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MicroRNAs Micromanage Themselves

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Abstract

Since their discovery not long ago, microRNAs (miRNAs) have been extensively studied in hundreds of laboratories around the world. Initially thought of as merely cytoplasmic repressors of mRNA expression, it has since become more apparent that they also play regulatory roles in the nucleus. A recent study published in *Nature* introduces novel concepts in both miRNA regulation and function by showing that the *let-7* miRNA regulates its own expression.

miRNAs are key posttranscriptional regulators of gene expression. Over a thousand eukaryotic miRNAs have been discovered, many of which are predicted to target hundreds of mRNAs.[1,2] Binding of the mature ≈ 21-nucleotide miRNA to target mRNAs typically silences their expression, providing a complex layer of fine-tuned gene regulation. However, in the past few years, it has become clear that miRNAs are themselves subjected to sophisticated regulatory mechanisms. In a recent *Nature* article, Zisoulis et al[3] reported the first example of a direct miRNA autoregulatory loop. They showed that the *Caenorhabditis elegans* (*C. elegans*) *let-7* miRNA induces its own maturation in an Argonaute (ALG-1)-dependent manner. RNA immunoprecipitation assays revealed an ALG-1–binding site containing a *let-7*-complementary element near the 3′ end of the primary *let-7* (*pri-let-7*) transcript. Northern blots showed that mutant worms with the *pri-let-7*–ALG-1 interaction disrupted had increased levels of the primary transcript and lower levels of the precursor and mature forms, suggesting a defect in pri-miRNA processing. Furthermore, the introduction of a wild-type *let-7* gene into a *let-7* mutant strain was able to upregulate levels of the mutant miRNA. Together, these data point to an intriguing new model whereby mature *let-7* binds to a complementary element in the *pri-let-7* transcript, recruiting ALG-1 and promoting its own downstream processing. The findings by Zisoulis et al[3] introduce novel concepts in the regulation of *let-7* expression and represent an important contribution to our overall understanding of miRNA regulatory pathways.

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let-7 is one of the founding members of the miRNA family, first identified in *C. elegans* as a temporal regulator of cell fate decisions during development.[4,5] *let-7* promotes the transition from a larval to adult state by silencing genes that maintain stem-cell–like properties and inducing cell differentiation.[4,5] Importantly, *let-7* is highly conserved across animal species, both in sequence and function.[4] It is present in numerous copies throughout the human genome and has been implicated in countless pathologies, including cancers and cardiac disease.[1,4,6,7]

Canonical miRNA biogenesis begins with the transcription of the primary miRNA, typically by RNA polymerase II.[8–10] This transcript is 5′ capped and polyadenylated and cleaved by Drosha, an RNase III family member.[8–10] The product of this cleavage is the ≈ 70-nucleotide precursor miRNA, which is exported out of the nucleus by exportin-5.[8–10] In the cytoplasm, the precursor miRNA is further processed by Dicer, a Drosha-related RNase III that cleaves the precursor miRNA, producing an ≈ 21-bp miRNA duplex.[8–12] One of these strands, the guide strand, is bound by Argonaute and incorporated into the miRNA-induced silencing complex (mRISC).[8–11] Binding of this complex to target mRNAs via sequence complementarity with the guide strand usually results in transcript destabilization and translational repression.[8–11]

The findings from Zisoulis et al[3] move away from the canonical miRNA pathway in many respects. First, they implicate a role for mature *let-7*, as well as Argonaute, in the nucleus. Argonaute proteins are classically known as key components of the miRNA-induced silencing complex, which regulates mRNA expression in the cytoplasm.[8–11] However, using RNA immunopurification assays, Zisoulis et al[3] showed that ALG-1 actually interacts with nuclear *pri-let-7* in a *let-7*–dependent manner. They confirmed the nuclear localization of these factors by carrying their work from *C. elegans* into HeLa cells: ≈20% of total Argonaute and ≈50% of all mature *let-7* were found to be in the nucleus. In addition, *pri-let-7* co-purified with Argonaute from both whole cell and nuclear extracts, suggesting that this interaction observed in *C. elegans* was conserved in humans.

The authors also introduce a surprising noncanonical miRNA target: its own primary transcript. Furthermore, they show that *let-7* does not silence, but instead directly activates, its own expression. This conclusion is based on 2 pieces of evidence. The first is that mature *let-7* levels are reduced in worms missing the *let-7*–binding site in the primary transcripts. The second comes from elegant experiments using the *n2853 C. elegans* strain. These animals have a single G→A transition in the *let-7* seed sequence, which is predicted to disrupt binding of the mature miRNA to the complementary element in its own primary transcript. Northern blot analyses of *n2853* extracts revealed a defect in *pri-let-7* processing, which correlated with a lack of ALG-1 binding. Importantly, mature *n2853 let-7* levels were rescued by the introduction of wild-type *let-7*.

The findings by Zisoulis et al[3] not only describe the first direct miRNA autoregulatory loop, but also add to a growing body of evidence, suggesting nuclear roles for both mature miRNAs and Argonaute proteins. For instance, promoter-directed small interfering RNAs introduced into human cells have previously been shown to inhibit transcription of endogenous genes through various epigenetic mechanisms.[13,14] Studies in HeLa cells have

revealed that a hexanucleotide element at the 3′ end of mature miRNAs may direct nuclear import[15] and that small interfering RNAs can indeed mediate the specific degradation of nuclear RNAs.[16] Just last year, Tang et al[17] described the first example of a pri-miRNA transcript under direct miRNA regulation.

C. elegans research has been instrumental in elucidating RNA interference (RNAi) pathways as well. Recently discovered roles for *C. elegans* Argonaute proteins range from the nuclear transport of cytoplasmic small interfering RNAs[18] to mediating the inheritability of RNAi phenotypes.[19] Powerful genetic tools unique to *C. elegans* have facilitated the discovery of not only *let-7*, but also many of its regulatory targets.[20] In turn, some of these factors can regulate various stages of *let-7* expression, thus forming multiple feedback loops.[4] The work by Zisoulis et al[3] now adds an important positive auto-regulatory module to the *let-7* biogenesis pathway (Figure). Positive autoregulation is a mechanism that can result in the integration of graded inputs (eg, intercellular signaling) to a binary output (eg, self-renewal versus differentiation).[21] Given the pivotal role of *let-7* in cell fate decisions, it is not surprising that positive feedback loops have evolved to generate a bistable system where mature *let-7* levels are either very low or very high. Cells with low *let-7* expression will self-renew, whereas those with high expression will differentiate. Such autoregulatory modules are common cellular mechanisms used to ensure the irreversibility of cell fate specifications.[21]

In the mouse heart, *let-7* accounts for ≈14% of all expressed miRNAs.[1] If the autoregulatory loop described by Zisoulis et al[3] is also true for human *let-7*, then it may be at least, in part, responsible for this high level of expression. Microarray analyses reveal that *let-7* is significantly upregulated in many diseased heart tissues,[1] suggesting that this autoregulatory loop may be a useful therapeutic target. It is intriguing that a common feature of many cardiac diseases involves the re-expression of a fetal gene program.[21–23] In a simple model, we would have expected the downregulation of cardiac *let-7* to help induce this fetal expression profile, given its role in *C. elegans* and human embryonic stem cells to promote cell differentiation.[4,24] It is of course possible that in the context of certain cardiac expression profiles, *let-7* upregulation may have different biological consequences than in development.

There are also many other questions that remain unanswered. Zisoulis et al[3] have shown that mature *let-7* can induce the maturation of its own primary transcripts by binding to a complementary element near their 3′ end and recruiting ALG-1. But what are the molecular events upstream and downstream of this binding? How is *let-7* localized to the nucleus? Alternatively, is it possible that some miRNAs can mature without ever leaving the nucleus? How does ALG-1 promote *let-7* processing? Given the diverse roles of Argonaute family members, identifying Argonaute-interacting factors by immunoprecipitation and mass spectrometry may provide important insights into the molecular mechanisms of nuclear miRNA functions.

Perhaps most importantly, a combination of computational and experimental approaches will be required to apply the concept of *let-7* autoregulation to other miRNAs. Are such autoregulatory modules common in miRNA biogenesis pathways? How significant is the

nuclear role of miRNAs in general? When assaying for nuclear levels of mature *let-7*, Zisoulis et al[3] observed that some other miRNAs (miR-2, miR-244, miR-58) also displayed ≈ 20% nuclear localization. Could they be acting as an additional common layer of gene regulation? The answers to these questions will certainly lead the scientific community further away from the canonical view of miRNA biogenesis and function toward a more holistic understanding of miRNA-mediated gene regulation.

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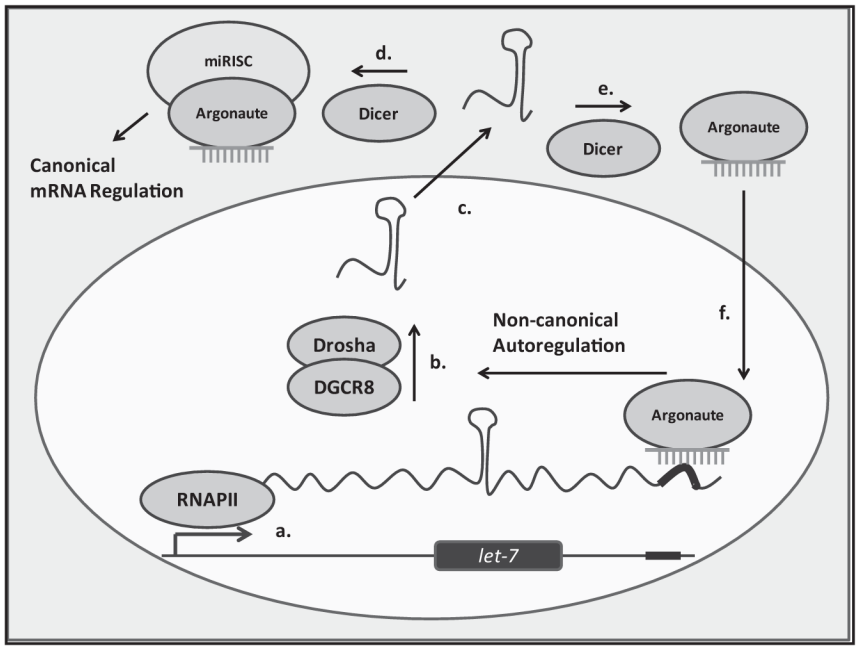

Figure. Positive autoregulation of *let-7* biogenesis

(**a**) Transcription of the primary microRNA (miRNA) by RNA polymerase II. (**b**) Processing and cleavage of the primary transcript by Drosha and DGCR8, producing the precursor miRNA. (**c**) Nuclear export of the precursor miRNA (dark green and light green represent the nucleus and cytoplasm, respectively). **(d**) The precursor miRNA undergoes the canonical pathway, processed by Dicer into the mature form and incorporated into the miRNA-induced silencing complex (miRISC). (**e**) and (**f**) A potential mode of mature miRNA nuclear transport by an Argonaute, after Dicer cleavage. The mature *let-7*-Argonaute complex binds to the complementary element (dark red) in the primary *let-7* transcript, upregulating its own processing and completing the feedback loop.