RNA Technologies in Cardiovascular Medicine and Research

Volker A. Erdmann • Wolfgang Poller
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Editors

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Preface

The heart may respond to chronic and acute injury by hypertrophic growth and pathological remodeling. Cardiomyocyte hypertrophy is a dominant cellular response to all kinds of hemodynamic overload, inherited mutations in many structural and contractile proteins, and other factors and may be compensatory or maladaptive. In the latter case pathological remodeling may result from diverse molecular pathomechanisms which are still incompletely understood.

Small deviations in a mechanism controlling cardiac morphology and function may lead to enormous negative effects including loss of function and, in severe cases, even death. Despite great progress in understanding various aspects of heart development, cardiovascular diseases remain a major problem for medicine. Therefore, there is a need for new diagnostic and therapeutic strategies to detect, classify and cure heart diseases.

Ribonucleic acid (RNA) in its many facets of structure and function is more and more understood, and therefore it is possible to design and use RNAs as valuable tools in molecular biology and medicine. An understanding of the role of RNAs within the cell has changed dramatically in recent years (Fig. 1). Its status expanded with reports on catalytic RNAs (ribozymes) 25 years ago, of endogenous RNA interference 15 years later, and other noncoding RNA very recently. Today, it is obvious that RNAs are not merely the intermediary molecules between DNA and proteins, but that they can also be functional end products. Large stretches of genomic DNA do not contain protein-coding sequences and have, therefore, been considered as 'junk'. However, a significant fraction of this noncoding DNA have actually been found to hold the information for some of these functional noncoding RNAs. Diverse eukaryotic organisms harbor a class of noncoding small RNAs which are thought to function as regulators of gene expression. Thus, RNAs can be the transmitters (mRNAs) of genetic information to the ribosome for proteins to be synthesized, and also the regulators in protein synthesis. The conclusion to be drawn is that RNA is much more than solely a messenger RNA, and therefore this class of molecules are truly renaissance molecules. Most of the noncoding DNA is occupied by various units of repeats, satellite sequences and transposons. These sequences have been sought to be epigenetic elements that control stability of gene expression programs, and organize heterochromatic domains at centromers and telomers. Their role appears to be mainly regulatory. Although the effects of antisense RNAs on the corresponding

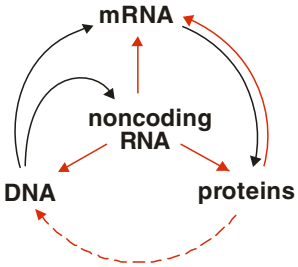

Fig. 1 Genetic information from DNA is transcribed into mRNA containing instruction for protein synthesis and regulatory RNAs which take part in systems controlling expression of genes

sense RNAs have not been clearly established, a number of examples indicate that they may exert control at various levels of gene expression, such as transcription, mRNA processing, splicing, stability, transport, and translation.

RNA has become a focus of investigations into novel therapeutic schemes. Ribozymes, antisense RNAs, RNA decoys, aptamers, micro RNAs and small interfering (siRNAs) have been used to down regulate undesired gene expression (Fig. 2). Multiple challenges, such as optimization of selectivity, stability, delivery and long term safety, have to be addressed in order for RNA drugs to become successful therapeutic agents. Not all RNA classes (e.g., ribozymes or RNA decoys) have been so far successfully developed as drugs. The recognition of the biological roles of small molecular weight RNAs have been one of the most significant discoveries in molecular biology. These RNA molecules influence the translation of messenger RNAs (mRNAs) in post-transcriptional manner that makes the regulation of RNAs even more complex.

The use of RNA-mediated interference (RNAi) for gene silencing has provided a powerful tool for loss-of-function studies in a variety of metazoans. SiRNA mediated gene silencing by degradation of target messenger RNAs have been widely used in gene function characterizations.

Compared with the laborious, time-consuming, and very costly gene knockout models, siRNA provides an efficient, specific and cheap solution for inhibiting expression of target genes. Efficient siRNA delivery is essential for the success of specific gene silencing. As the popularity of RNAi technology grows, so does the frustration it still causes for many researchers. Direct measurement at the mRNA level is always needed for direct verification that RNA interference is decreasing the amount of mRNA.

Because high doses of siRNAs may provoke an altered expression of many other genes, selections of optimal conditions are essential to minimize potential side effects. The most informative experiments in understanding the specificity of a siRNA would consider acquiring global gene expression of relevant genes, which unfortunately is lacking in many siRNA studies. These small RNAs of about 15–49 nucleotides in length guide the RNA-induced silencing complex (RISC). The beauty of the system lies in the application of short RNAs, which can be synthesized at reasonable cost and can evolve quickly, to regulate a large and complex protein synthesis.

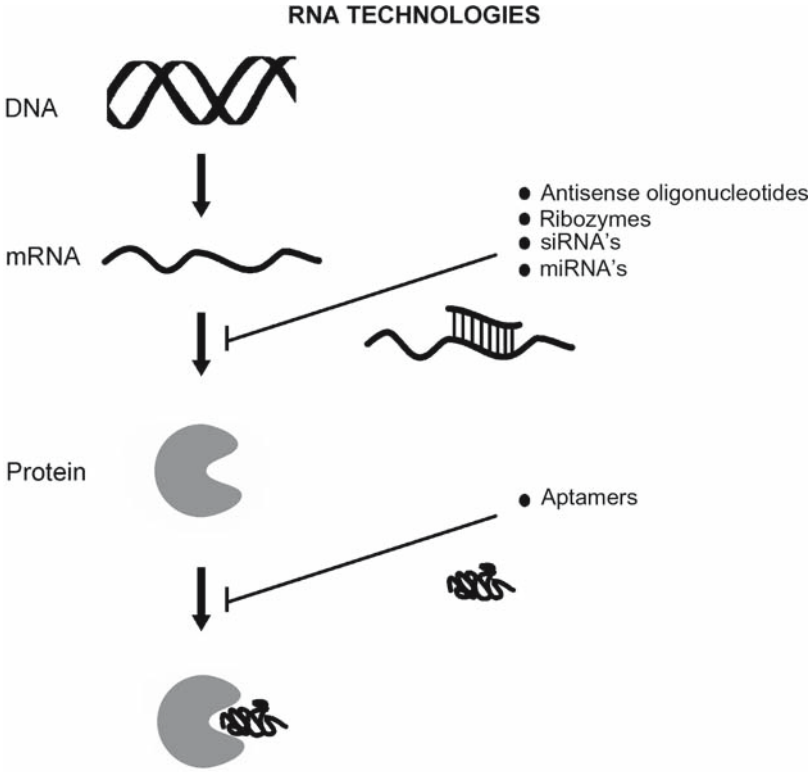

Fig. 2 The potentials of the different RNA technologies which can be applied to inhibit protein biosynthesis on RNA levels (*antisense oligonucleotides*, *ribozymes*, *short interference RNAs* and *microRNAs*) or protein functional level (*aptamers*)

In several recent studies, microarray analyses were performed to determine whether miRNAs are deregulated in hyperthrophic and failing hearts. The results implicate that miRNAs function as negative regulators of cell growth or as regulators of prosurvival pathways such that their downregulation predisposes the heart to pathological remodeling. A major challenge for the future will be to identify the mRNA targets of RNAs that participate in cardiac remodeling and to understand the functions of their target mRNAs.

Finally, the recent application of advanced vector technologies developed initially in the gene therapy field has had an enormous impact on the efficacy by which RNAi and microRNAs can be employed for therapeutic purposes in vivo. These most recent developments have brought clinical translation of certain RNA-based therapies within reach.

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Part I
MicroRNA

An Overview of MicroRNA

E. Wang

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Abstract Recently, microRNAs (miRNAs) have emerged as central posttranscriptional regulators of gene expression. miRNAs regulate many key biological processes, including cell growth, death, development and differentiation. This discovery is challenging the central dogma of molecular biology. miRNAs have been known to be involved in development, cell proliferation and apoptosis. Several reports have recently shown that miRNAs might also be involved in filtering out gene expression noise by regulating positive regulatory loops in cells. Loss- or gain-of-function of specific miRNAs appears to be a key event in the genesis of many diverse diseases. Recent studies have shown that miRNAs are important during heart development and adult cardiac physiology, and modulate a diverse spectrum of cardiovascular functions in vivo. miRNAs have been shown to regulate pathways controlled by genes like p53, MYC and RAS, which are closely related to cancer. Single-nucleotide polymorphisms (SNPs) of miRNA binding sites are associated with gene expression

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levels of the target alleles and cancer. Finally, these miRNA studies also have implications for understanding complex pathways, e.g., interactions between miRNAs, cell signaling and transcription factors, involved in human diseases, and can lead to potential opportunities in manipulating miRNAs as therapeutic targets.

1 Introduction

According to the central dogma of molecular biology, RNAs are passive messengers and only take charge of transferring genetic information, or carrying out DNA instructions, or code, for protein production in cells. However, this central dogma is getting challenged by the recent findings that tiny fragments of noncoding RNA typically ~22 nucleotides in length, namely microRNA (miRNA), are able to negatively regulate protein-coding genes by interfering with mRNA's original instructions. Recent studies indicate that miRNAs have emerged as central posttranscriptional repressors of gene expression. miRNAs suppress gene expression via imperfect base pairing to the 3′ untranslated region (3′UTR) of target mRNAs, leading to repression of protein production or mRNA degradation (Bartel 2004; Carthew 2006; Valencia-Sanchez et al. 2006). These noncoding regulatory RNA molecules have been found in diverse plants, animals, some viruses and even algae species, and it now seems likely that all multicellular eukaryotes, and perhaps some unicellular eukaryotes, utilize these RNAs to regulate gene expression.

Some researchers claimed that the human genome might encode more than 1,000 miRNAs (Bentwich et al. 2005). However, a recent sequencing survey of miRNA expression across 26 distinct organ systems and cell types of human and rodents validated that only 300+ miRNAs are present in humans and/or rodents (Landgraf et al. 2007). Computational predictions indicate that thousands of genes could be targeted by miRNAs in mammals (John et al. 2004; Krek et al. 2005; Lewis et al. 2005; Rajewsky 2006). Experimental analysis revealed that 100–200 target mRNAs are repressed and destabilized by a single miRNA (Krutzfeldt et al. 2005; Lim et al. 2005; Yu et al. 2007a). It is estimated that more than one-third of human genes are potentially regulated by miRNAs. These findings suggest that miRNAs play an integral role in genome-wide regulation of gene expression.

miRNAs regulate many key biological processes, including cell growth, death, development and differentiation, by determining how and when genes turn on and off. Animals that fail to produce certain mature miRNAs are unable to survive or reproduce (Bernstein et al. 2003; Cao et al. 2006; Forstemann et al. 2005; Ketting et al. 2001; Plasterk 2006; Shivdasani 2006; Wienholds et al. 2003). Thus, a single, malfunctioning microRNA can be sufficient to cause cancer in mice (Costinean et al. 2006). These discoveries offer new insights into another layer of gene regulation and at the same time underscore the powerful role that these tiny snippets of noncoding RNA play in cells. These discoveries indicate that it is no longer the genes, nor mRNAs themselves, that hold the most mystery, but the miRNAs that influence their behavior and the result that such gene regulation process produces. Thus, miRNA has become an important force in biology.

In this chapter, I will summarize the basic knowledge of miRNAs, including their biogenesis and functions, especially highlighting recently discovered miRNA functions, such as filtering out gene expression noise by miRNAs, and the potential function of maintaining tissue identity by miRNAs. The recent progress of miRNA involvements in heart development, heart physiology and heart diseases will be discussed. Finally, I will review the relationship of miRNAs to cancer.

2 miRNA Biogenesis

miRNAs as posttranscriptional regulatory molecules were first discovered to regulate expression of partially complementary mRNAs in *Caenorhabditis elegans* (Lee et al. 1993; Moss et al. 1997; Wightman et al. 1993). miRNAs are encoded in either intergenic regions of genomes or within introns of known protein-coding genes. miRNAs are transcribed by RNA polymerase II as long precursor transcripts, which are called primary miRNAs (pri-miRNAs). The pri-miRNAs are capped and polyadenylated, and can reach several kilobases in length (Cullen 2005; Kim 2005). A single pri-miRNA might contain one, or up to several, miRNAs. Several sequential steps of transcript processing are required to produce mature miRNAs from pri-miRNAs. In the nucleus, there is a microprocessor complex in which the major components are the RNase-III enzyme Drosha and its partner DGCR8/Pasha (Denli et al. 2004; Gregory et al. 2004; Landthaler et al. 2004), which initially recognize pri-miRNAs and then excise the stem–loop hairpin structure that contains the miRNA, a 60–80 nucleotide intermediate known as precursor miRNA (pre-miRNA) with pri-miRNAs. Exportin-5, a nuclear export factor, recognizes and transports the pre-miRNAs to cytoplasm (Bohnsack et al. 2004; Lund et al. 2004; Yi et al. 2003). In the cytoplasm, Dicer, a second RNase-III enzyme, cleavages the pre-miRNAs to generate double-stranded 18–24 nucleotide-long RNA molecules – miRNAs (Bernstein et al. 2003; Grishok et al. 2001; Hutvagner et al. 2001; Ketting et al. 2001; Knight and Bass 2001). RNA-induced silencing complex (RISC), the core component of which is the Argonaute protein (Kim 2005), incorporates one of these two strands – the guide strand of miRNAs. Finally, the miRNA guides the RISC complex to the target mRNA to suppress gene expression via imperfect base pairing to the 3′UTR of target mRNAs, leading to repression of protein production and, in some cases, mRNA degradation (Bartel 2004; Carthew 2006; Valencia-Sanchez et al. 2006).

3 Biological Functions of miRNAs

3.1 miRNA Emerges as a Central Regulator for Development

Several reports indicated that miRNAs repress a large set of targets so that the targets are expressed at low levels in the miRNA-expressing cells (Krutzfeldt and Stoffel 2006; Lim et al. 2005; Yu et al. 2007a). This might offer a second layer of regulation

to reinforce transcriptional controls at posttranscriptional level. A number of lines of evidence suggested that miRNAs are involved in regulating developmental processes. We conducted a genome-wide survey of transcription factor binding sites in the promoter regions of human genes and found that developmental genes are significantly regulated by more transcription factors (Cui et al. 2007a). Furthermore, we showed that the more transcription factors a gene is regulated by, the more miRNAs that gene is regulated by (Cui et al. 2007a). Certain miRNAs have been suggested to be essential regulators for developmental programs (Giraldez et al. 2005). For instance, without miR-430, zebrafish embryos develop defects, which can be rescued and complemented by supplying miR-430 (Giraldez et al. 2005). Genes in this process by miR-430 seem to be direct miR-430 targets based on miRNA seed matches, and are misregulated in the absence of miR-430 (Giraldez et al. 2006). Another example comes from the study of *C. elegans* miRNAs, lin-4 and let-7. Without lin-4, *C. elegans* is unable to make the transition from the first to the second larval stage due to a differentiation defect, which is caused by a failure to posttranscriptionally repress the lin-14 gene, which is the target gene of lin-4 (Lee et al. 1993; Wightman et al. 1993). Similarly, without let-7, a failure of larval-to-adult transition was observed (Reinhart et al. 2000). It is known that lin-41, hbl-1, daf-12 and the forkhead transcription factor pha-4 are the direct targets of let-7 during this transition (Abrahante et al. 2003; Grosshans et al. 2005; Slack et al. 2000).

3.2 miRNAs are Involved in Cell Proliferation and Apoptosis

miRNAs have been shown to regulate key genes for tumorigenesis and cancer progression, which coordinately controls cell proliferation and apoptosis. For instance, miRNA let-7 promotes tumorigenesis by regulation KRAS and NRAS transcripts (Johnson et al. 2005). miRNAs are known to regulate pathways controlled by genes such as p53, MYC and RAS. Furthermore, miR17-92 cluster has been shown to be able to act as a functional switch between cell proliferation and apoptosis.

3.3 miRNAs Act as Regulators for Noise Filtering and Buffering

Eukaryotic cells are noisy environments in which transcription often occurs in a bursting manner, causing the number of mRNAs per cell to fluctuate significantly (Blake et al. 2006; Golding et al. 2005; Raj et al. 2006). Moreover, such fluctuations can propagate through the network, e.g., fluctuations in the level of an upstream transcription factor can significantly induce the expression fluctuations of downstream genes (Pedraza and van Oudenaarden 2005; Rosenfeld et al. 2005). In positive regulatory loops, noise or stochastic fluctuations of gene transcripts and protein molecules leads to randomly switching cell phenotypes in yeast, while a negative regulator adding in the positive regulatory loops often helps in reducing such noise in biological systems and making a robust decision for cell development

(Acar et al. 2005). Because miRNAs can tune target protein levels more rapidly at the posttranscriptional level, they might significantly shorten the response delay and, in turn, provide more effective noise buffering. The miRNA miR-17 might play a role in preventing noise-driven transition from apoptosis to cell proliferation. c-Myc and E2F1 are known to reciprocally activate transcription of one another, establishing a positive feedback circuit (Fernandez et al. 2003; Leone et al. 1997). This architectural structure of the circuit makes it possible for miRNAs to support a shift from apoptosis toward proliferation by repressing E2F1. Expression of E2F1 promotes G1 to S phase progression by activating genes involved in cell cycle (Bracken et al. 2004). High expression of E2F1, however, is sufficient to induce apoptosis (Johnson et al. 1994a,b; Matsumura et al. 2003). In the absence of additional regulatory mechanisms, this circuit might be expected to overactivate E2F1, leading to apoptosis, when c-Myc simultaneously activates E2F1 transcription and miR17-92 cluster, which in turn negatively regulates E2F1. This might promote a proliferative signal but not an apoptotic signal. Another example is the fly miR-9a, which is suggested to set up a "threshold" for signals in a positive feedback loop, so that it can filter out noise (Li et al. 2006). Without miR-9a, flies produce extra sense organs (Li et al. 2006). During fly sensory organ development, a fly gene, *senseless* expression is activated by proneural proteins and feedbacks positively to reinforce proneural gene expression. If *senseless,* the target of miR-9a is highly expressed and the defects mentioned above occur. miR-9a has been suggested to set a threshold that *senseless* expression has to overcome to induce the normal developmental program. In agreement with these findings, we found that cross-species expression divergences of miRNA target genes are significantly smaller than those of other genes (Cui et al. 2007b). Similar observations have been found between human and chimpanzee, between human and mouse, between *Drosophila* species, and between *D. melanogaster* and *D. simulans* (Cui et al. 2007b). These results suggest that miRNAs might provide a genetic buffer to constrain gene expression divergence. We showed that miRNAs preferentially regulate positive regulatory loops (Cui et al. 2006). It is possible that miRNAs serve to buffer stochastically fluctuating expression of genes in positive regulatory loop. Given that positive feedback circuits are abundant in genomes (Brandman et al. 2005; Ferrell 2002), we surmise that miRNAs provide a common mechanism in buffering gene expression noise by frequently regulating positive regulatory loops (Fig. 1).

Buffering by miRNAs decreases the detrimental effects of errors in gene regulation. miRNA buffering might also provide a way for silence-accumulating mutations without being subjected to selective forces and thus might contribute to evolvability.

3.4 miRNAs Might Contribute to Maintaining Tissue Identity

We conducted a genome-wide analysis of the expression profiles of mRNA targets in human, mouse and *Drosophila* (Yu et al. 2007a). We found that the expression levels of miRNA targets are significantly lower in all mouse mature tissues and *Drosophila*

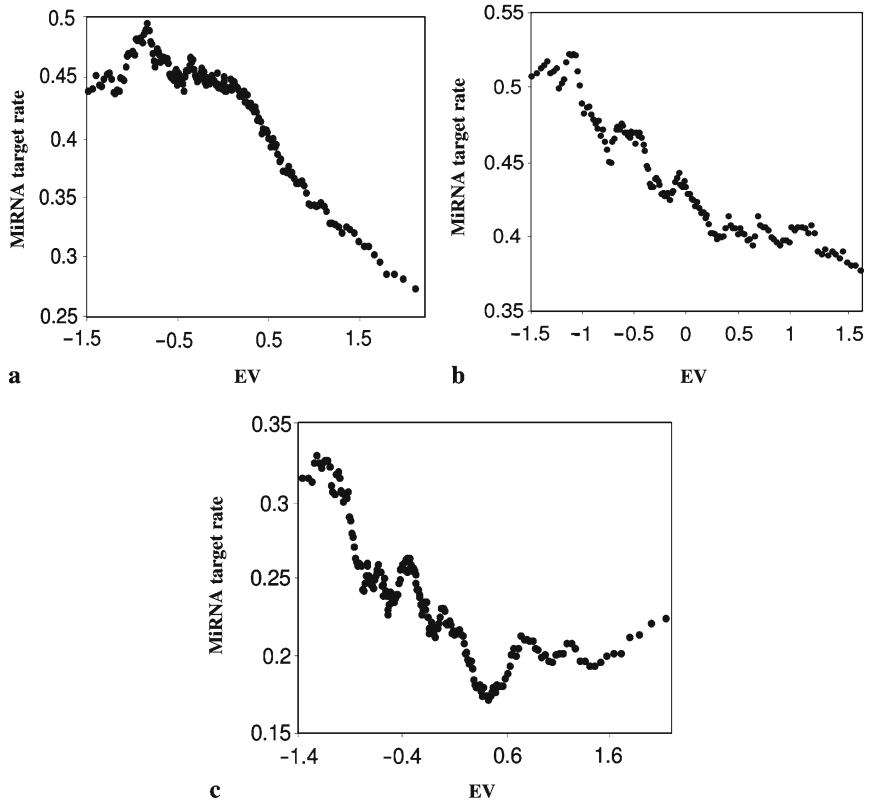

Fig. 1 Distribution of miRNA targets among the genes with different cross-species gene expression variations (*EVs*). Genes were grouped according to their EV values. The ratio of miRNA targets to total genes in each group (miRNA target rate) was calculated. (**a**) Human and chimpanzee. (**b**) Human and mouse. (**c**) *Drosophila melanogaster* and *D. simulans*

later life stages than in the embryos. These results indicate that miRNAs might play roles in determining the timing of tissue differentiation during the larva period of *Drosophila* development and maintaining the tissue identity during adulthood.

4 miRNAs in Human Disease

4.1 miRNAs and Heart Diseases

Loss- or gain-of-function of specific miRNAs appears to be a key event in the genesis of many diverse diseases. Recently, an interesting question how miRNAs influence heart development and disease has been addressed. Four recent papers highlighted

the role of miRNAs in the heart. These reports showed that miRNAs are essential for heart development and regulating the expression of genes which take part in cardiac function in vivo: the conductance of electrical signals, heart muscle contraction, and heart growth and morphogenesis.

It has been reported that there is a function for miR-1 in heart conductivity. miR-1 levels were positively correlated with coronary artery disease and rats after cardiac infarction (Yang et al. 2007). Loss-of-function of miR-1 prevented heart arrhythmia, whereas miR-1 overexpression caused heart arrhythmia in normal and infarcted hearts. They further showed that both gain- and loss-of-function of miR-1 affect conductivity through affecting potassium channels. These results suggest that miR-1 has a prominent effect on the development of cardiac arrhythmia, irregular electrical activity in the heart. A separate study also focused on miR-1 by creating mice that are deficient in a muscle-specific miRNA, miR-1-2 (Zhao et al. 2007). They showed that the miRNA-deficient embryos have cardiac failure and a variety of developmental defects, including pericardial edema and underdevelopment of the ventricular myocardium, an increase in cardiomyocyte proliferation and electrophysiological defects, a reduction in heart rate and the prolonging of ventricular depolarization. Interestingly, these phenotypes are similar to the defects during heart development in zebrafish embryos, when miRNAs are non-functional (Giraldez et al. 2005). Both studies identify miR-1 targets that might, at least in part, account for the manifestation of the associated diseases.

In another study, it was found that the muscle-specific miR-133 is a negative regulator of cardiac hypertrophy, which is an essential adaptive physiological response to mechanical and hormonal stress and heart size (Care et al. 2007). To understand the molecular mechanism by which miR-133 controls heart size, they showed that Rhoa, Cdc42 and Whsc2 are the direct targets of miR-133. Moreover, the heart-specific miRNA miR-208 also modulates the genes that are controlling the hypertrophic response (van Rooij et al. 2007). The main function of miR-208 seems to be mediating the switch from expression of the heavy chain of α-myosin to that of β-myosin during stress or thyroid-hormone-induced cardiac growth (van Rooij et al. 2007). These results suggest that miR-208 is an important regulator for cardiac growth and gene expression in response to stress and hypothyroidism.

Taken together, it seems clear that miRNAs have an important role in regulating gene expression in the heart. These studies indicate that miRNAs are important during heart development and adult cardiac physiology, and modulate a diverse spectrum of cardiovascular functions in vivo. The findings revealed a level of molecular control of heart physiology that is beyond the well-accepted regulatory role of signaling and transcription factor complexes in the heart. Furthermore, these studies also have implications for understanding complex pathways, e.g., interactions between miRNAs, cell signaling and transcription factors, involved in heart diseases, and can lead to potential opportunities in manipulating miRNAs as therapeutic targets.

4.2 miRNAs and Cancer

Human cancer studies are always the hotspots in life science research. Much significant progress in miRNAs and cancer has been made in the past few years. Genome-wide studies of miRNA expression profiling have shown that miRNA expression levels are altered in primary human tumors (Calin et al. 2004; Calin and Croce 2006; Lu et al. 2005). Significant signatures of miRNA expression profiles can be linked to various types of tumors, suggesting that miRNA profiling has diagnostic and perhaps prognostic potential (Lu et al. 2005; Calin and Croce 2006). Certain miRNAs could be tumor suppressors, because loss of these miRNAs is often associated with cancers. miR-15a and miR-16-1 genes are deleted in most cases of chronic lymphocytic leukemia (Calin et al. 2004). Loss of miRNA let-7 in lung tumors correlates with high RAS protein expression, suggesting that let-7 promotes tumorigenesis by regulation of popular oncogenes, KRAS and NRAS transcripts (Johnson et al. 2005). miR-372 and miR-373 have been shown to be able to overcome oncogenic Ras-mediated arrest and, therefore, induced tumorigenesis (Voorhoeve et al. 2006). miR-21 was demonstrated to be consistently upregulated in human glioblastoma tumor tissues, primary tumor cultures and established glioblastoma cell lines relative to normal fetal and adult brain tissue (Chan et al. 2005). Knockdown of miR-21 in glioblastoma cell lines led to activation of caspases and a corresponding induction of apoptotic cell death. Furthermore, two recent studies have placed the miR-24 family into the p53 tumor suppressor network. miR-24, regulating apoptosis and cell proliferation, has become an essential component of the p53 network (He et al. 2007; Raver-Shapira et al. 2007), which is closely associated with cancer.

4.3 Single-Nucleotide Polymorphisms (SNPs) of miRNA Binding Sites and Human Diseases

Mapping human SNP genotype data (25,000 SNPs) generated in the HapMap and Perlegen projects onto the 3′-UTR regions of human gene transcripts (Chen and Rajewsky 2006), uncovered that SNP density in conserved miRNA sites was lower than in conserved control sites. These results indicate that a large class of computationally predicted conserved miRNA target sites is under significant negative selection. Similarly, we showed the same trend when mining NCBI's dbSNP database (Yu et al. 2007b). These results have implications that SNPs located at miRNA-binding sites are likely to affect the expression of the miRNA target and might contribute to the susceptibility of humans to common diseases. Indeed, naturally occurring polymorphisms in miRNA binding sites have been documented in Tourette's syndrome in humans and muscularity in sheep (Abelson et al. 2005; Clop et al. 2006).

Motivated by this concept, we explored the effects of miRNA-binding SNPs on cancer susceptibility by genome-wide analysis of the data deposited in NCBI's

dbSNP database and human dbEST database (Yu et al. 2007b). Interestingly, we found that the frequencies of the minor alleles (non-target alleles) of the miRNA-binding site SNPs are extremely low. Furthermore, we showed that the average expression level of the non-target alleles of miRNA-binding site SNPs is significantly higher than that of the target alleles. Moreover, we identified a set of potential candidates for miRNA-binding site SNPs with an aberrant allele frequency present in the human cancer EST database. Finally, we experimentally validated them by sequencing clinical tumor samples.

Although the miRNA inducing disease studies are still in their infancy, miRNAs are known to regulate pathways controlled by genes such as p53, MYC and RAS. These findings emphasize the need to integrate the study of miRNA expression and function into other cellular processes, such as signaling, gene regulation, and others, in order to achieve a complete understanding of this group of disorders. Unraveling miRNA regulatory circuits, even miRNA regulation of cellular networks that are involved in disease development, is challenging, but is essential to gain a comprehensive understanding of the molecular mechanisms of the diseases. Luckily, there have been recent developments in technologies such as microarray and systemic delivery of small RNA systems that allow high-throughput studies of the function of miRNAs (Krutzfeldt et al. 2005; Soutschek et al. 2004). These approaches provide promise for understanding miRNA function at systems-level and eventually developing therapeutic strategies based on miRNA overexpression or inhibition.

5 Summary

miRNA research has been conducted for only a few years. There are still lots of unknown but exciting knowledge to be revealed about miRNAs. Here, we highlighted the recently discovered new functions of miRNAs. miRNAs might act as regulators for filtering out gene expression noise and letting cells make right decisions for normal development. In addition, miRNAs might maintain tissue identity, although further experimental evidence is needed to validate this hypothesis.

Although miRNAs have been thought to take part in many kinds of human diseases, it was still exciting to find that miRNAs become important players in heart development, affecting adult cardiac physiology, and modulating a diverse spectrum of cardiovascular functions in vivo. Many miRNAs have been implicated in tumorigenesis and cancer progression. A few studies in genome-wide microarray profiling of miRNAs of tumor samples suggested that miRNAs could be used as tumor biomarkers. More and more miRNAs have been documented in causing cancer, but it is still unclear whether and how miRNAs could cooperate to take part in cancer development.

Finally, most of the miRNA studies revealed a level of molecular control of human diseases that is beyond the well-accepted regulatory systems such as regulation by transcription factors and signaling proteins. Furthermore, these studies also have implications that, in a cell, a more complex regulatory system, e.g., interactions

between miRNAs, cell signaling and transcription factors, is involved in many cellular activities and human diseases. Therefore, it is essential to study the underlying interactions between miRNAs and cellular regulatory systems and pathways. Thus, it leads to understanding gene regulation in a more comprehensive manner and opening up new opportunities in manipulating miRNAs as diagnostic and therapeutic targets.

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References

Abelson JF, Kwan KY, O'Roak BJ et al (2005) Sequence variants in SLITRK1 are associated with tourette's syndrome. Science 310:317–320

Abrahante JE, Daul AL, Li M et al (2003) The *Caenorhabditis elegans* hunchback-like gene lin-57/hbl-1 controls developmental time and is regulated by microRNAs. Dev Cell 4:625–637

Acar M, Becskei A, van Oudenaarden A (2005) Enhancement of cellular memory by reducing stochastic transitions. Nature 435:228–232

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Bentwich I, Avniel A, Karov Y et al (2005) Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 37:766–770

Bernstein E, Kim SY, Carmell MA et al (2003) Dicer is essential for mouse development. Nat Genet 35:215–217

Blake WJ, Balazsi G, Kohanski MA et al (2006) Phenotypic consequences of promoter-mediated transcriptional noise. Mol Cell 24:853–865

Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10:185–191

Bracken AP, Ciro M, Cocito A et al (2004) E2F target genes: unraveling the biology. Trends Biochem Sci 29:409–417

Brandman O, Ferrell JE Jr, Li R et al (2005) Interlinked fast and slow positive feedback loops drive reliable cell decisions. Science 310:496–498

Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6:857–866

Calin GA, Liu CG, Sevignani C et al (2004) microRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA 101:11755–11760

Cao X, Yeo G, Muotri AR et al (2006) Noncoding RNAs in the mammalian central nervous system. Annu Rev Neurosci 29:77–103

Care A, Catalucci D, Felicetti F et al (2007) MicroRNA-133 controls cardiac hypertrophy. Nat Med 13:613–618

Carthew RW (2006) Gene regulation by microRNAs. Curr Opin Genet Dev 16:203–208

Chan JA, Krichevsky AM, Kosik KS (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 65:6029–6033

Chen K, Rajewsky N (2006) Natural selection on human microRNA binding sites inferred from SNP data. Nat Genet 38:1452–1456

Clop A, Marcq F, Takeda H et al (2006) A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat Genet 38:813–818

Costinean S, Zanesi N, Pekarsky Y et al (2006) Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci USA 103:7024–7029

Cui Q, Yu Z, Purisima EO, Wang E (2006) Principles of microRNA regulation of a human cellular signaling network. Mol Syst Biol 2:46
Cui Q, Yu Z, Pan Y et al (2007a) MicroRNAs preferentially target the genes with high transcriptional regulation complexity. Biochem Biophys Res Commun 352:733–738
Cui Q, Yu Z, Purisima EO, Wang E (2007b) MicroRNA regulation and interspecific variation of gene expression. Trends Genet 23:372–375
Cullen BR (2005) Human immunodeficiency virus: nuclear RNA export unwound. Nature 433:26–27
Denli AM, Tops BB, Plasterk RH et al (2004) Processing of primary microRNAs by the microprocessor complex. Nature 432:231–235
Fernandez PC, Frank SR, Wang L et al (2003) Genomic targets of the human c-Myc protein. Genes Dev 17:1115–1129
Ferrell JE (2002) Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. Curr Opin Cell Biol 14:140–148
Forstemann K, Tomari Y, Du T et al (2005) Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. PLoS Biol 3:e236
Giraldez AJ, Cinalli RM, Glasner ME et al (2005) MicroRNAs regulate brain morphogenesis in zebrafish. Science 308:833–838
Giraldez AJ, Mishima Y, Rihel J et al (2006) Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science 312:75–79
Golding I, Paulsson J, Zawilski SM et al (2005) Real-time kinetics of gene activity in individual bacteria. Cell 123:1025–1036
Gregory RI, Yan KP, Amuthan G et al (2004) The microprocessor complex mediates the genesis of microRNAs. Nature 432:235–240
Grishok A, Pasquinelli AE, Conte D et al (2001) Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. Cell 106:23–34
Grosshans H, Johnson T, Reinert KL et al (2005) The temporal patterning microRNA let-7 regulates several transcription factors at the larval to adult transition in *C. elegans*. Dev Cell 8:321–330
He L, He X, Lim LP et al (2007) A microRNA component of the p53 tumour suppressor network. Nature 447:1130–1134
Hutvagner G, McLachlan J, Pasquinelli AE et al (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293:834–838
John B, Enright AJ, Aravin A et al (2004) Human microRNA targets. PLoS Biol 2:e363
Johnson DG, Cress WD, Jakoi L et al (1994a) Oncogenic capacity of the E2F1 gene. Proc Natl Acad Sci USA 91:12823–12827
Johnson DG, Ohtani K, Nevins JR (1994b) Autoregulatory control of E2F1 expression in response to positive and negative regulators of cell cycle progression. Genes Dev 8:1514–1525
Johnson SM, Grosshans H, Shingara J et al (2005) RAS is regulated by the let-7 microRNA family. Cell 120:635–647
Ketting RF, Fischer SE, Bernstein E et al (2001) Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. Genes Dev 15:2654–2659
Kim VN (2005) MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 6:376–385
Knight SW, Bass BL (2001) A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. Science 293:2269–2271
Krek A, Grun D, Poy MN et al (2005) Combinatorial microRNA target predictions. Nat Genet 37:495–500
Krutzfeldt J, Stoffel M (2006) MicroRNAs: a new class of regulatory genes affecting metabolism. Cell Metab 4:9–12
Krutzfeldt J, Rajewsky N, Braich R et al (2005) Silencing of microRNAs in vivo with 'antagomirs'. Nature 438:685–689

Landgraf P, Rusu M, Sheridan R et al (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129:1401–1414
Landthaler M, Yalcin A, Tuschl T (2004) The human DiGeorge syndrome critical region gene 8 and Its *D. melanogaster* homolog are required for miRNA biogenesis. Curr Biol 14:2162–2167
Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854
Leone G, DeGregori J, Sears R et al (1997) Myc and Ras collaborate in inducing accumulation of active cyclin E/Cdk2 and E2F. Nature 387:422–426
Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20
Li Y, Wang F, Lee JA et al (2006) MicroRNA-9a ensures the precise specification of sensory organ precursors in *Drosophila*. Genes Dev 20:2793–2805
Lim LP, Lau NC, Garrett-Engele P et al (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433:769–773
Lu J, Getz G, Miska EA et al (2005) MicroRNA expression profiles classify human cancers. Nature 435:834–838
Lund E, Guttinger S, Calado A et al (2004) Nuclear export of microRNA precursors. Science 303:95–98
Matsumura I, Tanaka H, Kanakura Y (2003) E2F1 and c-Myc in cell growth and death. Cell Cycle 2:333–338
Moss EG, Lee RC, 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
Pedraza JM, van Oudenaarden A (2005) Noise propagation in gene networks. Science 307:1965–1969
Plasterk RH (2006) MicroRNAs in animal development. Cell 124:877–881
Raj A, Peskin CS, Tranchina D et al (2006) Stochastic mRNA synthesis in mammalian cells. PLoS Biol 4:e309
Rajewsky N (2006) microRNA target predictions in animals. Nat Genet 38(Suppl):S8–S13
Raver-Shapira N, Marciano E, Meiri E et al (2007) Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell 26:731–743
Reinhart BJ, Slack FJ, Basson M et al (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403:901–906
Rosenfeld N, Young JW, Alon U et al (2005) Gene regulation at the single-cell level. Science 307:1962–1965
Shivdasani RA (2006) MicroRNAs: regulators of gene expression and cell differentiation. Blood 108:3646–3653
Slack FJ, Basson M, Liu Z et al (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
Soutschek J, Akinc A, Bramlage B et al (2004) Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 432:173–178
Valencia-Sanchez MA, Liu J, Hannon GJ et al (2006) Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev 20:515–524
van Rooij E, Sutherland LB, Qi X et al (2007) Control of stress-dependent cardiac growth and gene expression by a microRNA. Science 316:575–579
Voorhoeve PM, le Sage C, Schrier M et al (2006) A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 124:1169–1181
Wienholds E, Koudijs MJ, van Eeden FJ et al (2003) The microRNA-producing enzyme Dicer1 is essential for zebrafish development. Nat Genet 35:217–218
Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75:855–862
Yang B, Lin H, Xiao J et al (2007) The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med 13:486–491

Yi R, Qin Y, Macara IG et al (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17:3011–3016

Yu Z, Jian Z, Shen SH et al (2007a) Global analysis of microRNA target gene expression reveals that miRNA targets are lower expressed in mature mouse and *Drosophila* tissues than in the embryos. Nucleic Acids Res 35:152–164

Yu Z, Li Z, Jolicoeur N et al (2007b) Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. Nucleic Acids Res 35:4535–4541

Zhao Y, Ransom JF, Li A et al (2007) Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 129:303–317

MicroRNAs and Their Potential

M. Abdellatif

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Abstract With the advent of microRNA (miRNA) we are compelled to revise our understanding of the mechanisms underlying gene regulation during health and disease. A miRNA is approximately 21 ribonucleotides long, genetically encoded, with a potential to recognize multiple mRNA targets guided by sequence complementarity and RNA-binding proteins. This class of molecules is functionally versatile, with the capacity to specifically inhibit translation initiation or elongation, as well as induce mRNA degradation, through predominantly targeting the 3′-untranslated regions of mRNA. Early on it was realized that the levels of individual miRNA varied under different developmental, biological, or pathological conditions, thus implicating these molecules in normal and pathological cellular attributes. In this chapter, we will discuss how the functions of miRNA relate to our existing knowledge on post-transcriptional regulation of gene expression that is the underlying mechanism of many diseases, including cardiac hypertrophy and failure, and their potential as biomarkers and therapeutic targets in diseases.

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1 The Importance of Being miRNA

In computer terms, a macro is a single command that is programmed to replace multiple separate commands that perform a series of actions, thus simplifying, expediting, and minimizing error. Similarly, the cell employs a single miRNA to regulate post-transcriptional expression of an array of genes that are involved in a particular cellular function. This mechanism will ensure synchronization of the regulatory effects and promptness of the response. Moreover, post-transcriptional regulation of multiple genes via a single miRNA circumvents the need for transcriptional regulation of individual genes and is, thus, potentially faster and more energy efficient. This is what Beyer et al. (2004) refer to as "translation on demand," not overlooking the fact that regulating RNA with miRNA is unhindered by the need for a translation product. Thus, it becomes clear that the functions of miRNA complement perfectly our current knowledge of post-transcriptional regulation of expression.

2 Post-Transcriptional Regulatory Mechanisms During Cardiac Hypertrophy

Compensatory cardiac hypertrophy is characterized by a change in the gene expression pattern that recapitulates the neonatal profile (Johnatty et al. 2000). This switch is triggered by regulation of transcriptional and post-transcriptional functions (Fig. 1). Transcription is regulated by selective accessibility of the promoter to the initiation complex and upstream enhancers and/or repressors. Some of the regulators that have been shown to play a role during cardiac hypertrophy include the histone variant, H2A.z (Chen et al. 2006), histone acetylases (Dai and Markham 2001; Gusterson et al. 2003), and deacetylase (Chang et al. 2004; Zhang et al. 2002). Once access to the promoter is granted the availability and/or activity of RNA polymerase II and transcriptional regulators, such as GATA4 (Molkentin et al. 1998), SRF (Zhang et al. 2001), and NKx2-5 (Chen et al. 1996), among others, will determine the level of mRNA produced. But it is well established that translation of mRNA is also a tightly controlled function. It is regulated by: targeted mRNA localization, mRNA stability (half-life), the rate of translation initiation, and, finally, when translation is completed, the rate of protein degradation will determine the extent of the functional outcome. MiRNA are a newly discovered class of post-transcriptional regulators that can inhibit translation and/or induce mRNA degradation (Fig. 2).

3 Post-Transcriptional Regulation by miRNA

Transcription of a gene does not connote automatic translation of the transcript. In other words, we cannot use relative mRNA levels as predictive measures of relative protein levels of a given gene (Gygi et al. 1999), which suggest post-transcriptional

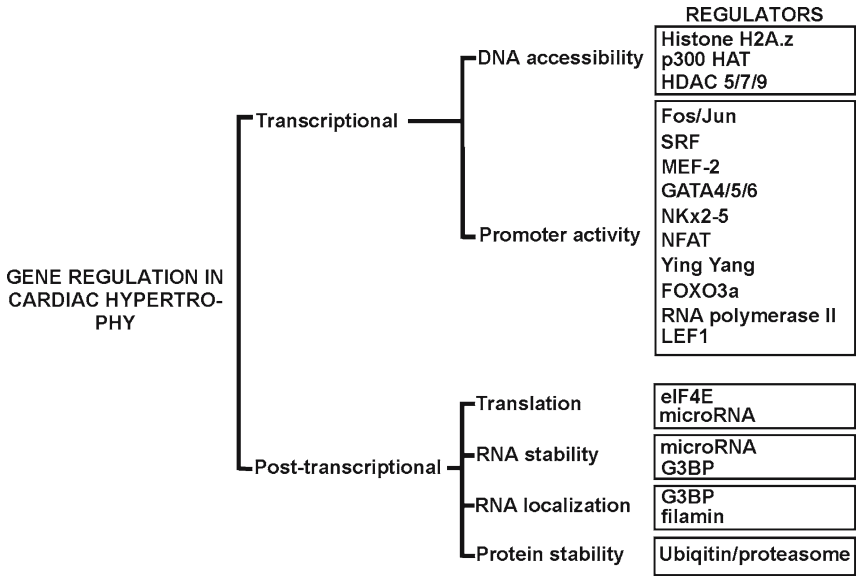

Fig. 1 A schematic showing gene expression regulatory mechanisms involved in cardiac hypertrophy. Gene expression is regulated by transcriptional and post-transcriptional mechanisms. Transcription is restricted by factors that regulate genomic DNA accessibility, while the activity of a promoter is regulated by the availability and activity of various transcription factors, some of which are highlighted in the diagram. Post-transcriptional mechanisms involve regulation of translation initiation and elongation, protein stability, and mRNA stability and localization. MicroRNA plays a role in both translation initiation and elongation, as well as, mRNA stability

regulation of gene expression. Post-transcriptional regulation refers to events that limit the availability or accessibility of mRNA for translation. The study by Beyer et al. (2004) confirms weak or no correlation between mRNA and protein abundance for the whole cell. Similar results were obtained when the calculations were repeated on separate cellular compartments. But, interestingly, a more positive correlation emerged when they grouped functionally related genes. From what we know, an individual miRNA (or a miRNA family) also has the potential to post-transcriptionally regulate a set of specific genes involved in a given cellular function. For example, we observed that miR-1 targets an array of growth-related genes that have been previously implicated in the development of cardiac hypertrophy, which include Ras GTPase-activating protein, cyclin-dependent kinase 9, endothelin, fibronectin, Ras homologue enriched in brain, eukaryotic initiation factor 4E, JunD, quaking, insulin-like growth factor, and Rap1 (Table 1). Concordantly, upon induction of cardiac hypertrophic growth, miR-1 is down-regulated, allowing for the up-regulation of these targets (Sayed et al. 2007). Similarly, when John at al. (2004) analyzed individual miRNA for gene ontology terms they observed that some favored certain terms. For example, miR-208 targets were biased toward “transcription factor,” while miR-105 had a preponderance of “small GTPase mediated signal transduction.” To the best of our knowledge, there is no other class of molecules identified to

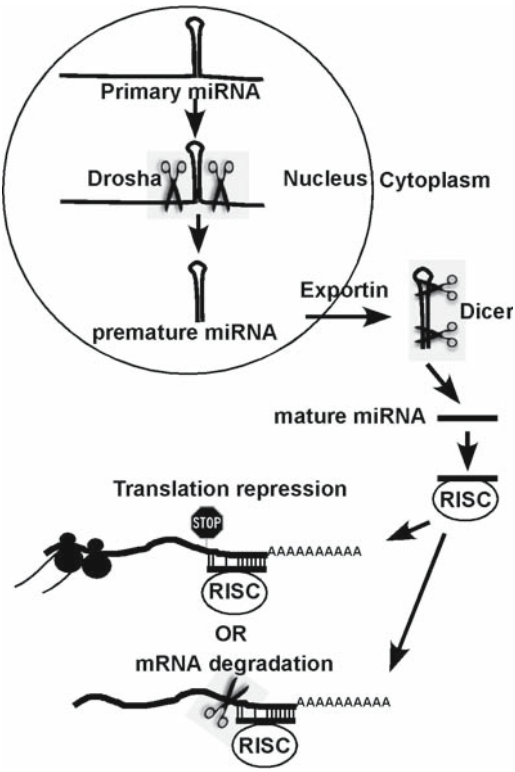

Fig. 2 A diagram showing the processing of microRNA and its fate. MicroRNA are expressed as part of a much longer transcript that matures via a two-step process, one in the nucleus and the second in the cytoplasm, by the enzymes Drosha and Dicer, respectively. Guided by sequence complimentarity and the RISC complex, the mature microRNA targets mRNA and has the potential to induce translation inhibition or mRNA degradation

date that has the potential to fulfill this function as globally and specifically as observed by miRNA. This supports the idea that miRNA are major post-transcriptional regulators.

Transcriptional regulation is the main first step in determining the availability of mRNA, but it immediately follows that the mRNA's half-life will dictate its temporal availability for translation of the open reading frame. Wang et al. (2002) have reported that in yeast the half-lives of mRNA ranged from 3 to 90 min. There was no correlation between the half-life and ribosome density (translational activity), but the half-lives of proteins that form a physical complex were very similar. For example, the 4 core histones have a decay rate of $t_{1/2} = 7 \pm 2$ min, while 131 ribosomal protein mRNA have a $t_{1/2} = 22 \pm 6$ min. Similarly, decay rates of mRNA of functionally related genes were also comparable. In general, genes related to metabolism had longer half-lives than those enrolled in regulatory functions, such