MicroRNA Interference Technologies

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Preface

MicroRNAs (miRNAs), endogenous noncoding regulatory mRNAs of around 22-nucleotides long, have rapidly emerged as one of the key governors of the gene expression regulatory program in cells of varying species. Accumulating evidence suggests that miRNAs constitute a novel, universal mechanism for fine regulation of gene expression in all organisms, "fine tuning" the cellular phenotype during delicate processes. Owing to their ever-increasing number in the mammalian cells and their ever-increasing implication in the control of the fundamental biological processes (such as development, cell growth and differentiation, cell death, etc), miRNAs have now become a research subject capturing major interest of scientists worldwide. Moreover, with recent studies revealing the macro roles of miRNAs in the pathogenesis of adult humans, we have now entered a new era of miRNA research. The exciting findings in this field have inspired us with a premise and a promise that miRNAs will ultimately be taken to heart for the therapy of human disease. Yet these mysterious tiny molecules still remain mystifying in their cellular function and pathological role. While miRNAs have been considered potential therapeutic targets for disease treatment, it remains obscure what strategies we can use to achieve this goal. Thorough understanding of these molecules is obviously a prerequisite for realizing their rousing promise, which calls for an urgent need to develop apt technologies for the purpose.

In the past years, we have witnessed the rapid development of many creative, innovative, inventive techniques and methodologies pertinent to miRNA research and applications. These technologies have convincingly demonstrated their efficacy and reliability in producing gain-of-function or loss-of-function of miRNAs, providing new tools for elucidating miRNA functions and opening up a new avenue for the development of new agents targeting miRNAs for therapeutic aims. These stimulating advances prompted me to propose the concept of microRNA interference (miRNAi): *Manipulating the function, stability, biogenesis or expression of miRNAs to interfere with the expression of their target protein-coding mRNAs to alter the cellular functions.* This new thought motivated me to write this book entitled *MicroRNA Interference Technologies (miRNAi Technologies)*.

The aim of this book is to provide comprehensive descriptions of the strategies and methodologies for interfering miRNA expression, biogenesis and function and their applications in miRNA research and new drug design using miRNAs as therapeutic targets. It is my expectation that from this book readers will be able to acquire a basic knowledge of miRNAs and the new concepts pertinent to miRNAi, gain insight into the principles of various miRNAi technologies and master the key steps of miRNAi protocols.

miRNAi Technologies contains 13 chapters. It begins with Chapter 1 on the updated knowledge of miRNA biology and their potential as therapeutic targets for human disease. Chapter 2 introduces four new concepts pertinent to miRNAs, which are of pivotal importance for our understanding and application of the miRNAi technologies. These new concepts are (1) the "miRNA Interference (miRNAi)" concept, (2) the "miRNA as a Regulator of a Cellular Function" concept, (3) the "One-Drug, Multiple-Target" concept and (4) the "miRNA Seed Family" concept. Chapter 2 also gives a laconic introduction of miRNAi strategies and the perspectives of miRNAi technologies in a general term. From Chaps. 3–13, each chapter introduces one of the miRNAi technologies with detailed descriptions of state-of-the-art design, step-by-step directive protocols, principles of action, applications to basic research, R&D and clinical therapy and advantages and limitations of the technologies. Chapters 3–6 describe various gain-of-function miRNAi technologies and chapters 7–13 introduce the loss-of-function miRNAi technologies.

Each chapter also contains illustrations, flowcharts and tables for easier and straightforward understanding of the contents. Though step-by-step protocols are provided for each miRNAi technology, it is not my attempt to give very detailed, problem-proof procedures by including information like compositions of solutions, conditions of reactions and materials. Instead, I intend to provide readers with a guideline for designing and setting up the protocols for their own particular uses. This also leaves room for readers to make their own improvements and innovations of the technologies.

This book is written for: (1) Fundamentalists (starting from graduate students to PI) in the field of studies involving miRNAs, in universities and research institutions; (2) Pharmacologists and gene therapists involving translational studies on drug development; (3) Pharmaceutical companies involving R&D in target searching and drug design and (4) Medical practitioners from residents to professors of various types of medical fields.

Canada Zhiguo Wang

Acknowledgement

Writing a science book on a very specialized subject is absolutely not a solo but an orchestral achievement; though I am the sole author, hundreds of workers whose studies are cited, or are left out by accident, in the reference lists have contributed importantly and enormously to this book by providing their invaluable information and sharing their fascinating findings in their wonderful publications. I wish to give my sincere thanks to all these people, whether I knew them in person or not; I know their names from their papers and their works by heart. Without their sweat and intellect, this book would have been absolutely impossible.

My special thanks go to Dr Stanley Nattel, my former PhD supervisor and boss, who has given me everything I need to start my independent scientific career and to Dr Baofeng Yang, my best friend, with whom I have the pleasure of sharing a stage and working with and whose words over the years has taught me much about myself and the mysterious ways of life, the scientific life.

I wish to give my gratitude to my dear wife Xiaofan Yang who has had to live with my fluctuating attitude as I wrote these chapters. She has stood by me and meanwhile taken care of our kids and the nuts and bolts of our household. She has been my inspiration and support that has driven me onwards. She has kept me focused on everything I have done. Thank you my darling.

Much of what I have learnt over the years came as the result of being a father to two wonderful and delightful children, Ritchie and Jennifer, both of whom, in their own ways inspired me and, subconsciously contributed to this book, despite that they do not (and may never) understand the contents. The joyfulness they give me is definitely another driving force for me to complete the mission-impossible book writing – thanks kids!

I wish to express my appreciation to my wonderful fellows and students, Guorong Chen, Haijun Zhang, Huixian Lin, Huizhen Wang, Jiangchun Zhang, Jiening Xiao and Xiaobin Luo (listed in alphabetic order), for their continuous motivation, intelligence, hard work and understanding. I am also particularly grateful to Dr Jiening Xiao who convinced me to commence the miRNA business. We have been able to openly share knowledge, ideas and numerous tips,all of which culminated in the

completion of this book. Of course, we went through some difficult and cheerful times too, sharing together the frustration and exuberance. Without everyone's creative studies, I would not be the author of this book. What an amazing research team! Thank you all, guys!

Contents

Chapter 1
miRNAs Targeting and Targeting miRNAs

Abstract With the recent advance of research into microRNAs (miRNAs), this category of endogenous noncoding small ribonucleic acids (19–25 nts in length) has rapidly emerged as one of the central regulators of expression of an extensive repertoire of genes. MiRNAs are an abundant RNA species constituting >2% of the predicted human genes (>1,000 genes), which regulates ∼30% of protein-coding genes. Some miRNAs are expressed at >1,000 copies per cell. Thousands of miRNAs have been identified in several organisms including humans, some of which are registered in the miRBase Registry (http://microrna.sanger.ac.uk/registry/; the Wellcome Trust Sanger Institute). Computational prediction suggests an even larger number of miRNAs (∼25,000 in humans) exist in mammalian genome that are still to be identified [Miranda KC, Huynh T, Tay Y, Ang YS, Tam WL, Thomson AM, Lim B, Rigoutsos I, Cell 126:1203–1217, 2006; Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LA Jr, Sjoblom T, Barad O, Bentwich Z, Szafranska AE, Labourier E, Raymond CK, Roberts BS, Juhl H, Kinzler KW, Vogelstein B, Velculescu VE, Proc Natl Acad Sci USA 103:3687–3692, 2006.]

The high sequence conservation across metazoan species suggests strong evolutionary pressure and participation of miRNAs in essential biological processes such as cell proliferation, differentiation, apoptosis, metabolism, stress and so forth [Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB, Cell 115:787–798, 2003; Lewis BP, Burge CB, Bartel DP, Cell 120:15–20, 2005; Jackson RJ, Standart N, Sci STKE 23:243–249, 2007; Nilsen TW, Trends Genet 23:243–249, 2007; Pillai RS, Bhattacharyya SN, Filipowicz W, Trends Cell Biol 17:18–126, 2005; Alvarez-Garcia I, Miska EA, Development 132:4653–4662, 2005; Ambros V, Nature 431:350–355, 2004.] MiRNAs are also critically involved in a variety of pathological processes including human disease, such as developmental malformations, cancer, cardiovascular disease, neuronal disorders, metabolic disturbance and viral disease. MiRNAs have been considered a part of the epigenetic program in organisms.

The initial discovery of small temporal RNAs (now known as miRNAs) is credited to the pioneer work described by Lee et al. [Cell 75: 843–854, 1993] and Wightman et al. [Cell 75: 855–562, 1993] in their effort to search for a

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protein responsible for the disruption of the timing of larval to adult developmental stages due to the lin-4 mutation in the nematode worm *C. elegans*. They identified a small RNA from the locus, which bound with partial complementarity to the 3′-untranslated region (3′UTR) of lin-14 mRNA and negatively regulated lin-14 expression posttranscriptionally. These studies however did not arouse major attention in the scientific community until the let-7 (lethal-7) mutation, which also resulted in disruption of developmental timing in *C. elegans*, was mapped to another small RNA [Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G, Nature 403:901–906, 2000]. From that point, researchers began to realize that the let-7 miRNA sequence, along with its expression during development, was conserved in animals from arthropods to humans [Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G, Nature 408:86–89, 2000], indicating that miRNAs represent an ancient mechanism of gene regulation. Thus, lin-4 represents the first founding member of the miRNA family that can downregulate the protein lin-14 and let-7 is the second miRNA mediating translational repression of lin-41. However, the tidal wave of miRNA that hit the field of biology was not stirred up until three hallmark papers were simultaneously published in the journal *Science*, which reported the presence of large numbers of small, noncoding RNAs in *Drosophila*, *Caenorhabditis elegans* and mammalian cells [Lau N, Lim L, Weinstein E, Bartel DP, Science 294:858–862, 2001; Lee RC, Ambros V, Science 294:862–864, 2001; Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T, Science 294:853–858, 2001.] Thereafter, this new class of small regulatory RNAs gained its big name, miRNA and began to garner interest of scientists worldwide.

1.1 miRNA Biology

1.1.1 miRNAs Biogenesis

Genes for miRNAs are located in the chromosomes and many of them are identified in clusters that can be transcribed as polycistronic primary transcripts. Some miRNAs are encoded by their own genes and others are encoded by the sequences as a part of the host protein-coding genes. Based on the genomic arrangement of miRNA genes, miRNAs can be grouped into two classes:

1. Intergenic miRNAs (miRNA-coding genes located in-between protein-coding genes),
2. Intragenic miRNAs (miRNA-coding genes located within their host protein-coding genes). Further, the intragenic miRNAs can be divided into the following subclasses:

   (a) Intronic miRNAs (miRNA-coding genes located within introns of their host protein-coding genes),

(b) Exonic miRNAs (miRNA-coding genes located within exons of host protein-coding genes),
(c) 3′UTR miRNAs (miRNA-coding genes located within 3′UTR of host protein-coding genes).
(d) 5′UTR miRNAs (miRNA-coding genes located within 5′UTR of host protein-coding genes).

According to our analysis, for the human miRNAs identified thus far, the majority of miRNAs belong to intergenic and intronic miRNAs being comprised of ∼42 and ∼44% of the total, respectively and the other three categories are rare with the exonic miRNAs being ∼7%, 3′UTR miRNAs being 1.5% and 5′UTR miRNAs being 1%.

Clearly, miRNAs either have their own genes or are associated with their host genes; accordingly, miRNAs are generated by two different mechanisms. Biogenesis of miRNAs can be summarized as a five-step process as detailed below (see also Figs. 1.1 and 1.2).

1. Generation of primary miRNAs: transcription of miRNA genes. The intergenic miRNA genes are first transcribed as long transcripts, called primary miRNAs (pri-miRNAs) mostly by RNA polymerase II or RNA polymerase III (Ying and Lin 2005). The pri-miRNAs are capped and polyadenylated and can reach several

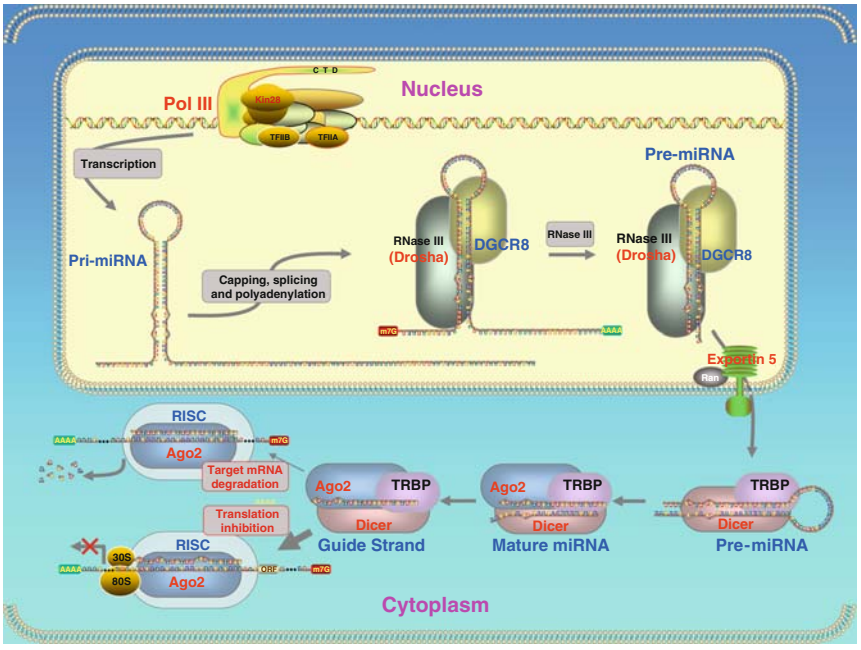

Fig. 1.1 Diagram illustrating the biogenesis pathway of intergenic miRNAs. Intergenic miRNAs have their own genes and their transcription is likely driven by Pol III. Pol III: polymerase III, Ago: Argonaute protein-2

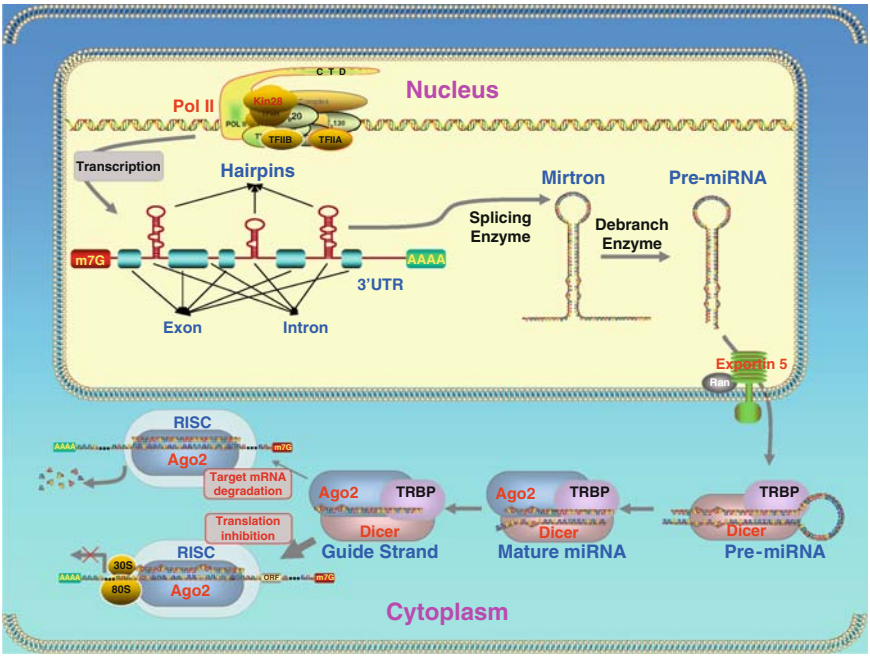

Fig. 1.2 Diagram illustrating the biogenesis pathway of intragenic miRNAs. Intragenic miRNAs are generated by the hairpin structures within host genes (mostly in introns) and they are normally transcripted along with their host genes by Pol II. Pol II: polymerase III, Ago: Argonaute protein-2

kilobases (kb) in length (Cullen 2004; Kim 2005). The clustered miRNA genes in polycistronic transcripts are likely to be coordinately regulated (Bartel 2004). The intronic miRNAs are processed by sharing the same promoter and other regulatory elements of the host genes. They are first transcribed along with their host genes by RNA polymerase II and then processed by Drosha independent pathway from excised introns by the RNA splicing machinery for their biogenesis in *Drosophila*, *C elegans* and mammals (Berezikov et al. 2007; Okamura et al. 2007; Ruby et al. 2007).

2. Generation of precursor miRNAs: endonuclease processing of pri-miRNAs. The pri-miRNAs are processed to precursor miRNAs (pre-miRNAs) by the RNase endonuclease-III Drosha and its partner DGCR8/Pasha in the nucleus (Lee et al. 2002b; Denli et al. 2004; Gregory et al. 2004; Landthaler et al. 2004). These pre-miRNAs are ∼60 to ∼100 nts with a stem-loop or hairpin secondary structure. Specific RNA cleavage by Drosha predetermines the mature miRNA sequence and provides the substrates for subsequent processing steps. Cleavage of a pri-miRNA by microprocessor begins with DGCR8 recognizing the single-stranded RNA (ssRNA)–double-stranded RNA (dsRNA) junction typical of a pri-miRNA (Han et al. 2006). Then, Drosha is brought close to its substrate through

interaction with DGCR8 and cleaves the stem of a pri-miRNA ∼11 nt away from the two single-stranded segments.

miRNA precursor-containing introns have recently been designated "mirtrons" (Miranda et al. 2006). Mirtrons are derived from certain debranched introns that fold into hairpin structures with 5′ monophosphates and 3′ 2-nt hydroxyl overhangs, which mimic the structural hallmarks of pre-miRNAs and enter the miRNA-processing pathway (Okamura et al. 2007; Ruby et al. 2007). The discovery of mirtrons suggests that any RNA, with a size comparable to a pre-miRNA and all the structural features of a pre-miRNA, can be utilized by the miRNA processing machinery and potentially give rise to a functional miRNA.

3. Nucleus to cytoplasm translocation of pre-miRNAs. Pre-miRNAs are then exported to the cytoplasm from the nucleus through nuclear pores by RanGTP and exportin-5 (Bohnsack et al. 2004; Lund et al. 2004; Yi et al. 2003). After a pre-miRNA is exported to the cytoplasm, RanGTP is hydrolyzed by RanGAP to RanGDP and the pre-miRNA is released from Exp-5.
4. Generation of mature miRNAs: endonuclease processing of pre-miRNAs. In the cytoplasm, pre-miRNAs are further processed by Dicer in animals, which is a highly conserved, cytoplasmic RNase III ribonuclease that chops pre-miRNAs into ∼22 nt duplexes of mature miRNAs containing a guide strand and a passenger strand (miRNA/miRNA*), with 2-nt overhangs at the 3′ termini (Kim 2005). Like other RNase III family proteins, Dicer interacts with double-stranded RNA-binding protein (dsRBP) partners. In mammalian cells, Dicer associates with transactivation-response element RNA-binding protein (TRBP) and protein activator of the interferon-induced protein kinase (PACT) (Chendrimada et al. 2005; Lee et al. 2006). In plants, miRNAs are cleaved into miRNA:miRNA* duplex possibly by Dicer-like enzyme 1 (DCL1) in the nucleus rather than in the cytoplasm (Bartel 2004; Lee et al. 2002a), then the duplex is translocated into the cytoplasm by HASTY, the plant ortholog of exportin 5 (Bartel 2004). The strands of this duplex separate and release mature miRNA of 19–25 nts in length (Bartel 2004; Lee et al. 2002a). Plant miRNAs undergo further modification by methylation at the 3′ end by HEN1 (Yu et al. 2005).
5. Formation of miRISC. Mature miRNAs become integrated into a RNA-induced silencing complex (RISC) to form the miRNA:RISC complex (miRISC). Only one strand of miRNA/miRNA*, the guide strand, is successfully incorporated into RISC, while the other strand, the passenger strand, is eliminated. Strand selection may be determined by the relative thermodynamic stability of two ends of miRNA duplexes (Khvorova et al. 2003; Schwarz et al. 2003). The strand with less stability at the 5′ end is favorably loaded onto RISC, whereas the passenger strand is released or destroyed. miRISC contains several proteins such as Dicer, TRBP, PACT and Gemin3 but the components directly associated with miRNAs are Argonaute proteins (Ago). These proteins contain four domains: the N-terminal, PAZ, middle and Piwi domains. The PAZ domain binds to the 3′ end of guide miRNA, while the other three domains form a unique structure, creating grooves for target mRNA and guide miRNA interactions (Liu et al.

2004; Song et al. 2004; Ma et al. 2005; Parker et al. 2005). In mammalian cells, four Ago proteins have been identified, all of which can bind to endogenous miRNAs (Meister and Tuschl 2004). Despite the sequence similarity among these Ago proteins, only Ago2 exhibits endonuclease activity to slice complementary mRNA sequences between positions 10 and 11 from the 5′- end of guide strand miRNA. Therefore, human Ago2 is a component not only of miRISC but also of siRISC (siRNA-induced silencing complex), a RISC assembled with exogenously introduced siRNA. The roles of various Ago proteins in mammalian RISC are ambiguous but the division of labor among Ago proteins in *Drosophila* is well-defined. *Drosophila* Ago1 and Ago2 have been shown by biochemical and genetic evidence to participate in two separate pathways: Ago1 interacts with miRNA in translational repression, whereas Ago2 associates with siRNA for target cleavage (Carmell et al. 2002; Okamura et al. 2004).

1.1.2 miRNAs Actions

miRNAs exist in double-stranded form (duplex), activate in single-stranded form (simplex) and act in complex form miRISC. Subsequent binding of a miRNA in the miRISC to the 3′ untranslated region (3′UTR) of its target mRNA through a Watson-Crick base-pairing mechanism with its 5′- end 2 to 8 nts exactly complementary to recognition motif within the target. This 5′- end 2 to 8 nt region is termed "seed sequence" or "seed site" as it is critical for miRNA actions (Lewis et al. 2003, 2005). Partial complementarity with the rest of the sequence of a miRNA also plays a role in producing posttranscriptional regulation of gene expression, presumably by stabilizing the miRNA:mRNA interaction. Moreover, the mid and 3′- end regions of a miRNA may also be important for forming miRISC. Studies have shown that in addition to 3′UTR, coding region and 5′UTR can also interact with miRNAs to induce gene silencing (Jopling et al. 2005; Luo et al. 2008; Tay et al. 2008).

In mammalian species, a miRNA can either inhibit translation or induce degradation of its target mRNA or both, depending upon at least the following factors (see Fig. 1.3):

1. The overall degree of complementarity of the binding site,
2. The number of recognition motif corresponding to 5′- end 2 to 8 nts of the miRNA, and
3. The accessibility of the bindings sites (as determined by free energy states) (Jackson and Standart 2007; Nilsen 2007; Pillai et al. 2007).

The greater the degree of complementarity of accessible binding sites, the more likely a miRNA degrades its targeted mRNA. Perfectly complementary targets (Full miRNA:mRNA interaction) are efficiently silenced by the endonucleolytic cleavage activity of some Argonaute proteins (Hutvágner and Zamore 2002; Yekta et al. 2004; Davis et al. 2005) but the vast majority of predicted targets in animals are only partially paired (Partial miRNA:mRNA interaction) (Lewis et al. 2003, 2005;

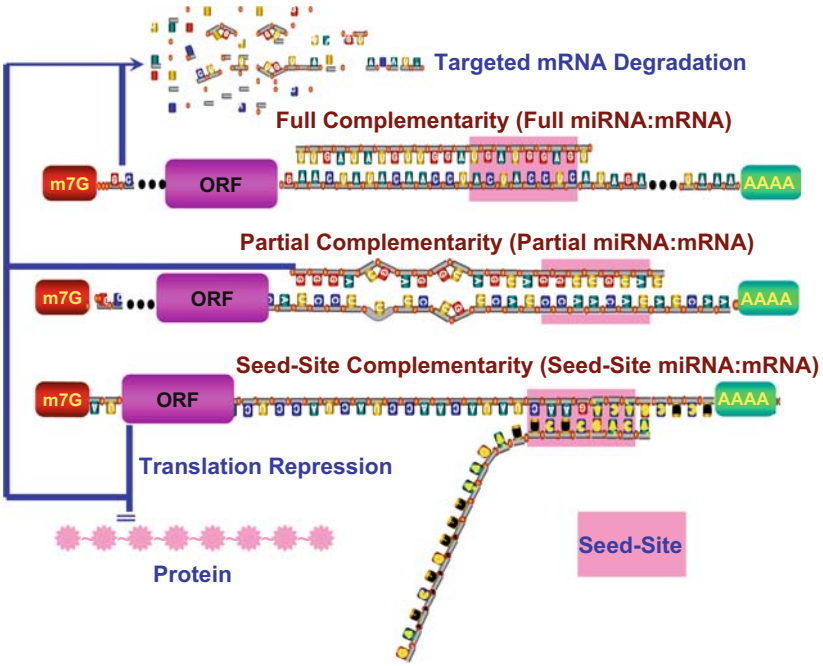

Fig. 1.3 Schematic illustration of mechanisms of action of miRNAs. Full complementarity between a miRNA and its target mRNA (Full miRNA:mRNA) results in targeted mRNA cleavage; Seed-site complementarity (Seed-Site miRNA:mRNA) leads to translation inhibition; and partial complementarity (Partial miRNA:mRNA) gives rise to both targeted mRNA degradation and protein translation repression

Grun et al. 2005; Krek et al. 2005; Rajewsky 2004; Brennecke et al. 2005) and can hardly be cleaved (Haley and Zamore 2004). Some miRNAs have only seed-site complementarity (Seed-Site miRNA:mRNA) and this interaction primarily leads to translation inhibition. And those miRNAs that display imperfect sequence complementarities with target mRNAs primarily result in translational inhibition (Lewis et al. 2003, 2005; Jackson and Standart 2007; Nilsen 2007; Pillai et al. 2007). The mechanisms for translational inhibition remain largely unknown, although inhibition of translation initiation has been identified as one such mechanism by several studies (Humphreys et al. 2005; Pillai et al. 2005). Greater actions may be elicited by a miRNA if it has more than one accessible binding site in its targeted miRNA, presumably by the cooperative miRNA:mRNA interactions from different sites. mRNA degradation by miRISC is initiated by deadenylation and decapping of the targeted mRNAs (Pillai et al. 2007). A recent study demonstrated, however, that miRNAs can also act to enhance translation when AU-rich elements and miRNA target sites coexist at proximity in the target mRNA and when the cells are in the state of cell-cycle arrest (Vasudevan et al. 2007).

In plants, miRNAs base-pair with their mRNA targets by precise or nearly precise complementarity (Wang et al. 2006).

It has been predicted that each single miRNA can have >1,000 target genes and each single protein-coding gene can be regulated by multiple miRNAs (Lewis et al. 2003, 2005; Jackson and Standart 2007; Nilsen 2007; Pillai et al. 2007; Alvarez-Garcia and Miska 2005; Ambros 2004). This is at least partially a result of a lax requirement of complementarity for miRNA::mRNA interaction (Lim et al. 2005). This implies that actions of miRNAs are sequence- or motif-specific but not gene-specific; different genes can have same binding motifs for a given miRNA and a given gene can have multiple binding motifs for distinct miRNAs. *Based on the characteristics of miRNA actions, I postulate that a miRNA should be viewed as a regulator of a cellular function or a cellular program, not of a single gene* (Wang et al. 2008).

1.2 miRNA Expression, Mutation and Polymorphism

1.2.1 miRNA Expression

Expression of some miRNAs is tissue restricted and of others is ubiquitous. The restriction can be qualitative (some miRNAs are expressed exclusively in certain tissue or cell types but not in others) or quantitative (some miRNAs are abundantly expressed only in certain tissue or cell types and modestly in others). For example, miR-122 accounts for 70% of the total miRNA population in the liver. miR-142 and miR-143 constitute ~30% of the total miRNAs in the colon and spleen, respectively (Lagos-Quintana et al. 2002). In the heart, miR-1 accounts for 45% of all murine miRNAs (Lagos-Quintana et al. 2002). The differential tissue distributions of miRNAs suggest tissue – or even cell type – specific functions of these molecules. For instance, the cell lineage-specific miRNA expression patterns may be required to control timing of development and tissue specification (Lagos-Quintana et al. 2002).

To be more appropriate, while each individual miRNA may not be expressed in a tissue/cell-specific manner, the expression profile of miRNAs appears to be tissue/cell-specific. Many miRNAs are enriched in a tissue/cell-specific manner (Landgraf et al. 2007): miR-1, miR-16, miR-27b, miR-30d, miR-126, miR-133, miR-143 and the let-7 family are abundantly but not exclusively expressed in adult cardiac tissue. In addition to cardiomyocytes, the heart contains many other 'noncardiomyocyte' cell types, such as endothelial cells, smooth muscle cells, fibroblasts and immune cells, which may have completely distinct miRNA expression profiles. Indeed (skin) fibroblasts mainly express miR-16, miR-21, miR-22, miR-23a, miR-24, miR-27a and others, an expression pattern that is highly different from that of cardiomyocytes. In artery smooth muscle the most abundant miRNAs are miR-145, let-7, miR-125b, miR-125a, miR-23 and miR-143 (Ji et al. 2007), despite that

the "muscle-specific" miR-1 and miR-133 are also expressed in artery smooth muscle. Other miRNAs, such as the let-7 family, miR-126, miR-221 and miR-222, are highly expressed in human endothelial cells (Kuehbacher et al. 2007; Harris et al. 2008). In addition, miRNA expression profiles can change during cardiac development and many miRNAs that are only normally expressed at significant levels in the fetal human heart are re-expressed in cardiac disease, such as heart failure (Landgraf et al. 2007; Bauersachs and Thum, 2007).

Probably more important is the fact that the expression profile of miRNAs is disease-dependent. A particular pathological process may be associated with the expression of a particular group of miRNAs; this is what the signature expression pattern of miRNAs implies. This issue is discussed in the following sections of this chapter.

1.2.2 miRNA Mutation

It is generally believed that the mechanisms that alter the expression of miRNAs are similar to those that change the expression levels of mRNAs of tumor suppressors and oncogenes, i.e., gross genomic aberrations, transcriptional deregulations, epigenetic changes, DNA copy number abnormalities, defects in the miRNA biogenesis machinery and minor mutations affecting the expression level, processing, or target-interaction potential of the miRNA. However, to the best of my knowledge, mutation within the miRNA genes is rare (Diederichs and Haber 2006).

One study (Li et al. 2005) demonstrated that lin-58 alleles contain point mutations in a gene regulatory element of miR-48, a let-7 family member. This mutation causes developmental timing defects in *C. elegans*.

Some miRNA genes are frequently located at fragile sites, as well as in minimal regions of loss of heterozygosity, minimal regions of amplification (minimal amplicons), or common breakpoint regions (Calin et al. 2004b). For example, miR-15a and miR-16-1 genes are located at chromosome 13q14, a region that is frequently deleted in pituitary tumors (Calin et al. 2004b; Bottoni et al. 2005). Deletion mutations in the 3′ flanking region of miR-16-1 transcript have been identified in families with two or more members with chronic lymphocytic leukemia (Calin et al. 2005). Raveche et al. and Colleagues (2007) reported a point mutation in the 3′ flanking sequence of miR-16-1 in a strain of mice prone to autoimmune and B lymphoproliferative disease. They reported decreased levels of expression of miR-16 residing in the mouse D14mit160 region (Raveche et al. 2007). The human region of synteny with mouse D14mit160 is the human 13q.14 where miR-15 and miR-16 reside and a region which, as reported earlier by Calin et al. (2002, 2005), is deleted in chronic lymphocytic leukemia.