microRNAs and muscle disorders

Jian-Fu Chen, Thomas E. Callis and Da-Zhi Wang*

Carolina Cardiovascular Biology Center, and Department of Cell and Developmental Biology, University of North Carolina, Chapel Hill, NC 27599-7126, USA

*Author for correspondence (e-mail: dawang@med.unc.edu)

Journal of Cell Science 122, 13-20 Published by The Company of Biologists 2009
doi:10.1242/jcs.041723

Summary

MicroRNAs (miRNAs) are a class of non-coding regulatory RNAs of ~22 nucleotides in length. miRNAs are highly conserved across a number of species, including plants, worms and humans. miRNAs regulate gene expression post-transcriptionally, primarily by associating with the 3′ untranslated region (UTR) of their regulatory target mRNAs. Recent work has begun to reveal roles for miRNAs in a wide range of biological processes, including cell proliferation, differentiation and apoptosis. miRNAs are expressed in cardiac and skeletal muscle, and dysregulated miRNA expression has been correlated with muscle-related disorders. Genetic studies have identified distinct roles for specific miRNAs during cardiogenesis, cardiac hypertrophy and electrical conduction. Furthermore, conditionally inhibiting the maturation of miRNAs in mouse cardiac and skeletal muscles has revealed that miRNAs are essential for the development and function of those muscles. These previously unrecognized regulators shed new light on the molecular mechanisms that underlie muscle development and pathology, and suggest the potential importance of miRNAs as diagnostic markers and therapeutic targets for muscle-related disease.

Key words: microRNA, Cardiac muscle, Skeletal muscle, Cardiac hypertrophy, Cardiomyopathy, Gene regulation, Muscle disease

Introduction

MicroRNAs (miRNAs) are emerging as a new layer of regulators of gene expression at the post-transcriptional level; approximately 22 nucleotides long, they generally inhibit translation or promote mRNA degradation by base-pairing to complementary sequences within the 3′ untranslated regions (UTRs) of regulatory target mRNAs (Ambros, 2004; Bartel, 2004) (Fig. 1). Hundreds of miRNAs have been identified and many of them are highly conserved across a number of species, including plants, worms and humans (Berezikov et al., 2006; Griffiths-Jones, 2004). Bioinformatic analyses indicate that miRNAs might target more than a third of human protein-coding genes (John et al., 2004; Lewis et al., 2003). Individual messenger RNAs (mRNAs) can be targeted by several miRNAs, and a single miRNA can regulate multiple target mRNAs. More importantly, miRNAs can coordinately regulate a set of genes encoding proteins with related functions, providing enormous complexity and the potential of gene regulation (Esau et al., 2006; Leung and Sharp, 2007).

The very first miRNA genes, *lin-4* and *let-7*, were identified in *Caenorhabditis elegans* by loss-of-function mutations that cause strong defects in developmental timing (Lee et al., 1993; Reinhart et al., 2000; Wightman et al., 1993). Additional genetic studies performed in *C. elegans* and *Drosophila melanogaster* further support the view that miRNAs have crucial roles in animal development by fine-tuning, or acting as on-off switches in, gene expression (Brennecke et al., 2003; Johnston and Hobert, 2003; Li and Carthew, 2005). The genetic evidence for roles for specific miRNAs in vertebrate development is accumulating slowly, largely because vertebrate miRNAs exist as families with similar or even identical sequences (Lagos-Quintana et al., 2002). However, inducing the loss of all miRNAs in mice by depleting Dicer (also known as Dicer1), a ribonuclease III enzyme responsible for miRNA maturation, caused arrested development during gastrulation before the body plan was fully configured (Bernstein et al., 2003), suggesting that miRNAs are crucial for early vertebrate development.

Interestingly, a subset of miRNAs are either specifically or highly expressed in cardiac and skeletal muscle (Chen et al., 2006; Lagos-Quintana et al., 2002; Wienholds et al., 2005), providing an opportunity to understand how gene expression in these muscle types is controlled at the post-transcriptional level by miRNAs. Muscle cells have provided a powerful system for understanding the genetic networks that control cell differentiation and organogenesis. In this Commentary, we discuss the roles of miRNAs in normal muscle development and function, and in muscle disorders, and consider the prospects for therapeutically exploiting muscle-miRNA biology.

Regulation of miRNA expression in cardiac and skeletal muscle

Cardiac- and skeletal-muscle development begins when mesoderm stem cells adopt muscle-specific fates in response to cues from adjacent tissues (Buckingham, 2006; Solloway and Harvey, 2003). Although both muscle types have distinct structural genes and different functions, cardiac and skeletal muscle share a subset of transcriptional factors and signals that control their specification and differentiation (McKinsey et al., 2002; Potthoff and Olson, 2007). The MADS (MCM1, agamous, deficiens, serum response factor)-box transcriptional factors, including SRF (serum response factor) and MEF2 (myocyte enhancer factor 2), control cardiac- and skeletal-muscle differentiation in combination with other transcriptional regulators. SRF and MEF2 activate skeletal-muscle-specific genes in combination with myogenic bHLH (basic helix-loop-helix) factors, including MyoD and myogenin. Differentiation of cardiac muscle is cooperatively controlled by interactions between SRF and MEF2 together with GATA transcription factors, the homeodomain protein Nkx2.5, the transcriptional coactivator myocardin and others (Liu and Olson, 2006; Olson, 2002).

The regulation of muscle gene expression by the well-known transcriptional networks involving SRF, MEF2 and other transcription factors is complex (Niu et al., 2007). However, the

Journal of Cell Science

Journal of Cell Science

Fig. 1. miRNA gene regulatory networks and unique miRNA features. (A) Control of gene expression involves transcriptional, miRNA-mediated post-transcriptional and translational regulation. The selective expression of primary miRNAs and regular messenger RNAs (mRNAs) starts at the transcription step. mRNAs are further post-transcriptionally regulated by miRNAs through base-pairing to complementary sequences within the 3′ untranslated regions (UTRs) of the mRNAs, resulting in mRNA degradation and/or translational repression. (B) Features of miRNAs. Large numbers of miRNAs, which are highly conserved across species, have been identified and many of them are abundantly expressed. A single miRNA is thought to have dozens of targets and one particular mRNA can be regulated by multiple miRNAs simultaneously. ORF, open reading frame.

discovery of miRNAs as a previously unrecognized component of these networks starts to add an entirely new layer of complexity at the post-transcriptional level to our understanding of how muscle gene expression is regulated. SRF and MEF2 both regulate the expression of two pairs of related muscle-specific miRNA genes: miR-1-1 and miR-133a-2 (miR-1-1/miR-133a-2; clustered on mouse chromosome 2), and miR-1-2 and miR-133a-1 (miR-1-2/miR-133a-1; clustered on mouse chromosome 18). Each miRNA pair is encoded by a bicistronic miRNA gene (Chen et al., 2006; Liu et al., 2007; Niu et al., 2007; Rao et al., 2006; Zhao et al., 2005) (Fig. 2). In skeletal muscle, SRF and MEF2 cooperate with MyoD and myogenin to activate the expression of these miRNAs, the upstream promoter and/or enhancers of which contain binding sites for the SRF and MEF2 transcription factors. In the heart, SRF directs the expression of these miRNAs in ventricular and atrial myocytes (Niu et al., 2008), whereas MEF2 binds to the intronic enhancer of these miRNA genes to activate their expression in ventricular myocytes. A third pair of related miRNAs, miR-206 and miR-133b (miR-206/miR-133b), are skeletal-muscle specific and are controlled by MEF2, MyoD, myogenin and other regulators (Kim et al., 2006; Rao et al., 2006; Rosenberg et al., 2006). miR-1-1 and miR-1-2 are identical in nucleotide sequence, whereas miR-206 differs from miR-1-1 and miR-1-2 by four nucleotides. miR-133a-1, miR-133a-2 and miR-133b have the same sequence except for one nucleotide at the 3′ end of miR-133b (Fig. 2). The sequence similarity of these muscle-specific miRNAs suggests that they share the same or similar regulatory mRNA targets and creates a substantial challenge to functionally study these miRNAs using conventional loss-of-function approaches.

The primary transcripts of both miR-1-1/miR-133a-2 and miR-206/miR-133b are derived from independent non-coding RNAs, whereas the precursor of miR-1-2/miR-133a-1 is derived from the intron of Mib1, an E3 ubiquitin ligase (Chen et al., 2006) (Fig. 2). Considering the ubiquitous expression pattern of Mib1, the muscle-specific expression of miR-1-2/miR-133a-1 must rely on its own promoter. In contrast to the miR-1, miR-133 and miR-206 miRNAs, which are expressed as independent transcriptional units, miR-208a is encoded by an intron of its host gene, α-myosin heavy chain (Myh6; also known as αMHC). Both miR-208a and αMHC are cardiac specific and are concurrently expressed during development, suggesting that their expression levels are controlled by a common regulatory element (van Rooij et al., 2007). In addition, miR-208b (another member of the miR-208 miRNA family), which shares a high level of sequence similarity with miR-208a, is located within the intron of the gene encoding β-myosin heavy chain (Myh7; also known as βMHC), whereas miR-499 is located in the intron of its host myosin heavy chain 7b (*Myh7b*) gene (Landgraf et al., 2007) (Fig. 2). Although their expression and function have not yet been described, our unpublished data show that miR-208b and miR-499 are co-expressed with their host myosin genes in cardiac muscle. Collectively, the expression of muscle-specific miRNAs is under

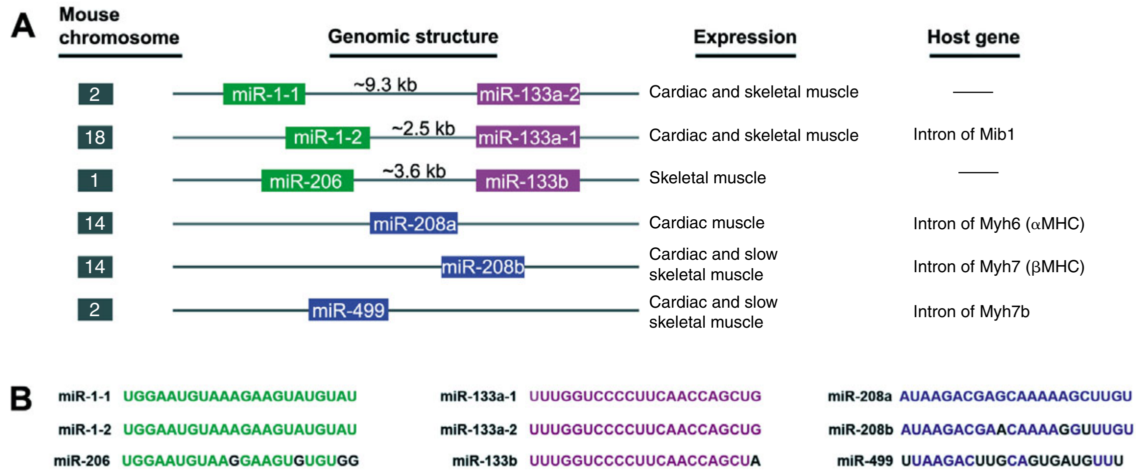

Fig. 2. Genomic structures of muscle-specific miRNAs and their sequence homologies. (A) The genomic locations of muscle-specific miRNA genes, including *miR-1-1/miR-133a-2*, *miR-1-2/miR-133a-1*, *miR-206/miR-133b*, *miR-208a*, *miR-208b* and *miR-499*, on mouse chromosomes. The expression of these miRNAs and the host genes in which they reside are also indicated. (B) Comparison of muscle-specific miRNA sequences (shown 5′-3′). Matching colors indicate the homology of miRNA gene families, whereas the black coloring marks nucleotide differences.

tight spatial and temporal regulation by transcriptional networks that are important for muscle gene expression.

miRNAs in skeletal-muscle proliferation and differentiation

The importance of miRNAs in normal skeletal-muscle development is highlighted by Dicer loss-of-function studies (O'Rourke et al., 2007). A conditional Dicer allele was specifically removed in the skeletal muscle by the expression of Cre recombinase under the control of MyoD regulatory elements, which direct gene expression in skeletal muscle as early as embryonic day 9.5 (E9.5). The elimination of Dicer in skeletal muscle disturbs embryonic and postnatal skeletal-muscle development, causing skeletal-muscle hypoplasia and perinatal death of mutant mice (O'Rourke et al., 2007).

The proliferation and differentiation of skeletal-muscle cells is mutually exclusive. The proliferating muscle cells (myoblasts) actively expand under growth conditions, whereas they quickly exit from the cell cycle in response to growth-factor depletion (differentiation conditions) and fuse to form terminally differentiated multinucleated myotubes (Parker et al., 2003; Pownall et al., 2002). By regulating the balance between the antagonistic activity of cell proliferation and differentiation, miRNAs are integral components of genetic regulatory circuits for skeletal-muscle development. Our laboratory reported that the muscle-specific miRNAs miR-1 and miR-133 modulate skeletal-muscle-cell proliferation and differentiation by repressing the activity of HDAC4 (histone deacetylase 4; a signal-dependent inhibitor of muscle differentiation) and SRF, respectively, thereby establishing negative-feedback loops for muscle-cell differentiation (Chen et al., 2006). Using a C2C12 myoblast model system, which faithfully mimics skeletal-muscle development in vitro, overexpression and knockdown experiments demonstrated that miR-1 enhances skeletal-muscle-cell differentiation. In further support of those in vitro results, the injection of single-cell *Xenopus laevis* embryos with miR-1 led to developmental defects in which miR-1 promoted the differentiation of muscle cells but inhibited their proliferation. Furthermore, miR-1 represses the expression of HDAC4, which represses MEF2 activity (Lu et al., 2000; McKinsey et al., 2000). MEF2, in turn, is a potent activator of miR-1 expression in skeletal and cardiac muscle (Liu et al., 2007; Rao et al., 2006). Therefore, the upregulation of miR-1 during skeletal-muscle development is an effective mechanism to dampen the expression of HDAC4 and promote muscle-cell differentiation through the activity of MEF2 (Fig. 3).

By contrast, miR-133 promotes the proliferation of myoblasts and inhibits their differentiation. miR-133 enhances myocyte proliferation, at least in part, by reducing protein levels of SRF, a crucial regulator of muscle-cell differentiation (Chen et al., 2006; Niu et al., 2007). miR-133 also inhibits the translation of polypyrimidine tract-binding protein (nPTB), which controls differential transcript splicing during skeletal-muscle-cell differentiation (Boutz et al., 2007). Paradoxically, miR-1 and miR-133 exert opposing effects on skeletal-muscle development despite originating from the same miRNA polycistronic transcript. Interestingly, miR-1 and miR-133 also produce opposing effects on apoptosis (Xu et al., 2007). Additionally, embryonic stem (ES)-cell differentiation towards cardiomyocytes is promoted by miR-1 and inhibited by miR-133 (Ivey et al., 2008). These studies support the view that miR-1 and miR-133 fine-tune key regulatory pathways for muscle-cell proliferation and differentiation in an antagonistic manner, with the balance being tipped one way or the other by additional regulators of gene expression.

miR-1 is highly conserved among worms, flies, fish and humans. Genetic studies in *Drosophila* revealed that miR-1-mutant larvae exhibit decreased locomotion, which ultimately progresses to death, accompanied by severe gross disruption of the larval musculature (Kwon et al., 2005; Sokol and Ambros, 2005). A subset of severely affected *miR-1*-null larvae exhibited an enlarged pool of cardiac progenitors (Kwon et al., 2005), indicating that miR-1 inhibits cell proliferation; this result is consistent with mammalian studies involving miR-1.

Similar to miR-1 in skeletal muscle, miR-206, which is specific to skeletal muscle and is not found in cardiac muscle, promotes myoblast differentiation. miR-206 has been shown to inhibit the expression of the gap-junction protein connexin 43 (Cx43), thereby decreasing the electrical coupling between myofibers via gap

junctions, which impedes the terminal differentiation of skeletal-muscle cells (Anderson et al., 2006). miR-206 also represses the translation of the p180 subunit of DNA polymerase-α (polA1), thereby inhibiting DNA synthesis and suppressing cell proliferation (Kim et al., 2006). In addition, miR-206 has been proposed to be the missing link that underlies MyoD-mediated gene repression during skeletal-muscle-cell differentiation, in which miR-206 mediates the inhibition of the genes encoding follistatin-like 1 (Fstl1) and utrophin (Utrn) in skeletal-muscle cells (Rosenberg et al., 2006).

In addition to muscle-specific miR-1, miR-133 and miR-206, several other miRNAs have also been implicated in skeletal-muscle-cell proliferation and differentiation. miR-181 is broadly expressed and upregulated in response to skeletal-muscle injury (Naguibneva et al., 2006). miR-181 is also upregulated during myocyte differentiation and represses homeobox protein Hox-A11, a repressor of muscle-cell differentiation, thereby allowing new muscle growth (Naguibneva et al., 2006). miR-214 is expressed in skeletal-muscle progenitor cells during zebrafish development and was shown to specify the muscle-cell type by modulating the response of muscle progenitor cells to Hedgehog signaling (Flynt et al., 2007). It will be interesting to test whether miR-214 has a similar role in mammalian skeletal-muscle development.

miRNAs in normal heart development

Two studies of heart-specific Dicer deletion illustrate that miRNAs have crucial roles during normal vertebrate heart development and function (Chen et al., 2008; Zhao et al., 2007). Conditional deletion of Dicer, a protein that is required for miRNA maturation, removed all cardiac-expressed miRNAs. The use of a Cre recombinase controlled by the predominantly postnatally expressed αMHC promoter to delete Dicer in the heart did not affect the specification and patterning of cardiac muscle. However, the hearts of those mice exhibited aberrant expression of cardiac contractile proteins and remarkable sarcomere disarray coupled with significantly reduced cardiac function; the mice progressed rapidly to dilated cardiomyopathy, heart failure and postnatal death (Chen et al., 2008). However, the use of Cre recombinase under the control of the *Nkx2.5* promoter, which directs Cre recombinase expression in the early embryonic heart, to delete Dicer resulted in early embryonic lethality with heart morphogenesis defects in some Dicer-null embryos (Zhao et al., 2007). Genetic elimination of Dicer at different heart developmental stages has provided strong evidence that miRNAs are required for normal heart development and function.

Genetic studies have also revealed crucial and distinct roles for individual miRNAs in cardiac development (Chen et al., 2006; van Rooij et al., 2006; van Rooij et al., 2007; Zhao et al., 2007). In developing mouse embryos, overexpression of miR-1 in the heart causes lethality during mid-embryogenesis owing to cardiomyocyte deficiency and subsequent heart failure (Zhao et al., 2005), whereas the introduction of miR-1 into developing *Xenopus* embryos also perturbs heart development, with defects in cell proliferation (Chen et al., 2006). Excessive numbers of cardiac cells were consistently found in miR-1-2-knockout hearts (Zhao et al., 2007), indicating that miR-1 inhibits cell proliferation during normal heart development. Furthermore, forced expression of miR-1 promotes ES-cell differentiation towards a cardiac lineage (Ivey et al., 2008). Although definitive functions of miR-133 in heart development are yet to be established, it is intriguing to speculate that miR-133 will also have distinct roles in heart development, as miR-1 and miR-133 have opposing functions in skeletal muscle (Chen et al., 2006). miR-208a is exclusively expressed in the heart but, interestingly, its deletion in the mouse heart did not cause any apparent gross developmental defects, suggesting that miR-208a is not crucial for normal heart development (van Rooij et al., 2007). The reported phenotypes of other transgenic mice that overexpress miRNAs range from benign to catastrophic: miR-214 overexpression led to no apparent cardiac defects, miR-195 overexpression caused hypertrophic growth in the adult heart and overexpression of miR-24 resulted in embryonic lethality (van Rooij et al., 2006). Together, these findings indicate that miRNAs are required for normal cardiogenesis, with individual miRNAs having specific roles.

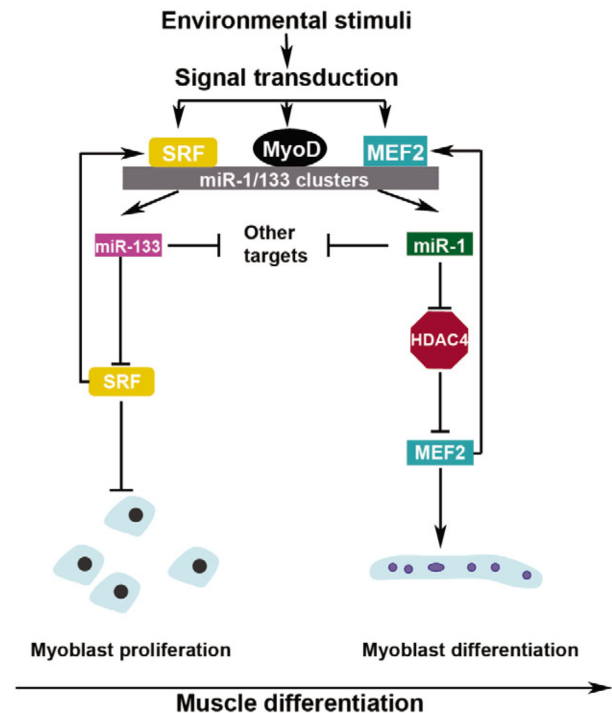

Fig. 3. Model of miR-1- and miR-133-mediated gene regulation during muscle proliferation and differentiation. Tissue-specific expression of *miR-1* and *miR-133* clusters is controlled by the transcription factors SRF, MEF2 and MyoD. miR-1 promotes muscle differentiation by repressing the expression of HDAC4 (histone deacetylase 4), a signal-dependent inhibitor of muscle differentiation that represses MEF2 activity. MEF2, in turn, potently activates the expression of myoblast-differentiation genes and *miR-1*. miR-133, however, reduces protein levels of SRF, a crucial regulator of muscle differentiation, thereby enhancing the proliferation of myoblasts and inhibiting their differentiation.

miRNAs in skeletal-muscle disorders

Primary skeletal-muscle disorders involve different groups of diseases, including muscular dystrophies, inflammatory myopathies and congenital myopathies. Currently, although the number of genes known to be involved in muscle disorders increases every year and histological pathology of disease tissue is well documented, the underlying molecular pathways remain poorly understood (Davies and Nowak, 2006). Recent studies have begun to link miRNAs and certain muscle-related diseases together (Eisenberg et al., 2007; McCarthy and Esser, 2007; McCarthy et al., 2007).

miR-206 levels are elevated in the diaphragm muscle of the *mdx* (also known as *Dmd*) mouse, an animal model of muscular dystrophy (McCarthy et al., 2007). Moreover, the expression levels

of miR-1 and miR-133a were decreased in a mouse model of skeletal-muscle hypertrophy (McCarthy and Esser, 2007). Comprehensive miRNA expression-profiling studies revealed that a total of 185 miRNAs were dysregulated in samples of diseased muscle tissue from ten different muscle disorders – five miRNAs (miR-146b, miR-221, miR-155, miR-214 and miR-222) were consistently regulated in almost all samples that were examined (Eisenberg et al., 2007), suggesting a possible involvement of common miRNA-mediated regulatory mechanisms in muscle disorders. In addition to those studies of miRNA expression in muscle disorders, a direct genetic link has connected miRNA function to muscle hypertrophy (Clop et al., 2006). A mutation that is responsible for the exceptional muscularity of Texel sheep has been mapped to a single G-to-A mutation within the 3′ UTR of the mRNA encoding myostatin, a member of the transforming growth factor-β (TGFβ) family; myostatin functions to repress muscle growth. This mutation creates a binding site for miR-1 and miR-206, leading to the translational repression of myostatin, which phenocopies the 'muscle doubling' that results from the loss of myostatin in mice, cattle and humans (Lee, 2004; Tobin and Celeste, 2005). These findings underscore the importance of miRNA-mediated regulation in diverse muscle biological processes and disease status.

miRNAs and cardiac hypertrophy

One of the major responses of the heart to biomechanical stress and pathological stimuli is to undergo extensive cardiac remodeling, known as cardiac hypertrophy, which is anatomically defined as an increase in the thickness of the cardiac ventricular wall, owing to enlargement of myocyte size and/or increased fibrosis (Ahmad et al., 2005). Cardiac hypertrophy is initially an adaptive response to maintain cardiac output; however, prolonged hypertrophic growth is associated with adverse consequences that might lead to heart failure and sudden death. Myocardial stiffness – which is associated with myocardial fibrosis – changes in relaxation and contractile properties, and alterations of cardiac cellularity, including perivascular inflammation, are all associated with prolonged hypertrophy (Berk et al., 2007). Cardiac hypertrophy is also accompanied by reactivation of a set of cardiac fetal genes, including those that encode atrial natriuretic peptide (ANP; also known as ANF), B-type natriuretic peptide (BNP), βMHC and others, suggesting that molecular events controlling heart development are redeployed to regulate hypertrophic growth (McKinsey and Olson, 2005).

In addition to their distinct roles in normal heart development, miRNAs also have significant functions in neonatal and adult hearts under both physiological and pathological conditions (Callis and Wang, 2008; van Rooij and Olson, 2007; Zhao and Srivastava, 2007) (Fig. 4). Recently, miRNA microarray analyses were performed to examine miRNA expression profiles in hypertrophic or failing hearts and revealed a collection of miRNAs that are dysregulated under those pathological conditions (Cheng et al., 2007; Sayed et al., 2007; Tatsuguchi et al., 2007; Thum et al., 2007; van Rooij et al., 2006). Furthermore, functional analyses using both gain- and loss-of-function approaches in mice began to establish the roles of miRNAs in cardiac hypertrophy.

miR-195 is upregulated during cardiac hypertrophy in both human and mouse hearts, and is sufficient to induce hypertrophic growth in cultured rat cardiomyocytes. Cardiac-specific overexpression of miR-195 induced hypertrophy within several weeks after birth. Continued miR-195 overproduction led to dilated cardiomyopathy and heart failure in young mice (van Rooij et al., 2006). Although the mechanisms that underlie the function of miR-195 are not yet clear owing to the lack of target verification, these studies suggest that miR-195 can actively contribute to the progression of cardiac disease.

miR-21 is also upregulated in response to agonist-induced cardiac hypertrophy in cell-culture experiments and in pressure-overload-induced hypertrophy in vivo (Cheng et al., 2007; Tatsuguchi et al., 2007; van Rooij et al., 2006). However, the defined function of miR-21 remains elusive. Inhibition of miR-21 using antisense oligonucleotides suppressed agonist-induced hypertrophic growth in primary cardiomyocytes (Cheng et al., 2007), whereas inhibition of miR-21 using locked nucleic-acid-modified miR-21 antisense oligonucleotides stimulated hypertrophic growth in vitro (Tatsuguchi et al., 2007). The basis for these differences is unclear, and further genetic studies and delineation of the underlying molecular mechanisms that are modulated by miR-21 in different biological systems are required to define the function of this miRNA.

Unlike miR-195 and miR-21, the expression of miR-1 and miR-133 is decreased in diseased human heart, as well as in three models of cardiac hypertrophy (Care et al., 2007; Sayed et al., 2007; van Rooij et al., 2006). Overexpression of miR-133 by infecting both neonatal and adult cardiomyocytes with an adenoviral vector expressing miR-133 inhibited the hypertrophic response to agonist stimulation. Conversely, prolonged inhibition of miR-133 in vivo using chemically modified oligonucleotides antisense to miR-133 produced remarkable hypertrophic growth accompanied by an induction of fetal gene expression (Care et al., 2007). These studies indicated that miR-133 has an active role in the inhibition of cardiac hypertrophy. Although miR-1 also inhibited the hypertrophic growth of cardiomyocytes in vitro (Sayed et al., 2007), additional genetic studies are required to confirm the regulatory roles of miR-1 in cardiac hypertrophy in vivo. Both miR-1 and miR-133 are proposed to regulate the expression of growth-related genes (Care et al., 2007; Sayed et al., 2007), indicating that these two miRNAs might act as growth repressors that are relieved during cardiac hypertrophy. Further validation of miR-1 and miR-133 targets and the molecular pathways involved are worthy of pursuit.

miR-208a is expressed specifically in the heart and was deleted recently from the mouse genome by van Rooij and colleagues (van Rooij et al., 2007). *miR-208a*-null mice were viable and appeared to be normal without any apparent developmental defects. However, microarray analyses of hearts from wild-type and mutant mice at 2 months of age revealed statistically significant upregulated expression of numerous skeletal-muscle contractile-protein genes, which are normally not expressed in the heart. Moreover, *miR-208a*-null animals exhibited a slight reduction in contractility at 2 months of age and a continued reduction in cardiac function in later life. These studies suggest that miR-208a normally maintains the cardiomyocyte contractile phenotype by repressing the expression of skeletal-muscle genes in the heart. The remarkable function of miR-208a is revealed by the aberrant hypertrophic response of *miR-208a*-null animals. *miR-208a*-null mice failed to develop hypertrophy of cardiomyocytes or fibrosis and were unable to induce fetal βMHC gene expression in response to constitutively activated calcineurin signaling or cardiac-pressure-overload-induced stress. miR-208a is suggested to regulate stress-dependent cardiac growth and gene expression through, at least in part, the repression of thyroid hormone receptor-associated protein 1 (Thrap1), a cofactor of the

Journal of Cell Science

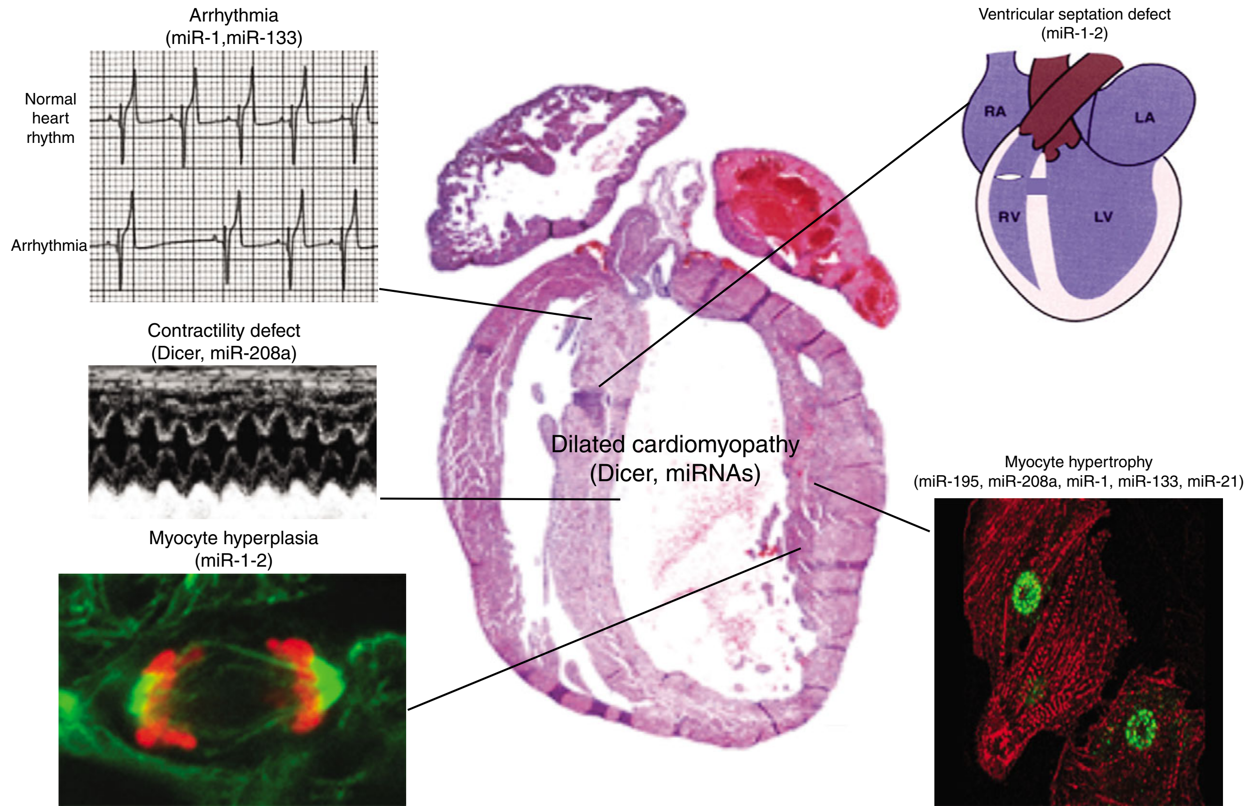

Fig. 4. Roles of miRNAs in heart development and function. Diagrams show some of the known roles of muscle-specific miRNAs in heart development and function. Cardiac-specific deletion of Dicer, a ribonuclease III enzyme that is responsible for miRNA maturation, causes dilated cardiomyopathy and a defect in cardiac contractility. miR-1 contributes to numerous heart abnormalities, including arrhythmias, myocyte proliferation and cardiac hyperplasia, ventricular septation defects, and cardiac hypertrophy, whereas miR-133 is associated with arrhythmias and cardiac hypertrophy. In addition, miR-195, mir-208a and miR-21 are implicated in cardiac hypertrophy, and miR-208a can cause a defect in cardiac contractility.

thyroid-hormone nuclear receptor. Collectively, miR-208a is important for the heart stress response but is not crucial for normal heart development, implying that miR-208a could be an attractive therapeutic target for βMHC-associated cardiac disorders.

miRNAs and cardiac electrical conduction

The cardiac electrical-conduction system comprises specialized myocyte cells and distinct sets of ion channels. Functional defects in the conduction system can result in arrhythmias that cause sudden and unexpected death. In addition to their function in cardiac development (Kwon et al., 2005; Zhao et al., 2007), and myoblast proliferation and differentiation (Chen et al., 2006), miR-1 and miR-133 also regulate the expression of cardiac conduction-system components and are arrhythmogenic (Xiao et al., 2007; Yang et al., 2007).

miR-1 expression is elevated in human hearts with coronary artery disease and in infarcted rat hearts. To study the role of miR-1 in cardiac pathogenesis, in vivo gene transfer was used to either enhance or inhibit the function of miR-1 in normal or infarcted rat hearts (Yang et al., 2007). The injection of miR-1 into the infarcted myocardium slowed cardiac conduction and led to arrhythmias, whereas specific knockdown of miR-1 inhibited arrhythmogenesis (Yang et al., 2007). These studies suggest that miR-1 is involved in electrical remodeling and arrhythmias, effects that were mediated, at least in part, through the repression of the *KCNJ2* and *GJA1* genes. *KCNJ2* encodes Kir2.1, the inward rectifying K^+ channel that is involved in setting and maintaining cardiac resting membrane potential (Diaz et al., 2004), whereas *GJA1* encodes Cx43, the main cardiac gap-junction channel protein, comprising the hemichannels that are responsible for intercellular conductance in the ventricle (Jongsma and Wilders, 2000).

miR-133 has a role in cardiac conductance abnormalities through the repression of ether-a-go-go-related gene (*ERG*; also known as *KNCH*), which encodes a cardiac K^+ channel protein that is important for myocyte repolarization and is associated with congenital arrhythmias (Xiao et al., 2007). miR-133 levels were found to be elevated in the heart in a rabbit model of diabetes, and this elevation occurred concurrently with lower ERG protein levels but without a reduction in *ERG* mRNA levels (Xiao et al., 2007). Furthermore, the introduction of miR-133 into isolated cardiomyocytes reduced ERG expression post-transcriptionally and, accordingly, delayed myocyte repolarization. Together, increasing evidence has established miRNAs as a novel class of key regulators of heart development and cardiac function.

miRNAs as novel therapeutic targets

The crucial and widespread roles of miRNAs in cardiac- and skeletal-muscle development and function raise interesting prospects for therapeutically exploiting miRNAs in a variety of muscle diseases. Several characteristics of miRNAs make them unique for clinical application. First, miRNAs are quite stable and technologies exist to precisely examine the expression profiles of hundreds of

Journal of Cell Science

miRNAs simultaneously (Bartel, 2004; Thomson et al., 2004), making miRNAs promising potential biomarkers or diagnostic indicators of muscle disorders. In fact, it has been reported that specific miRNA expression patterns are correlated with muscular dystrophies and other myopathies (Eisenberg et al., 2007). In addition, distinct miRNA expression signatures have been implicated in tumors of different types and origins (Garzon and Croce, 2008; Stahlhut Espinosa and Slack, 2006). Second, miRNAs are relatively small molecules, making their in vivo delivery feasible. This is particularly true when additional modifications, such as cholesterol modification, have been developed to help them transit across the cell membrane (Krutzfeldt et al., 2007; Krutzfeldt et al., 2005).

Individual miRNAs are predicted to have multiple mRNA targets and, most importantly, many of these miRNA regulatory targets seem to work in concert to control a common pathway and/or biological function (Esau et al., 2006; Leung and Sharp, 2007). This unique feature of miRNAs is likely to make them efficient tools for targeting a particular disease pathway or process. A notable example is the studies from *miR-208a*-knockout mice (van Rooij et al., 2007). These mice exhibited diminished hypertrophy, fibrosis and fetal gene activation in response to pathological stress, indicating that therapeutic suppression of miR-208a might restrain hypertrophic growth after acute or chronic stress. It is interesting to note that miR-133 represses cardiac hypertrophy (Care et al., 2007), raising the possibility that the introduction of synthetic miR-133 into a patient could control pathological hypertrophy. However, further studies also showed that elevated expression of miR-133 induces arrhythmias (Xiao et al., 2007), raising concern about the application of miR-133 in cardiac hypertrophy.

Chemically modified oligonucleotides can be used to either inhibit a specific miRNA or to disrupt the binding between an miRNA and its cognate targets. A promising approach in this regard is to use chemically modified RNA oligonucleotides, named antagomirs, which form complementary base pairs with miRNA and effectively inactivate miRNA function in animals in vivo (Krutzfeldt et al., 2007; Krutzfeldt et al., 2005). Recently, using the same principle, locked-nucleic-acid-modified oligonucleotide (LNA-antimiR) was demonstrated to effectively antagonize miRNAs in non-human primates (Elmen et al., 2008). With the development of new approaches to inactivate disease-associated miRNAs, it is important to bear in mind that manipulating miRNA function could also generate tremendous ‘off-target’ effects. Clearly, caution is warranted and further studies are required that are directed at understanding the pathways regulated by miRNAs before clinical treatments can be seriously considered.

Future prospects for miRNAs in muscle biology and muscle disorders

miRNA biology is in its infancy and, as an emerging field, there are many more questions than answers. Current studies, especially computational analyses, give us a very broad picture in terms of what miRNAs are likely to be doing. At least one third of human protein-coding genes are predicted to be regulated by as many as 1000 miRNAs, many of which are uncharacterized in terms of their expression pattern, function and regulatory targets. Given the vast number of miRNAs and the diverse functions in different biological processes observed in the relatively small number of miRNAs studied thus far, it is apparent that many new and unanticipated functions of miRNAs in normal muscle development, function and disorders are awaiting discovery. Considering both that many miRNAs fine-tune gene-expression programs and the intrinsic complexity of miRNA functional models, it will take many years and considerable effort to determine the specificity of miRNA-mediated gene regulation at whole-organism levels. miRNAs certainly present a major challenge for the future.

Research in our laboratory was supported by the March of Dimes Birth Defect Foundation, National Institutes of Health and Muscular Dystrophy Association. D.-Z.W. is an Established Investigator of the American Heart Association. Deposited in PMC for release after 12 months.

References

Ahmad, F., Seidman, J. G. and Seidman, C. E. (2005). The genetic basis for cardiac remodeling. *Annu. Rev. Genomics Hum. Genet.* **6**, 185-216.

Ambros, V. (2004). The functions of animal microRNAs. *Nature* **431**, 350-355.

Anderson, C., Catoe, H. and Werner, R. (2006). MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res.* **34**, 5863-5871.

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281-297.

Berezikov, E., Thuemmler, F., van Laake, L. W., Kondova, I., Bontrop, R., Cuppen, E. and Plasterk, R. H. (2006). Diversity of microRNAs in human and chimpanzee brain. *Nat. Genet.* **38**, 1375-1377.

Berk, B. C., Fujiwara, K. and Lehoux, S. (2007). ECM remodeling in hypertensive heart disease. *J. Clin. Invest.* **117**, 568-575.

Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., Mills, A. A., Elledge, S. J., Anderson, K. V. and Hannon, G. J. (2003). Dicer is essential for mouse development. *Nat. Genet.* **35**, 215-217.

Boutz, P. L., Chawla, G., Stoilov, P. and Black, D. L. (2007). MicroRNAs regulate the expression of the alternative splicing factor nPTB during muscle development. *Genes Dev.* **21**, 71-84.

Brennecke, J., Hipfner, D. R., Stark, A., Russell, R. B. 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.

Buckingham, M. (2006). Myogenic progenitor cells and skeletal myogenesis in vertebrates. *Curr. Opin. Genet. Dev.* **16**, 525-532.

Callis, T. E. and Wang, D. Z. (2008). Taking microRNAs to heart. *Trends Mol. Med.* **14**, 254-260.

Care, A., Catalucci, D., Felicetti, F., Bonci, D., Addario, A., Gallo, P., Bang, M. L., Segnalini, P., Gu, Y., Dalton, N. D. et al. (2007). MicroRNA-133 controls cardiac hypertrophy. *Nat. Med.* **13**, 613-618.

Chen, J. F., Mandel, E. M., Thomson, J. M., Wu, Q., Callis, T. E., Hammond, S. M., Conlon, F. L. and Wang, D. Z. (2006). The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat. Genet.* **38**, 228-233.

Chen, J. F., Murchison, E. P., Tang, R., Callis, T. E., Tatsuguchi, M., Deng, Z., Rojas, M., Hammond, S. M., Schneider, M. D., Selzman, C. H. et al. (2008). Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. *Proc. Natl. Acad. Sci. USA* **105**, 2111-2116.

Cheng, Y., Ji, R., Yue, J., Yang, J., Liu, X., Chen, H., Dean, D. B. and Zhang, C. (2007). MicroRNAs are aberrantly expressed in hypertrophic heart: do they play a role in cardiac hypertrophy? *Am. J. Pathol.* **170**, 1831-1840.

Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J. M., Eychenne, F. 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.

Davies, K. E. and Nowak, K. J. (2006). Molecular mechanisms of muscular dystrophies: old and new players. *Nat. Rev. Mol. Cell. Biol.* **7**, 762-773.

Diaz, R. J., Zobel, C., Cho, H. C., Batthish, M., Hinek, A., Backx, P. H. and Wilson, G. J. (2004). Selective inhibition of inward rectifier K+ channels (Kir2.1 or Kir2.2) abolishes protection by ischemic preconditioning in rabbit ventricular cardiomyocytes. *Circ. Res.* **95**, 325-332.

Eisenberg, I., Eran, A., Nishino, I., Moggio, M., Lamperti, C., Amato, A. A., Lidov, H. G., Kang, P. B., North, K. N., Mitrani-Rosenbaum, S. et al. (2007). Distinctive patterns of microRNA expression in primary muscular disorders. *Proc. Natl. Acad. Sci. USA* **104**, 17016-17021.

Elmen, J., Lindow, M., Schutz, S., Lawrence, M., Petri, A., Obad, S., Lindholm, M., Hedtjarn, M., Hansen, H. F., Berger, U. et al. (2008). LNA-mediated microRNA silencing in non-human primates. *Nature* **452**, 896-899.

Esau, C., Davis, S., Murray, S. F., Yu, X. X., Pandey, S. K., Pear, M., Watts, L., Booten, S. L., Graham, M., McKay, R. et al. (2006). miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab.* **3**, 87-98.

Flynt, A. S., Li, N., Thatcher, E. J., Solnica-Krezel, L. and Patton, J. G. (2007). Zebrafish miR-214 modulates Hedgehog signaling to specify muscle cell fate. *Nat. Genet.* **39**, 259-263.

Garzon, R. and Croce, C. M. (2008). MicroRNAs in normal and malignant hematopoiesis. *Curr. Opin. Hematol.* **15**, 352-358.

Griffiths-Jones, S. (2004). The microRNA Registry. *Nucleic Acids Res.* **32**, D109-D111.

Ivey, K. N., Muth, A., Arnold, J., King, F. W., Yeh, R. F., Fish, J. E., Hsiao, E. C., Schwartz, R. J., Conklin, B. R., Bernstein, H. S. et al. (2008). MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. *Cell Stem Cell* **2**, 219-229.

John, B., Enright, A. J., Aravin, A., Tuschl, T., Sander, C. and Marks, D. S. (2004). Human MicroRNA targets. *PLoS Biol.* **2**, e363.

Johnston, R. J. and Hobert, O. (2003). A microRNA controlling left/right neuronal asymmetry in Caenorhabditis elegans. *Nature* **426**, 845-849.

Jongsma, H. J. and Wilders, R. (2000). Gap junctions in cardiovascular disease. *Circ. Res.* **86**, 1193-1197.

Kim, H. K., Lee, Y. S., Sivaprasad, U., Malhotra, A. and Dutta, A. (2006). Muscle-specific microRNA miR-206 promotes muscle differentiation. *J. Cell Biol.* **174**, 677-687.

Krutzfeldt, J., Rajewsky, N., Braich, R., Rajeev, K. G., Tuschl, T., Manoharan, M. and Stoffel, M. (2005). Silencing of microRNAs in vivo with 'antagomirs'. *Nature* **438**, 685-689.

Krutzfeldt, J., Kuwajima, S., Braich, R., Rajeev, K. G., Pena, J., Tuschl, T., Manoharan, M. and Stoffel, M. (2007). Specificity, duplex degradation and subcellular localization of antagomirs. *Nucleic Acids Res.* **35**, 2885-2892.

Kwon, C., Han, Z., Olson, E. N. and Srivastava, D. (2005). MicroRNA1 influences cardiac differentiation in Drosophila and regulates Notch signaling. *Proc. Natl. Acad. Sci. USA* **102**, 18986-18991.

Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W. and Tuschl, T. (2002). Identification of tissue-specific microRNAs from mouse. *Curr. Biol.* **12**, 735-739.

Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., Iovino, N., Aravin, A., Pfeffer, S., Rice, A., Kamphorst, A. O., Landthaler, M. et al. (2007). A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* **129**, 1401-1414.

Lee, R. C., Feinbaum, R. L. and Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**, 843-854.

Lee, S. J. (2004). Regulation of muscle mass by myostatin. *Annu. Rev. Cell Dev. Biol.* **20**, 61-86.

Leung, A. K. and Sharp, P. A. (2007). microRNAs: a safeguard against turmoil? *Cell* **130**, 581-585.

Lewis, B. P., Shih, I. H., Jones-Rhoades, M. W., Bartel, D. P. and Burge, C. B. (2003). Prediction of mammalian microRNA targets. *Cell* **115**, 787-798.

Li, X. and Carthew, R. W. (2005). A microRNA mediates EGF receptor signaling and promotes photoreceptor differentiation in the Drosophila eye. *Cell* **123**, 1267-1277.

Liu, N. and Olson, E. N. (2006). Coactivator control of cardiovascular growth and remodeling. *Curr. Opin. Cell Biol.* **18**, 715-722.

Liu, N., Williams, A. H., Kim, Y., McAnally, J., Bezprozvannaya, S., Sutherland, L. B., Richardson, J. A., Bassel-Duby, R. and Olson, E. N. (2007). An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133. *Proc. Natl. Acad. Sci. USA* **104**, 20844-20849.

Lu, J., McKinsey, T. A., Zhang, C. L. and Olson, E. N. (2000). Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol. Cell* **6**, 233-244.

McCarthy, J. J. and Esser, K. A. (2007). MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J. Appl. Physiol.* **102**, 306-313.

McCarthy, J. J., Esser, K. A. and Andrade, F. H. (2007). MicroRNA-206 is overexpressed in the diaphragm but not the hindlimb muscle of mdx mouse. *Am. J. Physiol. Cell Physiol.* **293**, C451-C457.

McKinsey, T. A. and Olson, E. N. (2005). Toward transcriptional therapies for the failing heart: chemical screens to modulate genes. *J. Clin. Invest.* **115**, 538-546.

McKinsey, T. A., Zhang, C. L., Lu, J. and Olson, E. N. (2000). Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**, 106-111.

McKinsey, T. A., Zhang, C. L. and Olson, E. N. (2002). Signaling chromatin to make muscle. *Curr. Opin. Cell Biol.* **14**, 763-772.

Naguibneva, I., Ameyar-Zazoua, M., Polesskaya, A., Ait-Si-Ali, S., Groisman, R., Souidi, M., Cuvellier, S. and Harel-Bellan, A. (2006). The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. *Nat. Cell. Biol.* **8**, 278-284.

Niu, Z., Li, A., Zhang, S. X. and Schwartz, R. J. (2007). Serum response factor micromanaging cardiogenesis. *Curr. Opin. Cell Biol.* **19**, 618-627.

Niu, Z., Iyer, D., Conway, S. J., Martin, J. F., Ivey, K., Srivastava, D., Nordheim, A. and Schwartz, R. J. (2008). Serum response factor orchestrates nascent sarcomerogenesis and silences the biomineralization gene program in the heart. *Proc. Natl. Acad. Sci. USA* **105**, 17824-17829.

Olson, E. N. (2002). A genetic blueprint for growth and development of the heart. *Harvey Lect.* **98**, 41-64.

O'Rourke, J. R., Georges, S. A., Seay, H. R., Tapscott, S. J., McManus, M. T., Goldhamer, D. J., Swanson, M. S. and Harfe, B. D. (2007). Essential role for Dicer during skeletal muscle development. *Dev. Biol.* **311**, 359-368.

Parker, M. H., Seale, P. and Rudnicki, M. A. (2003). Looking back to the embryo: defining transcriptional networks in adult myogenesis. *Nat. Rev. Genet.* **4**, 497-507.

Potthoff, M. J. and Olson, E. N. (2007). MEF2: a central regulator of diverse developmental programs. *Development* **134**, 4131-4140.

Pownall, M. E., Gustafsson, M. K. and Emerson, C. P., Jr (2002). Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. *Annu. Rev. Cell Dev. Biol.* **18**, 747-783.

Rao, P. K., Kumar, R. M., Farkhondeh, M., Baskerville, S. and Lodish, H. F. (2006). Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc. Natl. Acad. Sci. USA* **103**, 8721-8726.

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.

Rosenberg, M. I., Georges, S. A., Asawachaicharn, A., Analau, E. and Tapscott, S. J. (2006). MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206. *J. Cell Biol.* **175**, 77-85.

Sayed, D., Hong, C., Chen, I. Y., Lypowy, J. and Abdellatif, M. (2007). MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circ. Res.* **100**, 416-424.

Sokol, N. S. and Ambros, V. (2005). Mesodermally expressed Drosophila microRNA-1 is regulated by Twist and is required in muscles during larval growth. *Genes Dev.* **19**, 2343-2354.

Solloway, M. J. and Harvey, R. P. (2003). Molecular pathways in myocardial development: a stem cell perspective. *Cardiovasc. Res.* **58**, 264-277.

Stahlhut Espinosa, C. E. and Slack, F. J. (2006). The role of microRNAs in cancer. *Yale J. Biol. Med.* **79**, 131-140.

Tatsuguchi, M., Seok, H. Y., Callis, T. E., Thomson, J. M., Chen, J. F., Newman, M., Rojas, M., Hammond, S. M. and Wang, D. Z. (2007). Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J. Mol. Cell. Cardiol.* **42**, 1137-1141.

Thomson, J. M., Parker, J., Perou, C. M. and Hammond, S. M. (2004). A custom microarray platform for analysis of microRNA gene expression. *Nat. Methods* **1**, 47-53.

Thum, T., Galuppo, P., Wolf, C., Fiedler, J., Kneitz, S., van Laake, L. W., Doevendans, P. A., Mummery, C. L., Borlak, J., Haverich, A. et al. (2007). MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation* **116**, 258-267.

Tobin, J. F. and Celeste, A. J. (2005). Myostatin, a negative regulator of muscle mass: implications for muscle degenerative diseases. *Curr. Opin. Pharmacol.* **5**, 328-332.

van Rooij, E. and Olson, E. N. (2007). MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J. Clin. Invest.* **117**, 2369-2376.

van Rooij, E., Sutherland, L. B., Liu, N., Williams, A. H., McAnally, J., Gerard, R. D., Richardson, J. A. and Olson, E. N. (2006). A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc. Natl. Acad. Sci. USA* **103**, 18255-18260.

van Rooij, E., Sutherland, L. B., Qi, X., Richardson, J. A., Hill, J. and Olson, E. N. (2007). Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* **316**, 575-579.

Wienholds, E., Kloosterman, W. P., Miska, E., Alvarez-Saavedra, E., Berezikov, E., de Bruijn, E., Horvitz, H. R., Kauppinen, S. and Plasterk, R. H. (2005). MicroRNA expression in zebrafish embryonic development. *Science* **309**, 310-311.

Wightman, B., Ha, I. and 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.

Xiao, J., Luo, X., Lin, H., Zhang, Y., Lu, Y., Wang, N., Zhang, Y., Yang, B. and Wang, Z. (2007). MicroRNA miR-133 represses HERG K+ channel expression contributing to QT prolongation in diabetic hearts. *J. Biol. Chem.* **282**, 12363-12367.

Xu, C., Lu, Y., Pan, Z., Chu, W., Luo, X., Lin, H., Xiao, J., Shan, H., Wang, Z. and Yang, B. (2007). The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. *J. Cell Sci.* **120**, 3045-3052.

Yang, B., Lin, H., Xiao, J., Lu, Y., Luo, X., Li, B., Zhang, Y., Xu, C., Bai, Y., Wang, H. et al. (2007). The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat. Med.* **13**, 486-491.

Zhao, Y. and Srivastava, D. (2007). A developmental view of microRNA function. *Trends Biochem. Sci.* **32**, 189-197.

Zhao, Y., Samal, E. and Srivastava, D. (2005). Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* **436**, 214-220.

Zhao, Y., Ransom, J. F., Li, A., Vedantham, V., von Drehle, M., Muth, A. N., Tsuchihashi, T., McManus, M. T., Schwartz, R. J. and Srivastava, D. (2007). Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell* **129**, 303-317.