Chapter 18

Master Regulators of Posttranscriptional Gene Expression Are Subject to Regulation

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Abstract

MicroRNAs (miRNAs) are small noncoding RNAs of 17–25 nt in length that control gene expression posttranscriptionally. As master regulators of posttranscriptional gene expression, miRNAs themselves are subject to tight regulation at multiple steps. The most common mechanisms include miRNA transcription, processing, and localization. Additionally, intricate feedback loops between miRNAs and transcription factors result in unidirectional, reciprocal, or self-directed elegant control mechanisms. In this chapter, we focus on the posttranscriptional regulatory mechanisms that generate miRNAs whose sequence might be slightly different from the miRNA-coding sequences. Hopefully, this information will be helpful in the discovery of novel miRNAs as well as in the analysis of deep-sequencing data and ab initio prediction of miRNAs.

Key words miRNA, miRNA regulation, Small RNAs, Posttranscription

1 Introduction

Regulation of the genetic information has been the key to the evolution of complex, multicellular organisms and the generation of biological diversity. Intricate molecular regulatory programs that span over DNA, RNA, and protein levels dictate the biological activity of any gene product. In 1993, a novel mechanism of posttranscriptional regulation of gene expression by small noncoding RNAs was discovered in animals [1]. Over time, the number of these regulatory RNAs has enormously increased, and they have been shown to be active from development to disease [2–4].

The estimated number of all microRNAs (miRNAs) reaches out to nearly 1–5 % of all the predicted genes in nematodes, flies, and mammals [5–7]. A large number of these miRNA genes are dispersed throughout the genome. Some miRNAs are found in clusters that are co-expressed as polycistronic units showing their functional relationships. More than half of miRNAs reside in introns of their host genes and are co-expressed with their neighboring

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protein-coding sequences. [7–9]. Recent developments in sequencing technologies (e.g., deep sequencing) have accelerated novel miRNA discoveries as it is now possible to identify rarely expressed miRNAs owing to the high coverage rate of deep sequencing technologies. This technology also allows for extensive and detailed comparison of miRNA expression under various physiological and pathological cellular states. However, single-nucleotide changes introduced into miRNAs during biogenesis or variations at 5′ and 3′ termini require careful bioinformatics analyses so as not to lose any valuable information that may be associated with the phenotype of interest. As an introduction to miRNAs in biological systems and the role of miRNAs in human diseases are covered in the first two chapters, we discuss the transcriptional regulation of miRNAs followed by a focus on the posttranscriptional regulatory mechanisms.

2 Transcriptional Regulation of MicroRNA Genes

Despite the initial predictions about the intergenic localization of miRNA genes, subsequent findings showed that the majority of mammalian miRNA genes are organized within transcription units [8, 10]. Based on their genomic location, miRNAs can be contained within intronic sequences in protein-coding or noncoding transcription units or exonic sequences in noncoding transcription units. Since Drosha processing precedes splicing, a single transcript can give rise to both miRNAs and an mRNA [9, 11, 12]. Thus, the transcriptional regulation of miRNAs depends upon the genomic localization of miRNA genes.

In most of the cases, miRNAs are transcribed by RNA polymerase II which initially generates a primary miRNA (pri-miRNA) transcript consisting of one or more hairpin structures. These hairpins are then processed both in the nucleus and the cytoplasm to generate the mature miRNA. Pri-miRNAs have 5′ cap, undergo splicing, may have poly A tail, and often generate more than one functional miRNA [13].

A tightly controlled multi-step process of transcription provides the first line of regulation of miRNA expression, RNA polymerase II being the major player (Fig. 1). The promoter structure of miRNA genes has been shown to be largely similar to that of protein-coding genes [14, 15]. Consequently, the members of the transcription machinery also largely overlap between protein and miRNA genes. Some transcription factors have been shown to regulate the expression of both proteins and miRNAs, c-myc, p53, and hypoxia-inducible factor (HIF) being potent examples. C-myc has been shown to bind to the E-boxes in the promoter and activate the transcription of the miR 17-92 cluster [16]. STAT1 is another transcription factor that has recently been reported to regulate about 9 % of the total

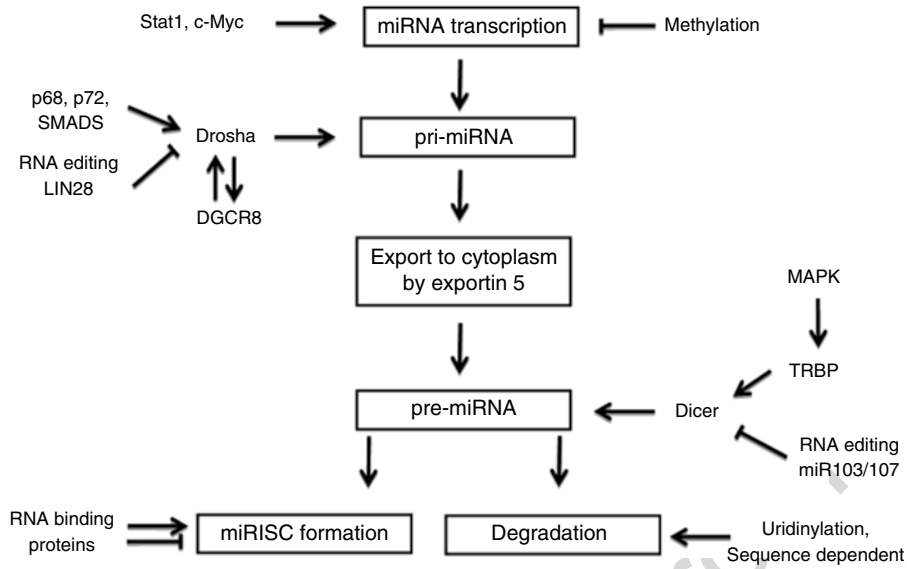

Fig. 1 A brief overview of transcriptional and posttranscriptional regulation of miRNAs. miRNA transcription itself might be subject to regulation by transcription factors and/or modulators of chromatin structure, e.g., methylation. The nuclear processing by Drosha might generate miRNAs with heterogeneous termini in addition to internal editing events. Similar terminal heterogeneity and internal editing events might be introduced in the cytoplasm during processing by Dicer. Additionally, modulation of intracytoplasmic RISC location and 3′-uridinylation adds further complexity to the cytoplasmic regulatory events that determine the fate of a miRNA

1,105 miRNAs in response to interferon γ stimulation in melanoma cells [17]. There may also be unilateral, reciprocal, or double-negative feedback loops between miRNAs and transcription factors [18]. For example, PITX3, a transcription factor involved in dopaminergic neuron differentiation, activates miR-133b transcription, which results in suppression of PITX3 expression through a negative autoregulatory mechanism [19].

Another mechanism of transcriptional control of miRNA biogenesis lies in their epigenetic regulation. Epigenetic modifications of miRNA loci may result in altered transcription of these genes. In several human ~~cancer~~ promoter regions of the genes encoding miR-9-1, -193a, -137, -342, -203, and -34b/c have been shown to be hypermethylated ([20], Chapter 19). Histone deacetylase (HDAC) inhibitors have been reported to increase the expression of some miRNAs including miR-1 in cancer cells [21, 22].

3 Regulation of Drosha Activity

In the nucleus, a multiprotein complex, called microprocessor complex, cuts the ~~pri-miRNA~~ into about 70 nt long hairpin structures called pre-miRNAs. Drosha, an RNase III enzyme, and

DGCR8 (Pasha), a double-strand RNA (dsRNA)-binding protein, are the vital components of the microprocessor complex along with cofactors including the DEAD box RNA helicases p68, p72, and heterogeneous nuclear proteins [23]. Drosha cleaves the pri-miRNA co-transcriptionally to generate a product with 2 nt overhang at the 3′ end [9].

There are many factors that modulate Drosha activity both positively and negatively. Two members of the DEAD box RNA helicase family, p68 and p72, have been reported as components of Drosha and DGCR8 [23]. Both single- and double-knockout p68 and p72 are lethal in mice [24]. These factors probably serve as scaffold proteins that recruit other modulatory proteins to the microprocessor complex to enhance pri-miRNA processing. LIN-28 and SMADs are other well-known examples of accessory proteins that modulate the processing efficiency of Drosha [18].

Regulation of the total protein levels of Drosha and DGCR8 also plays a vital role in the regulation of miRNA processing. DGCR8 has a stabilizing effect on the Drosha protein level [25]. In turn, Drosha determines the DGCR8 levels by processing hairpins present in the DGCR8 transcript. Consequently, the ratio of Drosha to DGCR8 appears to be crucial for the activity of the microprocessor complex. In addition to cleaving pre-miRNA hairpins, the microprocessor complex can also promote cleavage of hairpin structures within annotated protein-coding genes [25, 26], a more direct form of gene regulation than the indirect miRNA approach.

An important issue to consider while analyzing miRNA data generated from SAGE or deep sequencing is the heterogeneity at the miRNA termini. Not only Drosha but also Dicer generates nonuniform miRNA termini. Certainly this heterogeneity could have a dramatic effect on the duplex stability and strand selection [27]. As a result, miRNA:miRNA* ratio would change if extensive heterogeneity is introduced. The terminal heterogeneity also influences the target mRNA selection, particularly the heterogeneity at the 5′ end that affects the seed register of miRNAs. [AU1]

4 Export of Pre-miRNA to the Cytoplasm

Exportin 5 is responsible for the transfer of pre-miRNA from nucleus to the cytoplasm in a ran-GTP-dependent manner [28–30]. A 16–18 base pair long stem of pre-miRNA and modifications of 3′ overhang affect its binding to and ~~the (delete the)~~ transport efficiency by exportin 5 [31]. In the cytoplasm, pre-miRNA is released from exportin 5 by the hydrolysis of GTP and is processed further. This nuclear-to-cytoplasmic transfer may be differentially regulated under certain conditions. The precursor hairpins of miR-105, -108, and -31 are found at high levels in many cells, but [AU2]

mature miRNAs are not detectable [32]. A specific example of this phenomenon is miR-31. The nuclear form of this miRNA is almost equally expressed in both MCF7 and HS766T cell lines. In HS766T cells, high levels of mature miR-31 are found but are not detectable in MCF7. In HS766T cells, pre-miR31 shows cytoplasmic localization while it is accumulated in the nucleolus in MCF7 cells. This shows that the cytoplasmic export of miR-31 is cell type dependent and is regulated by some factors that are still to be unraveled.

5 Regulation of Dicer Activity

Once in the cytoplasm, another RNase III enzyme, Dicer, cuts the pre-miRNA into a ~22 nt long miRNA duplex with the help of dsRBD proteins TRBP/PACT [33–35]. The two miRNA strands are then separated, and one of the strands associates with Argonaute protein to form the RNA-induced silencing complex (RISC).

Regulation of miRNA processing by Dicer involves inhibition of Dicer activity. In colorectal tumor samples, miR-143 and miR-145 show very low expression as compared to the normal tissues despite the equal pre-miRNA levels. This suggests that the nuclear transcription and processing are similar, but the cytoplasmic processing by Dicer may be altered in colorectal tumor samples [36]. Another example includes developmental regulation of miR-138 processing. The mature form of this miRNA is only detectable in adult mouse brain and foetal liver, whereas pre-miR138 is expressed in all tissues [37].

Dicer activity is known to be modulated by several factors. Perhaps the best-characterized protein is TRBP, which is required for Dicer stability as mutations in TRBP lead to the impairment of Dicer function. TRBP in turn is stabilized by MAPK-mediated phosphorylation of serine residues [38], establishing a link between signalling pathways and Dicer-mediated modulation of cellular functions. Accessory proteins may regulate Dicer activity, resulting in the modulation of specific miRNAs, rather than modulating global Dicer activity. LIN-28 binds to the terminal loop of pri-let-7 in embryonic stem cells and blocks its processing by Dicer [39]. KSRP is another protein that promotes processing by Dicer [18].

The expression of Dicer may also be subject to regulation by miRNAs. The miR-103/107 family has been shown to target the expression of Dicer and down-regulate global miRNA biogenesis [40, 41]. The miR-103/107-mediated regulation of Dicer expression is associated with increased epithelial-mesenchymal transition (EMT) and metastasis. The amino terminal helicase domain of Dicer may have an autoinhibitory function, as removal of this domain increases the catalytic activity of Dicer processing [42].

6 RNA Editing

RNA editing is catalyzed by members of the adenosine deaminase acting on RNA (ADAR) protein family, found in most metazoans [43]. A-to-I editing may happen in all types of RNA where adenosine is converted to inosine through hydrolytic deamination. The stem–loop structures of both pri- and pre-miRNAs are potential targets for editing where editing of pri-miRNAs may occur at more than one adenosine (residue) [44–46]. Pri-miR-22 was the first miRNA to be reported as a miRNA edited at six different positions including sites within the mature miRNA. Editing of miRNAs may decrease their stability or inhibit their processing by Drosha or Dicer. For example, editing of pri-miR-142 at two adenosines close to the Drosha cleavage site not only inhibits its processing by Drosha but also makes it less stable. Editing of miR-151 inhibits its processing by Dicer but not by Drosha [47].

Editing events not only influence the processing efficiency of miRNAs, but it also affects the target mRNA selection. Because there is partial complementarity between the miRNAs and the 3′ untranslated region of target mRNAs, even the change in a single-nucleotide sequence may have a dramatic effect on the stability of a miRNA:mRNA pairing. Thus, it is quite important, during bioinformatics analyses, to consider potential editing events to obtain information at the maximum level. It is quite challenging, however, to identify the edited miRNAs as it is possible for the sequences with single-nucleotide mismatches to originate from other genomic loci. Thus, prior to calling a sequence an edited miRNA, it is imperative that the genomic origin of the sequences be identified unequivocally. Additionally, functional reporter assays would be required to illustrate the functional significance of these editing events.

7 Conclusion

Since the discovery of miRNAs way back in 1993, the number and scope of their function have tremendously increased over the years. They not only fulfil the duty of a carrier of information between DNA and protein but also fine-tune the various steps of posttranscriptional regulation to maintain the tight balance in protein expression. To achieve this goal, miRNAs are themselves very tightly regulated both at the levels of transcription and posttranscription. An extreme example of miRNA gene regulation comes from the chromatin studies. Cis-regulatory elements of gene expression, scaffold/matrix attachment regions (MARs), have been shown to define the cell-specific expression of let-7b, miR-93, miR-17, and miR-221 by tethering the chromatin to the nuclear matrix [48]. A minority of miRNAs has been found to

respond to circadian rhythm regulatory mechanisms. For example miR-219 is targeted by the CLOCK and BMAL1 complexes [49]. So the cell- and context-type specificity of miRNAs appear to involve a coordinated activity of cis-regulation, transcription factor binding, and chromatin modulation resulting in specific gene expression. This also involves the tightly regulated miRNA processing factors both in the nucleus and cytoplasm, loading of mature miRNA to the RISC complex, editing, and degradation to ensure a precise balance in the expression of miRNAs.

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Queries	Details Required	Author's Response
AU1	Please provide significance for "*" in the sentence "As a result, miRNA:miRNA …* if necessary.	
AU2	Please revise the sentence "A 16–18 base pair long stem of…" for clarity.	