, cognate mRNA targets promote miRNA trimming and tailing at their 3′ ends if the targets are extensively base paired with the miRNAs. In *C. elegans*, target mRNAs prevent the release of miRNAs from the bound miRISC.[22] These differing results may be due to differences in the biochemical mechanisms employed by different species, but it might also be attributed to the experimental systems used. While the observation that targets protect miRNAs has been tested both *in vitro* and *in vivo*, the observation that targets trigger miRNA trimming has been analyzed in cell free systems. More importantly, targets that are able to remodel miRISC and give rise to decay of miRNAs must almost perfectly base pair with miRNAs. However, most endogenous targets do not fulfill that requirement. Therefore, it is essential to clearly examine the interplay between miRNAs and their endogenous targets in the context of the mammalian cell.

Recently, a novel paradigm for mRNA function independent of protein coding was identified. Messenger RNAs, including pseudogene mRNAs, bind targeting miRNAs and thus alleviate miRNA-mediated repression on mRNAs whose 3′ UTRs have a common miRNA binding site (i.e., by acting as a sponge).[41] The genetic and genomic evidence suggests that pseudogenes of oncogenes and tumor suppressor genes have a significant impact on cancer biology.[42,43] Thus, due to the effect of target-directed protection or degradation on miRNAs, it is conceivable that pseudogene mRNAs may stabilize or destabilize their targeting miRNAs besides acting as a competitor between miRNAs and protein coding genes.

Factors That Alter miRNA Stability

The miRNAs undergo rapid decay dynamics in a few circumstances, such as miR-29b in cycling cells and a small subset of miRNAs in neurons. Which environmental stimuli or cellular factors can accelerate turnover of miRNAs? In fertilized zebrafish embryos, miR-430 plays a role in clearance of maternal mRNAs by directing them for destruction.[44] Then, how are maternal miRNAs cleared? Can the clearance of maternal miRNAs be attributed to active degradation or to simply dilution of miRNAs by cellular proliferation during embryonic development? In addition, miRNAs are often involved in stress responses such as hypoxia. It is possible that miRNA decay may contribute to the rapid dynamic regulation of miRNA abundance in such circumstances.

The most fascinating questions concerning miRNA decay are the specificity of degradation. For example, miR-29b, but not its paralog miR-29a, undergoes rapid turnover in cycling cells[18]; several retina-enriched miRNAs such as miR-183/96/182, miR-204, and miR-211 disappear quickly, while constitutively expressed miRNA miR-16 remains constant in dark adaptation.[21] How is such a high level of selection achieved? Since several neuron-enriched miRNAs do not possess the consensus sequences noted above, additional factors are likely to 'mark' these miRNAs for degradation.

Modifications, especially uridine addition to miRNA 3′ ends, are plausible candidates for 'tags' that promote miRNA degradation. As mentioned earlier, uridine addition can lead to instability or inactivation of miRNAs. Possibly both action modes can take effect. Alternatively, there is an intrinsic link between miRNA inactivation and instability since a deficiency of target accessibility may reduce target-directed protection. Another key point is that while a poly(U) tail can trigger mRNA decay by acting as a platform to assemble the degradation machinery,[45] oligo(U) addition to miRNAs may be too short to serve a similar function for miRNAs. Keeping in mind that miRNA termini are thought to be anchored by AGO, one can imagine that 3′-end modifications might alter miRNA affinity for miRISC and thereby promote or prevent release of miRNAs from the protein complex.

A compelling finding is that viral long noncoding RNAs base pair with some miRNAs and direct them for destruction. Endogenous long noncoding RNAs, large numbers of which were identified in neural systems,[46] might mimic viral noncoding RNAs to guide miRNA decay. However, many endogenous long noncoding RNAs are localized in the nucleus,[47] while mature miRNAs are predominantly restricted to the cytoplasm.[48] Hence, the scenario in which long noncoding RNAs and miRNAs interact will require further experimental consideration.

Degradation Machinery

XRN2 and XRN1 degrade miRNAs in *C. elegans*; PNPT1 preferentially degrades a small subset of miRNAs in response to interferon; and RRP41 and XRN1 are also likely to degrade miRNAs in 293T cells. It is an open question whether these ribonucleases are responsible for degradation of neuron-enriched miRNAs and miR-29b in cycling cells. None of them appear required for adhesion-dependent decay of miR-141 and constitutive degradation of miR-503.[27,25] The preference of PNPT1 for select miRNAs is due to unknown factors. Furthermore, XRN1 and XRN2 are 5′ → 3′ exoribonucleases, while RRP41 and PNPT1 are subunits of the exosome, which is a 3′ → 5′ degradation complex. The exosome is probably one site where cleavage of miRNAs occurs. However, hydrolysis of miRNAs can begin with either the 5′ or 3′ end, which implies a specific selection process for miRNA decay modes.

Regulation of miRNA Decay

In the documented cases of miRNA decay mentioned above, the changes in magnitude of miRNA abundance in cells is often quite small, typically a few fold. This change is relatively modest compared with that of mRNAs. Does this characteristic indicate that there is fine-tuned control of miRNA degradation? Indeed, miR-29b displays an exquisite, oscillating profile between cycling and mitotic phases of HeLa cells, and neuronal-enriched miRNAs precisely respond to light/dark adaptation. Characterizing the regulation of miRNA decay should continue to unveil the truly intricate mechanisms that regulate gene expression.

Acknowledgments

This work was supported in part by the National Natural Science Foundation of China (81130005, 30828006), Ministry of Science and Technology of China (No. 2010CB945600, 2011CB811304, and 2007CB947002), Chinese

Academy of Sciences (XDA01040306, KSCX2-YW-R-233, and KSCX2-YW-R-096), and Shanghai Pujiang Program (05PJ14105). G.B. was supported by NIH grant CA052443 and by a Visiting Professorship from the Institute of Health Sciences, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences.

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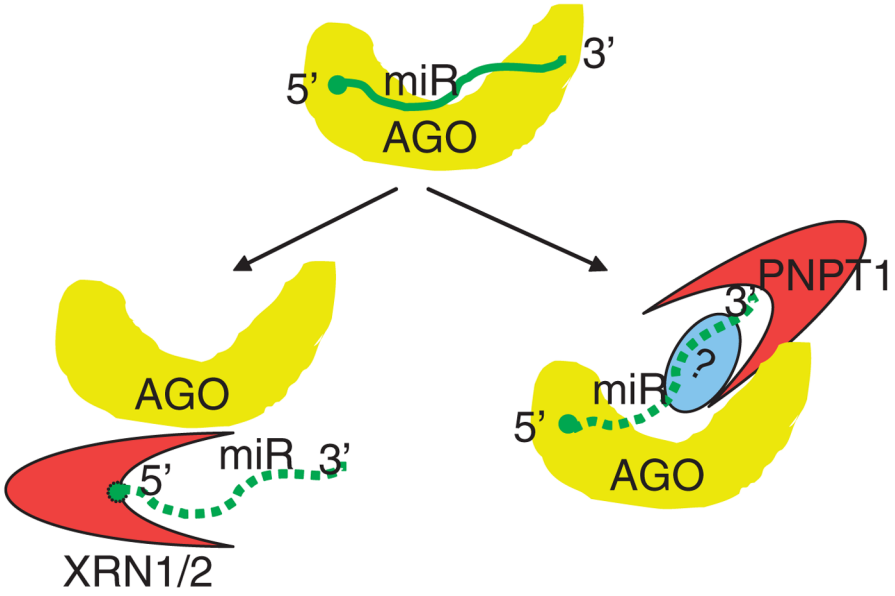

FIGURE 1.

A cartoon depicting biochemical pathways for miRNA decay. The miRNAs can be degraded from either 5′ end (left branch) or 3′ end (right branch). Exoribonuclease XRN1 and XRN2 facilitate the dissociation of miRNAs from miRISC and cut miRNAs in the 5′ → 3′ direction. PNPT1 can degrade specific miRNAs in the 3′ → 5′ direction. A potential factor (denoted as ‘?’) may cooperate with PNPT1 to achieve specificity.

TABLE 1

Documented miRNA Decay in Animals

miRNA	Specificity	Sequence	Cellular Context	RNase	Reference
let-7, miR-241	General	Sequence independent	*Caenorhabditis elegans*	XRN1, XRN2	22,23
let-7, bantam, miR-277 in fly, miR-16, -21 in human		Sequence independent	Fly S2 cells, HeLa	XRN1, XRN2	24
miR-29b	Specific	UUU (9-11)	HeLa	ND	6,18
miR-141		UGUCU(7-11)	Lack of cellular adhesion	ND	25
miR-27a		Seed sequence	Viral long noncoding RNA	ND	26
miR-382		GGAUUCG	HEK293T	RRP41, XRN1	5
miR-503		Seed sequence and at least 6 nucleotide at 3′ end	NIH3T3	ND	27
miR-221		ND	HO-1	PNPT1	28
miR-9, 183, 146a, 132, 125b		ND	Neurons	ND	19
miR-124		ND	Aplysia neurons; serotonin stimulated	ND	20
miR-183/96/182, -204, -211		ND	Differentiated neurons; dark adaption	ND	21

ND, not determined.

Wiley Interdiscip Rev RNA. Author manuscript; available in PMC 2013 July 01.