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MicroRNAs: History, Biogenesis and Modes of Action to Regulate Gene Expression

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Abstract

MicroRNAs (miRNAs) are short, non-coding RNAs that regulate post-transcriptional gene expression in animals and plants. Biogenesis of miRNAs is itself a highly complex process. miRNAs bind to the 3' untranslated region (UTR), 5' UTR or/and coding regions of their target mRNAs in a sequence specific manner. Targeting of mRNAs leads to the repression of protein synthesis by a mechanism that is yet to be fully determined. miRNA-mediated translational repression has been proposed to occur in distinct ways. Some reports have also shown miRNA-mediated translational activation. Details regarding the different modes of actions related to transcriptional and post-transcriptional regulation of miRNAs are still emerging. In this review, information regarding the history, biogenesis and different modes of actions of miRNAs are discussed.

Keywords: MicroRNAs, biogenesis, translational repression, translational activation, 3'UTR, AGO.

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Introduction

History

MicroRNAs (miRNAs) are small (21-24 nucleotide (nt) in length) non-coding RNAs, which are able to regulate gene expression at the post transcriptional level [1]. The first miRNA; lin-4 (22 nt long) was identified in a nematode; *Caenorhabditisis elegans* (*C. elegans*) in 1993 by the Ambros and Ruvkun laboratories simultaneously [2, 3]. It was found to negatively regulate the expression of the lin-14 protein product by targeting complementary sites in the 3' untranslated region (3'UTR) of the lin-14 mRNA via an antisense RNA-RNA interaction [3, 4]. Mutation in the lin-4 ORF did not affect its function, suggesting that lin-4 did not encode for a protein [2]. This discovery remained unrealized for almost seven years when Reinhart *et al*, identified a second miRNA; let-7 in *C. elegans* [5]. let-7 was found to interact with the 3'UTRs of lin-41 and lin57 mRNAs and inhibit their translation [6, 7]. These discoveries unveiled a new family of RNAs which later became known as microRNAs (miRNAs) [8]. It has since been shown miRNAs are expressed in large numbers and present in a diverse range of different species (including algae, arthropods, nematodes, protozoa, vertebrates, plants, and viruses) [1, 9, 10]. The latest miRBase release (v20, June 2013), contains 24521 miRNA loci, processed to produce 30424 mature miRNAs from 206 species [11] . The regulatory roles of miRNAs have been identified in various biological processes including determination of cell fate, proliferation, cell death, immune response and tumorigenesis [12-14].

MiRNA biogenesis

miRNA processing in the nucleus

The biogenesis of miRNAs is a multi-step process. It involves sequential processing and editing of transcribed miRNA genes (**Figure 1**). Then majority of miRNAs are derived from large RNA polymerase (pol) II transcripts, while a small proportion of miRNAs is derived from pol III transcripts [15, 16]. These primary transcripts (pri-miRNAs) are 5'end capped and 3' end poly adenylated and range from hundreds to thousands of nucleotides in length [17, 18]. Pri-miRNAs are transcribed from introns, exons, intergenic regions or in an antisense direction of annotated genes [17, 19, 20]. A single pri-miRNA transcript can either generate monocistronic miRNA or polycistronic clusters of miRNAs, under the influence of a single promoter or different promoters for individual miRNAs [15, 17, 21]. Approximately 40% of human miRNAs are co-transcribed as clusters encoding more than one miRNA sequences in a single pri-miRNA transcript [22, 23]. A pri-miRNA contains an imperfect double-stranded (ds) stem-loop structure flanked by single-stranded (ss) RNA. One arm of the stem-loop structure includes the mature miRNA [24].

The stem-loop structure and the flanking region of the pri-miRNAs direct the pri-miRNAs to a multiprotein complex called the microprocessor complex [25-28]. The microprocessor complex contains an RNase III enzyme called Drosha and its cofactor protein DiGeorge syndrome critical region gene 8 (DGCR8). DGCR8 interacts with the stem-loop structure and recruits Drosha, which then cleaves the pri-miRNAs precisely at the stem-loop structure

and liberates a 60-110 nt long RNA product called the precursor miRNA (pre-miRNA) with a 5' phosphate and a 2 nt overhang at the 3' end [24, 29, 30] (Fig. 1). The 3' overhang and adjacent stem of pre-miRNA is recognized by a heterodimer made up of Exportin 5 and Ran-GTP cofactor (Exportin/Ran complex). The Pre-miRNA interacts with the Exportin/Ran complex and is exported from the nucleus into the cytoplasm [31, 32].

MiRNAs processing in the cytoplasm

In the cytoplasm, hydrolysis of Ran-GTP to Ran-GDP causes the release of the pre-miRNA from the Exportin/Ran complex [33]. The pre-miRNA is then taken up by the RISC loading complex (RLC) made up of an RNase III enzyme called Dicer, its cofactors; TAR RNA-binding protein (TRBP) and protein activator of PKR (PACT) and the argonaute-2 (Ago-2) protein [34-37]. TRBP and PACT are not absolutely required for pre-miRNA processing but they seem to help in stabilizing Dicer, recruiting Ago-2 and in RLC formation [36, 38, 39]. Once in the RLC, the exported pre-miRNA is recognized by the PAZ (piwi-argonaute-zwille) and two RNase III domains (RNase IIIa and RNase IIIb) of Dicer [40-43]. Once bound, Dicer cleaves the pre-miRNA at the base of the stem-loop, leaving an ~22 nt miRNA duplex with a 5' phosphate and a 3' OH with 2 nt overhang [44].

Once pre-miRNA has been cleaved by Dicer, the resultant miRNA duplex directly interacts with an Ago protein (also called as elF2C2) to generate the effector complex; RNA induced silencing complex (RISC) [34, 45, 46]. The Ago family proteins are the key effector molecules of RISC [47] and are composed of PAZ, MID and PIWI domains. The PAZ domain recognizes and interacts with the 2 nt overhang at 3' end of the miRNA, whereas the MID domain anchors the 5' end of the miRNA [48-50]. The PIWI domain structure is similar to RNase H and is thought to play a role in cleaving of the target mRNA bound to the miRNA (also called slicer activity) [29, 51, 52]. In mammals four Ago proteins (Ago1-4) are associated with the miRNA but among those only Ago2 has been found to have an enzymatically competent PIWI domain with slicer activity to cleave the target mRNA strand that are perfectly complementary to the mature miRNA [53, 54].

After loading onto Ago proteins the miRNA duplex is unwound by helicases. One strand of the duplex remains in Ago and acts as a mature miRNA (the guide strand or miRNA), whereas the other strand (the passenger stand or miRNA*) is released for degradation or to be incorporate into another RISC as another mature miRNA [29, 45, 55, 56]. Relative thermodynamic stability of the two strands in the duplex, determines which strand is to be selected as the guide strand [57]. The strand with the less stable base pairing at the 5' end is incorporated into RISC and becomes the mature miRNA. If both strands of the duplex are used as mature miRNAs with similar frequency then 5p or 3p is added at the end of their names to denote which arm of the duplex, the mature sequence comes from [58]. Once the mature miRNA has become associated into RISC, the miRNA is used to guide and bind the complex to their complementary target sites located in the mRNA transcripts.

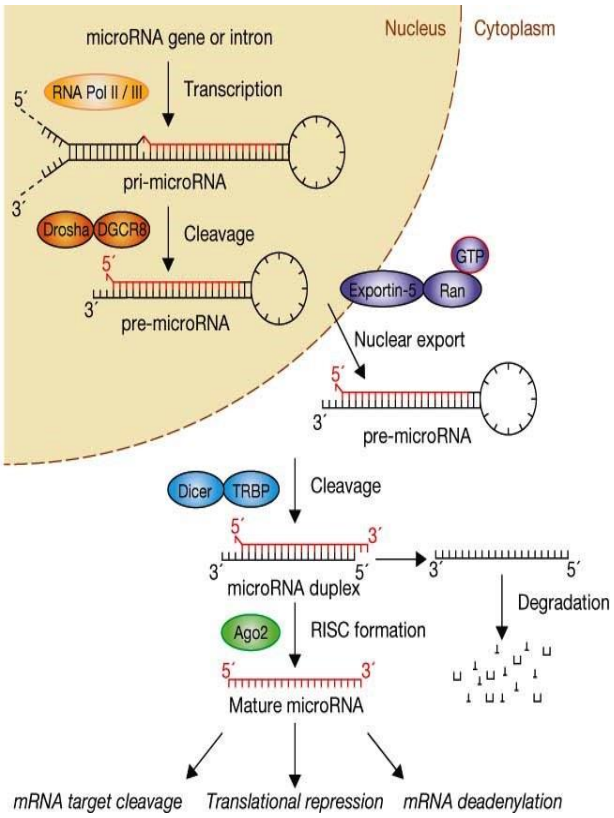

Fig. 0: Schematic diagram of miRNA biogenesis. (Adapted from [59].

Mode of action of miRNAs

miRNA target recognition

miRNAs generally regulate gene expression by binding to target mRNAs, at a post transcriptional level. Plants show perfect or near perfect complimentarity between miRNA and their target mRNA and induce translational repression through

degradation of their target transcripts [60] whereas in animals, miRNAs generally use a 6-8 nt sequence (seed region) out of ~21 nt of miRNA sequence to recognize the target mRNA [61]. The miRNAs seed region is located at nucleotide position 2-7 or 2-8 at the 5' end of the mature miRNA [62] and it is this region which is most conserved across metazoan miRNAs [63, 64]. The binding of most miRNAs includes the 5' seed region however the presence of non-seed interactions have also been reported e.g. at the 3' end of miRNAs and a site in the centre of miRNAs [65-67].

Helwak *et al* mapped human miRNAs and mRNAs interactions using a biochemical approach combined with bioinformatic analysis; Cross linking, Ligation and Sequencing of Hybrids (CLASH) and identified that approximately 60% of the interactions between the seed region and target sites in mRNAs were non-canonical containing bulged and mismatched nucleotides. In this study, 18% of the total miRNA-mRNA interactions involved the 3′ end of miRNAs, with little evidence for 5′ end contact [65]. In addition to seed region match, base pairing with target mRNA at the 3'end of miRNA is also possible and is called supplementary pairing [68]. The presence of mismatches or G:U pairing (refers to the pairing of a G with a U instead of a C) in the seed region is also acceptable, however target repression can be affected in this type of pairing [69-71].

A single miRNA can target several mRNAs by hybridizing to the target site/s (complementary to the miRNA seed region) located in the 5'UTRs, coding regions and/or 3'UTRs leading to translational inhibition or mRNA degradation [66, 70, 72-74]. miRNA-mediated mRNA cleavage, mediated most likely by Ago2 RNase H activity, is based on perfect complimentarity between the miRNA and its target mRNA [45, 75-77]. However an imperfect complementarity between miRNA and its target mRNA might lead to the initiation of the other mechanisms of miRNA mediated gene silencing; translational repression and mRNA degradation.

Translational repression

The process of mRNA translation initiates with the recognition of the 5' cap by eukaryotic translation initiation factor (eIF) 4E, along with other eIFs (eIF4G, eIF4A and eIF3). This interaction facilitates the recruitment of ribosomes to the 5′ end of mRNA and thus initiates translation.

It has been suggested in various studies that miRNA mediated translational repression can occur at the translational initiation stage or at the translational post-initiation stage [78-81].

mRNAs whose translation is not dependent on the presence of 5' cap i.e mRNAs containing an internal ribosome entry sites (IRES) and mRNA which has a non- functional 5' cap, have been found to show resistance to miRNA-mediated repression [82-86]. These studies suggest that miRNA-mediated silencing interferes with eIF4E function or the cap recognition process during the initiation of translation. Moreover, evidence also suggests that repression of cap-dependent translation can be mediated by inhibiting the formation of the mature ribosomal complex i.e. by inhibiting the recruitment of the 40S subunit and 80S initiation complex formation [87] or by inhibiting the joining of the 60S ribosomal subunit with the 40S subunit [81, 88]. In another study, Mathonnet *et al*, discussed the possibility that Ago2, as a part of the RISC, interacts with the 5'cap of the mRNA and interferes with the binding of eIF4E, which leads to the inhibition of translation initiation [83].

miRNA-mediated inhibition of translation at the post-initiation stage has also been proposed as another mechanism to target mRNAs. It was found that the lin-4 miRNA did not change the abundance of the target mRNA lin-14 in polysomal fractions, suggesting that translation was initiated normally and that miRNAs might act after translational initiation [89]. Various other studies also supported this mechanism of inhibition and provided evidence that repressed mRNAs were associated with actively translating polysomes [90-92]. miRNAs can also interfere with the elongation phase of translation either by causing degradation of the nascent polypeptide chain [91] or by initiating premature ribosome drop-off from the target mRNA [92].

miRNA mediated degradation of target mRNA

Although, previous studies suggested that miRNA mediated silencing results in the repression of translation of the target mRNA without changing the mRNA levels [89], recent studies have indicated that miRNA-mediated translational repression is associated with the destabilization and degradation of the target mRNA [93, 94].

Degradation of target mRNA by miRNA, requires deadenylation and/or 5'decapping of the target mRNA [82, 93]. The degradation of the target mRNA is thought to occur in the cytoplasmic P-bodies [95-97]. The Ago proteins, the poly(A) binding proteins (PABP) and the P-body protein GW182, are all involved in the deadenylation of the target mRNA [98-101]. GW182 protein recruits the deadenylase

complexes; CCR4-CAF1-NOT1 and PAN2-PAN3 through direct interaction with NOT1 and direct or indirect interaction with PAN3 and PABP respectively. These interactions are considered important for the deadenylation and degradation of the target mRNA in a 3'-to-5' direction. However, the exact mechanisms involved in the recruitment of the deadenylase complex to the RISC and subsequent deadenylation of the poly(A) tail are still not well understood [81, 98, 100-102].

The next step in miRNA-mediated degradation involves the 5' decapping of the target mRNA by the decapping-complex proteins DCP1 and DCP2 [103]. Knockdown of the decapping-complex proteins has been shown to lead to an accumulation of deadenylated mRNAs [95, 104]. A decapped mRNA is then degraded by the exonuclease activity of the major cytoplasmic 5'– 3' exonuclease XRN1 [103, 105].

MiRNA mediated translational activation

Several miRNAs have been reported to induce translational activation instead of repression under certain conditions or in specific cells [106-108]. Translational up-regulation by miRNAs could be achieved in two ways; activation by direct action of the miRNA or by the relief of repression where the action of a repressive miRNA is abrogated [108]. The translation of the CAT1 mRNA is repressed by a liver specific miRNA miR-122, in the P-bodies in human hepatoma cells. However following amino acid starvation the CAT1 mRNA is released from the P-bodies and interacts with the polysomes. This process depends on the binding of HuR, an AU rich-element binding protein, to the 3'UTR of the CAT1 mRNA and it is this binding that inhibits the repression by miR-122 [109]. Another miRNA miR-369-3 has been shown to target the 3'UTR of TNFα mRNA and repress its translation in proliferating cells, however in G1/G0 arrested cells translation of TNFα mRNA has been found to be up-regulated. It has been reported that under serum starvation conditions miR-369-3 in RISC, bound to TNFα mRNA could recruit the fragile X–related protein 1 (FXR1) and stimulate mRNA translation [107, 110]. Another miRNA, miR-10a which can interact with the 5′-terminal oligopyrimidine tract (5′-TOP) motif in the 5'UTR of many ribosomal proteins' mRNAs, has also been shown to up-regulate translation of these mRNAs under stress conditions or nutrient shortage [111].

Conclusion

The distinct modes of action of miRNAs have proved that contribution of miRNA towards gene expression regulation is highly significant. miRNAs have evolved as the critical regulators of cell type differentiation, proliferation and survival. Studies showed that alterations in the expression of miRNAs are clearly linked to the changes in numerous human, animal or plants disorders, cancer, in particular. However, the details regarding the regulation of their expression, biogenesis and transcriptional regulation are still in their infancy. Studies are required to investigate these details in order to enable a better understanding of miRNA regulation mechanisms. Based on increasing numbers of specific miRNA functional study, it is indispensable to construct a global view to understand miRNAs in different angles and their role in cell physiology and in various diseases.

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