UCSF

Title

Regulation of miRNA biogenesis and turnover in the immune system

Permalink

https://escholarship.org/uc/item/3m94g60m

Journal

Immunological Reviews, 253(1)

ISSN

0105-2896

Authors

Bronevetsky, Yelena
Ansel, K Mark

Publication Date

2013-05-01

DOI

10.1111/imr.12059

Peer reviewed

Regulation of miRNA biogenesis and turnover in the immune system

Yelena Bronevetsky and **K. Mark Ansel**
Sandler Asthma Basic Research Center, and Department of Microbiology & Immunology, University of California San Francisco, San Francisco, CA, USA

Summary

MicroRNAs (miRNAs) have emerged as important regulators of gene expression in diverse biological processes ranging from cell proliferation and survival to organ development and immunity. Here, we review mechanisms that regulate the expression of miRNAs themselves in the immune system. Like protein-coding genes, miRNAs can be regulated at the transcriptional level, downstream of signaling pathways and circuits that activate or inhibit transcription factors and chromatin remodeling. The resulting primary miRNAs are processed into active mature miRNAs through a series of biochemical steps, and miRNA abundance can be regulated at each step of this biogenesis pathway. Recent work has uncovered regulation of mature miRNA turnover in the immune system as well. A better understanding of these processes and their regulation by immunogenic stimuli is critical for integrating miRNAs into current models of gene expression networks that determine cell identity and immune function.

Keywords

miRNA processing; miRNA turnover; Argonaute

Introduction and basics of miRNA biogenesis

Since their discovery over 20 years ago, microRNAs (miRNAs) have been the subject of an ever-intensifying field of research (1). This work has revealed roles for miRNAs in most eukaryotes and in almost every mammalian cell and organ system that has been tested, including the immune system. miRNA-based therapeutics and diagnostic tests have advanced rapidly toward application (2). In parallel, many mechanistic details regarding how miRNAs are produced and how they repress the translation and stability of their mRNA targets have been elucidated (3, 4). As one might expect, it has become clear that the expression of the miRNAs themselves is subject to global and sequence-specific regulation through a variety of mechanisms that impact every step of their life cycle, from transcription through enzymatic processing and degradation (5, 6). All of these topics have been reviewed in detail elsewhere, but a basic understanding of miRNA biogenesis is particularly critical to understanding how miRNA expression is regulated in the immune system.

miRNAs are processed from long primary miRNA (pri-miR) transcripts that may contain one or several miRNAs embedded in stem loop hairpin structures (Fig. 1). These 60–80 nucleotide (nt) hairpin intermediates (pre-miRNAs) are cropped out of the pri-miRNA by the Microprocessor complex, which is comprised of the RNase III Drosha and its obligate

RNA-binding protein partner, DiGeorge syndrome critical region gene (DGCR8). Pre-miRNAs are exported from the nucleus to the cytoplasm by exportin 5. There, they are processed into short-lived double-stranded duplexes by the cytoplasmic RNase III Dicer, which also employs an RNA binding cofactor, TAR RNA-binding protein (TRBP). These duplexes are separated, and one strand is selected as the mature miRNA, while the other strand is rapidly degraded. Mature miRNAs are incorporated into Argonaute (Ago) protein-containing effector complexes, known as 'miRNP' (miRNA-containing ribonucleoprotein complex) or miRISC (miRNA-containing RNA-induced silencing complex). In mammals, miRNAs guide miRISC activity by complementary base pairing with imperfectly matched binding sequences in target mRNAs, leading to translational repression and accelerated mRNA decay.

Integrating miRNAs and transcriptional regulation in gene expression networks

Precise control of gene expression is a key feature of animal development. The formation of complex organisms made up of hundreds of distinct cell types requires that transcripts and their protein products be produced and maintained at tightly controlled concentrations in space and time. The first two miRNAs to be discovered, *Caenorhabtidis elegans* lin-4 and let-7, are both critical regulators of developmental timing (7, 8), and the control of development and cell differentiation has remained a common theme as the biological functions of miRNAs have been elucidated in plants and other animals (9). Organisms must also dynamically regulate gene expression in response to their environment, and miRNAs have also emerged as important regulators of stress signaling (10). Consistent with these themes, miRNAs have been shown to play critical roles in hematopoiesis and immune responses (11, 12). The current challenge is to integrate miRNAs into existing models of the gene expression networks that govern these processes. Meeting this challenge will require both a better accounting of miRNA targets and a better understanding of the control of miRNA expression.

Like any other gene product, miRNAs are subject to transcriptional regulation (13). However, investigation in this area has been slowed by limitations in the methodology to define the promoters and measure the transcription of pri-miRNA transcripts. Pri-miRNAs are necessarily unstable, since they are processed by the nuclear Microprocessor complex very soon after transcription. Therefore, they generally do not accumulate to great abundance in cells and are underrepresented in expressed sequence tag and RNA sequencing (RNA-seq) libraries. The lack of meaningful open reading frames also makes it harder to predict pri-miRNAs than mRNAs. Some miRNAs appear in the introns or 3′ untranslated region (UTR) of protein-coding genes, but even these are sometimes the product of distinct noncoding transcripts that utilize alternative promoters. Although their final products are very short, pri-miRNAs can be long, spliced transcripts that originate from promoters hundreds of thousands of base pairs (bp) away from the mature miRNA sequence.

These challenges have recently been largely overcome by epigenomic and transcriptomic experiments. Despite their low steady state abundance, spliced pri-miRNAs can often be assembled from RNA-seq data. One study took advantage of the fact that many pri-miRNAs accumulate in cells lacking Drosha to map pri-miRNAs in Drosha-deficient T cells using RNA-seq (14). In addition, this group and several others mined genome-wide chromatin mapping data to identify likely pri-miRNA promoters in mouse and human cells (15, 16). In one case, proximal promoters were predicted using a combination of analyses to detect nucleosome-depleted regions, high evolutionary conservation, CpG islands, transcription factor motifs within regions containing trymethylation of histone-3 lysine-4 (H3K4me3) or acetylation of histone-3 lysine-9 and -14 (H3K9/14Ac), and RNA polymerase binding to

determine the transcription initiation regions of 175 miRNAs (15). The combination of the H3K4me3 mark of transcription initiation and H3K36me3 or H3K79me2 mark of transcriptional elongation was also useful for mapping active pri-miRNA promoters in human and mouse T cells (14, 16). Further work is needed, especially in view of the fact that alternative promoters, splicing, and processing may generate diversity in pri-miRNA structure and miRNA expression in different cell types and conditions.

Even the identity of the RNA polymerase that transcribes miRNA genes was difficult to define. It was first presumed that transcription of miRNA genes would be similar to that of other small RNAs, such as tRNA, and would require RNA Polymerase III (Pol III). However, several studies revealed that many pri-miRNAs contain features typical of RNA Polymerase II (Pol II), including polyadenylation and splicing (17). More recent studies have determined that some miRNA genes, especially those interspersed among Alu repeats, are transcribed by Pol III (18). These findings have led to a model in which intragenic miRNAs (within introns or exons of protein-coding genes) on the same strand as their host gene are usually co-transcribed by Pol II, while intergenic miRNAs are transcribed from their own Pol II or Pol III promoter (13).

Transcriptional regulation involves the interplay of genomic *cis*-regulatory elements, transcription factors, co-activator and co-repressor complexes, chromatin modifications, and other epigenetic factors. Rather than attempt to review the full spectrum of our current knowledge of transcriptional regulation of miRNA expression, we focus on autoregulatory feedback loops in which miRNAs influence their own expression. Fidelity in gene expression is often maintained by mechanisms that allow a transcriptional product to feed back to increase or inhibit its own production. Because they are direct regulators of gene expression, miRNAs are particularly suited to participate in feedback circuits. Here we cite several cases in which miRNAs directly target mRNAs that encode factors involved in their own transcription in cells of the immune system (Fig. 2).

Myc

A number of key transcriptional regulators of hematopoiesis and the immune response transcriptionally target miRNA genes. The proto-oncogene product Myc was one of earliest identified regulators of miRNA transcription. Dysregulated Myc expression or function frequently occurs in human malignancies, including Burkitt's lymphoma and acute myeloid leukemia (19). Myc activity influences hematopoietic stem cell self-renewal and differentiation, and Myc stabilization induced in activated lymphocytes promotes their metabolic reprogramming and proliferation in immune responses (20).

Myc has many transcriptional targets and may act as a universal amplifier of transcriptionally active genes (21, 22). It binds directly to the miR-17~92 locus on human chromosome 13 and transactivates expression (23). This interaction may prove to be very important for Myc's role in oncogenesis and normal physiology. The miR-17~92 cluster is also overexpressed in many lymphomas and leukemias, and its overexpression in lymphocytes caused lymphoproliferative disease and the development of B-cell lymphomas (24, 25). Pri-miR-17~92 is transcriptionally upregulated in activated T lymphocytes, and individual miRNAs within the cluster support T-cell proliferation and survival (26–28), though the role of Myc or other transcriptional activators has yet to be tested in this setting. However, germline hemizygous deletion of either Myc or the miR-17~92 cluster causes Feingold syndrome in human patients and very similar skeletal and growth phenotypes in mouse models (29).

Myc also transcriptionally represses a broader set of miRNAs (30). Myc binding was confirmed at the promoters or likely promoters of 12 miRNA genes in human and mouse B-

cell lines, concomitant with repression of miRNA expression. Many of these repressed miRNAs are established or predicted tumor suppressors, and their enforced expression diminished the tumorigenic potential of the lymphoma cell lines. Though they do not appear to be direct transcriptional targets, let-7 miRNAs are also decreased in response to Myc (31). In turn, let-7 represses Myc expression in a manner that requires binding of both let-7 and the RNA-binding protein HuR to adjacent sites in the Myc 3′ UTR, forming a reciprocal negative feedback loop (32).

NF-κB

Nuclear factor κB (NF-κB) is a key transcriptional regulator linking inflammation and tumorigenesis, and its influence is also intertwined with miRNAs (33). Activation of the NF-κB pathway in numerous cell types leads to the production of cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and IL-8. NF-κB can be activated by many oncogenes, and mutations in the NF-κB pathway are implicated in activated B-cell (ABC)-like diffuse large B-cell lymphoma and multiple myeloma, among others. Exposure of human monocytic THP-1 cells to lipopolysaccharides (LPS), TNF-α, and IL-1β leads to rapid induction of miR-146a, probably mediated by NF-κB sites in the pri-miR-146a promoter (34). In turn, miR-146a directly targets TNF receptor-associated factor 6 (TRAF6) and interferon (IFN) receptor-associated kinase-1 (IRAK1), two key adapter molecules in the signaling pathway that leads to NF-κB activation, forming a negative feedback loop. This circuit is active in myeloid cells as well as both regulatory and conventional effector T cells, and it is required to prevent runaway immune responses and the spontaneous development of myeloid malignancies (35–37). Thus, a miR-146a-driven feedback loop maintains immune homeostasis in the aftermath of inflammatory stimuli.

NF-κB also directly upregulates the expression of the oncogenic miRNA miR-155 and indirectly increases the transcription of several other miRNAs, including miR-21, -181a, and -9. Interestingly, miR-9 can directly repress the NF-κB1 transcript, suggesting that this miRNA may also mediate negative feedback to dampen or limit the duration of NF-κB activation (38).

Other miRNA autoregulatory loops with transcription factors

The *Myb* proto-oncogene encodes a hematopoietic cell transcription factor associated with bone marrow megakaryocyte hyperplasia and thrombocytosis. Myb expression is highest in hematopoietic progenitors and its down-regulation is required for lineage differentiation. Myb binds to elements in the pri-miR-15a promoter, transactivating its expression in K562 human erythroleukemia cells (39). In turn, miR-15a can target the *Myb* transcript, and this interaction is important for miR-15a's ability to limit cell-cycle progression.

Another regulator of hematopoiesis, Runx1, participates in a similar regulatory loop. During megakaryocytic differentiation, Runx1 binds a region upstream of miR-27a and induces its expression (40). Concurrently, miR-27a and several other miRNAs can repress the *Runx1* mRNA. Human granulopoiesis is controlled by a regulatory circuit involving miR-223 and the transcription factors NFI-A and C/EBPα (41). Both factors compete for binding to the miR-223 promoter, with NFI-A repressing miR-223 transcription while C/EBPα induces it in response to retinoic acid-induced differentiation. miR-223 can target NFI-A mRNA, thus promoting its own expression during granulocyte differentiation.

Regulation of miRNA processing: bringing specificity to a common pathway

It has long been known that mature miRNA levels are not determined solely by their transcription. Parallel measurements of pri-miRs and their corresponding mature miRNAs in developing mouse embryos revealed poor correlation for many pri-miR/miRNA pairs, suggesting that specific miRNAs were subject to developmental regulation of their processing and/or stability (42). This observation was extended to a panel of tumor samples, which additionally displayed a global reduction in miRNA abundance relative to their corresponding healthy tissues. Similarly, only 25% of the fluctuations in miRNA expression in developing B cells could be attributed to changes in transcription as inferred by pri-miRNA abundance in the same cells (43). The mechanisms by which transcription factors confer specificity in their regulation of miRNA levels is straightforward, but it was at first less obvious how regulation of the miRNA processing pathway can lead to effects on individual miRNAs and not others. Many studies have now shown that RNA-binding accessory proteins impart specificity by interacting with pri-miRNAs or pre-miRNAs and regulating their association and accessibility for processing by Drosha or Dicer (44). Startlingly, several of these proteins turned out to be well-known transcription factors that have evolved dual functions in transcriptional and post-transcriptional regulation. The immune system features several examples of regulated miRNA processing, including several in which signaling molecules are recruited to a processing complex to modulate the expression of a restricted set of miRNAs (Fig. 3).

SMAD

Smad proteins are transcription factors that transduce extracellular signals from transforming growth factor-β (TGF-β) and bone morphogenetic protein (BMP) cytokines (45). Ligation of the receptors for these cytokines leads to receptor (R)-Smad phosphorylation, association with Co-Smad (a.k.a. Smad4), and translocation to the nucleus where they mediate changes in the transcription of target genes. This signaling pathway was the first to be found to regulate pri-miRNA processing, when it was discovered that TGF-β signaling enhances pri-miR-21 processing in smooth muscle cells (46). The effect of R-Smads on mature miR-21 expression is posttranscriptional and does not require Smad4. Instead, it requires interaction between R-Smads and the DEAD-box RNA helicase p68, an important accessory subunit of the Drosha microprocessor complex (47). This finding was expanded to include 20 TGF-β/BMP-regulated miRNAs, many of which contain a consensus sequence motif within the stem region of their pri-miRs (48). Smads were demonstrated to directly bind this sequence, and mutation of the sequence abrogated TGF-β/BMP-induced recruitment of Smads, Drosha, and DGCR8 to the pri-miR, impairing processing. Conversely, introduction of this sequence into unregulated pri-miRs was sufficient to recruit Smads and allow regulation of processing by TGF-β/BMP. Thus in this example, specificity for miRNA processing is determined by the interaction between a pri-miRNA-specific sequence and a signal transduction protein. This mechanism is of particular relevance to the immune system, where the TGF-β pathway plays critical roles in regulating inflammatory processes (49). Smads also regulate the transcription of the miR-302~367 cluster, demonstrating that this pathway can modulate miRNA expression at multiple levels (50).

p53

Another protein that regulates miRNA expression at multiple steps in their biogenesis is the tumor suppressor p53. Mouse models of p53 deficiency predominantly exhibit malignant lymphomas, and 19% of human lymphoid malignancies and 11% of myeloid malignancies exhibit mutations in the p53 coding sequence (51). p53 directly activates the transcription of

several miRNAs, including miR-34, miR-200, miR-15a and miR-16, and these miRNAs may mediate much of p53's tumor suppressive activity (52). miR-34 directly targets numerous cell cycle regulators including cyclin E2 and Myc, and ectopic expression of miR-34 arrests cells in G1. Further evidence of the important role that p53-induced miRNAs play in preventing unrestrained cell proliferation came from studies of the *DLEU2* locus, from which the pri-miRNA that contains miR-15a and miR-16-1 is transcribed. This region is frequently deleted in human chronic lymphocytic leukemia (CLL), and mouse models confirmed that the miRNAs are required for its role in preventing lymphoproliferation (53). miR-29 is also induced by p53 and participates in a positive feedback loop to reinforce p53 expression by targeting two negative regulators of p53, CDC42 and the p85α-regulatory subunit of phosphoinositide 3-kinase (PI3K) (54).

Besides regulating the transcription of miRNA genes, p53 also affects the processing of specific miRNAs. p53 associates with Drosha through direct interaction with p68, the same helicase recruited to pri-miRNAs by Smad proteins (55). This interaction enhances the processing of several pri-miRs, increasing the expression of mature miR-16-1, miR-143, miR-145, and miR-206. This capacity of p53 to promote the processing of specific pri-miRs is mediated by its DNA-binding domain, as tumor-derived transcriptionally inactive mutant p53 suppresses the interaction between Drosha and p68, resulting in decreased levels of mature miR-16-1, miR-143, and miR-206.

p53 can also function downstream of defective miRNA processing to prevent tumorigenesis. Complete loss of miRNA processing in any cell type almost universally decreases cell proliferation and increases the rate of cell death, but partial loss of Dicer function increases tumorigenesis, making it a haploinsufficient tumor suppressor (56). Dicer deficiency (and therefore incomplete miRNA maturation) induces p53 expression, which leads to reduced proliferation and premature senescence in mouse embryonic fibroblasts (MEFs) (57). This effect is dependent on p53, as deletion of p53 prevents premature senescence induced by Dicer deletion.

Recently another mechanism by which p53 regulates miRNA processing and function was identified. The RNA-binding protein, RNA-binding motif protein 38 (RBM38) is induced by p53 and is required for optimal induction of G1 cell cycle arrest following DNA damage (58). RBM38 functions by shielding the 3′ UTRs of several p53 targets, such as *CDKN1A* (which encodes p21) from repression by miRNAs. Taken together, these studies illustrate the importance of control of miRNA biogenesis and function for p53's tumor suppressive activity.

Lin28

The let-7 family constitutes one of the most abundant miRNA families in mammals, with high expression in virtually all adult tissues. In contrast, embryonic stem cells and hematopoietic progenitor cells of fetal origin express significantly less let-7. Unexpectedly, pri-let-7 expression remains essentially constant throughout development, indicating that a post-transcriptional mechanism of regulation exists (42). Immunoprecipitating proteins that associate with pre-let-7 identified the RNA-binding protein Lin28 and its homologue Lin28b as factors that block let-7 maturation in embryonic and progenitor cells (59). Lin28 represses both Drosha and Dicer processing of let-7 *in vitro* by binding to conserved sequences in the precursor loop. Interestingly, pri-let-7 and pre-let-7 were not found to accumulate in embryonic cells, suggesting that Lin28 functions through a turnover mechanism. In fact, Lin28 directly recruits a poly(U) polymerase, terminal (U) transferase-4 (TUT4), to pre-let-7 and oligo-uridylation of the precursor triggers degradation (60). The mechanism by which Lin28 blocks pri-let-7 processing remains uncertain. In another example of feedback

regulation, Lin28 is itself a target of let-7, with Lin28 expressed in a pattern reciprocal to that of mature let-7 during embryonic development (8, 61).

The Lin28-let-7 axis plays a deeply evolutionarily conserved role in developmental timing (62). Recent work has highlighted the key role of the Lin28-let-7 axis in hematopoiesis. The immune system develops in waves during ontogeny, such that it is first populated by cells generated from fetal hematopoietic stem cells (HSCs) and later by cells derived from adult HSCs. Lin28b is specifically expressed in mouse and human fetal liver and thymus, but not adult bone marrow or thymus (63). Moreover, ectopic expression of Lin28b reprograms hematopoietic stem and progenitor cells from adult bone marrow, allowing them to mediate multilineage reconstitution that mirrors fetal lymphopoieis. This includes increased development of B-1a B cells, marginal zone B cells, $\gamma\delta$ T cells, and natural killer T cells. These data extend our understanding of the Lin28-let-7 axis in developmental transitions to the hematopoietic system, and they recall the ability of Lin28 to cooperate with ES cell-specific transcription factors to reprogram somatic cells into induced pluripotent stem cells (64).

Other regulators of miRNA processing

Estrogens are steroid hormones that mediate sex differences, but they also affect the function of lymphocytes and monocytes and may contribute to the increased frequency of autoimmune disorders in females (65). Signaling through estrogen receptor α (ERα) inhibits the processing of certain miRNAs, including miR-16 and miR-125a (66). In the presence of its ligand estradiol (E_2), ERα is recruited to the Drosha complex, leading to dissociation of Drosha from ERα-targeted pri-miRNAs.

KH-type splicing regulatory protein (KSRP) is an RNA-binding protein that destabilizes mRNAs via adenylate/uridylate-rich elements (ARE) in the 3′UTR of transcripts. For example, KSRP regulates inflammation in the central nervous system by destabilizing transcripts encoding TNF-α, IL-1β, and others in astrocytes (67). In addition, KSRP associates with both Dicer and Drosha and increases the processing of several miRNAs including let-7 and miR-21 (68). In macrophages, KSRP contributes to the LPS-induced upregulation of miR-155 by enhancing the processing of miR-155 precursors. The mechanism of enhanced processing involves KSRP binding to 5′ guanosine-rich regions in the loop regions of specific miRNA precursors and the recruitment of Drosha and Dicer to the pri- and pre-miRNA, respectively.

Another protein that employs a similar mechanism of regulation is hnRNP A1, which has been implicated in many RNA processing and transport pathways (69). hnRNP A1 can enhance Drosha-mediated processing of miR-18a (70) by binding to the loop of pri-miR-18a and inducing relaxation of the stem region, which promotes cleavage by Drosha (71). Though miR-18a is just one of 6 miRNAs found within the polycistronic pri-miR transcribed from the miR-17~92 gene cluster (12), only miR-18a is affected by hnRNP A1. Moreover, miR-18b, which is part of the homologous miR106a~363 cluster, is also processed independently of hnRNP A1, highlighting the specificity of this regulation. Interestingly, approximately 14% of all pri-miRNAs have highly conserved loop regions, suggesting the existence of other auxiliary factors for processing of specific miRNAs (71).

Modulation of mature miRNA homeostasis

miRNA expression continues to be regulated after biogenesis is complete. Mature miRNA homeostasis is influenced by signals that modulate the stability of the miRISC complex, by nucleases that degrade miRNAs, and by the abundance of their mRNA targets. Early reports established that miRNAs are often surprisingly stable in cells, displaying half-lives up to 12

days long (72). Yet, cell differentiation and cell fate decisions are frequently marked by dramatic changes in mature miRNA expression. Recent reports have started to elucidate the mechanisms that provide flexibility to the cellular miRNA repertoire by actively eliminating mature miRNAs.

Argonaute stability, loading, and localization

Several studies have used Argonaute (Ago) deficiency, knockdown, or overexpression to show that these proteins are limiting factors that determine total cellular miRNA abundance. This makes intuitive sense, since Ago proteins are the core component of the miRISC complex and must be expressed at 1:1 stoichiometry with miRNAs to mediate their canonical function in gene regulation. The human and mouse genomes both encode four Ago proteins. Despite some tissue-specific distribution, most cells express more than one Ago family member. All four Ago proteins appear to serve overlapping roles in miRNA-mediated repression, with little or no miRNA sorting preferences between the proteins (73–75). However, only Ago2 retains 'slicer' activity, endowing it with a unique role in RNA interference as the enzyme that endonucleolytically cleaves perfectly complementary short interfering RNA targets. Ago2 is required for the maturation of B-cell and erythroid precursors independent of its slicer activity, and Ago2-deficient erythroblasts, fibroblasts, hepatocytes all display a global reduction in miRNA expression (76). In the skin, approximately 60% of the total miRNA pool is associated with Ago2, with an additional 30% associating with Ago1 and 10% with Ago3 (75). Deletion of both Ago1 and Ago2, but not either one alone, was sufficient to reduce miRNA expression far enough to cause defects in skin morphogenesis.

We found that Ago proteins are also limiting for miRNA expression in T cells (28). Deletion of even one copy of Ago2 reduced miRNA abundance in naive T cells, and Ago2-deficient T cells displayed an average 60% reduction of miRNA expression. Deletion of both Ago1 and Ago2 reduced miRNA expression even further (28, authors' unpublished data). miRNA expression is also reduced in Ago2-deficient mouse embryonic fibroblasts and in Ago-deficient *Xenopus* embryos (77, 78). Conversely, overexpression of Ago2 but not other proteins in the miRNA biogenesis pathway increases miRNA expression in HEK293 cells. Thus, changes in Ago protein expression and stability can have dramatic effects on mature miRNA expression within cells.

Several post-translational modifications regulate Ago2 localization, function, and stability. Phosphorylation of Ago2 at serine-387 was observed in HEK293T cells responding to cellular stress signals induced by sodium arsenite or anisomycin (79). Phosphorylation appears to be crucial for Ago2 localization, as mutating this residue to alanine abrogated Ago2 association with P-bodies. Serine-387 phosphorylation was dependent on p38 mitogen-associated protein kinase (MAPK). Tyrosine-529 is a crucial residue required for Ago2 binding to the 5′ end of small RNAs, and phosphorylation of this residue abrogates small RNA binding (80). In addition, prolyl hydroxylation of proline-700 is important for the stability of Ago2. Mutation of this residue or knockdown of type I collagen prolyl-4-hydroxylase destabilizes Ago2 (81). Finally, MAPK signaling downstream of the epidermal growth factor (EGF) receptor enhances Ago2 stability in human breast cancer cell lines (82). In this case, Ago2 abundance could also be increased by treatment with the proteasome inhibitor, MG132, indicating that Ago2 may be ubiquitinated and degraded in these cells. In fact, Ago2 can be ubiquitinated by the let-7 target mLin41 (a.k.a. Trim71), inducing Ago2 proteasomal degradation and loss of miRNA-mediated repression (83). However, studies in mouse neural progenitors and mouse ES cells showed that loss of mLin41 did not lead to changes in Ago2 stability, arguing against a required role for endogenous mLin41 in Ago2 turnover (84, 85). Indeed mLin41 cooperates with miRNAs to repress target gene

expression, and can directly bind and repress some mRNAs independent of its ubiquitin ligase activity (85, 86).

Our recent work in this area uncovered inducible post-transcriptional downregulation of both Ago proteins and their associated miRNAs in activated CD4^{+} T cells (28). We first observed that most miRNAs undergo rapid downregulation upon T-cell activation *in vitro* and *in vivo*. The average miRNA drops approximately 5–10-fold in abundance relative to total cellular RNA, rRNA, or tRNAs. Ago proteins are also post-transcriptionally downregulated to a similar degree and with similar kinetics, suggesting induced degradation of the entire miRISC complex. Indeed, Ago2 is ubiquitinated in activated T cells, and its downregulation can be rescued by treatment with the proteasome inhibitor MG132 for just 2–4 h. The E3 ligase that ubiquitinates Ago proteins in T cells remains to be identified, but we were able to rule out mLin41, which is not expressed in T cells (28, 87). Thus T-cell activation accelerates miRISC turnover, with continuous production of new miRNAs and Ago proteins coupled with rapid degradation that reduces miRNA half-life to less than 2 h. This together with miRNA gene-specific transcriptional changes rapidly reset the miRNA repertoire of activated T cells (Fig. 4).

Induced miRISC turnover likely has important consequences for activated T cells. Naive T cells lacking Dicer, Dgcr8, Drosha, or even Ago2 all exhibit increased sensitivity to signals that induce effector T-helper 1 (Th1) and Th2 differentiation and cytokine production (26, 28, 88, 89). Therefore, some subset of the miRNAs expressed in naive T cells form a significant barrier to effector T-cell differentiation. As one would expect, increased miRNA turnover in activated T cells quickly reprograms miRNA expression patterns in favor of miRNAs that are transcriptionally upregulated, while other miRNAs that are transcriptionally repressed are rapidly cleared from activated T cells. The few miRNA genes that are transcriptionally induced and therefore upregulated or held steady at the mature miRNA level include miR-155 and the miR-17~92 cluster, both of which are important for supporting T-cell-mediated immune responses (24, 26–28, 90, 91). In contrast, miR-29a and miR-29b inhibit Th1 cell differentiation (26, 92, 93), and these miRNAs are rapidly downregulated in activated T cells through combined post-transcriptional and transcriptional mechanisms (28). Taken together, these findings indicate that the antigen and cytokine signals that induce helper T-cell differentiation work in part by shifting the miRNA repertoire away from miRNAs that inhibit differentiation and toward those that support T-cell proliferation, survival, and acquisition of immune effector functions.

A similar phenomenon of global miRNA downregulation has been observed in human tumors, as compared to normal tissue (94, 95). In addition, poorly differentiated tumors have lower global levels of miRNA expression than more differentiated samples. These data are consistent with the idea that miRNA expression is closely linked to differentiation. Furthermore, global repression of miRNA maturation promotes transformation and tumorigenesis, and Dicer1 can function as a haploinsufficient tumor suppressor (56, 96). It will be interesting to determine whether Ago has similar properties and if decreased Ago protein abundance is also associated with tumorigenesis. Importantly, activation-induced Ago and mature miRNA downregulation in activated T cells depends on continuous signaling through the mammalian target of rapamycin (mTOR) pathway, suggesting that it may also be active in other activated immune cells and tumors of both immune and non-immune cell origin.

Extracellular miRNAs

Ago proteins interact with intracellular membranes associated with vesicle transport, including endosomes and multivesicular bodies (MVBs) (97, 98). Blocking MVB formation by depleting components of the endosomal sorting complex required for transport (ESCRT)

results in impaired miRNA silencing in human and *Drosophila* cells, indicating a required role for this physical association. RISC components and miRNAs have also been found in exosomes (99). Exosomes are small vesicles (~50nm in diameter), which form by invagination and budding from the limiting membrane of late endosomes, leading to the secretion of internal vesicles into the extracellular environment (100).

Exosomes have been isolated from the culture supernatant of many hematopoietic cells, including cytotoxic T lymphocytes, mast cells, and dendritic cells (DCs), and DC-derived exosomes have been shown to stimulate CD4+ T-cell activation and induce tolerance (101). Stimulation of THP-1 human monocytes with LPS, oleic acid/palmitic acid, advanced glycation end products and H_2O_2 led to dramatic changes in miRNA expression both in cells and in vesicles isolated from culture supernatants (102). These vesicles contained both Ago2 and miRNAs including miR-150, miR-21, and miR-26b, and vesicle-derived miR-150 could be delivered to recipient HMEC-1 human endothelial cells and repress target mRNAs in the recipient cells. Subsequent studies have suggested that vitamin D_3- and LPS-treated DCs release miRNA-containing exosomes that can be captured by other DCs during co-culture (103). *Staphylococcus* enterotoxin superantigen-E-pulsed Raji B cells were also shown to acquire exosome-derived J77 T cell-expressed miRNAs, which could then repress mRNA targets in the recipient cells (104). These findings illustrate another mechanism by which immune cell stimulation/activation can lead to significant changes in mature miRNA levels (Fig. 5A).

Interest in extracellular miRNAs in various body fluids grew quickly as early findings indicated their utility as readily accessible biomarkers. Circulating miRNAs have been studied in patient samples and animal models in the context of cardiovascular disease, liver injury, sepsis, cancer, and various other physiological and pathophysiological states (105). Tumor-specific miRNAs were first discovered in the serum of patients with diffuse large B-cell lymphoma, where high levels of miR-21 correlated with improved relapse-free survival (106). Since then more than 200 studies have assessed the use of serum or plasma miRNAs as biomarkers in different types of cancers and between patients with different prognoses. For example, a specific profile of plasma miRNAs was identified in patients with chronic lymphocytic lymphoma (CLL), as compared to those with multiple myeloma, hairy-cell leukemia, and healthy controls (107). This study indicated that circulating miRNAs could be correlated with the prognosis marker ζ-associated protein of 70 kDa (ZAP-70) status and could be used to stratify patients with CLL.

The origin of extracellular miRNAs is still poorly understood. Correlations between miRNAs highly expressed in solid tumors and those same miRNAs found at high levels in the circulation of patients with those tumor types have hinted that these miRNAs could be released by tumors themselves (108). However, blood cells appear to be a major contributor to circulating miRNAs (109). Hemolysis increases the abundance of plasma miRNAs found in red blood cells as much as 50-fold. In addition, high serum and plasma concentrations of miRNAs expressed selectively in granulocytes and lymphocytes correlated closely with the prevalence of those cells in the cellular component of the same blood samples. Although these results were presented as a cautionary note and a call for careful quality control in extracellular miRNA biomarker discovery studies, they also indicate that cells of the immune system are a major source of naturally released extracellular miRNAs.

It has also become clear that extracellular miRNAs exist in several distinct forms in human plasma (Fig. 5A). In addition to miRNAs encapsulated in vesicles such as exosomes, there are stable non-vesicular miRNAs that can be co-purified with Ago proteins that are accessible for direct immunoprecipitation from plasma samples (110). This finding indicates that circulating Ago2 complexes are sufficient to ensure the stability of extracellular

miRNAs. Vesicular miRNAs can be separated by differential sedimentation, are resistant to combined protease and nuclease treatment of plasma and may or may not be associated with Ago proteins. In addition to exosomes and apoptotic bodies, vesicular extracellular miRNAs include those associated with high-density lipoprotein (HDL) (111). Like exosomes, HDL-miRNA complexes could deliver miRNAs to recipient cells. Further research is urgently needed to clarify the cellular sources of miRNAs, the forms in which they are released, and whether this process is regulated during hematopoiesis or immune responses.

miRNA nucleases

As compared to miRNA transcription and processing, relatively little is known about the regulation of mature miRNA degradation. In *Arabidopsis thaliana,* miRNAs are protected from degradation by $2'$-O-methyl modification of their $3'$-terminal ribose mediated by the Hen1 methyltransferase (112). Unmethylated *Arabidopsis* miRNAs and siRNAs are subject to $3'$ polyuridylation (113) and degradation by $3'$-to-$5'$ exonucleases of the *SMALL DNA DEGRADING NUCLEASE* (*SDN*) family (114). In *C. elegans*, the $5'$-to-$3'$ exonuclease XRN2 degrades mature miRNAs (101). *In vitro* reconstitution experiments showed that larval lysates can efficiently extract miRNAs from the intact miRISC, making them accessible for XRN2-mediated degradation. However, target mRNA binding protects miRNAs from this process, suggesting a homeostatic role for mRNA targets in controlling miRNA stability.

The identity of the enzymes that degrade mature miRNAs in mammals remains unknown. However, at least two ribonucleases have been shown to negatively regulate the expression of mature miRNAs (Fig. 5B). IRE1α, an endoplasmic reticulum (ER) transmembrane RNase activated in response to ER stress, cleaves precursors corresponding to miR-17, miR-34a, miR-96, and miR-125b and mediates rapid decay of their expression in response to sustained cellular stress (115). This mechanism relieves miRNA-mediated repression of Caspase-2 mRNA, which encodes an early apoptotic switch upstream of the mitochondrial pathway of programmed cell death. Interestingly, ER stress contributes to inflammation by both NF-κB-dependent and –independent mechanisms, and ER stress has been implicated in chronic inflammatory diseases, such as diabetes, arthritis, and inflammatory bowel disease (116). Further work will be required to determine the role of these miRNAs and their regulation by IRE1α in inflammation.

Eri1 is a $3'$-to-$5'$ exoribonuclease with a double-stranded RNA binding SAP domain and a deeply conserved role in 5.8S rRNA $3'$ end processing (117, 118). Eri1 also has additional functions in the maturation and degradation of histone mRNAs (119, 120). In *C. elegans*, *eri-1* mutants exhibit enhanced RNA interference (121), and in mammals Eri1 also limits miRNA abundance in CD4$^+$ T cells and natural killer (NK) cells (122). Eri1-deficient mice have a 50% reduction in NK cell numbers in peripheral organs, and the remaining NK cells exhibit approximately twofold increased expression of all miRNAs, an altered antigen receptor repertoire, and an immature surface phenotype. Eri1-deficient NK cells also expand inefficiently in response to murine cytomegalovirus (MCMV) infection and are unable to mediate effective antiviral immunity. These findings demonstrate that Eri1 is required to promote NK cell homeostasis and immune function. It remains to be determined whether these phenotypes are caused by defective miRNA homeostasis or one or more of the other molecular functions of Eri1. In addition, further work is needed to determine whether Eri1 directly degrades pre-miRNAs, mature miRNAs, or perhaps mRNA target-associated miRNAs that are tagged for degradation. The latter possibility is particularly interesting in light of the recent finding that Eri1's role in histone mRNA turnover involves $3'$ to $5'$ degradation of oligouridylated histone mRNAs (120).

A theme has recently emerged in which the interaction between miRNAs and their targets can result in degradation of the miRNA (Fig. 5C). This is often accompanied by 3′ oligo(U) or (A) tailing and trimming of the miRNA (123). In *Drosophila*, extensive complementarity between a target RNA and Ago1-bound miRNA triggers miRNA tailing and 3′-to-5′ trimming. As in *Arabidopsis,* Hen1-mediated 2′-O-methylation of the 3′-terminus protects miRNAs from tailing and trimming. Sequence-specific degradation of miRNAs following addition of RNA targets has also been observed in mammalian cells. miRNA 'antagomirs' and 'sponges', two technologies used to specifically knockdown miRNA expression, both rely on high miRNA-to-target complementarity leading to miRNA degradation (124, 125). In HeLa cells, transfection of antagomirs or incubation with target mRNAs triggers 3′ tailing and degradation of the corresponding miRNA (123). Addition of mRNA targets into A70 proB cells also leads to depletion of the cognate miRNA (43). Several recent studies have also identified mechanisms by which viruses hijack this process in infected cells. During infection of Jurkat T cells with *Herpesvirus saimiri*, miR-27 abundance is dramatically decreased. This decrease is dependent on two virally encoded transcripts that contain several miR-27 binding sites (126). miR-27 is similarly regulated by a transcript encoded by MCMV in infected fibroblasts (127, 128). Finally, during poxvirus infection of mouse fibroblasts, a virally encoded poly(A) polymerase mediates 3′ polyadenylation of host miRNAs, leading to their degradation (129). Further work is needed to determine the extent to which miRNA expression is regulated by target mRNAs, and to determine the molecular mechanisms that mediate this final step in the control of miRNA expression.

References

1. Ambros V. The evolution of our thinking about microRNAs. Nat Med. 2008; 14:1036–1040. [PubMed: 18841144]

2. Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. Nature. 2009; 457:426–433. [PubMed: 19158789]

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

4. Djuranovic S, Nahvi A, Green R. A parsimonious model for gene regulation by miRNAs. Science. 2011; 331:550–553. [PubMed: 21292970]

5. Hoefig KP, Heissmeyer V. MicroRNAs grow up in the immune system. Curr Opin Immunol. 2008; 20:281–287. [PubMed: 18554884]

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

7. 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]

8. Reinhart BJ, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000; 403:901–906. [PubMed: 10706289]

9. Ebert MS, Sharp PA. Roles for microRNAs in conferring robustness to biological processes. Cell. 2012; 149:515–524. [PubMed: 22541426]

10. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell. 2012; 148:1172–1187. [PubMed: 22424228]

11. Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. Cell. 2009; 136:26–36. [PubMed: 19135886]

12. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010; 10:111–122. [PubMed: 20098459]

13. Schanen BC, Li X. Transcriptional regulation of mammalian miRNA genes. Genomics. 2011; 97:1–6. [PubMed: 20977933]

14. Kirigin FF, et al. Dynamic microRNA gene transcription and processing during T cell development. J Immunol. 2012; 188:3257–3267. [PubMed: 22379031]

15. Ozsolak F, et al. Chromatin structure analyses identify miRNA promoters. Genes Dev. 2008; 22:3172–3183. [PubMed: 19056895]

16. Barski A, et al. Chromatin poises miRNA-and protein-coding genes for expression. Genome Res. 2009; 19:1742–1751. [PubMed: 19713549]

17. Lee Y, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004; 23:4051–4060. [PubMed: 15372072]

18. Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol. 2006; 13:1097–1101. [PubMed: 17099701]

19. Delgado MD, Leon J. Myc roles in hematopoiesis and leukemia. Genes Cancer. 2010; 1:605–616. [PubMed: 21779460]

20. Wang R, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity. 2011; 35:871–882. [PubMed: 22195744]

21. Lin CY, et al. Transcriptional amplification in tumor cells with elevated c-Myc. Cell. 2012; 151:56–67. [PubMed: 23021215]

22. Nie Z, et al. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell. 2012; 151:68–79. [PubMed: 23021216]

23. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature. 2005; 435:839–843. [PubMed: 15944709]

24. Xiao C, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 2008; 9:405–414. [PubMed: 18327259]

25. He L, et al. A microRNA polycistron as a potential human oncogene. Nature. 2005; 435:828–833. [PubMed: 15944707]

26. Steiner DF, et al. MicroRNA-29 regulates T-box transcription factors and interferon-gamma production in helper T cells. Immunity. 2011; 35:169–181. [PubMed: 21820330]

27. Jiang S, et al. Molecular dissection of the miR-17-92 cluster's critical dual roles in promoting Th1 responses and preventing inducible Treg differentiation. Blood. 2011; 118:5487–5497. [PubMed: 21972292]

28. Bronevetsky Y, et al. T cell activation induces proteasomal degradation of Argonaute and rapid remodeling of the microRNA repertoire. J Exp Med. 2013 in press.

29. de Pontual L, et al. Germline deletion of the miR-17approximately 92 cluster causes skeletal and growth defects in humans. Nat Genet. 2011; 43:1026–1030. [PubMed: 21892160]

30. Chang TC, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet. 2008; 40:43–50. [PubMed: 18066065]

31. Chang TC, et al. Lin-28B transactivation is necessary for Myc-mediated let-7 repression and proliferation. Proc Natl Acad Sci USA. 2009; 106:3384–3389. [PubMed: 19211792]

32. Kim HH, Kuwano Y, Srikantan S, Lee EK, Martindale JL, Gorospe M. HuR recruits let-7/RISC to repress c-Myc expression. Genes Dev. 2009; 23:1743–1748. [PubMed: 19574298]

33. Boldin MP, Baltimore D. MicroRNAs, new effectors and regulators of NF-kappaB. Immunol Rev. 2012; 246:205–220. [PubMed: 22435557]

34. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA. 2006; 103:12481–12486. [PubMed: 16885212]

35. Lu LF, et al. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell. 2010; 142:914–929. [PubMed: 20850013]

36. Yang L, et al. miR-146a controls the resolution of T cell responses in mice. J Exp Med. 2012; 209:1655–1670. [PubMed: 22891274]

37. Zhao JL, Rao DS, Boldin MP, Taganov KD, O'Connell RM, Baltimore D. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. Proc Natl Acad Sci USA. 2011; 108:9184–9189. [PubMed: 21576471]

38. Bazzoni F, et al. Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. Proc Natl Acad Sci USA. 2009; 106:5282–5287. [PubMed: 19289835]

39. Zhao H, Kalota A, Jin S, Gewirtz AM. The c-myb proto-oncogene and microRNA-15a comprise an active autoregulatory feedback loop in human hematopoietic cells. Blood. 2009; 113:505–516. [PubMed: 18818396]

40. Ben-Ami O, Pencovich N, Lotem J, Levanon D, Groner Y. A regulatory interplay between miR-27a and Runx1 during megakaryopoiesis. Proc Natl Acad Sci USA. 2009; 106:238–243. [PubMed: 19114653]

41. Fazi F, et al. A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis. Cell. 2005; 123:819–831. [PubMed: 16325577]

42. Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev. 2006; 20:2202–2207. [PubMed: 16882971]

43. Kuchen S, et al. Regulation of microRNA expression and abundance during lymphopoiesis. Immunity. 2010; 32:828–839. [PubMed: 20605486]

44. Davis BN, Hata A. Regulation of MicroRNA Biogenesis: A miRiad of mechanisms. Cell Comm Signal. 2009; 7:18.

45. Blahna MT, Hata A. Smad-mediated regulation of microRNA biosynthesis. FEBS Lett. 2012; 586:1906–1912. [PubMed: 22306316]

46. Davis BN, Hilyard AC, Lagna G, Hata A. SMAD proteins control DROSHA-mediated microRNA maturation. Nature. 2008; 454:56–61. [PubMed: 18548003]

47. Fukuda T, et al. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. Nat Cell Biol. 2007; 9:604–611. [PubMed: 17435748]

48. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. Mol Cell. 2010; 39:373–384. [PubMed: 20705240]

49. Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. J Biochem. 2010; 147:781–792. [PubMed: 20410014]

50. Kang H, et al. Inhibition of microRNA-302 (miR-302) by bone morphogenetic protein 4 (BMP4) facilitates the BMP signaling pathway. J Biol Chem. 2012; 287:38656–38664. [PubMed: 22988237]

51. Xu-Monette ZY, et al. Dysfunction of the TP53 tumor suppressor gene in lymphoid malignancies. Blood. 2012; 119:3668–3683. [PubMed: 22275381]

52. Hermeking H. MicroRNAs in the p53 network: micromanagement of tumour suppression. Nature reviews Cancer. 2012; 12:613–626.

53. Klein U, et al. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Cancer Cell. 2010; 17:28–40. [PubMed: 20060366]

54. Park SY, Lee JH, Ha M, Nam JW, Kim VN. miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. Nat Struct Cell Biol. 2009; 16:23–29.

55. Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by p53. Nature. 2009; 460:529–533. [PubMed: 19626115]

56. Kumar MS, et al. Dicer1 functions as a haploinsufficient tumor suppressor. Genes Dev. 2009; 23:2700–2704. [PubMed: 19903759]

57. Mudhasani R, et al. Loss of miRNA biogenesis induces p19Arf-p53 signaling and senescence in primary cells. J Cell Biol. 2008; 181:1055–1063. [PubMed: 18591425]

58. Leveille N, et al. Selective inhibition of microRNA accessibility by RBM38 is required for p53 activity. NatCommun. 2011; 2:513.

59. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. Science. 2008; 320:97–100. [PubMed: 18292307]

60. Heo I, et al. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. Cell. 2009; 138:696–708. [PubMed: 19703396]

61. Rybak A, et al. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. Nat Cell Biol. 2008; 10:987–993. [PubMed: 18604195]

62. Mondol V, Pasquinelli AE. Let's make it happen: the role of let-7 microRNA in development. Curr Topics Cell Biol. 2012; 99:1–30.

63. Yuan J, Nguyen CK, Liu X, Kanellopoulou C, Muljo SA. Lin28b reprograms adult bone marrow hematopoietic progenitors to mediate fetal-like lymphopoiesis. Science. 2012; 335:1195–1200. [PubMed: 22345399]

64. Yu J, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318:1917–1920. [PubMed: 18029452]

65. Oertelt-Prigione S. The influence of sex and gender on the immune response. Autoimmun Rev. 2012; 11:A479–485. [PubMed: 22155201]

66. Yamagata K, et al. Maturation of microRNA is hormonally regulated by a nuclear receptor. Mol Cell. 2009; 36:340–347. [PubMed: 19854141]

67. Li X, et al. KSRP: A checkpoint for inflammatory cytokine production in astrocytes. Glia. 2012; 60:1773–1784. [PubMed: 22847996]

68. Trabucchi M, et al. The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. Nature. 2009; 459:1010–1014. [PubMed: 19458619]

69. He Y, Smith R. Nuclear functions of heterogeneous nuclear ribonucleoproteins A/B. Cell Mol Life Sci. 2009; 66:1239–1256. [PubMed: 19099192]

70. Guil S, Caceres JF. The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. Nat Struct Mol Biol. 2007; 14:591–596. [PubMed: 17558416]

71. Michlewski G, Guil S, Semple CA, Caceres JF. Posttranscriptional regulation of miRNAs harboring conserved terminal loops. Mol Cell. 2008; 32:383–393. [PubMed: 18995836]

72. van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. Science. 2007; 316:575–579. [PubMed: 17379774]

73. Azuma-Mukai A, et al. Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing. Proc Natl Acad Sci USA. 2008; 105:7964–7969. [PubMed: 18524951]

74. Hafner M, et al. Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell. 2010; 141:129–141. [PubMed: 20371350]

75. Wang D, et al. Quantitative functions of Argonaute proteins in mammalian development. Genes Dev. 2012; 26:693–704. [PubMed: 22474261]

76. O'Carroll D, et al. A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. Genes Dev. 2007; 21:1999–2004. [PubMed: 17626790]

77. Diederichs S, Haber DA. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. Cell. 2007; 131:1097–1108. [PubMed: 18083100]

78. Lund E, Sheets MD, Imboden SB, Dahlberg JE. Limiting Ago protein restricts RNAi and microRNA biogenesis during early development in Xenopus laevis. Genes Dev. 2011; 25:1121–1131. [PubMed: 21576259]

79. Zeng Y, Sankala H, Zhang X, Graves PR. Phosphorylation of Argonaute 2 at serine-387 facilitates its localization to processing bodies. Biochem J. 2008; 413:429–436. [PubMed: 18476811]

80. Rudel S, et al. Phosphorylation of human Argonaute proteins affects small RNA binding. Nucleic Acids Res. 2011; 39:2330–2343. [PubMed: 21071408]

81. Qi HH, et al. Prolyl 4-hydroxylationregulates Argonaute 2 stability. Nature. 2008; 455:421–424. [PubMed: 18690212]

82. Adams BD, Claffey KP, White BA. Argonaute-2 expression is regulated by epidermal growth factor receptor and mitogen-activated protein kinase signaling and correlates with a transformed phenotype in breast cancer cells. Endocrinology. 2009; 150:14–23. [PubMed: 18787018]

83. Rybak A, et al. The let-7 target gene mouse lin-41 is a stem cell specific E3 ubiquitin ligase for the miRNA pathway protein Ago2. Nat Cell Biol. 2009; 11:1411–1420. [PubMed: 19898466]

84. Chen J, Lai F, Niswander L. The ubiquitin ligase mLin41 temporally promotes neural progenitor cell maintenance through FGF signaling. Genes Dev. 2012; 26:803–815. [PubMed: 22508726]

85. Chang HM, Martinez NJ, Thornton JE, Hagan JP, Nguyen KD, Gregory RI. Trim71 cooperates with microRNAs to repress Cdkn1a expression and promote embryonic stem cell proliferation. Nat Commun. 2012; 3:923. [PubMed: 22735451]

86. Loedige I, Gaidatzis D, Sack R, Meister G, Filipowicz W. The mammalian TRIM-NHL protein TRIM71/LIN-41 is a repressor of mRNA function. Nucleic Acids Res. 2013; 41:518–532. [PubMed: 23125361]

87. Benoist C, Lanier L, Merad M, Mathis D. Consortium biology in immunology: the perspective from the Immunological Genome Project. Nat Rev Immunol. 2012; 12:734–740. [PubMed: 22955842]

88. Muljo SA, Ansel KM, Kanellopoulou C, Livingston DM, Rao A, Rajewsky K. Aberrant T cell differentiation in the absence of Dicer. J Exp Med. 2005; 202:261–269. [PubMed: 16009718]

89. Chong MM, Rasmussen JP, Rudensky AY, Littman DR. The RNAseIII enzyme Drosha is critical in T cells for preventing lethal inflammatory disease. J Exp Med. 2008; 205:2005–2017. [PubMed: 18725527]

90. Rodriguez A, et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007; 316:608–611. [PubMed: 17463290]

91. Thai TH, et al. Regulation of the germinal center response by microRNA-155. Science. 2007; 316:604–608. [PubMed: 17463289]

92. Ma F, et al. The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-gamma. Nat Immunol. 2011; 12:861–869. [PubMed: 21785411]

93. Smith KM, et al. miR-29ab1 deficiency identifies a negative feedback loop controlling Th1 bias that is dysregulated in multiple sclerosis. J Immunol. 2012; 189:1567–1576. [PubMed: 22772450]

94. Lu J, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435:834–838. [PubMed: 15944708]

95. Gaur A, et al. Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. Cancer Res. 2007; 67:2456–2468. [PubMed: 17363563]

96. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet. 2007; 39:673–677. [PubMed: 17401365]

97. Lee YS, et al. Silencing by small RNAs is linked to endosomal trafficking. Nat Cell Biol. 2009; 11:1150–1156. [PubMed: 19684574]

98. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol. 2009; 11:1143–1149. [PubMed: 19684575]

99. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007; 9:654–659. [PubMed: 17486113]

100. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002; 2:569–579. [PubMed: 12154376]

101. Zitvogel L, et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med. 1998; 4:594–600. [PubMed: 9585234]

102. Zhang Y, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol Cell. 2010; 39:133–144. [PubMed: 20603081]

103. Montecalvo A, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. Blood. 2012; 119:756–766. [PubMed: 22031862]

104. Mittelbrunn M, et al. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nat Commun. 2011; 2:282. [PubMed: 21505438]

105. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011; 8:467–477. [PubMed: 21647195]

106. Lawrie CH, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008; 141:672–675. [PubMed: 18318758]

107. Moussay E, et al. MicroRNA as biomarkers and regulators in B-cell chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2011; 108:6573–6578. [PubMed: 21460253]

108. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/hematol. 2011; 80:193–208.

109. Pritchard CC, et al. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res. 2012; 5:492–497.

110. Arroyo JD, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA. 2011; 108:5003–5008. [PubMed: 21383194]

111. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biology. 2011; 13:423–433.

112. Yu B, et al. Methylation as a crucial step in plant microRNA biogenesis. Science. 2005; 307:932–935. [PubMed: 15705854]

113. Li J, Yang Z, Yu B, Liu J, Chen X. Methylation protects miRNAs and siRNAs from a 3′-end uridylation activity in Arabidopsis. Curr Biol. 2005; 15:1501–1507. [PubMed: 16111943]

114. Ramachandran V, Chen X. Degradation of microRNAs by a family of exoribonucleases in Arabidopsis. Science. 2008; 321:1490–1492. [PubMed: 18787168]

115. Upton JP, et al. IRE1α cleaves select microRNAs during ER stress to derepress translation of proapoptotic caspase-2. Science. 2012; 338:818–822. [PubMed: 23042294]

116. Hasnain SZ, Lourie R, Das I, Chen AC, McGuckin MA. The interplay between endoplasmic reticulum stress and inflammation. Immunol Cell Biol. 2012; 90:260–270. [PubMed: 22249202]

117. Ansel KM, et al. Mouse Eri1 interacts with the ribosome and catalyzes 5. 8S rRNA processing. Nat Struct Mol Biol. 2008; 15:523–530. [PubMed: 18438418]

118. Gabel HW, Ruvkun G. The exonuclease ERI-1 has a conserved dual role in5. 8S rRNA processing and RNAi. Nat Struct Mol Biol. 2008; 15:531–533. [PubMed: 18438419]

119. Dominski Z, Yang XC, Kaygun H, Dadlez M, Marzluff WF. A 3′ exonuclease that specifically interacts with the 3′ end of histone mRNA. Mol Cell. 2003; 12:295–305. [PubMed: 14536070]

120. Hoefig KP, et al. Eri1 degrades the stem-loop of oligouridylated histone mRNAs to induce replication-dependent decay. Nat Struct Mol Biol. 2013; 20:73–81. [PubMed: 23202588]

121. Kennedy S, Wang D, Ruvkun G. A conserved siRNA-degrading RNase negatively regulates RNA interference in C. elegans. Nature. 2004; 427:645–649. [PubMed: 14961122]

122. Thomas MF, et al. Eri1 regulates microRNA homeostasis and mouse lymphocyte development and antiviral function. Blood. 2012; 120:130–142. [PubMed: 22613798]

123. Ameres SL, et al. Target RNA-directed trimming and tailing of small silencing RNAs. Science. 2010; 328:1534–1539. [PubMed: 20558712]

124. Krutzfeldt J, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature. 2005; 438:685–689. [PubMed: 16258535]

125. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods. 2007; 4:721–726. [PubMed: 17694064]

126. Cazalla D, Yario T, Steitz JA. Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. Science. 2010; 328:1563–1566. [PubMed: 20558719]

127. Marcinowski L, et al. Degradation of cellular mir-27 by a novel, highly abundant viral transcript isimportant for efficient virus replication in vivo. PLoS Pathogen. 2012; 8:e1002510. [PubMed: 22346748]

128. Libri V, et al. Murine cytomegalovirus encodes a miR-27 inhibitor disguised as a target. Proc Natl Acad Sci USA. 2012; 109:279–284. [PubMed: 22184245]

129. Backes S, et al. Degradation of host microRNAs by poxvirus poly(A) polymerase reveals terminal RNA methylation as a protective antiviral mechanism. Cell Host Microbe. 2012; 12:200–210. [PubMed: 22901540]
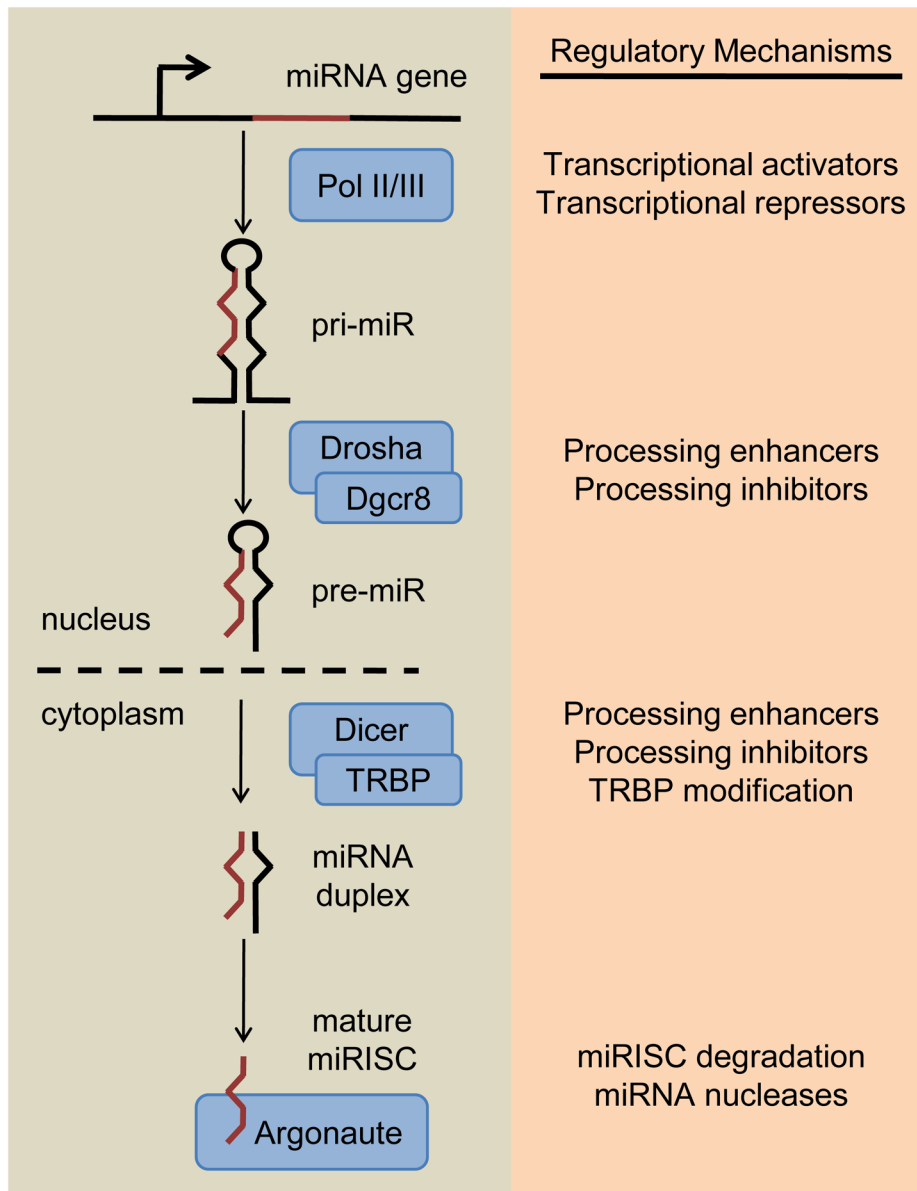
Fig. 1. Regulation of miRNA biogenesis
miRNAs are transcribed by RNA Polymerase II or III and processed from long primary transcripts (pri-miRs), which are cleaved into 60–80 nt hairpin intermediates (pre-miRs) by the nuclear microprocessor complex, comprised of Drosha and DGCR8. Pre-miRs are exported from the nucleus by the nuclear transport receptor, Exportin 5, and processed into an 18–22 nt double-stranded miRNA duplex by Dicer and its cofactor TRBP. The duplex is separated and one strand is selected as the mature miRNA, and the other strand is degraded. Mature miRNAs are incorporated into Argonaute (Ago) protein-containing effector complexes, known as miRISC (miRNA-containing RNA-induced silencing complex).
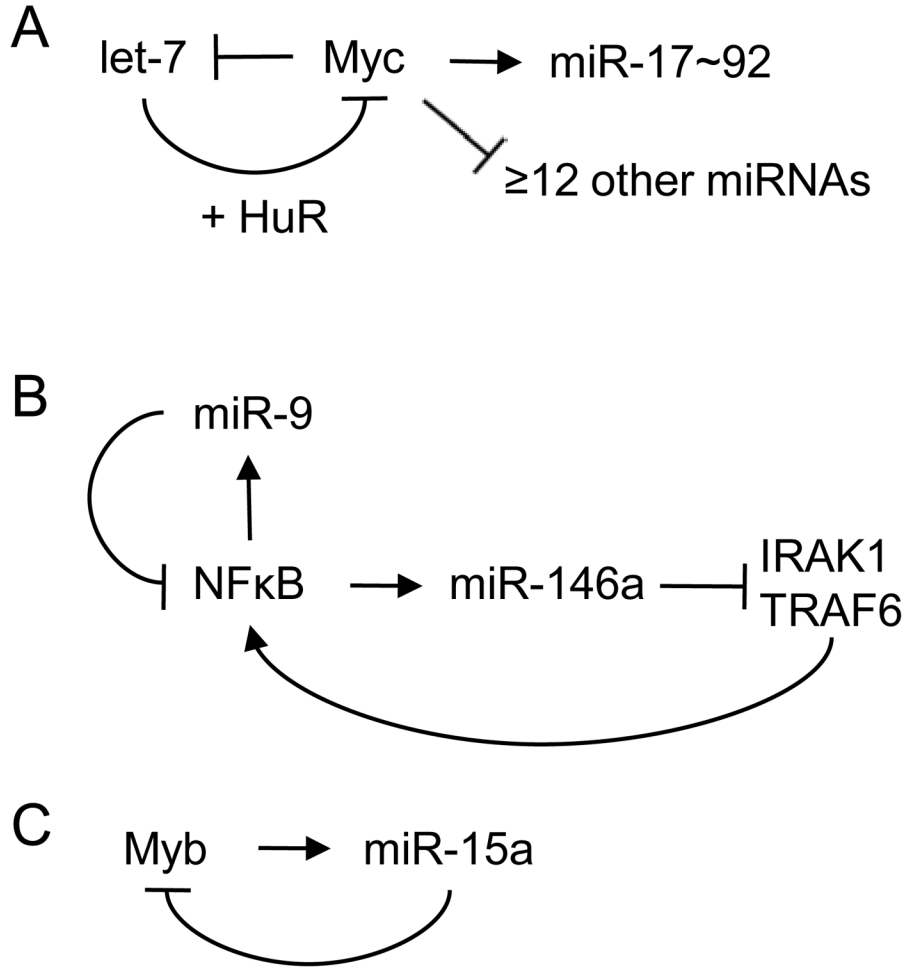
Fig. 2. MicroRNA and transcription factor feedback loops

(A) The Myc transcription factor represses the transcription of let-7 family miRNAs. In turn, *Myc* mRNA is repressed by let-7 in an HuR-dependent manner. Myc also enhances miR-17~92 transcription and represses the transcription of several other miRNA genes. (B) NF-κB is a transcriptional activator of miR-146a. miR-146 reciprocally decreases NF-κB activity by directly targeting TRAF6 and IRAK1, two adapter molecules in the NF-κB signaling pathway. NF-κB also induces the transcription of miR-9, which targets the *NFκB1* mRNA. (C) Myb transcriptionally activates miR-15a, which reciprocally represses *Myb* mRNA.
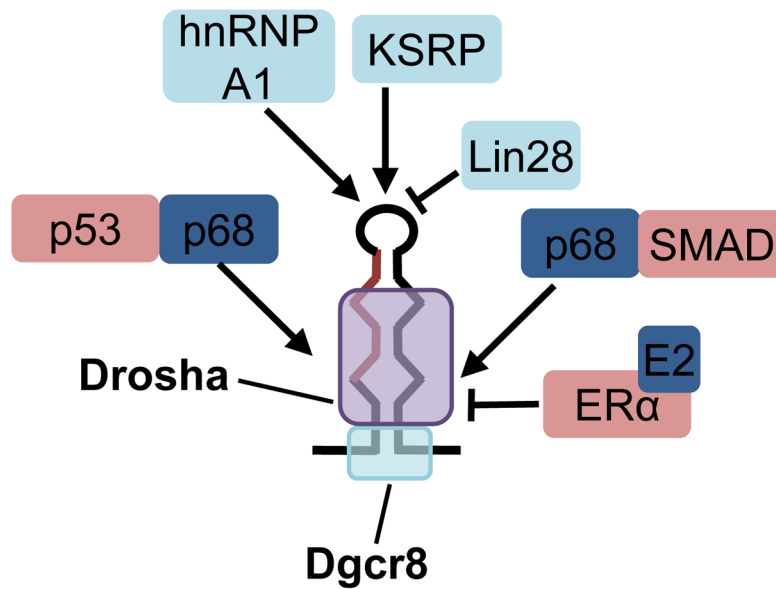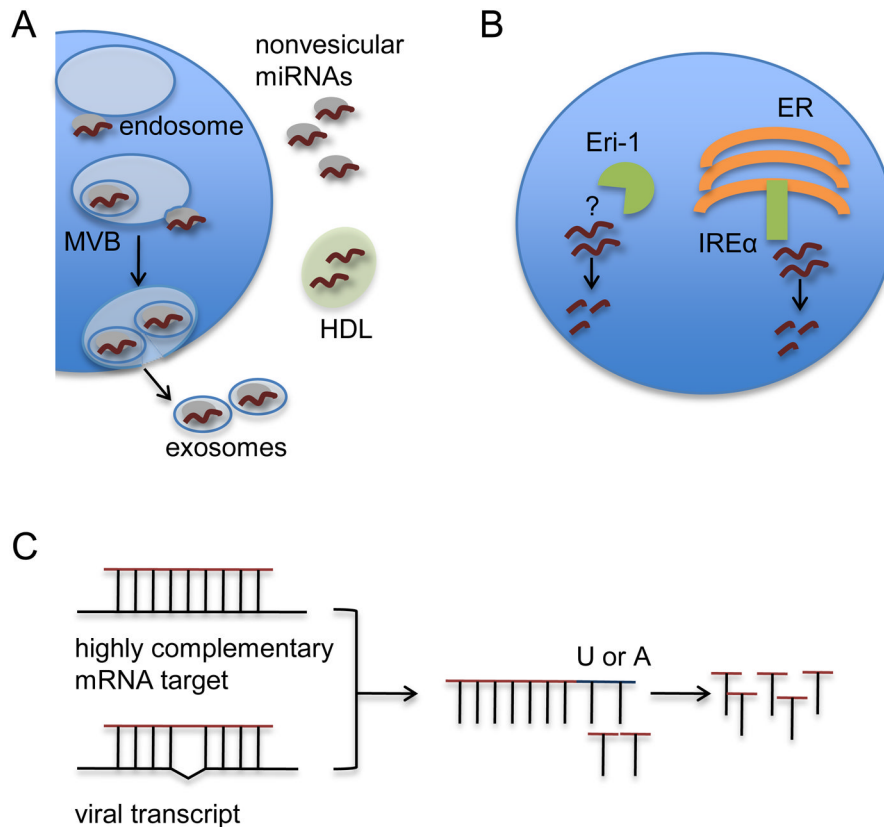
Fig. 3. Regulation of miRNA processing
Regulators of miRNA processing bind to the stem or loop regions of miRNA precursors and influence their processing by Drosha and/or Dicer. Smad proteins activated by TGF-β signaling bind a consensus sequence found within the stem region of numerous pri-miRNAs. Both Smads and p53 associate with Drosha via p68 and increases the processing of their specific target pri-miRs. The estrogen receptor also associates with Drosha in a ligand-dependent manner, but in this case the result is reduced Drosha processing of ERα-targeted pri-miRs. hnRNP A1 binds a conserved region of the pri-miR-18a loop and promotes its cleavage by Drosha. Similarly, KSRP binds sequences in the loop region of several miRNA precursors and recruits Drosha and Dicer to the pri- and pre-miRNA, respectively, enhancing their processing. In contrast, Lin28 represses both Drosha and Dicer processing of most let-7 family members by binding conserved sequences in their precursor loops. This mechanism involves Lin28 recruitment of TUT4 to pre-let-7, causing it to be oligo-uridylated and degraded.

Fig. 4. Activated T cells reset their miRNA repertoire
Upon activation, T cells increase their cellular metabolism to support cell growth, proliferation, and effector functions. Activated T cells also downregulate Argonaute proteins, the core component of the miRISC complex (grey ovals) through a process of ubiquitination (orange triangles) and proteasomal degradation. This leads to global miRNA depletion. Layered atop this post-transcriptional regulation is programmed modulation of miRNA gene transcription (arrows). Together, these mechanisms rapidly remodel the miRNA repertoire of activated T cells as they differentiate into effector cells.

Fig. 5. Mechanisms of miRNA turnover
(A) The miRISC interacts with intracellular membranes associated with vesicle transport, including endosomes and multivesicular bodies (MVBs). Ago protein and miRNAs have also been found in exosomes in blood serum or plasma. In addition to exosomes, extracellular miRNAs are found in non-vesicular Ago2 complexes, and in association with high-density lipoprotein (HDL). (B) Eri1 and IRE1α are ribonucleases that negatively regulate miRNA abundance in mammalian cells. Eri1 is a 3′-to-5′ exoribonuclease that limits mature miRNA levels in lymphocytes, but its direct substrate remains unknown. IRE1α is an endoplasmic reticulum (ER) transmembrane RNase activated that mediates rapid decay of miR-17, miR-34a, miR-96, and miR-125b expression in response to ER stress. (C) Highly complementary RNA targets induce miRNA degradation. Several viruses encode transcripts with miRNA binding sites that lead to degradation of one or more particular miRNAs. This process involves untemplated addition of U and/or A residues at the 3′ end of affected miRNAs.