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The biological functions of miRNAs: lessons from *in vivo* studies

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Despite their clear importance as a class of regulatory molecules, pinpointing the relevance of individual miRNAs has been challenging. Studies querying miRNA functions by overexpressing or silencing specific miRNAs have yielded data that are often at odds with those collected from loss-of-functions models. In addition, knockout studies suggest that many conserved miRNAs are dispensable for animal development or viability. In this review, we discuss these observations in the context of our current knowledge of miRNA biology and review the evidence implicating miRNA-mediated gene regulation in the mechanisms that ensure biological robustness.

An elusive role for miRNAs

miRNAs are small noncoding RNAs that regulate protein output post-transcriptionally [1]. Overwhelming evidence accumulated since their discovery [2,3] leaves little doubt regarding their importance. They comprise 1–2% of all genes in worms, flies, and mammals [1], and because each miRNA is predicted to regulate hundreds of targets, the majority of protein coding genes is thought to be under their control [4]. In practice, this means that virtually every biological process is subject to miRNA dependent regulation. As additional evidence of their functional relevance, miRNAs and their targets often display striking evolutionary conservation [5–7]. Lastly, animals carrying mutations that impair miRNA processing [8–12] are not viable, indicating that complete loss of miRNA activity is incompatible with life.

Despite their clear importance as a class of regulatory molecules, determining the biological relevance of individual miRNAs has proven challenging. For the most part, the physiological functions of specific miRNAs have been inferred from overexpression studies in animals and cultured cells, or from studies that used antisense molecules as a means of disrupting their pairing to targets. These experiments have attributed critical roles to miRNAs in processes such as cell proliferation, differentiation, and survival, and have implicated them as crucial players during normal development, homeostasis, and disease [13–17]. Surprisingly, the expectations raised by these early studies have been met by a growing number of knockout animals with very modest or no apparent phenotypes. Furthermore, so far only two miRNA genes (*miR-17∼92* and *miR-96*) have been shown to cause developmental defects in humans when mutated [18,19]. The absence of phenotypic consequences upon ablation of individual miRNAs seems to be the rule rather than the exception. In *Caenorhabditis elegans*, for example, systematic deletion of miRNAs indicates that less than 10% of them are individually required for normal animal development or viability [20], and this trend also seems to be true in mice [21] (Table 1).

In this review, we discuss these observations in the context of our current understanding of miRNA-mediated gene regulation and examine the evidence implicating miRNA activity in the processes that ensure robust animal development and homeostasis.

Endogenous miRNAs exert mild repression on many targets

miRNA processing has been extensively reviewed [22] and will be only briefly discussed here. MiRNAs are transcribed as long primary transcripts (pri-miRNAs) and cleaved in the nucleus by the Drosha/DGCR8 microprocessor complex. The resulting ∼70-nucleotide-long hairpin-shaped molecule – the pre-miRNA – is exported into the cytoplasm, where it is further processed by Dicer, bound by an Argonaute protein, and incorporated into an RNA-induced silencing complex (RISC). Metazoan miRNAs typically direct the RISC to target mRNAs through imperfect base pairing to their 3′ untranslated regions (3′UTR), leading to post-transcriptional repression mainly through mRNA destabilization, although a minor component of translation inhibition has also been detected [23] (Box 1).

Target recognition is primarily determined by the seed-sequence, a stretch of 6 nucleotides spanning nucleotides 2–7 on the 5′ end of the miRNA [24,25]. Accordingly, targets can be confidently predicted by searching for conserved matches to this sequence in the 3′UTR of messages [26]. Prediction accuracy increases further when this search is restricted to seven nucleotide-long motifs encompassing the seed [26], and when the sequence context within the 3′UTR is taken into consideration [27]. Noncanonical targeting through sites with mismatches to the seed has also been reported [28–32], but seems to be generally associated with lower levels of repression and its biological relevance remains unclear [4,30]. Because targeting requires the presence of such short conserved sequences, individual miRNAs have the potential to

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Table 1. miRNA knockout phenotypes in mice

miRNA gene	Knockout phenotype	Phenotype penetrance	Evidence of functional redundancy with family members	Phenotypes in response to external or internal perturbations	Refs
miR-155	Immunodeficiency and increased lung remodeling	Increased lung remodeling in ~56% of *miR-155*$^{-/-}$ animals	NA[a]	NA	[107]
miR-17~92	Perinatal lethality with heart, lung, B cell, and skeletal defects	100%	Co-deletion of *miR-17~92* with *miR-106~25* aggravates developmental phenotypes of *miR-17~92* single deletion	NA	[19,67]
miR-106a~363	No obvious phenotype	NA	Co-deletion of *miR-17~92* with *miR-106~25* and *miR-106a~363* aggravates developmental phenotypes of *miR-17~92* single deletion	NA	[67]
miR-106b~25	No obvious phenotype	NA	Co-deletion of *miR-17~92* with *miR-106~25* aggravates developmental phenotypes of *miR-17~92* single deletion	NA	[67]
miR-15a/16-1	B cell lymphoproliferative disorders	~24%	NA	NA	[108]
miR-144/451	Impaired late erythroblast maturation	Not specified, assumed to be 100%	NA	NA	[109]
miR-150	B1 cell expansion and increased humoral immune response	Not specified, assumed to be 100%	NA	NA	[110]
miR-223	Expanded granulocyte compartment	Not specified, assumed to be 100%	NA	NA	[111]
miR-1-2	Lethality at weaning with heart defects	~50%	NA	NA	[112]
miR-34a	No obvious phenotype	NA	Co-deletion of *miR-34* and *miR-449* leads to defects in cilliogenesis. Lethal for 60% of animals. Surviving adults are infertile	Loss of *miR-34* family members aggravates prostate neoplastic lesions caused by inactivation of *trp53*; loss of *miR-34a* improves cardiac function during aging and in response to stress	[42,65, 86,113]
miR-34b/c	No obvious phenotype	NA	Co-deletion of *miR-34* and *miR-449* leads to defects in cilliogenesis. Lethal for 60% of animals. Surviving adults are infertile	Loss of *miR-34* family members aggravates prostate neoplastic lesions caused by inactivation of *trp53*	[42,65,86]
miR-449	No obvious phenotype	NA	Co-deletion of *miR-34* and *miR-449* leads to defects in cilliogenesis. Lethal for 60% of animals. Surviving adults are infertile	NA	[65,66]
miR-290~295	Developmental delays; embryonic development outside yolk sac; embryonic lethality; germ cell deficiency in surviving adults	Lethality in 75% of animals	NA	NA	[114]
miR-10a	No obvious phenotype	NA	NA	NA	[115]
miR-208a	No obvious phenotype, but minor cardiac conduction defects reported	80%	NA	Reduced cardiac hypertrophy in response to stress and hypothyroidism	[84,116]
miR-208b	No obvious phenotype	NA	Decrease in type I myofibers in soleus muscle when *miR-499* is co-deleted	NA	[116]