Published in final edited form as:
Ageing Res Rev. 2013 January ; 12(1): 429–435. doi:10.1016/j.arr.2012.03.012.

Ageing and the Small, Non-Coding RNA World

Masaomi Kato and **Frank J. Slack***
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA

Abstract

MicroRNAs, a class of small, non-coding RNAs, are now widely known for their importance in many aspects of biology. These small regulatory RNAs have critical functions in diverse biological events, including development and disease. Recent findings show that microRNAs are essential for lifespan determination in the model organisms, *C. elegans* and *Drosophila*, suggesting that microRNAs are also involved in the complex process of ageing. Further, short RNA fragments derived from longer parental RNAs, such as transfer RNA cleavage fragments, have now emerged as a novel class of regulatory RNAs that inhibit translation in response to stress. In addition, the RNA editing pathway is likely to act in the double-stranded RNA-mediated silencing machinery to suppress unfavorable RNA interference activity in the ageing process. These multiple, redundant layers in gene regulatory networks may make it possible to both stably and flexibly regulate genetic pathways in ensuring robustness of developmental and ageing processes.

Keywords

Aging; microRNA; small non-coding RNA; RNA editing; *C. elegans*

1. Introduction

Recent advances in high-throughput technologies, such as a deep-sequencing, have facilitated studies of small, non-coding RNAs, including microRNAs (miRNAs). miRNAs make up a class of short, non-protein-coding RNA species, that negatively regulate expression of target genes at a post-transcriptional level via sequence-specific interactions (reviewed in (Kim et al., 2009; Stefani and Slack, 2008)). Since the discovery of *lin-4* and *let-7* miRNAs as regulators of developmental timing in *C. elegans* (Lee et al., 1993; Pasquinelli et al., 2000; Wightman et al., 1993), the last decade has seen the exponential growth of knowledge about the functions of miRNAs in broad areas of biological events, ranging from development to human disease, such as cancer, and ageing (Ambros, 2011; Boehm and Slack, 2005; Esquela-Kerscher and Slack, 2006)

The first evidence of a role for miRNAs in ageing was reported in a study of a developmental timing miRNA, *lin-4*, in *C. elegans* (Boehm and Slack, 2005). It now seems clear that additional miRNAs contribute to the normal lifespan by modulating the processes

*Corresponding author: Contact address: Frank Slack, Ph.D., Department of Molecular, Cellular and Developmental Biology, KBT 936, Yale University, P.O. Box 208103, 266 Whitney Ave, New Haven, CT 06520, Tel: 203-432-3492, Fax: 203-432-6161, frank.slack@yale.edu.

of DNA damage response, protein homeostasis and mitochondrial metabolism (Antebi, 2007; Boehm and Slack, 2005; de Lencastre et al., 2010; Ibanez-Ventoso et al., 2006; Kato et al., 2011; Kenyon, 2010). Furthermore, other types of small, non-coding RNA are also likely to function in stress response and ageing (Kato et al., 2011; Thompson and Parker, 2009; Tuck and Tollervey, 2011). In addition, the RNA editing machinery that modifies adenosine to inosine conversion in double-stranded RNA (dsRNA) transcripts seems to act with the dsRNA-mediated gene silencing pathway in lifespan determination (Sebastiani et al., 2009; Wu et al., 2011). These multiple layers in gene regulatory mechanisms may stabilize genetic networks in ageing. In this review we focus on recent studies of RNA-mediated regulatory roles in ageing in a model organism, *C. elegans*, and discuss their potential in ensuring robustness of genetic pathways against ageing and environmental perturbations.

2. The importance of miRNAs in lifespan determination

A developmental timing miRNA, *C. elegans lin-4*, was shown to be essential for normal lifespan (Boehm and Slack, 2005); animals carrying a deletion mutation of the *lin-4* miRNA displayed a shorter lifespan, while its over-expression caused a longer lifespan, showing that the *lin-4* miRNA promotes longevity and is required to prevent premature death in *C. elegans*. A temporal up-regulation of the *lin-4* miRNA in an early stage of development suppresses expression of its target gene, *lin-14*, to initiate the developmental stage transition (Lee et al., 1993; Wightman et al., 1993). Similarly, *lin-4* contributes to lifespan regulation through *lin-14* (Boehm and Slack, 2005). Specifically, both *lin-4* deletion mutants and gain-of-function mutants of *lin-14* (due to loss of a *lin-4* binding site in its 3' untranslated region), show the short-lived phenotype, while animals with over-expression of the *lin-4* miRNA or loss-of-function mutants of *lin-14* have extended lifespans.

Subsequent expression profiling of miRNAs using microarray and deep-sequencing technologies has demonstrated that many of the *C. elegans* miRNAs, in addition to *lin-4*, significantly change in expression level during adult life (de Lencastre et al., 2010; Ibanez-Ventoso et al., 2006; Kato et al., 2011). For example, *let-7* and miR-34, evolutionarily conserved miRNAs from *C. elegans* to human (Kato et al., 2009; Roush and Slack, 2008), show a dramatic decrease and increase in expression during ageing under normal conditions, respectively (de Lencastre et al., 2010; Ibanez-Ventoso et al., 2006; Kato et al., 2011). The small RNA sequencing work and the microarray work showed remarkably similar trends in cases where they profiled the same miRNAs.

In long-lived conditions (e.g. low incubation temperatures), many but not all of the miRNAs with increasing expression during ageing, such as miR-34, show a significant delay in expression change. Conversely, in short-lived conditions (e.g. higher incubation temperatures), they show an accelerated change in expression during ageing (Kato et al., 2011). These results suggest tight regulation of miRNAs during ageing, and that the age-associated miRNAs may serve as biomarkers of ageing (discussed below). All together, these observations suggest that in addition to *lin-4*, additional miRNAs might have a role in *C. elegans* ageing.

Recently several miRNAs with expression changes during ageing were indeed found to be necessary for normal lifespan in *C. elegans* (de Lencastre et al., 2010). A deletion mutation of *mir-71*, which is initially up-regulated in early adulthood and then gets down-regulated during ageing (de Lencastre et al., 2010; Kato et al., 2011; Pincus et al., 2011), caused a significantly shorter lifespan, indicating that miR-71 promotes longevity. In another example, a deletion mutation of *mir-239*, which knocks out a miRNA dramatically up-regulated during ageing, caused a longer lifespan, indicating that miR-239 antagonizes

lifespan (de Lencastre et al., 2010). Notably, over-expression of each miRNA exhibits the reciprocal effect on lifespan; animals with over-expression of miR-71 lead to a significant lifespan extension, while those with over-expression of miR-239 shorten lifespan. In addition to *mir-71* and *mir-239*, one of the evolutionary conserved miRNAs *mir-34* may also be involved in lifespan determination in *C. elegans* (Yang et al., 2011). As these mutants appear otherwise normal, these observations support the idea that the effect of loss of these miRNAs on lifespan is caused by a defect in adulthood rather than a developmental abnormality (Miska et al., 2007).

Consistent with the necessity of these individual miRNAs in ageing, such as *lin-4, mir-71* and *mir-239*, ALG-1, a core component in miRNA processing, is essential for normal adult lifespan in *C. elegans* (Kato et al., 2011). ALG-1 encodes an Argonaute protein necessary for the miRNA maturation and function, but not necessary for other known small, non-coding RNA pathways (Batista et al., 2008; Grishok et al., 2001). In this experiment, to further rule out the possibility of a developmental defect, *C. elegans* animals were treated with RNA interference (RNAi)-mediated *alg-1* knockdown only during adulthood, and they displayed an abnormal lifespan. In addition, under the condition of adult-specific *alg-1* loss, those age-associated miRNAs that increased expression during ageing, including miR-34 and miR-239, were disrupted in their expression (Kato et al., 2011). These observations suggest that the age-associated miRNAs are indeed important for the normal adult lifespan of *C. elegans*.

3. The miRNA-mediated regulation of ageing pathways

It is expected that these age-associated miRNAs modulate various genetic pathways in lifespan determination through the control of their target genes. The insulin-signaling pathway is well known for its central role in lifespan determination and stress response from yeast to human (Antebi, 2007; Fontana et al., 2010; Kenyon, 2010). In *C. elegans*, activation of the insulin receptor DAF-2 under normal conditions causes DAF-16, a homologue of the mammalian FOXO transcription factor, to be retained in the cytoplasm, whereas in response to stress (e.g. heat shock), or reduced levels of insulin-signaling (e.g. *daf-2* mutation), DAF-16 enters the nucleus where it activates longevity and stress resistance genes, leading to extended lifespan and stress resistant phenotypes (reviewed in (Antebi, 2007)). Age-associated miRNAs might regulate the insulin-signaling network, since some of the insulin-signaling pathway genes, such as *daf-16*, appear to have putative binding sites for miRNAs in their 3' untranslated regions (de Lencastre et al., 2010; Ibanez-Ventoso et al., 2006).

From genetic studies of lifespan, both miR-71 and miR-239 indeed likely function in the insulin-signaling pathway (Figure 1) since the longer lifespan induced by loss of *mir-239* is dependent on the normal DAF-16 function, and loss of *mir-71* significantly shortens the long lifespan induced by *daf-2* loss (de Lencastre et al., 2010). Moreover, in aged animals, the loss of *mir-239* causes a significant reduction of the messenger RNA (mRNA) levels of *age-1* and *pdk-1*, components in the insulin-signaling pathway (Antebi, 2007), suggesting that miR-239 may act upstream of these genes to promote insulin-signaling activity (de Lencastre et al., 2010). Conversely, the *mir-71* loss causes an increase in level of *pdk-1* mRNAs in aged animals, consistent with the interpretation that miR-71 promotes longevity by reducing the activity of genes in the insulin-signaling pathway (de Lencastre et al., 2010). Also, as the 3' untranslated region of *pdk-1* mRNA is likely to have potential binding sites for miR-71, expression of *pdk-1* may be directly suppressed by miR-71.

In addition to the insulin-signaling pathway, miR-71 may also act in a DNA damage response pathway (Figure 1) (de Lencastre et al., 2010). It has been shown that decreased activity of DNA checkpoint control proteins, such as CHK-1 (checkpoint kinase) and

members of CDC-25 family (phosphatase), causes a significant increase in lifespan (Olsen et al., 2006). The deletion mutation of *mir-71* completely suppresses the longevity phenotype induced by knockdown of *cdc-25.1* and *chk-1* (de Lencastre et al., 2010). The 3' untranslated region of *cdc-25.1* mRNA appears to have potential binding sites for miR-71, suggesting the possibility that miR-71 promotes longevity by directly suppressing the activity of *cdc-25.1*. In support of this, the mRNA levels of *cdc-25.1* are significantly elevated in aged animals lacking *mir-71* (de Lencastre et al., 2010). These observations suggest that miR-71 may act in not only the insulin-signaling pathway but also the DNA damage response pathway to modulate lifespan determination, although there is still no direct evidence that *mir-71* is necessary for the proper DNA damage response.

Similar to *mir-71* and *mir-239*, the *lin-4* miRNA and its target *lin-14* also function in the insulin-signaling pathway in ageing (Figure 1). As above, *lin-14* loss-of-function mutants exhibit an extended lifespan phenotype, and this longevity is completely suppressed by loss of *daf-16*, suggesting that *daf-16* activity is necessary for the *lin-14* loss-induced lifespan extension, and that *lin-14* acts upstream of *daf-16* (Boehm and Slack, 2005). Consistent with this, we recently found that the loss of *lin-14* activity caused up-regulation of DAF-16 downstream targets, such as *sod-3* (Lee et al., 2003; Murphy et al., 2003) (Kundu, S. and FJS, unpublished). Furthermore, *lin-4* and *lin-14* might act upstream of insulin-signaling DAF-2 (which is upstream of DAF-16) in lifespan regulation, since it has been shown that one of *C. elegans* insulin-like peptide genes, *ins-33*, appears to be a direct downstream target of LIN-14 in development (Hristova et al., 2005). On the other hand, there is a contradictory observation: the *lin-4* miRNA seems to act downstream of DAF-16 because *lin-4* expression is repressed by DAF-16 in a developmental arrest induced by food deprivation (Baugh and Sternberg, 2006). These results suggest the possibility of a negative feedback loop and imply the complexity in the miRNA-involved gene regulatory network. The role of miRNAs might be different among tissues, or between the processes of development and ageing.

Despite the extensive studies of molecular interaction with *lin-4* or *lin-14*, it is not clear exactly why loss of the *lin-4* miRNA shortens lifespan and accelerates the ageing process. However, a recent study may provide a clue to this question. *lin-4* deletion mutants have a remarkably reduced fat content compared to wild-type animals (Zhu et al., 2010). This is possibly due to lower mRNA levels of *sbp-1* (also named *lpd-1*), a key transcriptional factor in fat and sterol synthesis pathways (McKay et al., 2003). Mutations that cause abnormal lifespan may influence fat metabolism in *C. elegans*; for example, in the *daf-2* long-lived mutants, metabolism is shifted to the production of fat (Kimura et al., 1997). Also, many of the putative DAF-16 targets are known to be involved in fat accumulation (Oh et al., 2006). In addition to the abnormal fat metabolism, the loss of *lin-4* results in an increased accumulation of reactive oxygen species (ROS) (Zhu et al., 2010). ROS, which include free radicals such as superoxide, are mostly generated in mitochondria during the process of electron transport in energy production, and cause oxidative damage to macromolecules, such as nucleic acids, proteins and lipids, leading to functional declines (Hekimi et al., 2011). It is possible that in the *lin-4* mutants an imbalance between the mitochondrial ROS generation and repair/detoxification leads to deleterious effects on cellular components, resulting in loss of integrity and progression of the ageing process. (Note that the mitochondrial free radical theory of ageing is a long-held model; however, the relationship between ROS and the ageing process is much more complex than previously estimated, and recent studies present some conflicting findings for this. See (Hekimi et al., 2011) for details).

4. Additional miRNAs in ageing

The effect of miRNA loss on lifespan has been investigated using genetic knockouts for individual miRNAs with significant expression changes in ageing. However, only a few were clearly required for normal lifespan (e.g. *lin-4, mir-71, mir-246, mir-34* and *mir-239*; (Boehm and Slack, 2005; de Lencastre et al., 2010; Yang et al., 2011)). Since not all miRNA knockouts showed obvious abnormalities in lifespan despite their dynamic expression changes during ageing, their age-associated expression changes might be a consequence of ageing with no functional implications on the physiology of aged animals. However, as miRNAs have the potential to affect expression of multiple target genes, it remains possible that many age-associated miRNAs have subtle and/or redundant roles in lifespan determination.

A system biology approach, incorporating conventional miRNA target prediction algorithms with gene expression profiles of miRNAs and protein coding genes in ageing, reveals that many age-associated miRNAs appear to regulate genes in mitochondrial respiration and protein homeostasis (Kato et al., 2011). For example, many of these miRNAs, including miR-71 and some *let-7* family members, are likely to target genes in the TCA (tricarboxylic acid) cycle and oxidative phosphorylation, both of which are involved in energy production in the mitochondria (Wallace, 2005). Additional miRNAs, including *let-7* and miR-34, appear to target genes encoding proteases and Vacuolar-type H^+-ATPase, which compose of lysosomal compartments where damaged macromolecules, including misfolded or aggregated proteins, are degraded (Buchberger et al.; Nishi and Forgac, 2002), or autophagy proteins (Yang et al., 2011). These results suggest that additional miRNAs with age-associated expression changes may work together to maintain, for example, cellular energy balance and protein quality, possibly through a fine-tuning of metabolic genes in the ageing process. Indeed, a recent report indicates that loss of *Drosophila mir-34*, a miRNA with adult-onset expression in the brain, causes brain neurodegeneration and a short lifespan, whereby the *mir-34* loss leads to an increase in accumulation of mis-folded protein (Liu et al., 2012). Moreover, other *Drosophila* miRNAs *mir-278, mir-14* and *mir-8* contribute to energy homeostasis through the regulation of insulin-signaling and fat metabolism (Hyun et al., 2009; Teleman et al., 2006; Varghese et al., 2010; Xu et al., 2003).

Animals, even ones with a less redundant genetic background such as *C. elegans*, carrying deletions of individual miRNAs or all members of a miRNA family often do not display an observable phenotype, including lifespan changes (Alvarez-Saavedra and Horvitz, 2010; Miska et al., 2007). These observations seem to be consistent with the hypothesis that many miRNAs act to fine-tune gene expression to maintain protein levels of their target genes in an optimal range (Bartel and Chen, 2004), and in such cases, loss of individual miRNAs would not be expected to result in an observable phenotype under normal conditions. As an alternative explanation, mutant phenotypes might be masked by genetic and functional redundancies between miRNAs and also their target genes. In the case that the same miRNA controls genes in two or more pathways, studying miRNA mutants with loss of a gene in a predicted pathway (e.g. a mitochondrial gene for ageing; see above), might improve the ability to identify miRNA functions. Other more generally sensitized genetic backgrounds may also help. A recent report has demonstrated that combining deletion mutations of miRNAs with loss of *alg-1*, which reduces the activity of *all* miRNAs, can enhance or suppress developmental defects observed in the *alg-1* single mutants (Brenner et al., 2010). This suggests that in many cases the phenotypic consequences of loss of a single miRNA may only be evident in conditions in which other miRNAs are impaired. Similarly, studying the lifespan of miRNA deletion mutants in the context of adult specific *alg-1* knockdown may help to identify hidden functions of miRNAs in ageing, since we observed that the

proper *alg-1* activity was indispensable for the normal adult life in *C. elegans* (Kato et al., 2011).

5. miRNAs that respond to environmental perturbations

The phenotypic outcomes of the miRNA mutants observed in the loss of *alg-1* background (Brenner et al., 2010) imply that miRNAs may have important roles in ensuring the robustness of developmental pathways. This idea seems to be further supported by a recent observation that *C. elegans* animals with different life histories have a distinct expression pattern of miRNAs (Karp et al., 2011). In favorable conditions, *C. elegans* animals continuously develop into adulthood through larval stages. In contrast, in unfavorable conditions (e.g. food deprivation), they undergo distinct developmental processes, such as the dauer diapause, and when environmental conditions improve, the growth-arrested animals re-enter the developmental cycle and progress to adulthood (Hu, 2007). *C. elegans* experiencing such interrupted life histories have a different expression pattern of miRNAs, including miR-34, miR-71 and *let-7* family members, even at the same developmental stages after recovery from growth arrest (e.g. continuously developed to the 4th larval stage versus the same 4th larval stage but experiencing dauer diapause) (Karp et al., 2011). This suggests that these miRNAs might have the potential to buffer developmental programs in response to physiological alterations that are accompanied by stress or environmental perturbations. Consistent with this, it has recently been shown that an ageing miRNA *mir-71* is important in normal survival under starved condition and developmental recovery from starvation-induced diapause in *C. elegans* (Zhang et al., 2011). Notably, diapause state and food deprivation as well as mild stressors (e.g. relatively weak heat shock), can all prolong lifespan, depending on their degree – the hormesis effect, where mild stress exposure positively modulates biological processes, such as lifespan and stress resistance (Antebi, 2007; Cypser et al., 2006; Fontana et al., 2010; Honjoh et al., 2009; Kaeberlein et al., 2006). Although it remains unknown whether such life history-dependent miRNA expression changes (e.g. modified miR-71 levels) have an impact on the resulting lifespan, miRNAs might act to achieve better survival strategies by adapting animals to environmental changes in the natural condition.

6. MiRNA biomarkers of ageing

Almost all organisms, including *C. elegans*, have a broad range of lifespans, even in genetically identical backgrounds. That is, some sibling individuals live longer or shorter than others, even when they are cultured and grown in homogeneous conditions. Previous studies have shown that a quantitative evaluation of a transcription marker that declines with age (e.g. *sod-3*::GFP) as well as phenotypic changes (e.g. reduction in locomotion) in individual animals predicts their remaining lifespan (Herndon et al., 2002; Huang et al., 2004; Sanchez-Blanco and Kim, 2011). To examine how such ageing signatures detected in early life correlate with the future longevity, we developed a device that allows physiological and gene expression changes to be monitored for individual *C. elegans* over the course of their lives, and correlated with eventual longevity and health outcomes (Pincus et al., 2011). In addition to the conventional biomarkers of longevity, such as body size, movement rates and accumulation of age pigments, the expression profile of the age-associated miRNAs, including miR-71 and miR-239 (measured by promoter::GFP reporter constructs) was found to predict future longevity (Pincus et al., 2011). Animals with higher or longer-lasting levels of *mir-71*::GFP tend to live longer, consistent with the role of miR-71 in promoting lifespan (de Lencastre et al., 2010). Indeed, almost 50% of inter-individual variability in lifespan is explicable based on expression patterns of *mir-71*::GFP in the first four days of adulthood. Conversely, and consistent with miR-239 acting to antagonize longevity (de Lencastre et al., 2010), rapid increases in the levels of

mir-239::GFP expression predicted shorter lifespans. Many of these measures, and in particular *mir-71*::GFP levels, are predictive of future longevity even as early as the second day of adulthood (Pincus et al., 2011). As both of miR-71 and miR-239 regulate the insulin-signaling pathway (de Lencastre et al., 2010), these miRNAs not only predict for future longevity but also may directly determine the remaining lifespan by modulating insulin-signaling activity.

7. Additional small, non-coding RNAs with potential functions in ageing

miRNAs may only be a small portion of the regulatory RNAs involved in ageing. Recent deep-sequencing experiments have led to the discovery of a surprisingly large variety of small, non-coding RNAs. In addition to mature miRNAs, miRNA star strands, which are sequences with reverse-complimentary to mature miRNAs that are produced from the hairpin precursor during miRNA maturation (Lim et al., 2003), might have a biological role in ageing. Specifically, we found several cases where the star strands have distinct changes in abundance relative to their mature counterparts during ageing in *C. elegans* (Kato et al., 2011). Importantly, the star strands have a trend to preferentially associate with RDE-1, an Argonaute protein involved in dsRNA-induced siRNA pathway (i.e. RNAi machinery (Tabara et al., 1999)), while the mature strands are mostly incorporated into ALG-1 (Correa et al., 2010; Kato et al., 2011). Moreover, recent reports indicate that depletion of XRN-1 and -2, which encode 5'-to-3' exoribonucleases involved in miRNA turnover, causes an accumulation of star strands of certain miRNAs and also mature miRNA strands (Chatterjee et al., 2011; Chatterjee and Grosshans, 2009). Interestingly, the availability of a miRNA target also modulates the levels of a miRNA itself; the presence of the mRNA that has binding sites for the miRNA protects the miRNA from degradation, and increases stability of both mature and star miRNA strands, while the absence of target mRNA conversely destabilizes the miRNAs (Chatterjee et al., 2011). These observations suggest a dynamic and refined miRNA-associated gene regulatory mechanism.

Small RNAs derived from parental RNAs, notably transfer RNAs (tRNAs), are now recognized to have novel roles in diverse animal species. tRNAs are a classic, non-coding RNA species, which recognize specific codons during protein synthesis. Surprisingly, however, it now seems that this role is only part of the tRNA function: longer, parental tRNA transcripts are cleaved by a specific ribonuclease (e.g. angiogenin in mammalian cells) in response to stresses, such as heat shock, yielding roughly half-sized tRNA fragments (Figure 2, reviewed in (Thompson and Parker, 2009; Tuck and Tollervey, 2011)). These cleaved half-tRNAs may act to suppress protein synthesis since recent studies indicate that synthetic half-tRNA fragments inhibit translation *in vitro* (Ivanov et al., 2011), and promote the assembly of stress granules, cytoplasmic foci at which untranslated mRNAs in a protein complex are transiently concentrated (Emara et al., 2010). Stress-induced tRNA cleavage is evolutionarily conserved from yeast to human (Thompson and Parker, 2009), suggesting that it is a fundamental component of the stress response. In a preliminary observation, specific short tRNA reads were present in our deep-sequencing experiments in *C. elegans* with dramatic induction in response to heat stress; these smaller fragments may be derived from half-tRNAs, which would not have been observed due to our sequencing of only very short RNA species. Likewise, similar tRNA-derived short sequencing reads increased during ageing in *C. elegans* (Kato et al., 2011). Intriguingly, it is known that tRNA cleavage is also triggered by nutrient deprivation in some organisms (Thompson and Parker, 2009), implying that half-tRNA-mediated translational repression might be an adaptive response to environmental perturbation during development and ageing, similar to miRNAs.

8. Role of RNA editing in dsRNA-mediated gene regulation

dsRNA-mediated gene silencing, such as that exhibited by miRNAs and siRNAs, is likely to be further regulated by the RNA editing machinery. The predominant form of RNA editing is adenosine to inosine conversion that happens in a dsRNA duplex, mediated by a conserved enzyme, Adenosine Deaminase Acting on RNA (ADAR, reviewed in (Bass, 2006)). RNA editing has the capacity to generate functional diversity in protein products and/or altered target recognition by miRNAs or siRNAs. The *C. elegans* genome encodes two genes with ADAR activity, *adr-1* and *adr-2* (Tonkin et al., 2002). *C. elegans* carrying mutations in both *adr-1* and *adr-2* have a significantly shorter lifespan than wild-type animals (Sebastiani et al., 2009), indicating that ADAR activity has a critical role in lifespan determination in *C. elegans*, despite no other obvious abnormalities in *adr-1;adr-2* mutants (aside from a chemotaxis defect and a modulated response to transgene expression) (Knight and Bass, 2002; Tonkin and Bass, 2003). Notably, the short-lived phenotype observed in *adr-1;adr-2* mutants is suppress by loss of *rde-1*, although the *rde-1* mutation alone does not affect lifespan (Sebastiani et al., 2009). These results suggest an interaction between RNA editing and RNAi machineries in lifespan regulation.

The initial finding of the connection between RNA editing and RNAi machineries is from experiments using transgenes in *C. elegans* (Knight and Bass, 2002). In *C. elegans*, high-copy-number transgenes are reliably silenced in the germline but not in somatic tissues; however in *adr-1;adr-2* mutants, such transgenes are also often silenced in the soma, likely via an RNAi mechanism triggered by small amounts of aberrant double-stranded transcript (Knight and Bass, 2002). The authors' interpretation of these results is that the RNA editing modifies these aberrant dsRNA species so that they no longer form a perfectly complementary dsRNA, and thus preventing an RNAi response (which entails amplification of the RNAi template via an RNA-dependent RNA polymerase (Gent et al., 2010; Pak and Fire, 2007)). Thus, this result suggests that the RNA editing pathway may act to release and inactivate extensive populations of dsRNA products that are potentially capable of entering the RNAi pathway, possibly in order to prevent further unfavorable gene inactivation (Figure 3). As *adr-1;adr-2*-induced short lifespan was suppressed by the loss of RNAi activity, this competitive relationship now seems to extend to the regulation of endogenous genes as well.

A recent finding that *adr-1;adr-2* mutants accumulate many small RNAs, corresponding to diverse genomic loci, supports this hypothesis (Wu et al., 2011). This increase of small RNAs depends on an RNAi component, RDE-4, which encodes a dsRNA-binding protein acting in the initial phase of RNAi where longer-triggering-dsRNAs are processed into the shorter form of dsRNAs (i.e. primary siRNAs, (Parker et al., 2006)). This suggests that the effectiveness of the RNAi machinery in the absence of RNA editing activity arises as a consequence of the production of the primary siRNAs from longer dsRNA species. Although most of these short RNA sequences differentially expressed in the *adr-1;adr-2* mutants overlap transposons or inverted repeats, a significant proportion also overlap annotated transcripts (Wu et al., 2011). Further study indicates that such transcripts show the greatest up-regulation of their corresponding antisense siRNAs in *adr-1;adr-2* mutants, with a concomitant decrease in mRNA levels. Interestingly, these transcripts include a large class of histone genes (Wu et al., 2011) and some genes known to be necessary for the normal ageing in *C. elegans*, such as *cst-1*, a homologue of mammalian Ste20-like kinase gene, and *zfp-1*, a zinc finger and PHD/LAP domain protein gene, both of which interact with DAF-16, and shorten lifespan when they are lost (Lehtinen et al., 2006; Oh et al., 2006). The short lifespan phenotype in the loss of RNA editing activity might be partially due to inappropriate inactivation of these genes.

Approximately ~15,000 genomic positions are estimated as putative RNA editing sites including those for a histone gene, since these sites are present in wild-type but not in *adr-1;adr-2* mutants (Wu et al., 2011). It would be interesting to see whether the levels or pattern of RNA editing, and the ADAR gene activity are changed during development or ageing, or among different tissues. Moreover, although it has not yet been investigated in *C. elegans* whether these editing sites include miRNA loci, it seems plausible because miRNA precursors containing a hairpin duplex structure would also be a substrate of ADAR. Indeed, some examples of RNA editing are known in human miRNAs (Blow et al., 2006), yielding diversity in miRNA and target interaction.

9. Perspective

It is now becoming clear that miRNAs have a critical, yet sometimes subtle, role in lifespan determination. Such subtle, "fine-tuning" functions of miRNAs, the general redundancy in miRNAs and their target genes, and their frequent roles in feedback regulation (e.g. *let-7* and LIN-28, (Krol et al., 2010)), both make it plausible that these genes act as "buffers" to increase overall homeostatic stability, and make it difficult to study the function of any miRNA individually. In addition, the RNA editing machinery acts as an RNA surveillance mechanism to modulate dsRNA-mediated silencing pathways, such as miRNAs and siRNAs.

The small, non-coding RNA world is much more diverse than we had imagined even a few years ago. Growing evidence suggests that RNA cleavage fragments, do not always simply reflect RNA degradation, but can represent a novel class of molecules that have a distinct function from their parental RNAs. tRNA-derived half-sized fragments are now recognized as a new class of regulatory RNAs that inhibits translation in response to stress. Small nucleolar RNA (snoRNA)-derived fragments may also have another function different from their original transcripts (Tuck and Tollervey, 2011). Additional short RNA species, such as mRNA fragments, localizing in nuclei, might further act as regulatory RNAs possibly directly targeting on genomic DNAs or chromatin structure (Tuck and Tollervey, 2011). Since recent studies indicate that genes involved in methylation of histone H3 at lysine 4, an active mark of chromatin modifications, play an important role in lifespan determination in *C. elegans* (Greer et al., 2010; Greer et al., 2011), it is not implausible to imagine that small RNA control of chromatin structure may similarly influence lifespan. Studying ageing and small RNA biology in model organisms, including *C. elegans*, will lead to further understanding of our human ageing and age-associated diseases.

Acknowledgments

We thank Drs. Zachary Pincus and Alexandre de Lencastre for critical reading of this manuscript. This work was supported by a fellowship from the Uehara Life Science Foundation to M.K. and a grant from the NIH (R01 AG033921) to F.J.S.

References

Alvarez-Saavedra E, Horvitz HR. Many families of C. elegans microRNAs are not essential for development or viability. Curr Biol. 2010; 20:367–373. [PubMed: 20096582]

Ambros V. MicroRNAs and developmental timing. Curr Opin Genet Dev. 2011; 21:511–517. [PubMed: 21530229]

Antebi A. Genetics of aging in Caenorhabditis elegans. PLoS Genet. 2007; 3:1565–1571. [PubMed: 17907808]

Bartel DP, Chen CZ. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. Nat Rev Genet. 2004; 5:396–400. [PubMed: 15143321]

Bass BL. How does RNA editing affect dsRNA-mediated gene silencing? Cold Spring Harb Symp Quant Biol. 2006; 71:285–292. [PubMed: 17381308]

Batista PJ, Ruby JG, Claycomb JM, Chiang R, Fahlgren N, Kasschau KD, Chaves DA, Gu W, Vasale JJ, Duan S, et al. PRG-1 and 21U-RNAs interact to form the piRNA complex required for fertility in C. elegans. Mol Cell. 2008; 31:67–78. [PubMed: 18571452]

Baugh LR, Sternberg PW. DAF-16/FOXO regulates transcription of cki-1/Cip/Kip and repression of lin-4 during C. elegans L1 arrest. Curr Biol. 2006; 16:780–785. [PubMed: 16631585]

Blow MJ, Grocock RJ, van Dongen S, Enright AJ, Dicks E, Futreal PA, Wooster R, Stratton MR. RNA editing of human microRNAs. Genome Biol. 2006; 7:R27. [PubMed: 16594986]

Boehm M, Slack F. A developmental timing microRNA and its target regulate life span in C. elegans. Science. 2005; 310:1954–1957. [PubMed: 16373574]

Brenner JL, Jasiewicz KL, Fahley AF, Kemp BJ, Abbott AL. Loss of individual microRNAs causes mutant phenotypes in sensitized genetic backgrounds in C. elegans. Curr Biol. 2010; 20:1321–1325. [PubMed: 20579881]

Buchberger A, Bukau B, Sommer T. Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell. 40:238–252. [PubMed: 20965419]

Chatterjee S, Fasler M, Bussing I, Grosshans H. Target-mediated protection of endogenous microRNAs in C. elegans. Dev Cell. 2011; 20:388–396. [PubMed: 21397849]

Chatterjee S, Grosshans H. Active turnover modulates mature microRNA activity in Caenorhabditis elegans. Nature. 2009; 461:546–549. [PubMed: 19734881]

Correa RL, Steiner FA, Berezikov E, Ketting RF. MicroRNA-directed siRNA biogenesis in Caenorhabditis elegans. PLoS Genet. 2010; 6:e1000903. [PubMed: 20386745]

Cypser JR, Tedesco P, Johnson TE. Hormesis and aging in Caenorhabditis elegans. Exp Gerontol. 2006; 41:935–939. [PubMed: 17067771]

de Lencastre A, Pincus Z, Zhou K, Kato M, Lee SS, Slack FJ. MicroRNAs both promote and antagonize longevity in C. elegans. Curr Biol. 2010; 20:2159–2168. [PubMed: 21129974]

Emara MM, Ivanov P, Hickman T, Dawra N, Tisdale S, Kedersha N, Hu GF, Anderson P. Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. J Biol Chem. 2010; 285:10959–10968. [PubMed: 20129916]

Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6:259–269. [PubMed: 16557279]

Fontana L, Partridge L, Longo VD. Extending healthy life span--from yeast to humans. Science. 2010; 328:321–326. [PubMed: 20395504]

Gent JI, Lamm AT, Pavelec DM, Maniar JM, Parameswaran P, Tao L, Kennedy S, Fire AZ. Distinct phases of siRNA synthesis in an endogenous RNAi pathway in C. elegans soma. Mol Cell. 2010; 37:679–689. [PubMed: 20116306]

Greer EL, Maures TJ, Hauswirth AG, Green EM, Leeman DS, Maro GS, Han S, Banko MR, Gozani O, Brunet A. Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans. Nature. 2010; 466:383–387. [PubMed: 20555324]

Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, Lim JP, Benayoun BA, Shi Y, Brunet A. Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans. Nature. 2011; 479:365–371. [PubMed: 22012258]

Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell. 2001; 106:23–34. [PubMed: 11461699]

Hekimi S, Lapointe J, Wen Y. Taking a “good” look at free radicals in the aging process. Trends Cell Biol. 2011; 21:569–576. [PubMed: 21824781]

Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M. Stochastic and genetic factors influence tissue-specific decline in ageing C. elegans. Nature. 2002; 419:808–814. [PubMed: 12397350]

Honjoh S, Yamamoto T, Uno M, Nishida E. Signalling through RHEB-1 mediates intermittent fasting-induced longevity in C. elegans. Nature. 2009; 457:726–730. [PubMed: 19079239]

Hristova M, Birse D, Hong Y, Ambros V. The Caenorhabditis elegans heterochronic regulator LIN-14 is a novel transcription factor that controls the developmental timing of transcription from the insulin/insulin-like growth factor gene ins-33 by direct DNA binding. Mol Cell Biol. 2005; 25:11059–11072. [PubMed: 16314527]

Hu PJ. Dauer. WormBook. 2007:1–19. [PubMed: 17988074]

Huang C, Xiong C, Kornfeld K. Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. Proc Natl Acad Sci U S A. 2004; 101:8084–8089. [PubMed: 15141086]

Hyun S, Lee JH, Jin H, Nam J, Namkoong B, Lee G, Chung J, Kim VN. Conserved MicroRNA miR-8/miR-200 and its target USH/FOG2 control growth by regulating PI3K. Cell. 2009; 139:1096–1108. [PubMed: 20005803]

Ibanez-Ventoso C, Yang M, Guo S, Robins H, Padgett RW, Driscoll M. Modulated microRNA expression during adult lifespan in Caenorhabditis elegans. Aging Cell. 2006; 5:235–246. [PubMed: 16842496]

Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P. Angiogenin-induced tRNA fragments inhibit translation initiation. Mol Cell. 2011; 43:613–623. [PubMed: 21855800]

Kaeberlein TL, Smith ED, Tsuchiya M, Welton KL, Thomas JH, Fields S, Kennedy BK, Kaeberlein M. Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell. 2006; 5:487–494. [PubMed: 17081160]

Karp X, Hammell M, Ow MC, Ambros V. Effect of life history on microRNA expression during C. elegans development. RNA. 2011; 17:639–651. [PubMed: 21343388]

Kato M, Chen X, Inukai S, Zhao H, Slack FJ. Age-associated changes in expression of small, noncoding RNAs, including microRNAs, in C. elegans. RNA. 2011; 17:1804–1820. [PubMed: 21810936]

Kato M, Paranjape T, Muller RU, Nallur S, Gillespie E, Keane K, Esquela-Kerscher A, Weidhaas JB, Slack FJ. The mir-34 microRNA is required for the DNA damage response in vivo in C. elegans and in vitro in human breast cancer cells. Oncogene. 2009; 28:2419–2424. [PubMed: 19421141]

Kenyon CJ. The genetics of ageing. Nature. 2010; 464:504–512. [PubMed: 20336132]

Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol. 2009; 10:126–139. [PubMed: 19165215]

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science. 1997; 277:942–946. [PubMed: 9252323]

Knight SW, Bass BL. The role of RNA editing by ADARs in RNAi. Mol Cell. 2002; 10:809–817. [PubMed: 12419225]

Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010; 11:597–610. [PubMed: 20661255]

Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75:843–854. [PubMed: 8252621]

Lee SS, Kennedy S, Tolonen AC, Ruvkun G. DAF-16 target genes that control C. elegans life-span and metabolism. Science. 2003; 300:644–647. [PubMed: 12690206]

Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villen J, Becker EB, DiBacco S, de la Iglesia N, Gygi S, Blackwell TK, et al. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell. 2006; 125:987–1001. [PubMed: 16751106]

Lim LP, Lau NC, Weinstein EG, Abdelhakim A, Yekta S, Rhoades MW, Burge CB, Bartel DP. The microRNAs of Caenorhabditis elegans. Genes Dev. 2003; 17:991–1008. [PubMed: 12672692]

Liu N, Landreh M, Cao K, Abe M, Hendriks GJ, Kennerdell JR, Zhu Y, Wang LS, Bonini NM. The microRNA miR-34 modulates ageing and neurodegeneration in Drosophila. Nature. 2012; 482:519–523. [PubMed: 22343898]

McKay RM, McKay JP, Avery L, Graff JM. C elegans: a model for exploring the genetics of fat storage. Dev Cell. 2003; 4:131–142. [PubMed: 12530969]

Miska EA, Alvarez-Saavedra E, Abbott AL, Lau NC, Hellman AB, McGonagle SM, Bartel DP, Ambros VR, Horvitz HR. Most Caenorhabditis elegans microRNAs are individually not essential for development or viability. PLoS Genet. 2007; 3:e215. [PubMed: 18085825]

Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature. 2003; 424:277–283. [PubMed: 12845331]

Nishi T, Forgac M. The vacuolar (H+)-ATPases--nature's most versatile proton pumps. Nat Rev Mol Cell Biol. 2002; 3:94–103. [PubMed: 11836511]

Oh SW, Mukhopadhyay A, Dixit BL, Raha T, Green MR, Tissenbaum HA. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. Nat Genet. 2006; 38:251–257. [PubMed: 16380712]

Olsen A, Vantipalli MC, Lithgow GJ. Checkpoint proteins control survival of the postmitotic cells in Caenorhabditis elegans. Science. 2006; 312:1381–1385. [PubMed: 16741121]

Pak J, Fire A. Distinct populations of primary and secondary effectors during RNAi in C. elegans. Science. 2007; 315:241–244. [PubMed: 17124291]

Parker GS, Eckert DM, Bass BL. RDE-4 preferentially binds long dsRNA and its dimerization is necessary for cleavage of dsRNA to siRNA. RNA. 2006; 12:807–818. [PubMed: 16603715]

Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature. 2000; 408:86–89. [PubMed: 11081512]

Pincus Z, Smith-Vikos T, Slack FJ. MicroRNA predictors of longevity in Caenorhabditis elegans. PLoS Genet. 2011; 7:e1002306. [PubMed: 21980307]

Roush S, Slack FJ. The let-7 family of microRNAs. Trends Cell Biol. 2008; 18:505–516. [PubMed: 18774294]

Sanchez-Blanco A, Kim SK. Variable pathogenicity determines individual lifespan in Caenorhabditis elegans. PLoS Genet. 2011; 7:e1002047. [PubMed: 21533182]

Sebastiani P, Montano M, Puca A, Solovieff N, Kojima T, Wang MC, Melista E, Meltzer M, Fischer SE, Andersen S, et al. RNA editing genes associated with extreme old age in humans and with lifespan in C. elegans. PLoS One. 2009; 4:e8210. [PubMed: 20011587]

Stefani G, Slack FJ. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol. 2008; 9:219–230. [PubMed: 18270516]

Tabara H, Sarkissian M, Kelly WG, Fleenor J, Grishok A, Timmons L, Fire A, Mello CC. The rde-1 gene, RNA interference, and transposon silencing in C. elegans. Cell. 1999; 99:123–132. [PubMed: 10535731]

Teleman AA, Maitra S, Cohen SM. Drosophila lacking microRNA miR-278 are defective in energy homeostasis. Genes Dev. 2006; 20:417–422. [PubMed: 16481470]

Thompson DM, Parker R. Stressing out over tRNA cleavage. Cell. 2009; 138:215–219. [PubMed: 19632169]

Tonkin LA, Bass BL. Mutations in RNAi rescue aberrant chemotaxis of ADAR mutants. Science. 2003; 302:1725. [PubMed: 14657490]

Tonkin LA, Saccomanno L, Morse DP, Brodigan T, Krause M, Bass BL. RNA editing by ADARs is important for normal behavior in Caenorhabditis elegans. EMBO J. 2002; 21:6025–6035. [PubMed: 12426375]

Tuck AC, Tollervey D. RNA in pieces. Trends Genet. 2011; 27:422–432. [PubMed: 21741109]

Varghese J, Lim SF, Cohen SM. Drosophila miR-14 regulates insulin production and metabolism through its target, sugarbabe. Genes Dev. 2010; 24:2748–2753. [PubMed: 21159815]

Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet. 2005; 39:359–407. [PubMed: 16285865]

Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell. 1993; 75:855–862. [PubMed: 8252622]

Wu D, Lamm AT, Fire AZ. Competition between ADAR and RNAi pathways for an extensive class of RNA targets. Nat Struct Mol Biol. 2011; 18:1094–1101. [PubMed: 21909095]

Xu P, Vernooy SY, Guo M, Hay BA. The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr Biol. 2003; 13:790–795. [PubMed: 12725740]

Yang J, Chen D, He Y, Melendez A, Feng Z, Hong Q, Bai X, Li Q, Cai G, Wang J, et al. MiR-34 modulates Caenorhabditis elegans lifespan via repressing the autophagy gene atg9. Age (Dordr). 2011

Zhang X, Zabinsky R, Teng Y, Cui M, Han M. microRNAs play critical roles in the survival and recovery of Caenorhabditis elegans from starvation-induced L1 diapause. Proc Natl Acad Sci U S A. 2011; 108:17997–18002. [PubMed: 22011579]

Zhu C, Ji CB, Zhang CM, Gao CL, Zhu JG, Qin DN, Kou CZ, Zhu GZ, Shi CM, Guo XR. The lin-4 Gene Controls Fat Accumulation and Longevity in Caenorhabditis elegans. Int J Mol Sci. 2010; 11:4814–4825. [PubMed: 21614175]

Hightlights

- MicroRNAs regulate lifespan through the control of conserved genetic pathways.
- MicroRNAs respond to environmental perturbations.
- Age-associated microRNAs predict for future longevity.
- tRNA-derived shorter fragments have a new regulatory role in the stress response.
- The RNA editing machinery acts in double-stranded RNA-mediated gene silencing.
- These multiple layers in gene regulatory mechanisms may stabilize genetic networks in ageing.

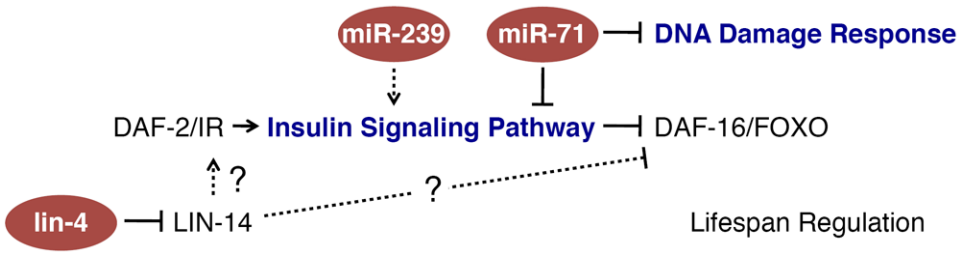

Figure 1.
A model for miRNA-mediated regulation of ageing pathways: The *lin-4* miRNA and its target *lin-14*, and miR-239 seem to regulate lifespan indirectly by modifying the insulin-signaling pathway (dashed lines). miR-71 appears to directly affect the insulin-signaling and DNA damage response pathways.

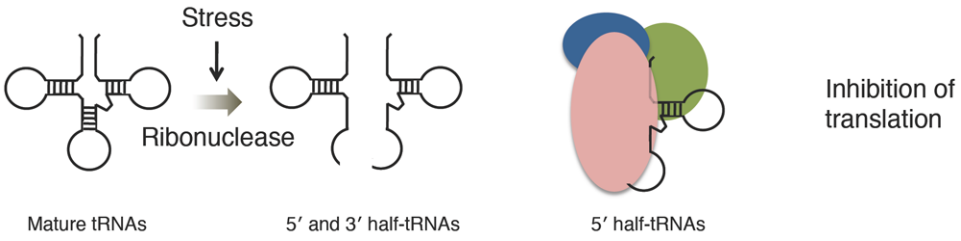

Figure 2.
A novel non-coding RNA role for tRNA-derived RNA fragments: A specific ribonuclease cleaves tRNA transcripts in response to stress (e.g. heat or oxidative stress), producing a nearly half-sized tRNA fragments (half-tRNAs). A subset of 5' half-tRNAs cooperates with a translational silencer complex to inhibit translational initiation.

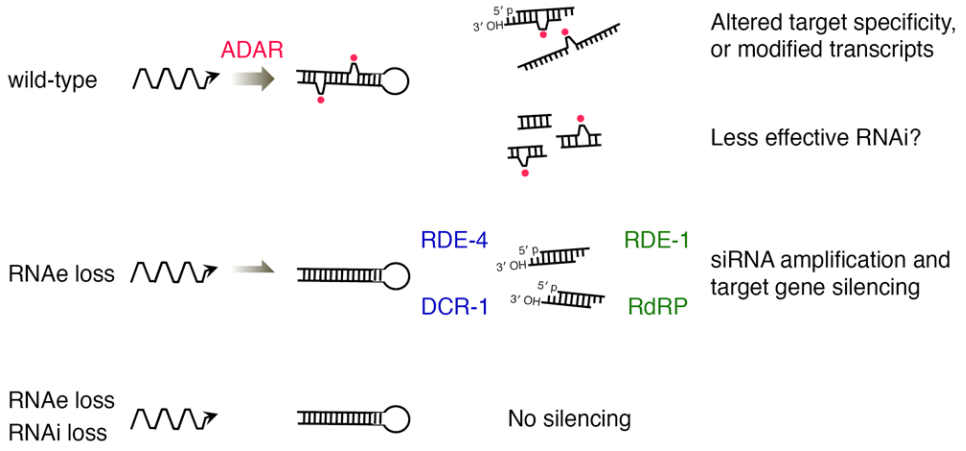

Figure 3.

Antagonistic function of RNA editing in RNAi machinery: ADAR activity can modify target specificity of miRNAs or siRNAs, or transcripts of protein-coding genes. In addition, ADAR activity may prevent dsRNAs from entering the RNAi pathway by introducing RNA modifications (shown by red circles) that reduce the self-complementarity of the dsRNAs – a requirement for effective RNAi. This hypothesis is based on the observation that loss of RNA editing activity (RNAe) results in an accumulation of siRNA species and an increase in certain forms of RNAi-mediated gene silencing, but the silencing activity is suppressed by loss of an RNAi component.