Review Article

MicroRNAs: The Mega Regulators in Eukaryotic Genomes

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ABSTRACT

MicroRNAs (miRNAs) are endogenous, small, noncoding RNAs of 18-25 nucleotide (nt) in length that negatively regulate their complementary messenger RNAs (mRNAs) at the transcriptional and posttranscriptional level in many eukaryotic organisms. By affecting the gene regulation, miRNAs are likely to be concerned with most biological processes. Majority of the miRNA genes are found in intergenic regions or in anti-sense orientation to genes and have their own miRNA gene promoter and regulatory units. In contrast to their name and size, the miRNAs perform mega functions in eukaryotic organisms. They perform important functions in plants and animals during growth, organogenesis, transgene suppression, signaling pathway, environmental stresses, disease development and defense against the invading viruses. miRNAs are evolutionarily conserved from species to species within the same kingdom. However, there is a controversy among scientists about their conservation from animals to plants. Their conserved nature becomes an important logical tool for homologous discovery of miRNAs in other species. This review is aimed at describing some basic concepts regarding biogenesis and functions of miRNAs.

Keywords: Gene silencing, MicroRNA, Non coding RNAs, Translation suppression

Abbreviations

Caenorhabditis elegans (*C. elegans*)

Double-stranded RNA (dsRNA)

Lethal-7 (let-7)

Lineage-14 (lin-14)

Lineage-4 (lin-4)

Messenger RNAs (mRNAs)

MicroRNAs (miRNAs)

Nucleotide(s) (nt)

Precursor miRNAs (pre-miRNAs)

Primary miRNA (pri-miRNA)

RNA induced silencing complex (RISC)

Transfer RNA (tRNA)

Untranslated regions (UTRs)

MicroRNA

MicroRNA; once called small temporal RNA [1] is an important class of non-coding regulatory RNAs. In 1993 Lee et al, [2] discovered the first miRNA, lineage-4 (lin-4) during the study of gene lineage-14 (lin-14) in *Caenorhabditis elegans* (*C. elegans*) development. Later in 2000, Reinhart et al., [3] discovered lethal-7 (let-7) miRNA in *C. elegans*. In 2001, Lau et al., [4] discovered a large class of small RNAs in *C. elegans*, invertebrates and vertebrates with potential regulatory roles and named them microRNA. In 2002, Reinhart et al., [5] discovered miRNAs in plants. Since the discovery of the first miRNA, now thousands of miRNAs have been reported in many organisms including humans. The non-coding RNAs such as miRNA, transfer RNA (tRNA) and others constitute 3% out of the total 5% functional genome [6]. The human genome may encode over 1000 miRNAs [7]. In humans the miRNAs have been reported in mitochondria as well [8].

The miRNAs are endogenous, small, noncoding RNAs of 18-25 nt in length that negatively regulate

their complementary mRNAs at transcriptional [9] and posttranscriptional level in many eukaryotic organisms [10]. They feature in the genomes of most eukaryotic organisms, from the brown algae [11] to the other plants and animals. By affecting the gene regulation, miRNAs are likely to be concerned with most biological processes [12].

Biogenesis

The miRNAs are synthesized endogenously (Fig. 1) [13]. Most of the genes of miRNA are found in intergenic regions or in anti-sense orientation to genes and have their own miRNA gene promoter and regulatory units [4]. The genes of miRNAs are usually transcribed by RNA polymerase II [14]. The product is called a primary miRNA (Pri-miRNA) which may be thousands of nt in length and contains one or more miRNA stem loops [15]. This transcript is capped with a specially modified nt at the 5' end, polyadenylated with multiple adenosines at 3' end [14] and spliced. The polymerase often binds to the promoter found near the DNA sequence encoding the hairpin loop of precursor miRNA (pre-miRNA). When a stem loop precursor is found in the 3' UTR, a

transcript may serve as a pri-miRNA and a mRNA [16]. RNA polymerase III also transcribes some miRNAs especially those with upstream Alu sequences, tRNAs and mammalian wide interspersed repeat (MWIR) promoter units [17].

Primary transcripts of mature miRNAs fold into a stable stem loop structure forming pre-miRNAs. The pri-miRNAs are processed in the nucleus by the microprocessor complex, consisting of the RNase III enzyme Drosha, and the double-stranded RNA (dsRNA) binding protein, Pasha/DGCR8 [18]. After being processed the pri-miRNA is changed into pre-miRNA. The resulting precursor miRNAs are approximately 70 nt in length in case of animals and in case of plants its length may be 90-140 nt. These are then folded into imperfect stem-loop structures. The pre-miRNA hairpins are exported to the cytoplasm where they are further processed into unstable, 19-25 nt miRNA duplex structures by the RNase III protein Dicer [19] and in case of plants it is processed by a Dicer like enzyme [20]. The less stable of the two strands in the duplex is incorporated into a multiple-protein nuclease complex, the RNA induced silencing complex (RISC), which is also known as a microRNA ribonucleoprotein complex: (miRNP) [21]. The RISC negatively regulates gene expression either by inhibiting translation elongation or by triggering mRNA destruction on the basis of the degree of complementarity of miRNA within its target [22;23].

The animal mRNA target has many weak miRNA complementary sites, so miRNA imperfectly match to these sites and suppress gene expression [24; 25]. A 'seed region' of about 6-8 nt in length at the 5' end of an animal miRNA is thought to be an important determinant of target specificity [26]. The plant mRNA target has single and perfect or near perfect miRNA complementary site, so miRNAs perfectly match this site and trigger the mRNA degradation [27]. Usually miRNA target sites are present at the 3' untranslated regions (UTRs) of the mRNAs [24]. Most commonly animal miRNAs are complementary to a site in the 3' UTR whereas plant miRNAs are usually complementary to the coding regions of mRNAs [28]. A single miRNA can target several genes and several miRNAs can regulate a single gene [29].

Some miRNAs derived from short intronic hairpins are termed "mirtrons" and their nuclear biogenesis

appears to bypass Drosha cleavage, which is essential for miRNA biogenesis [13]. The introns which act as miRNAs are called mirtronic introns. Once it was thought that "mirtrons" only exist only in *Drosophila* and *C. elegans*, but later mirtrons were also found in mammals [30].

In some cases a microRNA gene is transcribed simultaneously with its host gene; providing a mean for coupled regulation of miRNA and protein-coding gene [31]. The primary transcripts of miRNAs commonly produce more than one functional product, by at least three different mechanisms. The miRNAs are often produced from polycistronic transcripts together with other miRNA precursors [32].

Function

In contrast to their name and size, the miRNAs perform mega functions in eukaryotic organisms. They perform important functions in plant and animals during growth [33], organogenesis, [33; 26], transgene suppression [34] signaling pathway, [35], environmental stresses, [36; 37], disease development [38], and defense against the invading viruses [39].

In plants miRNAs are implicated in diverse aspects of plant growth and development including leaf morphology and polarity, lateral root formation, hormones signaling, transition from juvenile to adult vegetative phase and vegetative to flowering phase, flowering time, floral organ identity and reproduction [40].

An increasing body of evidence has shown that miRNAs function not only during development [41] but also in disease progression [38]. Another study led by researchers at The Wistar Institute showed that miRNAs can undergo a kind of molecular editing with significant physiological consequences. A single substitution in their sequence can redirect these miRNAs to target and silence entirely different sets of genes from their unedited counterparts. Further, errors in the editing can lead to serious health problems. Perhaps as many as 16% of pri-miRNAs may be altered through nuclear RNA editing [42].

A recent study showed that miRNAs not only control the activity of genes within a cell but also can move from cell to cell to send signals that influence gene expression on a broader scale [43].

Figure 1. Explaining the biogenesis of a miRNA.
Liu et al. [13]

Conservation

The miRNAs are evolutionarily conserved from species to species within the same kingdom. However there is a controversy among scientists about their conservation from animals to plants [44; 45]. The miRNA genes in one species may exist as orthologs in other species [44]. Their conserved nature becomes an important logical tool for homologous discovery of miRNAs in other species [46]. MicroRNA researchers are interested to study the conserved miRNAs in many organisms. To study the conservation and divergence of plant miRNA genes, Zhang et al. [47], have done valuable work. In their study, they identified 481 miRNAs in 71 different plant species and found solid evidence that miRNAs are highly conserved in the plant kingdom, irrespective of the time of evolutionary divergence. They found that eighteen families of miRNAs had orthologs in more than 10 different plant species, spanning the breadth of green plant phylogeny. Among these, miRNA 156/157, miRNA 172 and miRNA 170/171 had orthologs in 45, 24 and 22 different plant species which belong to 21, 12 and 12 plant families respectively. The miRNAs found in more than 10 different plant families were considered

as highly conserved miRNAs. In addition to these three miRNA families, miRNA 165/166, miRNA 159/319, miRNA 396, miRNA 168, miRNA 160 and miRNA 390 were also found in at least 10 plant families; these six miRNA families are also considered as highly conserved miRNAs. Ten miRNA families (miRNAs 394, 164, 169, 167, 162, 398, 414, 393, 397 and 163) were found in 5–9 different plant families. Zhang et al., [47] classified these miRNA families as moderately conserved miRNAs. This suggests that these miRNAs play important and conserved functions in plant development, such as flower and leaf development. Zhang et al., [47] also found that some miRNAs are less conserved or non-conserved in plants. These non-conserved miRNAs may play roles in more species-specific characteristics in plant development, such as cotton fiber differentiation, elongation and development.

In a previous study, miRNAs 163 and 158 were identified as non-conserved miRNAs in plants [48]. After expressed sequence tags (EST) analysis, five and four plant species have been identified as having homologs of these two miRNAs, respectively. They

could actually be classified as moderately or low conserved miRNA families.

Sunkar and Jagdeeswaran [40] also found conserved miRNA families in a large number of diverse plant species. For instance, they found 23 miRNA families in maize, 19 in sorghum, 15 in wheat, and 14 in *Citrus spp.* Other notable miRNA families were found in some important plant species: 12 in grapes, 11 in tomato, 10 in sugarcane and 7 in potato. They also found five families (miR159, miR160, miR164, miR166 and miR168) conserved in gymnosperms and two (miR396 and miR408) in *Selaginella*. Besides this miR156/157, miR165/166, miR169, miR319 and miR394 homologs were found in 45, 40, 41, 51, and 40 diverse plant species, respectively. Six families (miR159, miR160, miR167, miR170/171, miR396 and miR399) were found in 30–39 diverse plant species. Similarly, seven families (miR164, miR168, miR172, miR393, miR395, miR398 and miR408) were found in 20–29 diverse plant species. The families, miR162, miR390, miR397, miR403 and miR437 were found in 10–19 diverse plant species.

By the work of many researchers it is evident that a comparatively lesser number of annotated MIRNA gene families are conserved between plant families, while the majority of these are family or species-specific, suggesting that most known MIRNA genes arose relatively recently in evolutionary time.[49]

MicroRNA Databases

With the discovery of a large number of miRNAs and their target genes, some public resources have been constructed to store their sequences e.g. miRBase (<http://microrna.sanger.ac.uk/>), provides a set of precursor and mature miRNAs discovered in many plants [50]. Similarly, *Arabidopsis thaliana* Small RNA Project (ASRP) (<http://asrp.cgrb.oregonstate.edu/>) lists miRNAs and their target genes in *Arabidopsis* [51]; Cereal small RNAs Database (CSRDB) (<http://sundarlab.ucdavis.edu/smrnas/>) is a collection of miRNAs identified in maize and rice [52]; Rfam (<http://rfam.sanger.ac.uk/>) provides secondary structures of miRNA precursors in many plant species [53]. The Plant Micro RNA Database (PMRD) (bioinformatics.cau.edu.cn/PMRD) is a collection of 8433 miRNAs taken from 121 plant species [54].

MicroRNA and Human Diseases

Besides being involved in the normal functioning of the eukaryotic cells, the miRNA deregulation is also the cause of many diseases. In humans their deregulation leads to chronic lymphocytic leukemia [55], and many other types of cancer [56], hearing loss [56], Schizophrenia [57], and heart diseases [58]. There is evidence that many age-related diseases are

associated with a decreased control of cell signaling that occurs in mid-life [59]. There is a publicly available databank, miR2Disease [60], which documents known relationships between miRNA deregulation and human disease.

Conclusion

Even after the 20 years of their discovery, our knowledge regarding the biology of these tiny molecules is at initial stages while the number of discovered miRNAs is increasing day by day. Their application in therapeutics, agriculture and Medicine is being promised by the scientists and their regulatory pathways are now being studied in detail. Since the miRNAs play very important roles in the eukaryotic organisms, these may truly be called the “**mega regulators**” of the eukaryotic genomes.

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