

Chapter 2

Biogenesis of Mammalian miRNA

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Abstract MicroRNA (miRNA) are small noncoding molecules approximately 22 nucleotides in length that mediate translational repression of target messenger RNA (mRNA) transcripts. miRNA play important roles in cellular differentiation and proliferation, regulation of cellular metabolism, and are associated with enhanced tumorigenesis observed with many cancers. These small RNA molecules function by binding with imperfect complementarity to sequences located in the 3' untranslated region (UTR) of target mRNA. MiRNA are expressed from both intergenic or intronic regions of the genome in an RNA polymerase II-dependent manner and their expression is subject to both feed-forward and negative feedback regulatory loops. Nascent transcripts known as primary miRNA (pri-miRNA) undergo nuclear processing to generate ~ 60 –80 nt precursor miRNA (pre-miRNA) containing imperfect stem-loop structures. Pre-miRNA are subsequently exported out of the nucleus whereupon they are further processed by the RNase III enzyme Dicer resulting in the formation of a ~ 20 –24 nt miRNA duplex. Thermodynamic stability of each strand determines which strand of the miRNA duplex will be retained within the RNA-induced silencing complex (RISC) to function as the mature miRNA. This mature miRNA then guides the RISC to sequences within the 3'UTR of target mRNAs to repress protein translation. Due to the roles that miRNA have in regulating cellular proliferation and metabolism, they could potentially interfere with recombinant protein expression in cell-based systems.

Keywords MicroRNA · Biogenesis · Transcription · Translational repression

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2.1 Introduction

MicroRNAs (miRNAs) are small non-coding regulatory RNA molecules (~ 22 -nt) that post-transcriptionally regulate gene expression by mechanisms involving translational repression and/or decreasing mRNA stability. For the purposes of this review, the focus will be on regulation of mammalian miRNA biogenesis and mechanism of action. Because an individual miRNA species can regulate many targets, and because of the heterogeneity observed in mature miRNA sequences, miRNA serve an essential role in controlling the expression of transcripts and proteins involved in cellular development, differentiation, and metabolism. Furthermore, alterations in either miRNA expression or the expression/activity of components required for miRNA processing and target recognition may contribute to the pathogenesis of a variety of cancers and other chronic diseases. Thus, characterizing the factors that are responsible for controlling miRNA biogenesis is beneficial for enhancing our understanding of mechanisms allowing cells to respond to constantly changing environmental conditions in addition to providing therapeutic opportunities to prevent or treat chronic diseases.

2.2 Principles of MicroRNA Biogenesis

miRNA-dependent regulation of gene expression is thought to control the expression of as much as 60 % of the protein-coding genes expressed in mammals (Friedman et al. 2009). miRNA represent about ~ 1 –2 % of the eukaryotic microtranscriptome and have diverse roles in modulating cellular processes including cellular proliferation, differentiation, and metabolism in addition to being involved disease pathogenesis (Liu et al. 2008; Williams 2008; Esau et al. 2006; Farazi et al. 2011).

miRNAs are transcribed primarily in an RNA polymerase II-dependent process from intergenic regions of the genome or from introns and exons of protein-coding genes (Fig. 2.1) (Kim and Nam 2006; Lee et al. 2004). The genomic organization of miRNA genes is the subject of an excellent review article and will only be briefly described here (Olena and Patton 2010). Approximately one-third of miRNA genes are located in the introns of protein-coding genes suggesting that the expression of miRNA and mRNA may be coordinately regulated (Westholm and Lai 2011; Rodriguez et al. 2004; Baskerville and Bartel 2005). In mammals ~ 36 –46 % of known miRNA are clustered as polycistronic genes (Griffiths-Jones et al. 2008). The expression of miRNA from these clusters suggests that multiple miRNAs affecting multiple targets could be produced in response to environmental or developmental cues. As an additional level of complexity, targets of polycistronic miRNA are often regulated in such a way that multiple protein-protein interactions may be regulated by the induction of miRNA expression and subsequent translational control (Yuan et al. 2009). Expression of miRNA transcripts may also play an important role in autoregulatory loops. For example, one of the targets of hsa-miR-613 is the nuclear hormone

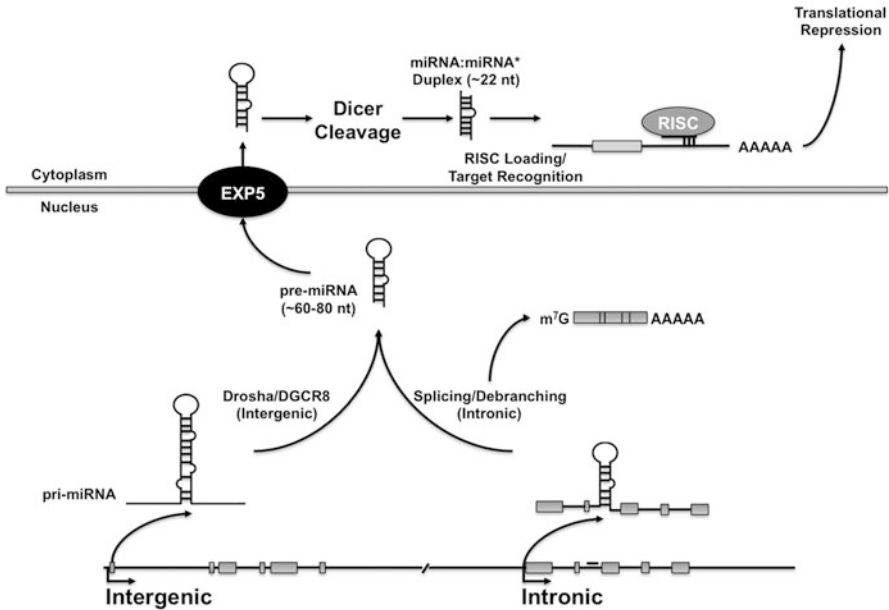


Fig. 2.1 Overview of mammalian miRNA biogenesis and function. The primary (pri-) miRNA transcripts that can adopt hairpin-like structures are transcribed from miRNA loci. Pri-miRNA transcripts from miRNA genes are processed to 60–80 nt pre-miRNA transcripts by a complex containing Drosha and DGCR8 in the nucleus. Alternatively, pre-miRNA may be derived from intronic regions of protein-coding genes in a Drosha/DGCR8 independent process requiring both the spliceosome and a debranching enzyme known as the lariat debranching enzyme. Both the canonical Drosha-dependent processing and intronic processing pathways generate a pre-miRNA with a hairpin-like structure that is then exported out of the nucleus into the cytoplasm through Exportin 5 (EXP5). Along with Argonaute (Ago) proteins, Dicer processes the pre-miRNA transcript into a mature miRNA duplex. The strand in the duplex with the least thermodynamically stable 5' end (guide strand) is retained by an Ago protein in mammals. The passenger strand (miRNA*) is generally released and degraded. Upon target recognition by the RNA-induced silencing complex (RISC) based on the seed region complementarity with the target mRNA, the target mRNA undergoes translational repression

receptor liver X receptor- α (LXR α) (Ou et al. 2011). The sterol response element binding protein (SREBP), a target of LXR α , binds an SREBP response element in the promoter of hsa-miR-613 and enhances its expression, thus down-regulating the expression of LXR α (Ou et al. 2011). Similarly, the expression of the clustered miRNA hsa-miR-2861 and hsa-miR-3960 is regulated by runt-related transcription factor 2 (Runx2), a key transcription factor that promotes osteoblast differentiation (Hu et al. 2011). Runx2 enhances the transcription of hsa-miR-2861 which results in the repression of histone-deacetylase 5 thereby inhibiting the repression of Runx2 to further promote osteoblast differentiation (Li et al. 2009). Runx2 also enhances the expression of hsa-miR-3960. A target of hsa-miR-3960 is homeobox a2 (Hoxa2), a protein that suppresses Runx2 expression (Hu et al. 2011). Thus, the

Runx2-mediated increase in miR-2861 and miR-3960 expression plays a key role in promoting osteoblast differentiation by suppressing inhibitors of Runx2 expression.

Similar to other protein-coding transcripts, these miRNA transcripts known as primary (pri-) miRNA undergo processing that promotes involving the addition of a 5'-m⁷G cap structure and polyadenylation (Cai et al. 2004). These pri-miRNA transcripts are then processed to a ~65-nt precursor (pre-) miRNA in the nucleus by the “Microprocessor” complex consisting of the RNase III-like enzyme Drosha and its co-factor DiGeorge Syndrome Critical Region 8 (DGCR8; Pasha in *D. melanogaster* and *C. elegans*) (Lee et al. 2003; Denli et al. 2004). Pre-miRNA containing hairpin-like structures are exported from the nucleus in a RanGTP-dependent process involving exportin 5 (Exp5) before undergoing additional cytosolic processing by Dicer to generate a mature miRNA (Bohnsack et al. 2004; Lund et al. 2004; Yi et al. 2003).

2.3 Nuclear Processing by Drosha

Drosha is a nuclear protein of 130–160 kDa containing two RNase III domains (RIIID) and a double-stranded RNA binding domain (dsRBD). Mammalian pri-miRNA transcripts contain dsRNA hairpins consisting of a ~33 base pair (bp) stem, a terminal loop, and 5' and 3' flanking ssRNA. Although Drosha exhibits RNase III activity in the absence of its co-factor DGCR8, it requires DGCR8 in order to bind target RNA (Han et al. 2004). Instead, Drosha interacts with DGCR8 to form the core Microprocessor complex that is competent for RNA binding and cleavage. DGCR8 recognizes the ssRNA-dsRNA junction of target RNA through two dsRBDs and facilitates substrate cleavage ~11 bp from the junction (Han et al. 2006; Zeng and Cullen 2005). The two RIIIDs of Drosha cleave the 5' and 3' strands of the stem resulting in the formation of a ~65-nt (60–80-nt) pre-miRNA with a 2-nt 3' overhang (Basyuk et al. 2003). The flanking sequence containing the 3' overhang is an important factor for recognition by EXP5 and subsequent nuclear export for additional cytosolic processing (Bohnsack et al. 2004; Lund et al. 2004; Yi et al. 2003). After binding the pre-miRNA, the EXP5 co-factor GTP-bound nuclear Ran undergoes cytoplasmic hydrolysis releasing the pre-miRNA into the cytosol.

2.4 Cytosolic Processing by Dicer

Following generation of the pre-miRNA by the Microprocessor complex, the RNase III-like protein Dicer generates an RNA duplex containing the mature miRNA. Dicer, an ~200 kDa protein containing DEAD-box RNA helicase domain, PAZ domain, two RIIIDs, and a dsRBD, cleaves the pre-miRNA near the loop to generate an ~22-nt duplex that contains both the mature miRNA (guide strand) and a similarly sized RNA (passenger strand) derived from the opposite stem of the hairpin (Bernstein et al. 2001; Grishok et al. 2001; Hutvagner et al. 2001; Macrae et al. 2006).

Similar to Drosha in the nucleus, Dicer interacts with other proteins forming larger complexes involved in loading of the RNA induced silencing complex (RISC). The dsRNA binding protein TAR RNA binding protein (TRBP) and protein activator of PKR (PACT) are not required for pre-miRNA processing by Dicer, but contribute instead to the stability of the complex and formation of the RISC (Haase et al. 2005; Lee et al. 2006; Chendrimada et al. 2005; Melo et al. 2009; Paroo et al. 2009). The mature miRNA duplex is therefore determined both by recognition of the pri-miRNA by Drosha in the nucleus and cleavage by Dicer in the cytosol. The mature miRNA may be located on either side of the pre-miRNA hairpin duplex.

2.5 Targeting is Influenced by The 5' End of The MicroRNA

The selection of cleavage sites by Drosha and Dicer may produce miRNA with multiple isoforms depending on the sequence of the 5' and 3' ends (Carthew and Sontheimer 2009). As explained below, differences in nucleotide composition can alter the “seed” sequence ultimately affecting which protein-coding mRNAs are targeted by the mature miRNA (Chiang et al. 2010). The ability of miRNA to repress translation is largely a function of the thermodynamic stability of base-pairing between the miRNA and complementary sequences generally found in the 3'UTR of target mRNA. In particular, nucleotides at positions 2–8 at the 5' end of the miRNA comprise the so-called “seed” sequence for miRNA target recognition (Doench and Sharp 2004). Although both the 5' and 3' ends of miRNA can vary depending on the addition or deletion of 1–2 nt, alterations in the 5' sequence can alter the seed sequence resulting in potential changes in miRNA target specificity. Additionally, thermodynamic stability of the 5' and 3' ends may affect which strand of the duplex is incorporated into the RISC (Krol et al. 2010). Differential selection of cleavage sites by Drosha and Dicer can therefore alter both the stability of the duplex and determine which strand is incorporated into the RISC as a mature miRNA.

2.6 RISC Assembly

Following cleavage by Dicer, the ~22-nt RNA duplex containing both the mature miRNA (guide strand) and the passenger strand (also referred to as the miRNA* strand) is incorporated into the RISC. In order to function in miRNA-mediated regulation of gene expression, the duplex is separated and the guide strand is retained in the RISC whereas the passenger strand is removed. RISC assembly occurs in two steps: first, ~22-nt RNA duplexes are incorporated into Argonaute (Ago) proteins and second, RNA duplexes are unwound or separated within Ago proteins (Kawamata and Tomari 2010). Argonaute proteins may be divided into three groups based on sequence similarity and the nature of their substrates: the AGO subfamily, the Piwi subfamily, and the *C. elegans*-specific WAGO subfamily (Czech and Hannon

2011). This review will focus on the role of AGO proteins on the processing and function of miRNA in mammals. Although the precise mechanism of assembly in mammals is not yet known, RNA duplex incorporation into AGO proteins and ATP-dependent RISC assembly appears to be a Dicer-independent process in contrast to the well-characterized Dicer-dependent RISC assembly involving Ago2 in flies (Kawamata and Tomari 2010; Schwarz et al. 2003).

Strand selection by the RISC is a regulated process dependent upon the thermodynamic stability of the nucleotides at the 5' termini at each end of the duplex (Schwarz et al. 2003; Khvorova et al. 2003). This asymmetric strand selection due to the relative stability of the 5' end is known as the "asymmetry rule". The strand with the least stable 5' end (miRNA or guide strand) is retained within the Ago-associated complex, whereas the passenger strand is unwound from the duplex and degraded (Kawamata and Tomari 2010; Schwarz et al. 2003; Khvorova et al. 2003). Recent deep sequencing studies in *Drosophila* revealed that miRNA* species for a few miRNAs are abundantly expressed and dynamically regulated suggesting that miRNA* might in fact play important gene regulatory roles in a cell-type or cell-stage specific manner (Okamura et al. 2008). Indeed, miRNA* are capable of regulating the expression of luciferase reporter constructs containing the target sites for miRNA* suggesting that the passenger strands likely play an important role in mammalian gene regulatory networks (Yang et al. 2011).

All four AGO proteins expressed in humans (AGO1–4) can bind miRNA duplexes containing central mismatches (Yoda et al. 2010). In contrast to flies, there does not appear to be strict small RNA sorting system involved in RISC assembly in mammals. As a result of mismatches in base-pairing in the duplex, the two strands are unwound by a cleavage-independent (i.e., nuclease-independent) mechanism (Kawamata et al. 2009). Whereas the passenger strand appears to be susceptible to rapid nucleolytic degradation, the guide strand is retained in the RISC. Upon the formation of a mature or active RISC, the mature miRNA is then capable of exhibiting a regulatory function by altering protein translation or mRNA stability.

2.7 MicroRNA Repress Protein Translation

Due to the combinatorial nature of 5' sequence heterogeneity in the seed region of the miRNA as a result of pri- and pre-miRNA processing, strand selection, and AGO association, the appropriate target recognition by mature miRNA is essential to its ability to participate in regulatory networks. Furthermore, miRNA relative expression levels and subcellular localization contribute to the complexity of miRNA-dependent regulation of gene expression. Although all four AGO proteins in humans evolved as ribonucleases, only AGO2 retains its catalytic activity (i.e., Slicer activity) required for the cleavage of target mRNA (Meister et al. 2004). Active RISC containing either AGO 1, 3, or 4 loaded with guide miRNA is able to recognize target sequence in the 3'UTR of target mRNA resulting in translational repression but could also decrease mRNA stability by promoting transcript degradation (Bartel 2009; Eulalio

et al. 2008; Chekulaeva and Filipowicz 2009). mRNA targets subject to translational repression and degradation may be localized to cytoplasmic processing (P-) bodies that are associated with both mature RISC and target mRNA (Parker and Sheth 2007; Berezchna et al. 2011; Pothof and van Gent 2011). By contrast to widely characterized translational inhibition, miRNA can also increase or activate translation of target mRNA in a cell-cycle dependent manner (Vasudevan et al. 2007; Niepmann 2009; Roberts et al. 2011). For example, AU-rich elements in the 3'UTR of Tumor Necrosis Factor- α mRNA that are usually associated with promoting enhanced mRNA decay can recruit miRNA to either mediate translational up-regulation under serum-starved condition or repress translation during cellular proliferation (Vasudevan et al. 2007). These results suggest that miRNA can control both translational repression and activation of target mRNA in a cell-cycle specific manner thereby allowing cells to adapt to changes in metabolic or environmental conditions.

As mentioned above, miRNA interact with target mRNA by a mechanism involving imperfect base-pairing. Although there is little tolerance for mismatches in the seed region (nt 2–8 of the mature miRNA), targets containing mismatches in the seed region have nonetheless been described (Vella et al. 2004; Lindenblatt et al. 2009). Mismatches in the central portion of the miRNA repress endonucleolytic cleavage of target mRNA while simultaneously allowing the formation of a mature RISC by increasing the rate of strand dissociation (Yoda et al. 2010; Kawamata et al. 2009). Rather than a one miRNA: one mRNA model of repression, there are usually multiple miRNA and/or target sequences required for maximal repression (Doench and Sharp 2004; Hon and Zhang 2007). While most miRNA target sites located in the 3'UTR serve as the basis of a number of target prediction tools (Lhakhang and Chaudhry 2011; Huang et al. 2011; Hsu et al. 2011; Liu et al. 2010; John et al. 2004), target sites within the 5'UTR have also been described (Forman and Collier 2010; Grey et al. 2010; Moretti et al. 2010).

mRNA whose translation are cap-independent or mRNA lacking a 5'-m⁷G cap structure are less robustly repressed than those mRNA that are cap-dependent (Pillai et al. 2005; Mathonnet et al. 2007). Additionally, internal ribosome entry site (IRES)-containing mRNA are poorly repressed by miRNA (Pillai et al. 2005; Humphreys et al. 2005). These results suggest that optimal repression may be dependent on disrupting the interaction between the cap binding eukaryotic initiation factor 4E (eIF4E) and the cap structure. Although the mechanisms are still not fully understood, alterations in protein-protein interactions are likely to result in an arrest of translational initiation. For example, GW182, a protein that is a component of the RISC, may interact with the poly(A) binding protein (PABP) thereby preventing PABP from binding to the scaffold protein eIF4G resulting in a block in translation initiation (Wakiyama et al. 2007; Wang et al. 2008; Fabian et al. 2009). Interestingly, both polyadenylated and deadenylated mRNA are subject to miRNA-mediated repression suggesting the existence of both poly(A)-dependent and independent mechanisms (Pillai et al. 2005; Beilharz et al. 2009). Some of these mRNA can also be found in P-body subcellular compartments providing a location wherein the repressed mRNA may be subsequently decapped and degraded (Parker and Sheth 2007; Cannell et al. 2008). Nonetheless, one of the molecular mechanisms through

which miRNA modulate protein abundance is through the repression of translation by impairing translation initiation.

Existing evidence also suggests that miRNA function to repress translation at a point following translational initiation. Using sucrose gradient fractionation to examine polysomal distribution of mRNA, results indicate that both synthetic and endogenous miRNA target messages may be associated with polysomes (Petersen et al. 2006; Nottrott et al. 2006; Gu et al. 2009). Furthermore, synthetic mRNA containing a cap-independent IRES may be subject to repression by miRNA and provides evidence that the regulatory step occurs at some point after the initiation of translation (Petersen et al. 2006). In fact, numerous miRNA have been found associated with polysomal fractions indicating that mature miRNA control protein expression at a point beyond initiation and is consistent with a model wherein miRNA may also bind to and repress target mRNA at a post-initiation step (Kim et al. 2004; Nelson et al. 2004; Maroney et al. 2006; Kong et al. 2008). The precise mechanism controlling the miRNA-induced repression via regulating elongation or termination steps remains unknown.

2.8 Target Regulation by Altering Transcript Stability

Regulation of post-transcriptional gene expression by miRNA also likely occurs through the modulation mRNA stability or turnover. Following recognition of the target mRNA, miRNA promote the deadenylation and decapping of the message resulting in its destabilization (Fabian et al. 2010). Deadenylation may be, at least in part, dependent on the interaction between PABP and GW182 of the RISC thereby impairing the interaction between PABP and eIF4G (Fabian et al. 2009). It does not appear that deadenylation alone results in enhanced decay, but instead suggests that deadenylation may be requirement for decapping and degradation by either a 3'–5' or 5'–3' exoribonuclease. Intriguingly, recent work using rabbit reticulocyte lysate suggests that the poly(A) tail, but not the 5'-m⁷G cap, of target mRNA is required for miRNA-mediated effects (Ricci et al. 2011). In fact, in these studies using rabbit reticulocyte lysates, translational repression was independent of either deadenylation or decreased mRNA stability. Despite examples of deadenylation and decay occurring to varying degrees, the extent to which these processes play an important role in mammalian cells remains unclear and may indicate that the primary means of regulation, at least in mammals, is through a mechanism involved in the regulation of translation initiation (Fabian et al. 2009).

2.9 Summary

Similar to protein-coding genes, miRNA gene expression is usually regulated through the recruitment of RNA polymerase II to produce a primary miRNA transcript. This nascent transcript undergoes nuclear processing to generate 60–80 nt pre-miRNA transcripts that are then exported out of the nucleus and further processed by the

RNase III enzyme Dicer to generate a miRNA duplex. The strand of the duplex with the least thermodynamically stable 5' terminus is subsequently incorporated into the RISC and directed towards recognition of the 3'UTR of target mRNA. In mammals, miRNA regulate protein expression primarily by repressing protein translation. Thus, alterations in miRNA expression allow cells to fine-tune protein expression to more appropriately control cellular fate and metabolism. Although there is an enhanced appreciation for the roles of miRNA in regulating cellular fate and metabolism, much remains to be known about the mechanisms controlling aspects of miRNA expression, miRNA turnover, and stoichiometry of miRNA: target ratios required for target repression.

References

- Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136(2):215–233. doi:10.1016/j.cell.2009.01.002
- Baskerville S, Bartel DP (2005) Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *Rna* 11(3):241–247. doi:10.1261/rna.7240905
- Basyuk E, Suavet F, Doglio A, Bordonne R, Bertrand E (2003) Human let-7 stem-loop precursors harbor features of RNase III cleavage products. *Nucleic Acids Res* 31(22):6593–6597
- Beilharz TH, Humphreys DT, Clancy JL, Thermann R, Martin DI, Hentze MW, Preiss T (2009) microRNA-mediated messenger RNA deadenylation contributes to translational repression in mammalian cells. *PLoS One* 4(8):e6783. doi:10.1371/journal.pone.0006783
- Berezchna SY, Supekova L, Sever MJ, Schultz PG, Deniz AA (2011) Dual regulation of hepatitis C viral RNA by cellular RNAi requires partitioning of Ago2 to lipid droplets and P-bodies. *Rna* 17(10):1831–1845. doi:10.1261/rna.2523911
- Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409(6818):363–366. doi:10.1038/35053110
- Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *Rna* 10(2):185–191
- Cai X, Hagedorn CH, Cullen BR (2004) Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *Rna* 10(12):1957–1966. doi:10.1261/rna.7135204
- Cannell IG, Kong YW, Bushell M (2008) How do microRNAs regulate gene expression? *Biochem Soc Trans* 36(Pt 6):1224–1231. doi:10.1042/BST0361224
- Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. *Cell* 136(4):642–655. doi:10.1016/j.cell.2009.01.035
- Chekulaeva M, Filipowicz W (2009) Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. *Curr Opin Cell Biol* 21(3):452–460. doi:10.1016/j.ceb.2009.04.009
- Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 436(7051):740–744. doi:10.1038/nature03868
- Chiang HR, Schoenfeld LW, Ruby JG, Auyeung VC, Spies N, Baek D, Johnston WK, Russ C, Luo S, Babiarz JE, Bilelloch R, Schroth GP, Nusbaum C, Bartel DP (2010) Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes Dev* 24(10):992–1009. doi:10.1101/gad.1884710
- Czech B, Hannon GJ (2011) Small RNA sorting: matchmaking for Argonautes. *Nat Rev Genet* 12(1):19–31. doi:10.1038/nrg2916
- Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ (2004) Processing of primary microRNAs by the Microprocessor complex. *Nature* 432(7014):231–235. doi:10.1038/nature03049

- Doench JG, Sharp PA (2004) Specificity of microRNA target selection in translational repression. *Genes Dev* 18(5):504–511. doi:10.1101/gad.1184404
- Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP (2006) miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 3(2):87–98. doi:10.1016/j.cmet.2006.01.005
- Eulalio A, Huntzinger E, Izaurralde E (2008) Getting to the root of miRNA-mediated gene silencing. *Cell* 132(1):9–14. doi:10.1016/j.cell.2007.12.024
- Fabian MR, Mathonnet G, Sundermeier T, Mathys H, Zipprich JT, Svitkin YV, Rivas F, Jinek M, Wohlschlegel J, Doudna JA, Chen CY, Shyu AB, Yates JR, 3rd, Hannon GJ, Filipowicz W, Duchaine TF, Sonenberg N (2009) Mammalian miRNA RISC recruits CAF1 and PABP to affect PABP-dependent deadenylation. *Mol Cell* 35(6):868–880. doi:10.1016/j.molcel.2009.08.004
- Fabian MR, Sonenberg N, Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* 79:351–379. doi:10.1146/annurev-biochem-060308-103103
- Farazi TA, Spitzer JJ, Morozov P, Tuschl T (2011) miRNAs in human cancer. *J Pathol* 223(2):102–115. doi:10.1002/path.2806
- Forman JJ, Collier HA (2010) The code within the code: microRNAs target coding regions. *Cell Cycle* 9(8):1533–1541
- Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19(1):92–105. doi:10.1101/gr.082701.108
- Grey F, Tirabassi R, Meyers H, Wu G, McWeeney S, Hook L, Nelson JA (2010) A viral microRNA down-regulates multiple cell cycle genes through mRNA 5'UTRs. *PLoS Pathog* 6(6):e1000967. doi:10.1371/journal.ppat.1000967
- Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: tools for microRNA genomics. *Nucleic Acids Res* 36(Database issue):D154–158. doi:10.1093/nar/gkm952
- Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC (2001) Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. *Cell* 106(1):23–34
- Gu S, Jin L, Zhang F, Sarnow P, Kay MA (2009) Biological basis for restriction of microRNA targets to the 3' untranslated region in mammalian mRNAs. *Nat Struct Mol Biol* 16(2):144–150. doi:10.1038/nsmb.1552
- Haase AD, Jaskiewicz L, Zhang H, Laine S, Sack R, Gatignol A, Filipowicz W (2005) TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. *EMBO Rep* 6(10):961–967. doi:10.1038/sj.embor.7400509
- Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* 18(24):3016–3027. doi:10.1101/gad.1262504
- Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, Sohn SY, Cho Y, Zhang BT, Kim VN (2006) Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell* 125(5):887–901. doi:10.1016/j.cell.2006.03.043
- Hon LS, Zhang Z (2007) The roles of binding site arrangement and combinatorial targeting in microRNA repression of gene expression. *Genome Biol* 8(8):R166. doi:10.1186/gb-2007-8-8-r166
- Hsu JB, Chiu CM, Hsu SD, Huang WY, Chien CH, Lee TY, Huang HD (2011) miRTar: an integrated system for identifying miRNA-target interactions in human. *BMC Bioinformatics* 12:300. doi:10.1186/1471-2105-12-300
- Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY, Guo LJ, Xie H, Zhou HD, Wu XP, Luo XH (2011) A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. *J Biol Chem* 286(14):12328–12339. doi:10.1074/jbc.M110.176099
- Huang J, Townsend C, Dou D, Liu H, Tan M (2011) OMIT: A Domain-Specific Knowledge Base for MicroRNA Target Prediction. *Pharm Res* 28(12):310–314. doi:10.1007/s11095-011-0573-8
- Humphreys DT, Westman BJ, Martin DI, Preiss T (2005) MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc Natl Acad Sci U S A* 102(47):16961–16966. doi:10.1073/pnas.0506482102

- Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 293(5531):834–838. doi:10.1126/science.1062961
- John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS (2004) Human MicroRNA targets. *PLoS Biol* 2(11):e363. doi:10.1371/journal.pbio.0020363
- Kawamata T, Seitz H, Tomari Y (2009) Structural determinants of miRNAs for RISC loading and slicer-independent unwinding. *Nat Struct Mol Biol* 16(9):953–960. doi:10.1038/nsmb.1630
- Kawamata T, Tomari Y (2010) Making RISC. *Trends Biochem Sci* 35(7):368–376. doi:10.1016/j.tibs.2010.03.009
- Khvorova A, Reynolds A, Jayasena SD (2003) Functional siRNAs and miRNAs exhibit strand bias. *Cell* 115(2):209–216
- Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G (2004) Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci U S A* 101(1):360–365. doi:10.1073/pnas.2333854100
- Kim VN, Nam JW (2006) Genomics of microRNA. *Trends Genet* 22(3):165–173. doi:10.1016/j.tig.2006.01.003
- Kong YW, Cannell IG, de Moor CH, Hill K, Garside PG, Hamilton TL, Meijer HA, Dobbyn HC, Stoneley M, Spriggs KA, Willis AE, Bushell M (2008) The mechanism of micro-RNA-mediated translation repression is determined by the promoter of the target gene. *Proc Natl Acad Sci U S A* 105(26):8866–8871. doi:10.1073/pnas.0800650105
- Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 11(9):597–610. doi:10.1038/nrg2843
- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN (2003) The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425(6956):415–419. doi:10.1038/nature01957
- Lee Y, Hur I, Park SY, Kim YK, Suh MR, Kim VN (2006) The role of PACT in the RNA silencing pathway. *Embo J* 25(3):522–532. doi:10.1038/sj.emboj.7600942
- Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN (2004) MicroRNA genes are transcribed by RNA polymerase II. *Embo J* 23(20):4051–4060. doi:10.1038/sj.emboj.7600385
- Lhakhang TW, Chaudhry MA (2011) Current approaches to micro-RNA analysis and target gene prediction. *J Appl Genet* 53(2):149–158. doi:10.1007/s13353-011-0060-2
- Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, Luo XH (2009) A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest* 119(12):3666–3677. doi:10.1172/JCI39832
- Lindenblatt C, Schulze-Osthoff K, Totzke G (2009) IkappaBzeta expression is regulated by miR-124a. *Cell Cycle* 8(13):2019–2023
- Liu H, Yue D, Chen Y, Gao SJ, Huang Y (2010) Improving performance of mammalian microRNA target prediction. *BMC Bioinformatics* 11:476. doi:10.1186/1471-2105-11-476
- Liu X, Fortin K, Mourelatos Z (2008) MicroRNAs: biogenesis and molecular functions. *Brain Pathol* 18(1):113–121. doi:10.1111/j.1750-3639.2007.00121.x
- Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U (2004) Nuclear export of microRNA precursors. *Science* 303 (5654):95–98. doi:10.1126/science.1090599
- Macrae IJ, Zhou K, Li F, Repic A, Brooks AN, Cande WZ, Adams PD, Doudna JA (2006) Structural basis for double-stranded RNA processing by Dicer. *Science* 311(5758):195–198. doi:10.1126/science.1121638
- Maroney PA, Yu Y, Fisher J, Nilsen TW (2006) Evidence that microRNAs are associated with translating messenger RNAs in human cells. *Nat Struct Mol Biol* 13(12):1102–1107. doi:10.1038/nsmb1174
- Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L, Murata T, Biffo S, Merrick WC, Darzynkiewicz E, Pillai RS, Filipowicz W, Duchaine TF, Sonenberg N (2007) MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4 F. *Science* 317(5845):1764–1767. doi:10.1126/science.1146067

- Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol Cell* 15(2):185–197. doi:10.1016/j.molcel.2004.07.007
- Melo SA, Ropero S, Moutinho C, Aaltonen LA, Yamamoto H, Calin GA, Rossi S, Fernandez AF, Carneiro F, Oliveira C, Ferreira B, Liu CG, Villanueva A, Capella G, Schwartz S, Jr., Shiekhattar R, Esteller M (2009) A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet* 41(3):365–370. doi:10.1038/ng.317
- Moretti F, Thermann R, Hentze MW (2010) Mechanism of translational regulation by miR-2 from sites in the 5' untranslated region or the open reading frame. *Rna* 16(12):2493–2502. doi:10.1261/rna.2384610
- Nelson PT, Hatzigeorgiou AG, Mourelatos Z (2004) miRNP:mRNA association in polyribosomes in a human neuronal cell line. *Rna* 10(3):387–394
- Niepmann M (2009) Activation of hepatitis C virus translation by a liver-specific microRNA. *Cell Cycle* 8(10):1473–1477
- Nottrott S, Simard MJ, Richter JD (2006) Human let-7a miRNA blocks protein production on actively translating polyribosomes. *Nat Struct Mol Biol* 13(12):1108–1114. doi:10.1038/nsmb1173
- Okamura K, Phillips MD, Tyler DM, Duan H, Chou YT, Lai EC (2008) The regulatory activity of microRNA* species has substantial influence on microRNA and 3' UTR evolution. *Nat Struct Mol Biol* 15(4):354–363. doi:10.1038/nsmb.1409
- Olena AF, Patton JG (2010) Genomic organization of microRNAs. *J Cell Physiol* 222(3):540–545. doi:10.1002/jcp.21993
- Ou Z, Wada T, Gramignoli R, Li S, Strom SC, Huang M, Xie W (2011) MicroRNA hsa-miR-613 targets the human LXRalpha gene and mediates a feedback loop of LXRalpha autoregulation. *Mol Endocrinol* 25(4):584–596. doi:10.1210/me.2010-0360
- Parker R, Sheth U (2007) P bodies and the control of mRNA translation and degradation. *Mol Cell* 25(5):635–646. doi:10.1016/j.molcel.2007.02.011
- Paroo Z, Ye X, Chen S, Liu Q (2009) Phosphorylation of the human microRNA-generating complex mediates MAPK/Erk signaling. *Cell* 139(1):112–122. doi:10.1016/j.cell.2009.06.044
- Petersen CP, Bordeleau ME, Pelletier J, Sharp PA (2006) Short RNAs repress translation after initiation in mammalian cells. *Mol Cell* 21(4):533–542. doi:10.1016/j.molcel.2006.01.031
- Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, Filipowicz W (2005) Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* 309(5740):1573–1576. doi:10.1126/science.1115079
- Pothof J, van Gent DC (2011) Spatiotemporal Aspects of MicroRNA-Mediated Gene Regulation. *Adv Exp Med Biol* 722:75–85. doi:10.1007/978-1-4614-0332-6_5
- Ricci EP, Limousin T, Soto-Rifo R, Allison R, Poyry T, Decimo D, Jackson RJ, Ohlmann T (2011) Activation of a microRNA response in trans reveals a new role for poly(A) in translational repression. *Nucleic Acids Res* 39(12):5215–5231. doi:10.1093/nar/gkr086
- Roberts AP, Lewis AP, Jopling CL (2011) miR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. *Nucleic Acids Res* 39(17):7716–7729. doi:10.1093/nar/gkr426
- Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A (2004) Identification of mammalian microRNA host genes and transcription units. *Genome Res* 14(10A):1902–1910. doi:10.1101/gr.2722704
- Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD (2003) Asymmetry in the assembly of the RNAi enzyme complex. *Cell* 115(2):199–208
- Vasudevan S, Tong Y, Steitz JA (2007) Switching from repression to activation: microRNAs can up-regulate translation. *Science* 318(5858):1931–1934. doi:10.1126/science.1149460
- Vella MC, Choi EY, Lin SY, Reinert K, Slack FJ (2004) The C. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev* 18(2):132–137. doi:10.1101/gad.1165404

- Wakiyama M, Takimoto K, Ohara O, Yokoyama S (2007) Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. *Genes Dev* 21(15):1857–1862. doi:10.1101/gad.1566707
- Wang B, Yanez A, Novina CD (2008) MicroRNA-repressed mRNAs contain 40 S but not 60 S components. *Proc Natl Acad Sci U S A* 105(14):5343–5348. doi:10.1073/pnas.0801102105
- Westholm JO, Lai EC (2011) Mirtrons: microRNA biogenesis via splicing. *Biochimie* 93(11):1897–1904. doi:10.1016/j.biochi.2011.06.017
- Williams AE (2008) Functional aspects of animal microRNAs. *Cell Mol Life Sci* 65(4):545–562. doi:10.1007/s00018-007-7355-9
- Yang JS, Phillips MD, Betel D, Mu P, Ventura A, Siepel AC, Chen KC, Lai EC (2011) Widespread regulatory activity of vertebrate microRNA* species. *Rna* 17(2):312–326. doi:10.1261/rna.2537911
- Yi R, Qin Y, Macara IG, Cullen BR (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 17(24):3011–3016. doi:10.1101/gad.1158803
- Yoda M, Kawamata T, Paroo Z, Ye X, Iwasaki S, Liu Q, Tomari Y (2010) ATP-dependent human RISC assembly pathways. *Nat Struct Mol Biol* 17(1):17–23. doi:10.1038/nsmb.1733
- Yuan X, Liu C, Yang P, He S, Liao Q, Kang S, Zhao Y (2009) Clustered microRNAs' coordination in regulating protein-protein interaction network. *BMC Syst Biol* 3:65. doi:10.1186/1752-0509-3-65
- Zeng Y, Cullen BR (2005) Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences. *J Biol Chem* 280(30):27595–27603. doi:10.1074/jbc.M504714200

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