General Impact Factor (GIF): 0.875
Scientific Journal Impact Factor: 1.205

e- ISSN: 2394-5532
p- ISSN: 2394-823X

International Journal of Applied And Pure Science and Agriculture

www.ijapsa.com

THE MICRO WORLD OF microRNA

Lekshmi R. S. and Dr. Soni K. B.

Department of Plant Biotechnology, College of Agriculture, Kerala Agricultural University, Vellayani, Trivandrum

1. Introduction

Four decades ago, when central dogma was formulated RNAs have been looked as simple molecules that merely convert genetic information into protein. It has been estimated that almost 97% of the genome does not encode proteins in higher eukaryotes; but are transcribed and its hard to believe these vast transcripts have no function. Now, putative non-coding RNAs (ncRNAs) have been discovered that function directly as RNA rather than encoding proteins. ncRNAs seem to be particularly suited for roles that require highly specific nucleic-acid recognition. The view of RNA has changed from the purely informational medium to a variety of structural, informational and even catalytic molecules in the cell. Many other ncRNAs that function as regulators have also been discovered, but their number and importance seem marginal. Recently, a remarkably large number of tiny ncRNA genes have been identified. Some of the small non-coding or nontranslated RNAs in the cell are (Grosshans et al., 2002)

1. transfer RNAs (tRNA) – Functions as adapters in translation,
2. small nuclear RNAs (snRNA) – involved in mRNA splicing
3. small nucleolar RNA (snoRNA) – directs modification of ribosomal RNA
4. small interfering RNA (siRNA)
5. micro RNA (miRNA) }
6. small temporally regulated RNA (stRNA) } – down regulation of gene
7. piwi interacting RNA (piRNA) } expression by RNAi
8. repeat associated siRNA (rasiRNA) pathway
9. natural antisense siRNA (nat-siRNA)- expressed specifically from overlapping genes during specific environmental constraints

2. microRNA (miRNA)

In the DNA of humans, flies and worms are genes for short (21–24 nucleotides) noncoding RNAs, dubbed microRNAs (miRNAs). The Tuschl lab came to know about miRNAs while they were searching for the RNA interference (RNAi) products and its mechanism which were also about 22 nucleotide (nt) long (Lau et al., 2001). Later the Bartel and Ambros also suspected the presence of too short RNAs other than those already known to control development of the organism. Later these 3 groups found nearly 100s of new miRNA genes, and also some genes which are conserved in flies worms and humans (Moss, 2002).

miRNA have regulatory roles in developmental timing, patterning, embryogenesis, differentiation, organogenesis, stem cell and germline proliferation, growth control, apoptosis,endocrine function (Long et al., 2007) fat metabolism in flies (Brennecke et al., 2003; Xu et al., 2003), neuronal patterning, modulation of haematopoetic lineage differentiation in mammals (Chen et al.,2004), control of leaf and flower differentiation in plants (Aukerman and Sakai, 2003)

2.1 The First MicroRNAs: lin-4 and let-7

The *Caenorhabditis elegans* genes for the miRNAs, lin-4 and let-7 were discovered by the way of their mutant phenotypes (Chalfie et al., 1981; Reinhart et al., 2000). Strains carrying a mutation in either of these genes display retarded development, with some cells failing to divide and

differentiate on schedule (Ambros, 2000). When these genes were cloned, they were found to encode unrelated 21–22 nucleotide RNAs (Reinhart et al., 2000; Lee et al., 1993). lin-4 miRNA controls hypodermal cell-fate decisions during early larval development by negatively regulating lin-14 and lin-28 mRNAs (Lin et al., 2003). The let-7 miRNA controls hypodermal cell-fate decisions during late-larval development by regulating the lin-41 and hbl-1 daf-12 and pha-4 mRNAs (Slack et al., 2000; Lin et al., 2003).

Both of these RNAs are believed to act by basepairing with the RNA of the 3′ untranslated regions (UTRs) of one or more target genes in the developmental timing pathway (Whitman et al., 1993; Slack et al., 2000). Mechanisms was not fully understood but this interaction leads to repression of the target genes at a post-transcriptional step (Olsen et al., 1999; Seggerson et al., 2002). Later it was found that *let-7*-like RNA can be found in diverse animals, including humans, demonstrating that these tiny RNAs are not peculiar to worms. Because they are different from any non-coding RNAs described previously, in their size and activity on mRNAs, *lin-4* and *let-7* represent a new class of RNAs, recently dubbed small temporal RNAs (stRNAs) for their roles in developmental timing.

2.2 Why were not more genes for tiny RNAs like *lin-4* and *let-7* found before?

The genetic approaches that identified *lin-4* and *let-7* relied on finding mutant animals by chance. But the genes for these RNAs present very small targets for mutagens. In all the many genetic screens conducted with *C. elegans* to find mutants with defective larval development, a mutation in *lin-4* appears to have turned up only once, so it could have easily been missed. When taking a biochemical approach to finding an unknown regulator of a gene's expression, most seek protein factors and would not think to look for small RNAs, which would require very different assays than are normally used. A gene-finding approach that relies on detecting RNA transcripts would also miss the miRNAs because they run off the bottom of gels most people use for northern blots. Bioinformatics approaches are also currently limited (Eddy, 1999). Without a significant open reading frame (which would reveal a protein-encoding gene) or significant similarity to genes for known RNAs, such as tRNAs and snRNAs, genes for noncoding RNAs lie camouflaged against the background of intronic and intergenic sequences.

3. Genomics

3.1 The miRNA genes

The 3 labs which were initially working on miRNA reported a total of one hundred genes for this tiny ncRNAs, approximately 20 new genes in Drosophila, 30 in human and over 60 in C. elegans (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). A registry has been set up to catalog the miRNAs and facilitate the naming of newly identified genes (Griffiths-Jones, 2004).

- Most miRNA genes (in C. elegans) come from regions of the genome quite distant from previously annotated genes, implying that they derive from independent transcription units (Lagos-Quintana et al 2001; Lau et al., 2001; Lee and Ambros, 2001).
- A sizable minority (about a quarter of the human genes) are in the introns of pre-mRNAs They are preferentially found in the same orientation as the predicted mRNA, suggesting that most of these miRNAs are not transcribed from their own promoters but are instead processed from the introns (Aravin et al., 2003; Lagos-Quintana et al., 2003; Lai et al., 2003; Lim et al., 2003). This arrangement provides a convenient mechanism for the coordinated expression of a miRNA and a protein.
- Other miRNA genes are clustered in the genome with an arrangement and expression pattern implying transcription as a multi-cistronic primary transcript. some genomic loccation include Hox clusters which function in animal development (Aravin et al., 2003; Lagos-

Quintana et al., 2003). other loci include mir-15-a and mir- 16 clusters found in human chromosome 13 thought to harbor a tumor suppressor gene (Calin et al., 2002).

3.2 miRNA expression

miRNAs have intriguing expression patters. For example miR-1 is found in mammalian heart (Lee and Ambros, 2001); miR-122 in granulocytes and macrophages of mouse bone marrow (Chen et al., 2004); mir-35 – mir42 cluster are found in C. elegans embryo (Lau et al., 2001); mir-290-mir-295 cluster in mouse embryonic stem cell but not in differentiated cells (Houbaviy et al., 2003). Expression technology has been adapted to examine miRNAs and has revealed distinct expression patterns in different developmental stages and in different tissues (Krichevsky et al., 2003). With all the different genes and expression patterns it can be proposed that every metazoan cell type at each developmental stagemight have a distinct miRNA expression profile-providing ample opportunity for 'microimaging' the output of the transcriptome.

Sheer abundance of certain miRNAs in the cells can be seen at times. This can be attributable to very robust transcription or to slow decay. Some miRNAs are expressed in much lower levels and this might be due to low expression in many cells or high expression in just a few cells (Lagos-Quintana et al., 2002).

3.3 Gene number

With these scoring tools the estimated number of miRNA genes in the genomes of human– 200 -255 miRNA genes (Lim et al., 2003b); *C. elegans* – 103-120 (Lim et al., 2003b); Drosophila – 96-124 (Lai et al., 2003).

4. Biogenesis

4.1 miRNA transcription

Two candidates of RNA polymerases for pri-miRNA transcription pol ll and pol lll.
miRNAs processed from the introns of protein coding host genes are transcribed by pol ll. These primary miRNA transcripts are called pri-miRNA and are much longer than structure with mant stem and loops (Lee et al., 2002).

Most of the miRNAs are transcribed by pol ll (Proved by certain observations) (Ohler et al., 2004)

1. The pri-miRNA can be quite longer (>1 kb) than typical pol lll transcripts
2. These pri-miRNAs have internal runs of uridine residues, which would be expected to prematurely terminate pol lll transcription
3. Many miRNAs are differentially expressed during development observed for pol ll and not with pol lll products
4. miRNA primary transcripts are capped pol ll transcripts

4.2 miRNA maturation

The first step is the nuclear cleavage of the pri-miRNA, which liberates a 60-70 nt stem loop intermediate, known as the miRNA precursor, or pre-miRNA (Lee et al., 2002; Zeng and Cullen, 2003). The processing is performed by the Drosha RNase III endonuclease, which cleaves both strands of the stem at sites near the base of the primary stem loop. Drosha cleaves the RNA duplex with a staggered cut typical of RNase III endonucleases, and thus the base of the pre-miRNA stem loop has a 5'phosphate and a 2 nt 3; overhang (Basyuk et al., 2003; Lee et al., 2003). This pre-

miRNA is actively transported from the nucleus to the cytoplasm by Ran-GTP and the export receptor exportin-5 (Yi et al., 2003; Lund et al., 2004)

The Drosha defines only end of the mature miRNA during the nuclear cut. The other end is processed in the cytoplasm by the enzyme Dicer, an RNase III endonuclease. It first recognizes the double-stranded portion of pre-miRNA with particular affinity for a 5' phosphate ans 3' overhang at the base of the stem loop. Then at about 2 helical turns away from the base of the stem loop, it cuts both strands of the duplex. This lops off the terminal base pairs and loop of the pre-miRNA, leaving the 5' phosphate and 2 nt 3' overhang characteristic of an RNase III.

4.3 RISC assembly

The mature miRNA eventually become incorporated as single stranded RNAs into a ribonucleoprotein complex, known as the RNA-induced silencing complex (RISC) (Hammond et al., 2000; Elbashir et al., 2001; Schwarz et al; 2002). The RISC identifies target messages based on perfect (or nearly perfect) complementarity measuring from the 5' end of the miRNA cutting between the nucleotides pairing to residues 10 and 11 of the miRNA

When RISC has been purified from various organisms it contained a member of the Agronaute protein family. This protein has been found to be a core component of the complex Ago and homologs (approx100kDa) are often called PPD proteins PPD proteins share the PAZ and PIWI domains. PAZ domain (First recognized in Piwi, Agronaute, and Zwille/Pinhead proteins) Has a stable fold and a β barrel core that togeather with a side appendage appears to bind weakly to ssRNA atleast 5 nt in length and also to dsRNA This dual binding ability suggests that the Agronaute protein could be directly associated with the miRNA before and after it recognizes the mRNA target. Other RISC-associated proteins include the suspected RNA binding proteins VIG and Fragile X-related protein and the nuclease Tudor-SN (Caudy et al., 2002, 2003; Ishizuka et al., 2002)

4.4 Role of Agronaute protein as mediator of mRNA cleavage

miRNAs, when perfectly base-paired to their target mRNA, direct cleavage of a single phosphodiester bond in the target mRNA. The severed bond lies between the residues paired to miRNA nucleotides 10 and 11 (counting from the miRNA 50 end) (Elbashir et al. 2001). This cleavage is a result of the ''Slicer'' activity in the RISC. Argonautes form the core of the RISC and are highly conserved _100-kDa proteins with members present even in archaea and eubacteria. They contain the signature PAZ and PIWI domains (Carmell et al. 2002). Argonaute PAZ domain has an oligonucleotide-binding fold that anchors the single-stranded 30 end of small RNAs (Lingel and Sattler 2005).

Most importantly, the PIWI domain of two archaeal proteins was shown to have a fold similar to that of RNase H (Parker et al. 2004; Song et al. 2004), an enzyme that cleaves the RNA strand in DNA–RNA hybrids. This suggested that the Ago protein could harbor the mRNA-cleaving ''Slicer'' activity. Information regarding recognition of the small RNAs came from cocrystal structures of an archaeal Ago protein with siRNA mimics, which revealed that the PIWI domain has a conserved binding pocket for the 50 phosphate of small RNAs (Parker et al. 2005; Ma et al. 2005). These studies suggest a model in which the small RNA is wedged between the PAZ and PIWI domains of the Ago protein, positioning the target mRNA scissile bond in close proximity to the catalytic center.

5. Mechanism

5.1 mRNA cleavage

MicroRNAs can direct the RISC to downregulate gene expression by either of two post transcriptional mechanisms: mRNA cleavage or translational repression. The choice of post transcriptional mechanisms is determined by the identity of the target: Once incorporated into a

cytoplasmic RISC, the miRNA undergo Watson-Crick base pairing at the 'seed sequence' to form a A-form helix with the target mRNA that will specify cleavage between the nucleotides pairing to residues 10 and 11 of the miRNA, if mRNA has sufficient complementarity to the miRNA, or it will repress productive translation if mRNA does not have sufficient complementarity to be cleaved. After cleavage of the mRNA, the miRNA remains intact and can guide the recognition and destruction of additional messages (Hutvagner and Zamore, 2002; Tang et al., 2003).

A mojor part of bulk mRNA degradation occurs via de-adenylation followed by decapping and 5'-3' exonuclease digestion. Proteins required for this pathway such as decapping enzymes, Dcp1 and Dcp2 and 5'-3' exonuclease Xrn1 are concentrated in specialized cytoplasmic processing bodies (PBs) (Bashkirov et al., 1997; Parker and Song 2004)

5.2 Translational repression

The miRNA can repress translation at a step after translation initiation,in a manner that does not perceivably alter the density of the ribosomes on the message, by the slowing or stalling of all the ribosomes on the message. An alternate possibility is that translation continues at the same rate but is non reproductive because the newly synthesized polypeptide is specifically degraded by proteolytic enzymes.

5.3 Target recognition

The importance of complementarity to the 5' portion of metazoan miRNAs has been noted since the observation that the lin-14 UTR has 'core elements' of complementarity to the 5' region of the lin-4 miRNA (Whitman et al., 1993). Observations supporting this idea are

1. Residues 2-8 (seed sequence) of several invertebrate miRNA are perfectly complementary to 3'UTR elements mediating post transcriptional repression (Lai 2002)
2. Within the miRNA complementary sites of the first validated targets of invertebrate miRNAs, mRNA residues that pair with the residues 2-8 of the miRNA are perfectly conserved in orthologous messages of the other species
3. Residues 2-8 of the miRNA are the most conserved among homologous metazoan miRNAs (Lewis et al., 2003; Lim et al., 2003)
4. When predicting targets of mammalian miRNAs requiring perfect pairing to the heptamer spanning residues 2-8 of the miRNA is much more productive than is requiring pairing to any other heptamer of the miRNA (Lewis et al., 2003)

5.4 5' end of the small RNA universally important regardless of the mechanism

RISC presents this core region to nucleate pairing to the mRNAs. Presentation of these nt prearranged in the geometry of an A-form helix would preferentially enhance the affinity with matched mRNA segments. Presentation of a preformed helical segment of this length would be a reasonable compromise between topological difficulties associated with longer prearranged helical geometry and the drop in initial binding specificity that would result from a shorter core. In this scenario mismatches with the core region inhibits initial target recognition and thus prevent cleavage or translational repression regardless of the degree of complementarity elsewhere in the complimentary sites. It there is sufficient additional pairing after the remainder of the miRNA is allowed to participate, cleavage ensues. Core pairing supplemented by just a few flanking pairs appears to be sufficient to mediate translational repression in cooperation with other RISCs bound to the message (Lewis et al., 2003). The ability of the Agronaute PAZ domain to bind both double and single stranded RNAs would make it suitable candidate for presenting the core and stabilizing the core pairing.

6. Function

6.1 Regulatory roles of miRNAs

miRNAs that have reported functions based on in vivo experimentation are listed below. MicroRNAs and Their Functions: Examples for which Phenotypic Consequences of Disrupted or Ectopic miRNA Regulation Are Known

miRNA	Target Gene(s)	Biological Role of miRNA/Target Gene
Nematodes		
lin-4 RNA	*Ce lin-14* probable transcription	Timing of early larval developmental factor transitions
	Ce lin-28 cold shock domain protein	Timing of early larval developmental transitions
let-7 RNA	*Ce lin-41* probable RNA-binding protein	Timing of late larval developmental transitions
	Ce hbl-1 transcription factor	Timing of late larval developmental transitions
lsy-6 RNA	*Ce cog-1* transcription factor	Left/right asymmetry of chemoreceptor expression
Insects		
bantam miRNA	*Dm hid* pro-apoptotic protein	Apoptosis and growth control during development
miR-14	unknown	Apoptosis and fat metabolism
Mammals		
miR-181	unknown	Hematopoietic differentiation 11
Plants		
miR165/166	*At REV* and relelated transcription factors	Axial meristem initition and leaf development
miR172	*At AP2* and related transcription factors	Flower development; timing transition to flowerring
miR-JAW	*At TCP4* and releated transcription factors	Leaf development, embryonic patterning
miR159	*At MYB33* and related transcription factors	Leaf development

Species abbreviations: *Caenorhabditis elegans*, *Ce*; *Drosophila melanogaster*, *Dm*; *Arabidopsis thaliana*, *At.* (Lee et al., 1993); (Wightman et al., 1993); (Moss et al., 1997); (Reinhart et al., 2000); (Slack et al., 2000); Abrahante et al., 2003); (Lin et al., 2003); (Johnston and Hobert, 2003); (Brennecke et al., 2003); (Xu et al.,2003); (Chen et al., 2004); (Rhoades et al.,2002); (Tang et al., 2003); (Emery et al., 2003); (Park et al., 2002); (Kasschau et al., 2003); (Chen, 2003); (Aukerman andSakai, 2003); (Palatnik et al., 2003)

For some cases fuction was determined by phenotypic consequences of a mutated miRNA or an altered miRNA complementary site, either of which can disrupt miRNA regulation. In some other cases function was inferred from effects of mutations or transgenic constructs that lead to ectopic expression of the miRNA. Computational approaches are being developed to find the regulatory targets of the miRNAs, providing clues to miRNA function based on known roles of the targets (Rhodes et al., 2002; Enright et al., 2003; Lewis et al.,2003; Stark et al., 2003).

The experiments supporting the identity of these targets typically fall into two classes. In cases where the miRNA is thought to specify mRNA cleavage, the cleavage products can be reverse transcribed, cloned, and sequenced; a preponderance of sequences that end precisely at the predicted site of cleavage provides experimental validation that this mRNA is the cleavage target of the complementary miRNA (Llave et al., 2002; Kasschau et al., 2003; Xie et al., 2003). To enable detection of both translational repression and mRNA cleavage, heterologous reporter assays can be used in which the miRNA complementary sites are fused to e reporter gene and expression is examined relative to control constructs, or in the presence and absence of the miRNA (Lewis et al., 2003). A positive result in the heterologous reporter assay indicates that determinants needed for miRNA regulation are present within the mRNA fragment fused to the reporter, which together with evolutionary conservation of both the miRNA and its complementary sites can provide reasonable evidence of a regulatory relationship.

7. Role of the precursor in miRNA function

The abundance of stem-loop structures in pre-miRNAs, as well as the significant conservation of these structures in multiple organisms where homologous miRNAs were found, suggests that these structures might be a prerequisite for miRNA maturation and possibly for function. One attractive hypothesis is that transcription as a precursor might allow another level of regulation of miRNA activity . Northern blot analysis suggest that pre-miRNA is continously transcribed but maturation to miRNA occurs only at distinct stages of development. Structured pre-miRNA is exported to processing machinery in the cytoplasm by dedicated tRNA nuclear export factor, Xpo-t. Proteins that participate in processing are involved in delivery of the mature miRNAs to the site of action so that miRNAs are handed over from one biogenesis factor to the next without intermittent release.

8. Roles of plant miRNAs

In plants miRNA pair with mRNA with near-perfect complementarity and lead to mRNA cleavage (Tang et al., 2003; Jones-Rhoades et al., 2006). miRNA:mRNA pairing in plants is evolutionarily conserved. Plant miRNAs target transcription factor gene families for those with roles in developmental patterning or cell differentiation. Many of the plant miRNAs function during cellular differentiation by mediating the degradation of key regulatory gene transcripts in specific daughter cell lineages (Rhodes et al., 2002). During differentiation, certain genes specifying a less differentiated state might need to be turned off. This can be achieved by repressing transcription. A gene is not fully off until its message stops making protein. Thus to more quickly stop expression of such a gene, the differentiating cell can deploy a miRNA that specifies the cleavage of that mRNA. In this respect, miRNA regulation would be analogous to ubiquitin- dependent protein degradation.

9. Roles of animal miRNAs

Animal miRNAs do not pair with mRNA with near-perfect complementarity. In contrast to plant miRNA mammalian miRNAs do not appear to be primarily involved at the upper levels of the gene regulatory cascades but appear to be operating at many levels to regulate the expression of a diverse set of genes, many of which do not go on to directly influence the expression of other genes (Lewis et al., 2003). Animal miRNAs imprecisely match their targets and cause target mRNA destabilization by other (non-slicer) mechanisms, such as deadenylation and decapping (wu et al., 2006; Giraldez et al., 2006), and some form of translational repression (Olsen and Ambros 1999).

10. miRNAs and gene knockdown

Elisa Izaurralde (Max Planck Institute) studies whether there is any effect of specific miRNAs or associated 3' UTRs have context-dependent effects on knockdown by using natural 3' UTRs fused to a luciferase reporter to perform genome-wide RNA interference screens for proteins required for miRNA function and she isolated components of processing bodies (PBs) and de-adenylation and decapping enzymes (Behn- Ansmant et al., 2006). Only certain targets were

repressed by miRNA in the absence of de-adenylation or decapping enzymes, which indicates that decapping of de-adenylated mRNAs is the primary mode of repression by some, but not all, miRNA-target interactions.

11. miRNAs and cancer

microRNA expression patterns appear to be more reliable than mRNA expression patterns for distinguishing the tissues of origin of human tumors (Lu et al.,2005). Croce reported that both *MIR-15* and *MIR-16* are tumor suppressors in chronic lymphocytic leukemia. Role of *MIR-21* (also known as *MIRN21* – Human Gene Nomenclature Database) in mammalian brain tumors is determined. The pretreatment of human brain tumor cells with anti-*MIR-21* oligonucleotides inhibits their tumorigenicity when transplanted into mice. *MIR-21* overexpression is a hallmark of certain classes of brain tumors, so these results suggest that *MIR-21* could indeed contribute to the malignancy of these tumor cells, probably via the repression of one or more tumor suppressor genes.

The converse situation, where tumor-suppressive microRNAs may function to repress oncogenes, was illustrated by David Bartel (Whitehead Institute and MIT, Cambridge, MA, USA). The oncogenic behavior of certain mutated forms of High mobility group A2 (HMGA2) in humans is induced by the deletion of the *HMGA2* 3' UTR (Mayr et al., 2007), which contains several evolutionarily conserved sites for *LET-7*, a microRNA with potential tumorsuppressive activity (Johnson et al., 2005).

12. The question of specificity

Proteins or mRNA structure could also facilitate recognition of the authentic mRNA targets my means of elements in the mRNA eg: -Fragile X-related protein, Drosophila RISC component that is related to proteins known to bind specific mRNAs (Caudy et al., 2002; Ishizuka et al., 2002). The quality and stability of base pairing is an important determinant of specificity. Pairing outside the 7 nt core site confers additional specificity. The cooperative action of homotypic and heterotypic miRNA: UTR interaction provides an additional mechanism of regulatory specificity (Bartel, 2004).

1. Genomics: Computational approaches

Homology searches have revealed orthologs and paralogs of known miRNA genes (Pasquinelli et al., 2000;Lagos-Quintana et al., 2001;Lau et al., 2001; Lee and Ambros., 2001). Search the vicinity of known miRNA genes for other stem loops representing additional genes of a genomic cluster (Lau et al., 2001; Arvin et al., 2003; Seitz et al., 2003; Ohler et al., 2004).Gene finding approach does not depend on homology or proximity to known genes. Does not need any rior idea about the genome. Start by identifying conserved genomic segments that fall outside of the predicted protein coding regions and potentially could form stem loops. Then these candidate miRNA stem loops are scored for the patterns of conservation and pairing that characterize known miRNA genes.

Two most sensitive computational scoring tools are MiRscan and miRseeker.

1. **MiRscan** – applied to nematode and vertebrate candidates (Lim et al., 2003a, 2003b),
2. **miRseeker** – applied to insect candidates (Lai et al., 2003)
3. **miRFinder 4**.0 - high throughput and good performance computational pre-miRNA prediction tool. The tool was designed for genome-wise, pair-wise sequences from two related species. In our experiments, the MiRFinder has demontracted reasonably better sensitivity and speed comparing to similar tools. Hosted by Huazhong Agricultural University, Wuhan, China.

There is yet another program called **Sfold** which can be used to investigate the effect of target secondary structure on the efficacy of repression by miRNAs. Using structures predicted by Sfold

program, the interaction between an miRNA and a target as a two-step hybridization reaction. Nucleation at an accessible target site followed by hybrid elongation to disrupt local target secondary structure and form miRNA target duplex. The reliable performance of the model strongly suggests a potent effect of mRNA secondary structure on target recognition by miRNAs.

15. miRNA target databases

(www.ncRNA.org)

miRanda - miRNA target prediction for human, drosophila and zebrafish genomes by using miRanda.

miRBase - Fromer "microRNA Registry", which is a comprehensive repository for miRNAs and their predicted targets. The target prediction is performed by using miRanda software.

miRNAMap - miRNAMap is a genomic maps of microRNA genes and their target genes in mammalian genomes. The target prediction is performed with miRanda.

TarBase - TarBase is a comprehensive database of experimentally supported animal microRNA targets.

microInspector - A web server for microInspector that detects miRNA binding sites. No prediction results available.

PicTar - Micro RNA targets for vertebrates, fly and nematodes are available (no batch download). PicTar on-line service is available. Software is not available for download.

TargetScanS - TargetScanS is a software for microRNA targets. Prediction results for human genome are available. No batch download and no software download.

RNAhybrid - Program source code is available. Web service is available. Perl modules and Java Beans are available. No prediction results available.

miRNA - Target Gene Prediction at EMBL - This website provides access to our 2003 and 2005 miRNA-Target predictions for Drosophila miRNAs with their original experimental method.

DIANA MicroTest - Micro RNA target prediction software provided as a web service. Query (miRNA) and Subject (genes) sequences are required to be input. No software download. No prediction results available.

16. miRNA sequence and annotation database

16.1 miRBase

Hosted by Faculty of life sciences, University of Manchester and funded by BBSRC, previously hosted and supported by Wellcome Trust Sanger Institute. It is a searchable database of published miRNA sequence and annotation. Each entry in the miRBase sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database) with information on the location and sequence of the mature miRNA (termed miR). Both hairpin and mature sequences are available for searching and browsing and entries can also be retrieved by name, keywords, references and annotations. All sequences and annotation data are also available for download.

miRBase registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results.

17. Conclusion:

The miRNAs play an important role in gene silencing either through transcriptional or translational regulation. These small RNA species have shown their roles in the developmental process of the animals and plants and in various disease conditions. As miRNAs act by interfering with the target mRNA through gene knock down mechanism rather than the gene knck out. Insilco approaches are being used for the identification of miRNAs involved in these processes which may help to regulate the gene expression. miRNA induced RNAi pathway can be exploited for a vast range of applications in various fields. This is a booming area of research today.

Bibliography

[1] Ambros, V. 1993. The functions of animal microRNAs. *Nature*. 431: 350-355.
[2] Ambros, V. 2003. MicroRNA pathways in flies and worms: Growth, Death, Fat, Stress, and Timing. *Cell*. 113: 673-676.
[3] Ambros, V. 2003. A uniform system for microRNA annotation. *RNA*. 9: 277-279.
[4] Ambros, V. and Horvitz, H. R. 2004. Heterochronic mutants of the nematode *Caenorhabditis elegans. Science*. 226: 409-416.
[5] Ambos, V., Lee, R. C., Lavanway, A., Williams, P. T. and Jewell, D. 2003. MicroRNAs and other tiny endogenous RNAs in *C. elegans*. *Curr. Biol*. 13: 807-818.
[6] Aravin, A. A., Lagos-Quintana, M. Yalcin, A., Zavolam, M., Marks, D., Snyder, B. and Meyer, 2003. The small RNA profile during *Drosophila melanogaster* development. *Dev. Cell*. 5, 337-350.
[7] Aukerman, M. J. and Sakai, H. 2003. Regulation of flowering time and floral organ identity by a microRNA and its APETALA 2- like target genes. *Plant Cell*. 10: 709-717.
[8] Bashkirov, V. I., Scherthan, H., Solinger, J. A., Buerstedde, J. M., and Heyer, W. D. 1997. A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates. *J. Cell. Biol*. 136: 761-773.
[9] Brennecke, J., hipfner, D. R., Stark, A. and Cohen, S. M. 2003. Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in *Drosophila*. *Cell*. 113: 25-36.
[10] Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldier, H. and Rai, K. 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci*. USA. 99: 15524-15529.
[11] Carrington, J. C. and Ambros, V. 2003. Roleof microRNA in plant and animal development. *Science*. 301: 336-338.
[12] Carmell, M. A., Xuan, Z., Zhang, M. Q. and Hannon, G. J. 2002. The Agronaute family: Tentacles that reach into RNAi, developmental control, stem call maintenance, and tumorogenesis. *Genes and Dev*. 16: 2733-2742.
[13] Chalfie, M., Horvitz, H. R. and Sulston, J. E. 1981. Mutations that lead to reiteration in the cell lineages of *C. elegans*. *Cell*. 24: 59-69.
[14] Chen, C. Z., Li, L., Lodish, H. F. and Bartel, D. P. 2004. MicroRNAs modulate hematopoetic lineage differentiation. *Science*. 303: 83-86.
[15] Doench, J. G., Peterson, C. P. and Sharp, P. A. siRNAs can function as miRNAs. *Genes Dev.* 17: 438-442
[16] Eddy, S. R. 1999. Noncoding RNA genes. *Curr. Opin. Genet. Dev*. 9: 695-699.
[17] Elbashir, S. M., Martinez, J., Patkaniowska, A., Lendeckel, W. and Tuschl, T. 2001. Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J*. 21: 6877- 6888.
[18] Giraldez, A. J., Mishima, Y., Rihel, J., Grocock, R. J., Van Dongen, S., Inoue, K., Enright, A. J. and Schier, A. F. 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science*. 312: 75-79.
[19] Grosshans, H. and Slack, F. J. 2002. Micro-RNAs: small is plentiful. *J. Cell Biol*. 156: 17-21.
[20] Hammond, S. C. Bernstein, E., Beach, D. and Hannon, G. J. 2000. An RNA-directed nuclease mediates posttranscriptional gene silencing in *Drosophila* cells. *Nature*. 404: 293-296.
[21] Hodgkin, J., Papp, A., Pulak, R., Ambros, V. and Anderson, P. 1989. Anew kind of informational suppression in the nematode *Caenorhabditis elegans*. *Genetics*. 123: 301-313.
[22] Hutvagner, G. and Zamore, P. D. 2002. A microRNA in a multiple-turnover RNAi enzyme complex. *Science*. 297: 2056-2060.
[23] Jones-Rhodes, M. W., Bartel, D. P. and Bartel, B. 2006. MicroRNA and their regulatory roles in plants. *Annu. Rev. Plant Biol*. 57: 19-53.
[24] Ke, X., Liu, C., Liu, D. and Liang, C. 2003. MicroRNAs: key participants in gene regulatory networks. *Curr. Opinion in Che. Biol*. 7: 516-523.
[25] Lagos-Quintana, M., Rauhut, R., Lendeckel, W. and Tuschl, T. 2001. Identification of novel genes coding for small expressed RNAs. *Science*. 294: 853-858.
[26] Lai, E. C., Tomancak, P., Williams, R. W. and Rubin, G. M. 2003. Computational identification of *Drosophila* microRNA genes. *Genome Biol*. 4(42): 1-20.

[27] Lau, N. C., Lim., L. P., Weinstein, E. and Bartel, D. P. 2001. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science.* 294: 858-862.
[28] Lee, R. C., Feinbaum, R. L. and Ambros, V. 1993. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complemenrarity to lin-14. *Cell.* 75: 843-854.
[29] Lee, Y., Jeon, K., Lee, J. T., Kim, S. and Kim, V. N. 2002. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO. J.* 21: 4663-4670.
[30] Lim, L. P. Glasner, M. E., Yekta, S., Burge, C. B. and Bartel, D. P. 2003. Vertebrate microRNA genes. *Science*. 4: 991-1008.
[31] Lin, S. Y., Johnson, S. M., Abraham, M., Vella, M. C. and Pasquilini, A. 2003. The *C. elegans* hunchback homolog, hbl-1 controls temporal patterning and is probable microRNA target. *Dev. Cell.* 4: 639-650.
[32] Lingel, A. and Sattler, M. 2005. Novel modes of protein-RNA recognition in the RNAi pathway. *Curr. Opin. Stuct. Biol.* 15: 107-115.
[33] Long, D., Lee, R., Williams, P., Chan, C. Y., Ambros, V. and Ding, Y. 2007. Potent effect of target structure on microRNA function. *Nature Structural and Mol Bio*. 14(4): 287-294.
[34] Ma, J. B., Yuan, Y. R., Meister, G., Pei, Y., Tuschl, T. and Patel, D. J. 2005. Structural basis for 5' end pecific recognition of guide RNA by the A. fulgidus Piwi protein. *Nature*. 434: 666-670.
[35] Mayr, C., Henmann, M. T. and Bartel, D. P. 2007. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science*. 16: 1576-1579.
[36] Miska, E. A., Alvarez-Saavedra, E., Abbott, A. L., Lau, N. C., Hellman, A. B., McGonagle, S. M., Bartel, D. P., Ambros, V. R. and Horvitz, H. R. 2007. Most Caenorhabditis elegans miroRNAs are individually not essential for development or viability. *Plos Genetics.* 3(12): 2395-2403.
[37] Moss, E. G. 2002. MicroRNAs: Hidden in the genome. *Curr. Biol.* 12: 138-140.
[38] Moss, E. G., Lee, R. C. and Ambros, V. 1997. The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the lin-4 RNA. *Cell*. 88, 637-646.
[39] Olsen, P. H. and Ambros, V. 1999. The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev.Biol*. 216: 671-680.
[40] Parker, J. S., Roe, S. M. and Barford, D. 2004. Crystal structure of a PIWI protein suggests mechanisms for siRNA recognition and slicer activity. *EMBO J.* 23: 4727-4737.
[41] Pillai, R. S. 2013. MicroRNA function: Multiple mechanisms for a tiny RNA?. *RNA.* 11(12): 1753-1761.
[42] Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., Horvitz, H. R. and Ruvkun, G. 2000. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature*. 403: 901-906.
[43] Schwartz, D. S., Hutvagner, G., Haley, B. and Zamore, P. D. 2002. Evidence that siRNAs function as guides, not primers, in the *Drosophila* and human RNAi pathways. *Mol. Cell*. 10: 537-547.
[44] Sempere, L. F., Sokol, N. S., Dubrovsky, E. B., Berger, E. M. and Ambros, V. 2003. Temporal regulation of microRNA expression in *Drosophila melanogaster* mediated by hormonal signals and Broad-complex gene activity. *Dev. Biol.* Article in press.
[45] Sledz, C. A., and Williams, B. R. G. 2012. RNA interference in biology and diseases. *Blood J.* 106(3): 787-794.
[46] Slack, F. J., Basson, M., Liu, Z., Ambros, V, and Horvtiz, H. R. 2000. The lin-41 RBCC gene acts in the *C. elegans* heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. *Mol. Cell.* 5: 659-669.
[47] Song, J. J., Smith, S. K., Hannon, G. J, and Joshua, T. 2004. Crystal structure of Agronaute and its implications for RISC slicer activity. *Science.* 305: 1434-1437.
[48] Tang, G., Reinhart, B. J., Bartel, D. P. and Zamore, P. D. 2003. A biochemical framework for RNA silencing in plants. *Genes Dev*. 17: 49-63.
[49] Voinnet, O. 2002. RNA silencing: Small RNAs as ubiquitous regulators of gene expression. *Curr. Opinion in mol. Biol*. 5: 444-451
[50] Whightman, B., Ruvkun, G. 1993. Post transcriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell.* 75: 855-862.
[51] Wu, L., Fan, J. and Belasco, J. G. 2006. MicroRNAs direct `rapid deadenylation of miRNA. *Proc. Natl. Acad. Sci.* USA. 103: 4034-4039.
[52] Xu, P., Vernooy, S. Y., Guo, M. and Hay, B. A. 2003. The *Drosophila* microRNA mir-14 supresses cell death and is required for normal fat metabolism. *Curr. Biol.* 13: 790-795.
[53] Yi, R., Qin, Y., Macara, I. G. and Cullen, B. R. 2003. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes. Dev*. 17: 3011-3016.
[54] Zeng, Y., Yi, R. and Cullen, B. R. 2003. MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. *Proc. Natl. Acad. Sci.* USA. 100: 9779-9784.

