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# miRNA regulates noncoding RNA: a noncanonical function model

Xi Chen, Hongwei Liang, Chen-Yu Zhang, and Ke Zen

Jiangsu Engineering Research Center for microRNA Biology and Biotechnology, State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, 22 Hankou Road, Nanjing 210093, China

**It has long been assumed that miRNAs can only target protein-coding mRNAs in the cytoplasm. Recent studies, however, reveal miRNAs are also transported from the cytoplasm to the nucleus, where they function in a noncanonical manner to regulate noncoding RNAs. Here, we highlight the working model of these noncanonical miRNAs.**

## Expression of mature miRNAs in the nucleus

miRNAs are small noncoding RNAs (ncRNAs) that are involved in post-transcriptional gene regulation [1]. According to the canonical model, miRNAs can recognize protein-coding mRNAs and exert their roles predominantly in the cytoplasm (Figure 1a). However, accumulating evidence has demonstrated that certain mature miRNAs can re-enter the nucleus [2–4]. Consistently, studies have also reported the presence of active miRNA effectors, such as Argonaute proteins, in the nucleus [5]. These findings strongly argue that there are mechanisms to guide the cytoplasmic–nuclear transport of miRNAs and Argonaute proteins. Although these mechanisms remain unknown at this stage, several studies have suggested that importins and exportins might be involved in these processes. Castanotto *et al.* [6] have provided the first evidence that exportin-1 allowed the nuclear–cytoplasmic shuttling of mature miRNAs in a complex containing Argonaute proteins. Zisoulis *et al.* [7] have shown that exportin-1 might serve as a pore for the translocation of Argonaute proteins from the cytoplasm to the nucleus. Furthermore, Weinmann *et al.* [8] have reported that Argonaute2 is transported into the nucleus by importin-8 in human cells. The re-entry of miRNAs and their effectors (e.g., Argonautes) into the nucleus is a prerequisite for the function of these miRNAs, therefore, it is of crucial importance to identify the molecular basis governing the cytoplasmic–nuclear shuttling of mature miRNAs. Nevertheless, discovering the presence of miRNAs and Argonautes in the nucleus has prompted an intriguing hypothesis that nuclear miRNAs might function through a mechanism other than classic post-transcriptional repression. Indeed, recent studies have highlighted that several nuclear miRNAs can act in a noncanonical manner to regulate the biogenesis and function of ncRNAs, including miRNAs and long ncRNAs [7,9,10].

*Corresponding authors:* Zhang, C-Y. (cyzhang@nju.edu.cn); Zen, K. (kzen@nju.edu.cn).

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## Noncanonical function model I: miRNA directly regulates miRNA

Our recent study provided the first evidence that one miRNA can control the biogenesis of other miRNAs by directly targeting their primary transcripts in the nucleus (Figure 1b). In particular, we have shown that miR-709, which is predominantly located in the mouse nucleus, specifically binds to a 19-nucleotide recognition element on the miR-15a/16-1 cluster and blocks the processing of miR-15a/16-1 primary transcript (pri-miR-15a/16-1) into miR-15a/16-1 precursor (pre-miR-15a/16-1), leading to the suppression of miR-15a/16-1 maturation [9]. Thus, nuclear miR-709 negatively regulates miR-15a/16-1 maturation at the post-transcriptional level and, more specifically, at a stage after pri-miRNA transcription but before pre-miRNA cleavage. miR-15a/16-1 is involved in cell apoptosis by targeting the antiapoptotic protein B-cell lymphoma 2 (Bcl-2), therefore, nuclear miR-709 can participate in the regulation of cell apoptosis through the miR-15a/16-1 pathway [9]. These results suggest that certain miRNAs might affect the expression level of many genes through regulating the biogenesis of other miRNAs.

More interestingly, a miRNA can also control its own expression (Figure 1b). Zisoulis *et al.* [7] have reported that protein Argonaute-like gene 1 (ALG-1) is associated with the let-7 primary transcript (pri-let-7) in nuclear fractions from *Caenorhabditis elegans*. Within the ALG-1-binding region of pri-let-7, they have also identified a conserved complementary site for mature let-7. Their results showed that the association of ALG-1 with pri-let-7 requires mature let-7, because when they disrupted the pairing of ALG-1 with mature let-7 by introducing a point mutation into the mature let-7 sequence, the pri-let-7 transcripts were no longer associated with ALG-1 [7]. Furthermore, they demonstrated that disrupting ALG-1–pri-let-7 binding resulted in the accumulation of pri-let-7 but a reduction of mature let-7 in animals [7]. This study reveals the first example of a direct miRNA autoregulatory loop, in which mature let-7, with the help of Argonaute proteins, binds and promotes the processing of its own primary transcript.

## Noncanonical function model II: miRNA regulates long ncRNA

Long ncRNAs are generally considered to be non-protein-coding transcripts longer than 200 nucleotides. Hansen *et al.* [10] have presented the first experimental evidence that long ncRNAs are functional miRNA targets (Figure 1c). They have observed that the antisense transcript of the cerebellar degeneration-related protein 1

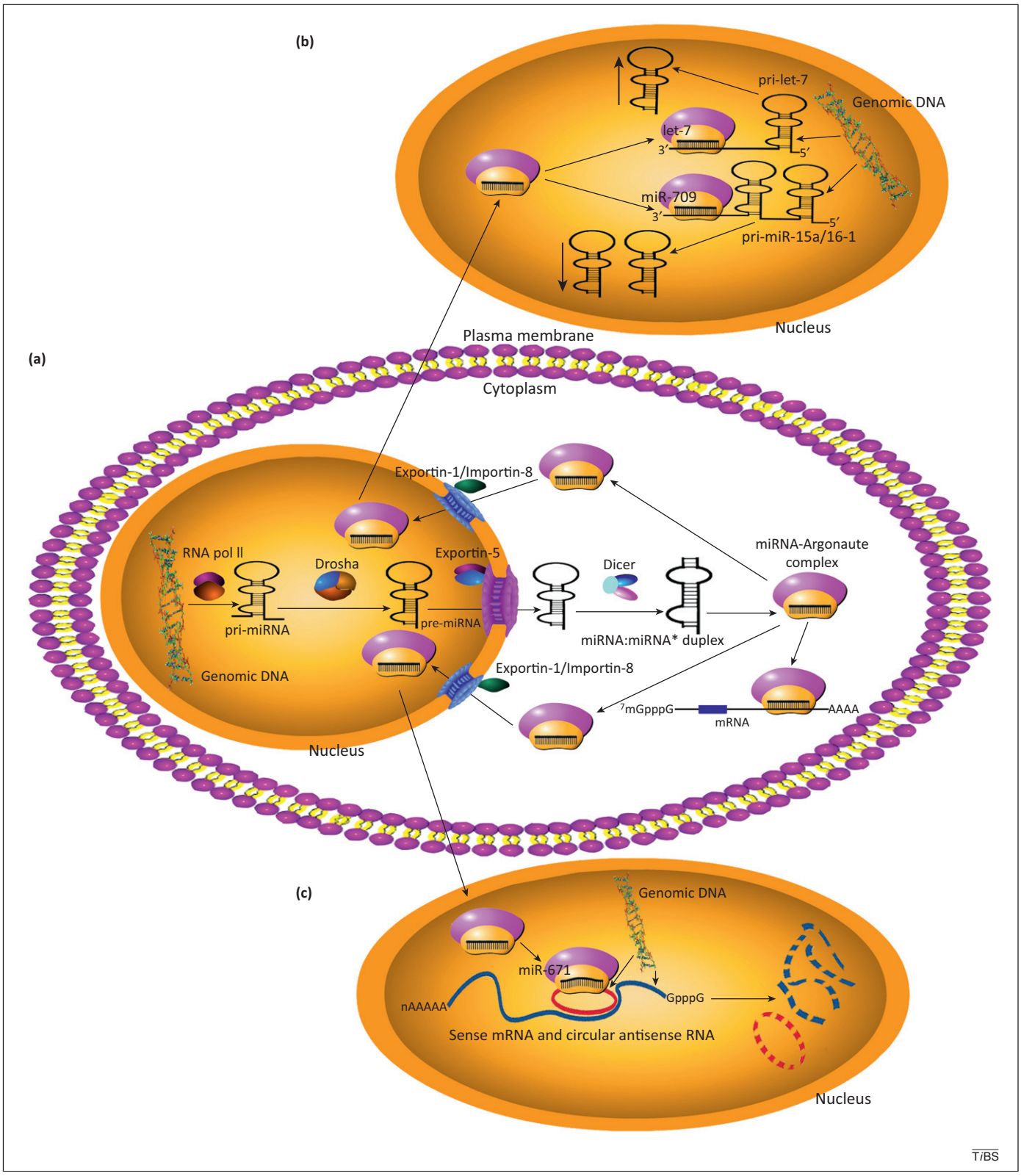


**Figure 1**. Canonical and noncanonical function models of miRNAs. **(a)** Biogenesis and canonical function of miRNAs. In the conventional linear processing pathway, a miRNA gene is transcribed by RNA polymerase II to yield a long primary transcript (pri-miRNA) within the nucleus. The pri-miRNA is then cleaved by the enzyme Drosha to generate hairpin miRNA precursor (pre-miRNA). Subsequently, pre-miRNA is exported by exportin-5 in a RanGTP-dependent manner to the cytoplasm, where it is further cleaved by Dicer to generate an asymmetric duplex. Then, one strand of the duplex, named 'guide strand' or 'mature miRNA', is combined with Argonaute proteins to form an RNA-induced silencing complex (RISC). Eventually, the complex binds to the complementary binding sites located in the 3′ untranslated region (UTR) of the target mRNA, thus inhibiting protein synthesis by either repressing translation and/or inducing mRNA degradation. According to this model of action, miRNAs can only recognize sequences in protein-coding mRNAs and execute their functions predominantly in the cytoplasm. By contrast, some specific miRNAs (e.g., miR-709 in mouse, let-7 in *Caenorhabditis elegans*, and miR-671 in humans) are preferentially imported into the nucleus. These miRNAs are usually combined with Argonaute proteins to form a complex, which could be imported, perhaps, via exportin-1, importin-8 or other carrier factors. **(b)** miRNA regulation of miRNA. In the mouse nucleus, Argonaute-associated miR-709 specifically binds to a 19-nucleotide recognition element on pri-miR-15a/16-1 transcripts. This prevents the processing of pri-miR-15a/16-1 into pre-miR-15a/16-1 and therefore suppresses mature miRNA biogenesis. Using a similar but not identical mechanism, Argonaute-associated let-7 binds to a specific site in the let-7 primary

(CDR1), a circular ncRNA, is nearly perfectly complementary to a nucleus-enriched miRNA, miR-671. Furthermore, they have shown that miR-671 directs the cleavage of a CDR1 antisense transcript in an AGO2-dependent manner [10]. Furthermore, downregulation of the circular antisense ncRNA is accompanied by a corresponding decrease in the CDR1 sense transcript [10]. This study reveals the stabilization of a sense mRNA by a circular noncoding antisense RNA, and destabilization of the antisense RNA by a nuclear-localized miRNA via AGO2-mediated cleavage.

## Biological relevance of noncanonical miRNAs

Although the canonical functions of miRNAs are well established, the role of noncanonical miRNAs in the nucleus is just starting to be revealed. An intriguing idea raised previously is that the recycling of miRNAs to the nucleus simply serves to sequester them from the cytoplasm and fine-tune their stability and impact on translational regulation. However, current findings argue that we might have underestimated the potential function of these nuclear miRNAs. Based on the vast volume of literature in miRNA research so far, we propose that miRNAs have two different functional models: in the cytoplasm, they bind to protein-coding mRNAs and regulate target gene translation (canonical pathway), whereas in the nucleus, miRNAs target ncRNAs, including miRNAs and long ncRNAs, and modulate their biogenesis and function (noncanonical pathway). Considering the complexity and diversity of miRNA–target interactions, it is reasonable to imagine that miRNAs and their target ncRNAs (miRNA and long ncRNA) form a complex regulatory network within the nucleus. Through this network, miRNAs can control ncRNA homeostasis and balance the tightly regulated, equilibrated state of miRNAs and other ncRNAs.

In summary, although the study of nuclear miRNAs is still at an early stage, and many fundamental questions about this physiological event remain to be answered, the discovery of the miRNA-mediated regulation of ncRNAs in the nucleus opens a new and exciting field of miRNA research. We fully anticipate that this novel model of nuclear miRNA function will become a hot research topic and the findings thus derived will significantly extend our understanding of gene regulation in eukaryotes.

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transcript in the nucleus of *C. elegans*. Through this manner of interaction, let-7 promotes the processing of its own primary transcript. **(c)** miRNA regulation of long ncRNA. Circular cerebellar degeneration-related protein 1 (CDR1) noncoding antisense RNA (red circle) pairs with sense CDR1 mRNA (blue line) in the nucleus and stabilizes it for export to the cytoplasm, in which the mRNA can be translated. In the presence of the miR-671-AGO2 complex in the nucleus, the antisense RNA is degraded, destabilizing the sense mRNA.