Another "loophole" in miRNA processing.

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Abstract

Here we preview, in the context of miRNA processing and function, the intriguing finding published in this issue of *Molecular Cell* which shows that an RNAse that has important role in immunity regulates miRNA processing in cancer and inflammation by cleaving the terminal loops of many miRNAs.

miRNAs repress the expression of numerous target genes that are involved in a variety of cellular systems, therefore the homeostatic control of miRNA biosynthesis and activity is important for mediating diverse physiological processes such as differentiation, development, immune response and the cell cycle. Consequently, the misregulation of miRNA function is associated with several pathologies, including cardiovascular diseases, neurological disorders and cancer. miRNA levels are controlled by the rates of transcription, processing and turnover. This sequence of steps is subject to complex regulation via mechanisms that can either have a global effect on miRNA generation, or specifically modulate the synthesis of a particular miRNA. In this issue of *Molecular Cell*, Suzuki et al. provides further evidence that miRNA activity is regulated by mechanisms that target post-transcriptional stages of the maturation of miRNAs (Suzuki et al., 2011).

miRNA processing

The production of mature, single-stranded, ~22 nt miRNAs involves many processing steps. Generally, a primary transcript (pri-miRNA) is synthesised by RNA polymerase II. The pri-miRNA is endonucleolytically cleaved by the Microprocessor complex, which is composed of two proteins: Drosha, an RNase III enzyme and DGCR8 (DiGeorge syndrome critical region protein 8), which binds dsRNA. The microprocessor cleaves the characteristic stem-loop structure of pri-miRNA into the precursor (pre)-miRNA hairpin. Subsequent to nuclear processing, the pre-miRNA is transported into the cytoplasm by Exportin-5 (XPO5), which is also thought to protect the pre-miRNA from degradation. Dicer, another RNase III enzyme, mediates the cytoplasmic processing of the majority of pre-miRNAs and forms the miRNA/miRNA* duplex (reviewed in Kim, et al., 2009). One strand of the Diced miRNA is incorporated into an Argonaute protein, forming the core of the RNA Induced Silencing Complex (RISC) (Figure 1).

Regulation of miRNA biosynthesis

Similar to protein-coding genes, the rate of pri-miRNA transcription is controlled by promoter sequences, transcription factors and chromatin state. However there is increasing evidence that diverse mechanisms regulate the post-transcriptional stages of miRNA maturation. Thus far, the discovered regulatory mechanisms either modulate the activity of proteins that are involved in miRNA biosynthesis, or mediated by proteins that bind directly to miRNA(s) and interfere with the processing of individual and/or groups of miRNAs (reviewed in Newman & Hammond, 2010).

The miRNA loop as a platform for regulation

Although the terminal loops of miRNA precursors are not essential for efficient processing, the discovery that loop sequences are highly conserved in many miRNA families suggests that they may have regulatory functions (Michlewski, et al., 2008). Indeed, increasing evidence shows that regulatory proteins that can either facilitate or inhibit miRNA processing frequently target the loop.

Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) binds specifically to a conserved sequence in the loop of pri-miR-18a. This interaction is thought to cause a conformational change in the RNA which promotes Drosha-mediated cleavage of the primary transcript (Guil & Cáceres, 2007). Another identified protein that binds to specific sequences of the terminal loop is the KH-type splicing regulatory factor (KSRP). Its association with the loops of a group of miRNAs enhances their biogenesis, possibly by aiding the positioning of the processing complexes, and/or promoting the recruitment of Drosha and Dicer to the pri-miRNA and pre-miRNA, respectively (Trabucchi et al., 2009) (Figure 1).

Lin28, the negative regulator of *let-7* miRNA processing, also uses the loop sequence as a binding platform and functions by inhibiting microprocessor association to pri-miRNA (Viswanathan, et al., 2008). Additionally, cytoplasmic Lin28 represses Dicer cleavage of pre-*let-7* and promotes miRNA turnover by recruiting a uridylyl transferase enzyme TUT4 (terminal U transferase 4) to the precursor RNA. Pre-*let-7* is subsequently polyuridylated at its 3' end, which triggers its degradation. This mechanism is important for embryonic stem cell maintenance and differentiation (Heo et al., 2009) (Figure 1).

Suzuki et al. have identified MCPIP1 (monocyte chemoattractant proteins (MCP)-1-induced protein 1) as a novel inhibitor of miRNA processing. MCPIP1 is a nuclease that degrades multiple miRNAs by directly cleaving the terminal loop. It has already been shown that mature miRNA degradation is mediated by RNases (Chatterjee & Großhans, 2009; Ramachandran & Chen, 2008), however this is the first time that a nuclease has been implicated in the degradation of pre-miRNAs (Figure 1).

The authors identified two major pathways in which the MCPIP1-mediated modulation of miRNA activity could be important. They found an antagonistic relationship between Dicer and MCPIP1 in two lung carcinoma cohorts, suggesting that MCPIP1 contributes to the general down regulation of miRNAs that is considered to be a general characteristic of many cancers. Additionally, they have shown that elevated MCPIP1 expression is associated with poor survival in these cohorts. Furthermore, Suzuki et al. connected the role of MCPIP1 in inflammation and its role in the regulation of miRNAs. They demonstrated that MCPIP1 indirectly regulates IL-4 level by modulating the expression of miR-155, a target of which is the transcription factor c-Maf that induces IL-4 expression upon different stimuli. MCPIP1 binds to and regulates the expression of a range of mRNAs that are involved in inflammation, and the lack of MCPIP1 expression leads to autoimmune disease in mice. This suggests that MCPIP1 has a crucial role in controlling the timely expression of pro- and anti-inflammatory mRNAs. As MCPIP1 recognizes a range of pre-miRNA terminal loops and its expression is highly induced by infection, it is very tempting to speculate that MCPIP1 may have a more direct role in the innate immune response, for example, by recognizing and destroying small RNA precursors deployed by viruses.

One can predict that more, as yet undiscovered, proteins can modulate miRNA expression and activity by recognising and binding to the loop of miRNA precursors. The loop sequence of multiple miRNA families have been conserved over the course of evolution, suggesting that the terminal loop could act as a platform that binds regulatory proteins. Moreover, microarray and sequencing data suggest that pre-miRNAs and miRNAs are subject to independent regulation during differentiation, in response to diverse stimuli, or in diseases. The appearance of an increasing number of loop sequences in deep sequencing data sets could also indicate that these sequences

are bound by proteins, thereby preventing their quick degradation and allowing them to be detected (Berezikov et al., 2011). As the majority of these proteins remain elusive, it is possible that they regulate miRNA stability at very specific stages of biological pathways, which makes them very difficult to catch in action.

Figure legend:

Figure 1: Processing and regulation of miRNA processing via the loop.

Figure 1 shows the basic steps of the canonical miRNA processing from transcription until one strand of a miRNA is associated with an Argonaute protein. The proteins that regulate this process by directly binding to the loop sequence of miRNA(s) have also been shown. Green arrows mean stimulating miRNA processing. Pri-miRNA: Primary miRNA, pre-miRNA: precursor miRNA. RISC: RNA Induced Silencing Complex.

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