The primary target of *let-7* microRNA

Amy E. Pasquinelli[1]
Division of Biology, University of California, San Diego, La Jolla, CA 92093-0349, U.S.A.

Abstract

The *let-7* miRNA (microRNA) is an essential regulator of development from nematode worms to humans. Altered expression of *let-7* results in larval arrest or lethality in *Caenorhabditis elegans.* Likewise, under- or over-expression of *let-7* in human cells can result in cellular overproliferation or halted cell division respectively. Thus the biogenesis of this critical miRNA is controlled at multiple levels. An unexpected mechanism for regulating the initial processing of *let-7* was recently found to involve the *let-7* miRNA itself. The mature *let-7* miRNA along with its effector protein, Argonaute, were shown to bind to a site in the primary transcripts produced by the *let-7* gene. This interaction enhances processing through a novel auto-regulatory feedback loop. This discovery highlights a new role for the miRNA complex in regulating miRNA biogenesis and enriches the classes of RNAs targeted by Argonaute.

let-7 miRNA (microRNA)

The founding members of the miRNA family, *lin-4* and *let-7*, were discovered as genes important for temporal development in the nematode *Caenorhabditis elegans* [1,2]. Well over 1000 miRNA genes are now recognized as regulators of gene expression across much of multicellular phylogeny. The *let-7* miRNA exemplifies one of the rare cases of an miRNA with perfect sequence conservation over distant species [3]. The human genome contains three distinct *let-7* genes with mature sequences matching that of the nematode worm *let-7*, as well as nine other genes encoding *let-7* miRNAs that differ by one to four nucleotides [4]. The general expression and function of the *let-7* miRNA also seem to be conserved. Typically, *let-7* is absent from pluripotent cells and starts to accumulate only as cells take on more differentiated fates, which is consistent with its role in repressing genes that promote cell division and proliferation [4]. Thus, in disease states associated with abnormal cellular growth, such as cancer, *let-7* miRNA is often found to be down-regulated through mechanisms that are not entirely clear. At least in some cases, this alteration in *let-7* levels appears to be critical for oncogenesis, as reintroduction of *let-7* miRNA has been demonstrated to halt tumour growth in mouse models of lung and breast cancer [5–7]. Additionally, *let-7* has been found to regulate several metabolic genes, and disruption of *let-7* expression alters insulin sensitivity in mouse models [8].

miRNA biogenesis

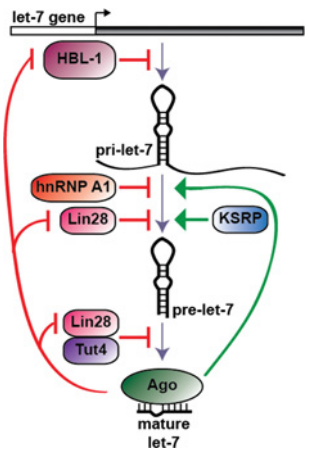
Biogenesis of *let-7*, and most other animal miRNAs, initiates with the synthesis of miRNA primary transcripts by RNA polymerase II [9]. In nematode worms, *let-7* is intergenic, and transcription produces two nascent RNAs and a third spliced primary transcript isoform [10]. Several of the human *let-7* genes are also intergenic, whereas others are located in the introns of protein-coding mRNAs; the nascent primary transcripts encoded by the human *let-7* genes have not yet been well characterized [11]. The RBP (RNA-binding protein), DGCR8 (DiGeorge syndrome critical region gene 8) (also known as Pasha) and RNase III enzyme Drosha comprise the core of the Microprocessor, which cleaves the hairpin precursor miRNA from the primary transcript [9]. In vertebrates, the released precursor miRNA is exported to the cytoplasm by the nuclear transporter XPO5 (exportin 5) [9]. As nematode worms lack an obvious XPO5 homologue, it is unclear how miRNA precursors are delivered to the cytoplasm. Once out of the nucleus, the RNAse III Dicer removes the loop from the hairpin precursor, producing the mature ~22 nt miRNA strands [9]. In the case of *let-7*, the 5′ half of the mature duplex is retained as the guide strand, whereas the 3′ half is degraded. Incorporation of the mature guide miRNA into an Argonaute-containing complex, referred to as the miRISC (miRNA-induced silencing complex), stabilizes the miRNA and positions it to serve as the specificity guide for target mRNA recognition and regulation [9]. In nematode worms, the exonucleases XRN-1/-2 destabilize mature *let-7*, and other miRNAs, upon their disassociation from Argonaute [9]. The role of the human homologues of these or other nucleases in the decay of mammalian miRNAs has yet to be determined.

Considering its significant role in development, metabolism and disease, it is not surprising that expression of the *let-7* miRNA is regulated at multiple levels (Figure 1). Transcription of the nematode worm *let-7* gene is controlled by multiple *cis*-acting elements and a variety of transcription factors that drive oscillating levels of pri-*let-7* (primary *let-7*) transcripts in most somatic tissues during nematode worm development [12–16]. Less is understood about the transcriptional regulation of the various *let-7* genes in

Key words: Argonaute, biogenesis, *Caenorhabditis elegans*, *let-7*, microRNA (miRNA).
Abbreviations used: ALG-1, Argonaute-like gene 1; CLIP, cross-linking and immunoprecipitation; DGCR8, DiGeorge syndrome critical region gene 8; HBL-1, hunchback-like 1; hnRNPA1, heteronuclear ribonucleoprotein A1; miRNA, microRNA; miRISC, miRNA-induced silencing complex; pri-*let-7*, primary *let-7*; RBP, RNA-binding protein; XPO5, exportin 5.
[1]email apasquinelli@ucsd.edu

Figure 1 | Regulation of *let-7* biogenesis

Depicted are a few examples of factors that regulate the expression of *let-7* miRNA. Red lines indicate inhibitory effects, and green arrows indicate positive regulation. Additionally, mature *let-7* miRNA indirectly regulates its own expression by repressing the expression of HBL-1 and Lin28 and directly stimulates biogenesis by recruiting Argonaute (Ago) to its own primary transcripts. KSRP, KH-type splicing regulatory protein.



humans. In nematode worms and mammalian cells, the RBP LIN-28 binds pri-*let-7* transcripts and prohibits processing by the Drosha complex [17–20] (Figure 1). This inhibition is relieved as LIN-28 is subject to down-regulation by other miRNAs when cells become more differentiated. hnRNPA1 (heteronuclear ribonucleoprotein A1) recognizes the terminal loop of human pri-*let-7a* and also blocks Drosha processing [21] (Figure 1). Processing inhibition by LIN-28 and hnRNPA1 is antagonized by KSRP (KH-type splicing regulatory protein), which binds to the loop region in *let-7* and other miRNA primary transcripts and enhances their processing [22] (Figure 1). Dicer cleavage of *let-7* precursor RNAs is also subject to inhibition by LIN-28, which recruits RNA uridyltransferases, such as TUT4 [23–27] (Figure 1). The addition of U-tails to *let-7* precursor miRNAs impedes Dicer cleavage and acts as a signal for rapid degradation. In addition to the factors that act specifically on *let-7* transcripts, numerous other proteins positively and negatively regulate the biogenesis of *let-7* and other miRNAs generally [9].

Regulation of biogenesis by miRNAs

There are several examples of miRNAs indirectly regulating their own expression. During nematode worm development, the transcription factor HBL-1 (hunchback-like 1) represses the expression of *let-7* in hypodermal cells until the *hbl-1* mRNA is targeted for down-regulation by *let-7* and its sister miRNAs in the last larval stages [16,28–30] (Figure 1). In mammalian cells, *let-7* miRNA represses the expression of Lin28, thus alleviating inhibition of its own biogenesis by this protein [31] (Figure 1). In each of these cases, the miRNA complex recognizes complementary sites in protein-coding mRNA sequences and induces repression through typical mRNA degradation and translational inhibition pathways. In contrast, a couple of recent studies have demonstrated that miRISC can also bind non-coding miRNA primary transcripts and regulate their processing through yet to be elucidated mechanisms [32,33].

Through studies aimed at identifying endogenous Argonaute-binding sites, we discovered a surprising role for *let-7* miRNA in the regulation of its own processing [33] (Figure 1). We utilized CLIP (cross-linking and immunoprecipitation) assays to isolate sequences bound by ALG-1 (Argonaute-like gene 1), the Argonaute predominantly responsible for miRNA function in nematode worms [34]. Thousands of ALG-1 sites were identified in mRNAs, but a closer examination of the data revealed a strong target site in pri-*let-7*. This site resides ~500 nt downstream of the mature *let-7* sequence and ~100 nt before the polyadenylation signal [33]. Centred within the region demarcated by the ALG-1 CLIP results is a sequence with complementarity to mature *let-7* miRNA. Notably, *let-7* genes in other Caenorhabditae species also harbour potential *let-7* complementary sites 400–600 nt downstream of the mature sequence. We confirmed that mature *let-7* directs ALG-1 to its own primary transcript by demonstrating that wild-type, but not a mutated mature *let-7* sequence, enables ALG-1 to bind the transcript [33].

Investigation of the function of ALG-1 binding to pri-*let-7* revealed another surprise. Originally, we predicted that this interaction might be important for pruning the levels of primary transcripts that had escaped processing. Since pri-*let-7* RNAs are capped and polyadenylated, recognition by miRISC might induce destabilization of the transcripts, as is often the case for targeted mRNAs [35]. This possibility was supported by the increased levels of pri-*let-7* transcripts that resulted when ALG-1 was prohibited from binding them [33]. However, these data were also consistent with a model where association with ALG-1 promotes processing of the primary transcripts. In the degradation scenario, higher levels of primary transcripts would be expected to produce higher, or at least the same, level of mature *let-7*. In contrast, if ALG-1 enhances processing, then the absence of this interaction would result in increased levels of the pri-*let-7* substrate and reduced levels of the mature product. In three different genetic backgrounds, i.e. loss of ALG-1, inability of mature *let-7* to recognize the target sequence, or deletion of the ALG-1-binding site, we observed increased levels of pri-*let-7* and decreased levels of the mature product [33]. We also showed that wild-type *let-7* enhanced the processing of a mutant version of *let-7* that was unable to recognize the complementary site in its own primary transcripts. Taken together, these results support a new role for miRISC in promoting miRNA processing. It is also the first example of an miRNA directly auto-regulating its own expression.

Regulation of primary miRNA processing by miRISC is not limited to *let-7*. In mouse cells, *miR-709* was found to bind the primary transcript for the clustered *miR-15a* and *miR-16-1* miRNAs [32]. This interaction is through a site ~800 nt downstream of the mature miRNA sequences

that is almost perfectly complementary to *miR-709* [32]. In contrast with the effect of miRISC binding to pri-*let-7* in nematode worms, *miR-709* represses the processing of *miR-15a/miR-16-1* primary transcripts. This regulation seems to be important for the cellular response to serum starvation. During this stress, the largely nuclear localization of *miR-709* shifted to the cytoplasm, alleviating its inhibitory effect on *miR-15a/miR-16-1* processing [32]. The resulting increased levels of mature *miR-15a/miR-16-1* targeted the anti-apoptotic gene, *Bcl-2*, for down-regulation, triggering cellular apoptosis programmes [32]. This study demonstrates that miRNAs can directly regulate the processing of other miRNAs, broadening the repertoire of targets under the control of miRISC.

Conclusions

The pri-*let-7* transcripts add to the growing list of RNAs regulated by *let-7* and establish non-coding RNAs as direct targets of miRISC. The generality of miRNA biogenesis regulation by miRISC remains to be determined. We found that primary transcripts for several of the *let-7* genes in human cells also associate with Argonaute, raising the possibility that they are subject to regulation similar to the nematode worm pri-*let-7* [33]. Although *miR-709* is restricted to mice, its predominant nuclear residence and potential to form base pairs with sequences in the primary transcripts of several miRNA genes suggests that it might have a broader role than just in the inhibition of *miR-15a/miR-16-1* processing [32]. The mechanism of positive or negative processing regulation by miRISC is an outstanding mystery. It is notable that the target sites for *let-7* and *miR-709* are each hundreds of nucleotides downstream of the miRNA precursors embedded in the primary transcripts. Thus long-range interactions between miRISC and the site of processing may be important for the effect on Drosha activity. Alternatively, Argonaute may facilitate transport of the primary transcripts to cellular locations that are more or less favourable for processing. Ultimately, as examples of miRISC binding and regulating non-coding RNAs are just starting to emerge, traditional views of miRNA targets and functions will need to be reconsidered.

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