

# MINIREVIEW

## Muscling Through the microRNA World

THOMAS E. CALLIS,\* ZHONGLIANG DENG,† JIAN-FU CHEN,\* AND DA-ZHI WANG\*<sup>1</sup>

\**Carolina Cardiovascular Biology Center, Department of Cell & Developmental Biology, University of North Carolina, Chapel Hill, NC 27599; and †Department of Orthopaedic Surgery, The Second Affiliated Hospital, Chongqing University of Medical Sciences, Chongqing 400010, P.R. China*

**microRNAs (miRNAs) are a class of highly conserved small non-coding RNAs that negatively regulate gene expression post-transcriptionally. The emerging field of miRNA biology has begun to unravel roles for these regulatory molecules in a range of biological functions, including cardiac and skeletal muscle development, as well as in muscle-related disease processes. In this paper, we review the role of miRNAs in muscle biology. Recent genetic studies have demonstrated that miRNAs are required for both proper muscle development and function, with crucial roles for miRNAs being identified in regulating muscle cell proliferation and differentiation. Furthermore, dysregulated expression of miRNAs has been correlated to certain muscle-related diseases, including cardiac hypertrophy, cardiac arrhythmias, and muscular dystrophy. Exp Biol Med 233:131–138, 2008**

**Key words:** cardiac muscle; skeletal muscle; microRNA; gene expression; cardiovascular disease; cell proliferation; cell differentiation

### Introduction

There are three major muscle types: cardiac, skeletal, and smooth. All of them are derived from the embryonic mesoderm layer during early embryogenesis. The heart is the first functioning organ to form during mammalian development and cardiac precursor cells come from a population of cells in the anterior lateral plate mesoderm in

early embryos (1). Skeletal muscle arises from paraxial mesoderm that gives rise to the somites along the anteroposterior axis of the embryo (2). Somites become compartmentalized into the myotome, sclerotome, and dermatome, which give rise to skeletal muscle, axial skeleton, and dermis, respectively (2, 3). In contrast to cardiac and skeletal muscle cells, which exit cell cycle and undergo terminal differentiation, smooth muscle cells (SMCs) are highly plastic and can modulate their phenotypes between proliferative and differentiated states in response to extracellular cues (4, 5). Much of our understanding of muscle gene expression regulation during development is at the level of transcription. In skeletal muscle, MyoD family of myogenic transcription factors and MEF2 family of MADS-box transcription factors play a central role in controlling myogenesis (2, 6). In cardiac muscle, Nkx2.5, GATA4 and T-box family transcription factors along with MEF2 are key regulators for cardiac gene expression and cardiomyocyte development (7). By contrast, SRF and myocardin are the known essential factors in smooth muscle-related gene expression and SMC differentiation (5, 8).

microRNAs (miRNAs or miRs) are an increasingly important class of small non-coding RNAs that negatively regulate gene expression post-transcriptionally. Their discovery and subsequent analysis has produced another layer of complex mechanisms appearing to ‘fine-tune’ protein dosages of key regulators in a variety of biological processes. Recent studies have shown that muscle miRNAs regulate the expression of transcription factors and signaling mediators important for muscle biology, including such fundamental processes as the regulation of proliferation and differentiation during myogenesis. Aberrant miRNA expression has been observed during muscle diseases, including cardiac hypertrophy as well as in muscular dystrophy. Genetics studies demonstrate that some of these

---

Research in the Wang lab was supported by the March of Dimes Birth Defects Foundation, Muscular Dystrophy Association, and NIH.

---

<sup>1</sup>To whom correspondence should be addressed at dawang@med.unc.edu

---

DOI: 10.3181/0709-MR-237  
1535-3702/08/2332-0131\$15.00  
Copyright © 2008 by the Society for Experimental Biology and Medicine

---

dysregulated miRNAs are sufficient to induce hypertrophy, while others are required for the process. Here, we review the wide range of emerging roles for miRNAs in muscle biology.

**miRNA Biogenesis and Its Requirement for Proper Development.** miRNAs arise endogenously from independent transcriptional units or from within the introns of messenger RNA (mRNA) transcripts (9). miRNAs are initially part of immature primary transcripts that undergo extensive post-transcriptional processing to yield mature miRNAs, whose lengths are approximately 18 to 24 nucleotides. The lengths of the primary transcripts range from several hundred to several thousand nucleotides and may harbor a single miRNA or sometimes several (10). Mature miRNAs become part of the RNA-induced silencing complex (RISC) that facilitates miRNA-mediated regulation of gene expression through complementary base-pairing between a miRNA and sequence(s) within the 3' untranslated region (UTR) of targeted mRNAs (11, 12). The majority of animal miRNAs base pair imperfectly to their targeted mRNAs, which generally results in suppression of translation (9). The mechanism underlying this suppression is thought to occur at the initiation step of translation, where the RISC component Ago2 precludes binding of eIF4E, an essential translation factor, to the 7-methylguanosine cap of a targeted mRNA (13). Interestingly, miRNAs have also been shown to affect stability of targeted mRNAs and mediate their degradation (14). Furthering our understanding of gene expression regulation by miRNAs has been their connection to discrete cytoplasmic foci called processing bodies (P-bodies), which are sites of programmed mRNA degradation (15–19). Components of RISC, miRNAs and their targeted mRNAs have been shown to co-localize to P-bodies, suggesting that miRNAs may mediate both translation suppression and mRNA degradation by directing targeted mRNAs to P-bodies. Moreover, release of mRNAs targeted by miRNAs from P-bodies and subsequent re-expression of those mRNAs indicate that P-bodies may also function as mRNA storage centers (20).

To date, more than 500 human miRNA genes have been identified, of which many are evolutionarily conserved (21). Many miRNA genes cluster into families based on their sequence similarity, with special weight given to the second through eighth 5' nucleotides termed the 'seed region.' The base pairing between the seed region of a miRNA and its mRNA target site is generally perfectly complementary, thus miRNAs with identical seed regions may target the same sets of genes. For example, miR-1, a cardiac and skeletal muscle miRNA, and miR-206, found only in skeletal muscle, both belong to the same miRNA family, a potential caveat for their genetic analysis since they have overlapping expression patterns and may regulate the same mRNA targets.

Genetic studies removing all functional mature miRNAs by disrupting miRNA biogenesis has provided convincing evidence that miRNAs are required for animal

development (22–25). Dicer is an endonuclease encoded by a single locus in vertebrates and is required to fully process all miRNAs to their mature, active form. Dicer deletion in mice caused arrested development during gastrulation before the body plan was fully configured, suggesting that miRNA function is critical for early development. Similarly, creation of maternal-zygotic Dicer zebrafish mutants resulted in abnormal morphogenesis during gastrulation with somitogenesis and heart development both proving abnormal (26). Circumventing the early lethality of Dicer deletion in mice, conditional Dicer knockout studies using the Cre-loxP system have demonstrated that Dicer, therefore miRNAs, is required for the morphogenesis of the limb (27), skin (28), lung (29), and heart (30). Although these Dicer deletion studies do not provide insight into the precise functions of specific miRNAs, collectively they establish that miRNAs overall play an essential role in animal development.

**Regulation of miRNA Expression in Skeletal and Cardiac Muscle Tissues.** Several microRNA genes are specifically expressed or highly enriched in skeletal and/or cardiac muscle. The expression of muscle-specific miRNAs miR-1, miR-133, miR-206, and miR-208, appears largely regulated by well-established and evolutionarily conserved muscle transcriptional networks involving SRF, MyoD, Twist, MEF2, and myocardin (31–34). For example, miR-1 was highly conserved during evolution and, in addition to mouse and human, it is found in the genomes of organisms as diverse as worm, fly, zebrafish, and chicken. The pathways controlling miR-1 expression also appear highly conserved: *Drosophila* miR-1 expression in the presumptive and early mesoderm occurs downstream of Twist and MEF2, two transcription factors that are major regulators of mammalian muscle development (33, 34). In vertebrates, there are two polycistronic genes that encode miR-1 along with miR-133 (31). Accordingly, the expression of miR-1 and miR-133 mirror one another in skeletal and cardiac muscle, where they are solely expressed. Their muscle-specific expression pattern is explained by promoter analyses demonstrating that both miR-1/miR-133 loci have upstream enhancers with SRF binding sites, and that myocardin activity increases the expression of those promoters in the heart, whereas as their skeletal muscle expression is controlled by MyoD (31, 33). Similarly, MyoD, a transcription factor sufficient to activate the program of skeletal muscle differentiation, stimulates the skeletal muscle-specific expression of miR-206 (35).

In contrast to miR-1, miR-133, and miR-206, which are expressed as independent transcriptional units, miR-208 is encoded by an intron of its host gene alpha myosin heavy chain ( $\alpha$ MHC) (36). More than 127 human miRNAs have been identified within the introns of protein-coding genes, and findings support the idea that these intronic miRNAs are generally co-expressed with their host genes (10, 36–38). In agreement, both miR-208 and  $\alpha$ MHC are heart-specific and concurrently expressed during development, suggesting that

their expression is controlled by a common regulatory element. The promoter region of the  $\alpha$ MHC gene contains several binding elements important for muscle-specific gene expression, such as GATA4 and MEF2 site, and thyroid hormone signaling is also known to play an important role in controlling  $\alpha$ MHC expression. Collectively, these studies indicate that muscle miRNA expression is under tight spatial and temporal regulation by transcriptional networks important for muscle gene expression.

**miRNAs Participate in Skeletal Muscle Proliferation, Differentiation, and Regeneration.** Skeletal muscle cells arise from embryonic mesoderm during embryonic development, where they exist as proliferating myoblasts or terminally differentiated myotubes that have exited the cell cycle. Multiple regulatory factors, including those of transcription factors and cellular signaling molecules play a critical role in the control of muscle proliferation and differentiation (2). Muscle proliferation and differentiation process can be faithfully mimicked *in vitro* in tissue-cultured cells. The C2C12 myoblast cell line will maintain an undifferentiated state and continue to proliferate when cultured in growth medium, where serum is provided. However, they rapidly differentiate into large multinucleated myotubes that express muscle-specific marker proteins when they are switched to differentiation medium, in which serum is removed (39). Interestingly, the expression of miR-1, miR-133 and miR-206 is significantly up-regulated when C2C12 myoblasts were induced to differentiate into myotubes (31, 40); therefore the C2C12 cell line offers an excellent tool to study the biological function and molecular mechanism of miRNAs in regulating muscle development.

Using the C2C12 model system, overexpression and knockdown experiments have been applied to study the function of those muscle miRNAs (31, 40). While miR-1 and miR-206 enhanced myogenesis, overexpression of miR-133 repressed myoblast differentiation and promoted myoblast proliferation. Conversely, inhibition of endogenous miR-1, -133, or -206 led to opposite effects on skeletal muscle proliferation and differentiation. In further support of those *in vitro* results, injection of *Xenopus* embryos with miR-1 or miR-133 led to developmental defects: once again, miR-1 enhances muscle differentiation and inhibits cell proliferation, whereas introduction of miR-133 induces cell proliferation (31). It is interesting to note that miR-1/-206 and miR-133 have opposing effects although miR-1 and miR-133 derive from the same miRNA polycistron and are transcribed together, which further support the view that miRNAs may play an important role in tipping the balance between cell proliferation and differentiation. Of further note, the expression levels of miR-1 and miR-133 were decreased in a functional model of skeletal muscle hypertrophy in the mouse (41). Such altered expression of miR-1 and miR-133 suggests their involvement in the adaptive response to skeletal muscle overload. Among the experimentally verified targets for miR-1, histone deacetylase 4

(HDAC4) has been shown to inhibit muscle differentiation and skeletal muscle gene expression, mainly by repressing MEF2C, an essential muscle-related transcription factor (42, 43). By contrast, miR-133 enhances myoblast proliferation, at least in part, by reducing protein levels of serum response factor (SRF), a critical factor for muscle proliferation and differentiation *in vitro* and *in vivo* (4).

Most recently, miR-1 and miR-133 were shown to play regulatory roles in apoptosis in rat cardiomyocytes: miR-1 mediated a pro-apoptotic effect, while the effect of miR-133 was anti-apoptotic (44). Thus, in addition to their role in regulating muscle cell proliferation and differentiation, miR-1 and miR-133 also seem to play opposing roles in regulating muscle cell apoptosis. The opposing effects of miR-1 and miR-133 during apoptosis are likely explained by which genes are targeted: miR-1 reduced protein levels of HSP60 and HSP70, while miR-133 repressed caspase-9 expression (44). Though a clear picture of which genes are regulated by miRNAs is desperately needed to fully understand the roles of miRNAs in muscle biology, the main theme that has emerged thus far is that miRNAs indeed participate in regulatory networks to modulate muscle gene expression, muscle cell proliferation, differentiation, and apoptosis (31, 44, 45).

Similar to miR-1, miR-206 has also been shown to promote myoblast differentiation (40, 46). Importantly, gap junction protein connexin43 (Cx43) and the p180 subunit (Pola1) of DNA polymerase alpha have been identified as regulatory targets of miR-206 in those studies. Although Cx43 is required for the initial phase of myogenesis, it is rapidly downregulated post-transcriptionally after the induction of differentiation (47), thus miR-206 is suggested to decrease communication between developing muscle fibers by decreasing Cx43 expression (46). Downregulation of Pola1 by miR-206 during early differentiation reduces DNA synthesis and contributes to the suppression of cell proliferation during myotube formation (40). miR-206 is also suggested to mediate MyoD-dependent inhibition of follistatin-like 1 (Fstl1) and Utrophin (Utrn) genes in myoblasts (35). In this case, MyoD activates the expression of miR-206, which in turn represses Fstl1 and Utrn gene expression post-transcriptionally. This study could presumably explain some of the previous observations in which MyoD, known as a transcriptional activator, repressed Fstl1 and Utrn gene expression. In support of a role for miR-206 in muscle pathophysiology, the expression level of miR-206 was elevated in the diaphragm muscle of *mdx* mice, a model of muscular dystrophy (48). While Utrn expression was repressed by miR-206 during myoblast differentiation (35), its expression was upregulated in *mdx* diaphragm muscle (48). This result is seemingly inconsistent with the increase of miR-206 expression found in *mdx* diaphragm muscle, however this phenomenon might reflect decreased efficiency of miRNA-mediated translational repression during a diseased state.

miR-214 is expressed in skeletal muscle cell progen-

itors during zebrafish development and was shown to specify muscle cell type during somitogenesis by modulating the response of muscle progenitors to Hedgehog signaling (49). Blocking miR-214 activity by injecting chemically-modified antisense oligonucleotides into zebrafish embryos decreased in the number of slow-muscle cell types present in the developing somites and distinctly changed the gross morphology of the somites in manner previously associated with attenuated Hedgehog signaling. This phenotype was attributed to relief of miR-214-mediated inhibition of *suppressor of fused (su(fu))* expression (49), a fine-tuner of Hedgehog signaling essential for proper specification of muscle cell types during somitogenesis (50). It will be interesting to test whether miR-214 plays a similar role in mammalian skeletal muscle development. Collectively, these studies indicate that miRNAs function as regulators of gene expression important for myoblast proliferation and differentiation and may play decisive roles in specifying cell types during development.

In contrast to other muscle miRNAs discussed, which are specifically expressed in a tissue-restricted manner, miR-181 is broadly expressed. Interestingly, the expression of miR-181 was increased in the regenerating muscle from an *in vivo* mouse model of muscle injury (51). Further analysis using the C2C12 cell line demonstrated that miR-181 depletion reduced MyoD expression and inhibited myoblast differentiation. One of the genes targeted by miR-181 is homeobox protein Hox-A11, which in turn represses MyoD expression. The proposed mechanism underlying miR-181 function is that miR-181 becomes up-regulated upon differentiation and targets a repressor (Hox-A11) of the differentiation process to allow new muscle growth. This study suggests that miRNAs can play roles in establishing a differentiated phenotype and alludes to the potential role of miRNAs in skeletal muscle regeneration. In addition to myogenesis, miR-181 was shown to modulate hematopoietic lineage differentiation in another study (52), which suggests that individual miRNAs may play very diverse biological roles depending upon their cellular context.

Intriguingly, a genetic link has recently connected miRNA function to muscular hypertrophy. In a study to identify the genetic basis underlying the exceptional muscularity of Texel sheep, the quantitative trait locus responsible was fine-mapped to an interval on chromosome 2 containing the myostatin gene (53). However no polymorphisms were detected in the open reading frame of myostatin, but surprisingly, a G to A transition in the 3' UTR of the myostatin gene created a target site for miR-1 and miR-206. Both miR-1 and miR-206 are strongly expressed in muscle tissues, suggesting that a gain-of-function miRNA target site created by this polymorphism negatively regulates the expression of the Texel myostatin gene. Indeed, myostatin expression/translation was dramatically repressed in Texel sheep. Further supporting the involvement of miRNAs, the 3' UTR containing the polymorphism conferred repression to a reporter gene *in*

*vitro* when miR-1 or miR-206 was co-expressed. Since loss of myostatin in mice, cattle, and human leads to muscle doubling, decreased myostatin expression by miRNAs explained the molecular mechanism underlying the muscle hypertrophy in Texel sheep. The discovery that a mutation in the non-coding region of an important gene created a miRNA target site underscores that importance of miRNAs in diverse biological processes and disease status.

**Evolutionarily Conserved miR-1 Is Essential for Muscle Development.** miR-1 is muscle-specifically expressed and highly evolutionarily conserved, present in worm, fly, fish, mouse, and human. Recent genetic studies of miR-1 in *Drosophila* and mouse provide convincing evidences about the function of this miRNA in muscle development (30, 34, 54). Genetic deletion revealed *Drosophila* miR-1 as an essential gene for viability (34, 54). Homozygous miR-1 mutant larvae exhibit decreased locomotion that ultimately progressed to death accompanied by severe gross disruption of the larval musculature (34), supporting the view that miR-1 plays a critical role in muscle development and function. A subset of severely affected miR-1 null embryos exhibited an enlarged pool of cardiac progenitors, suggesting that miR-1 may modulate differentiation of heart (54). The miR-1 loss-of-function phenotype could be partially rescued by re-introduction of miR-1 into mutant fly embryos, strongly supporting a muscle-specific role for miR-1 (34, 54).

Consistent with the role for miR-1 in muscle differentiation, overexpression of miR-1 in the developing mouse heart resulted in reduced ventricular myocyte expansion and decreased the number of proliferating myocytes (33). This phenotype was explained in part by the presence of a miR-1 target site in the 3' UTR of the Hand2 gene, an important cardiac transcription factor (33), whose genetic ablation in the mouse produced a similar failure in ventricular myocyte expansion (55). Similarly, introduction of miR-1 into *Xenopus* embryos interfered with cardiac and skeletal muscle development (31).

In vertebrates, the genes miR-1-1 and miR-1-2 both produce the mature miR-1 product and appear to have largely overlapping expression patterns (30, 31). Nevertheless, genetic deletion of miR-1-2 in mouse caused half of miR-1-2 null animals to die by weaning age and suffer defects indicative of abnormal cardiac morphogenesis, including incomplete ventricular septation and pericardial edema (30). This distinct phenotype suggests that miR-1-2 plays non-redundant roles with miR-1-1 in the heart despite their overlapping expression patterns and/or that a particularly fine balance of miR-1 levels is required for proper cardiac development. Interestingly, miR-1-2 deletion did not appear to affect skeletal muscle development, which may reflect a difference in the genes affected by miR-1 in cardiac versus skeletal muscle.

**miRNAs Modulate Hypertrophic Growth in Response to Cardiac Stress.** Cardiac myocytes proliferate rapidly during embryogenesis, but adult cardiac



**Table 1.** Roles of miRNAs in Muscle Biology

microRNA	Expression pattern	Biological roles	Validated targets <sup>a</sup>	References
miR-1	Heart, skeletal muscle	Apoptosis, cardiogenesis, conduction, myogenesis, skeletal muscle hypertrophy	Cdk9, Delta, Fibronectin, GDF8, GJA1, Hand2, Irf5, KCNJ2, HDAC4, HSP60, HSP70, KCNE1, nPTB, RasGAP, Rheb	(31–34, 41, 44, 53, 54, 70, 73)
miR-21	Heart, spleen, small intestine, colon	Apoptosis, cardiac hypertrophy, tumorigenesis,	PTEN, TPM1	(74, 75)
miR-133	Heart, skeletal muscle	Apoptosis, conduction, myogenesis, skeletal muscle hypertrophy	Caspase-9, Cdc42, ERG, KCNQ1, nPTB, RhoA, SRF, WHSC2	(31, 32, 41, 44, 70, 76)
miR-181	Brain, heart, lung, kidney, skeletal muscle, bone marrow, spleen, thymus	Myogenesis and regeneration, hematopoiesis	Hox-A11	(51, 52)
miR-195	Heart, lung, kidney, skin	Cardiac hypertrophy	None reported.	(60, 77)
miR-206	Skeletal muscle	Myogenesis	Cx43, GDF8, Fstl1, nPTB, Pola1, Utrn	(35, 40, 46, 53, 70)
miR-208	Heart	Cardiac hypertrophy	Thrap1	(36)
miR-214	Somites	Myogenesis	Su(fu)	(49)

<sup>a</sup> Abbreviations: Cdc42, Cell division cycle 42; Cdk9, Cyclin-dependent kinase 9; ERG, Ether-a-go-go potassium channel; GDF8, myostatin; GJA1, Gap junction protein alpha 1; Hand2, Heart and neural crest derivatives expressed 2; HSP60, heat-shock protein 60; HSP70, heat-shock protein 70; HDAC4, Histone deacetylase 4; Irf5, iroquois homeobox protein; KCNE1, Potassium voltage-gated channel, Isk-related family, member 1; KCNJ2, Potassium inwardly-rectifying channel, subfamily J, member 2; KCNQ1, Potassium voltage-gated channel, KQT-like subfamily, member 1; nPTB, polypyrimidine tract-binding protein 2; PTEN, phosphatase and tensin homolog; RasGAP, Ras GTPase-activating protein; Rheb, Ras homolog enriched in brain; RhoA, Ras homolog A; SRF, Serum response factor; Su(fu), suppressor of fused; Thrap1, thyroid hormone receptor associated protein 1; TPM1, tropomyosin 1; WHSC2, Wolf-Hirschhorn syndrome candidate 2.

myocytes lose their proliferative capacity and respond to mechanical and pathological stimuli by hypertrophic growth (56). Hypertrophic growth helps to sustain cardiac output in the face of such stress and is defined by an increase in myocyte size and/or myofibrillar volume without a change in myocyte number. Cardiac hypertrophy is also accompanied by re-activation of fetal cardiac genes normally expressed in the heart before birth. The reactivation of cardiac fetal genes in post-natal cardiomyocytes suggests the molecular events that control cardiac gene expression during development are redeployed to regulate hypertrophic cardiac growth or heart regeneration (57). Although cardiac hypertrophy induced by pathological stimuli is an adaptive mechanism that is beneficial in the short term, prolonged hypertrophy has adverse consequences associated with heart failure and sudden death. As such, much effort for understanding the complex genetic pathways required for myocardial hypertrophy has been made towards the ultimate goal of improving heart patient prognosis. Recent studies have found miRNA expression profiles changes during cardiac hypertrophy and that specific miRNAs are able to modulate the cellular response to cardiac stress.

Using miRNA microarrays, several reports have found the global miRNA expression profile regulated in models of physiological and pathological cardiac hypertrophy (58–60). Several groups document that expression of only a relatively small fraction of miRNAs were changed in response to cardiac hypertrophy (59, 60), while another group reports expression level changes for more than half of the miRNAs

in the heart (58). Furthermore, dysregulated miRNA expression has been shown in human patients with failing hearts (60–63), suggesting that miRNAs may contribute to heart disease by mediating pathological changes in gene expression.

Consistent with the observation that miRNA expression is dynamically regulated in physiologically- and/or pathologically-induced cardiac hypertrophy, functional analyses using both gain- and loss-of-function approaches have established a correlation between miRNAs and cardiac hypertrophy (36, 58–60, 64, 65). miR-1 and miR-133 are both down-regulated during cardiac hypertrophy and are proposed to be necessary for the expression of targeted growth-related genes and induction of hypertrophic growth (64, 65). In support, ectopic expression of miR-1 or miR-133 inhibited target gene expression and the hypertrophic growth response in a tissue-culture model of cardiac hypertrophy (64, 65). Conversely, blocking endogenous miR-133 function in isolated cardiomyocytes augmented agonist-induced hypertrophy (64). Furthermore, prolonged inhibition of miR-133 *in vivo* using chemically-modified oligonucleotides antisense to miR-133 caused a marked hypertrophic response (64). It should be pointed out that while the expression of miR-1 appears dysregulated in cardiac hypertrophy (65), there is not yet direct genetic evidence supporting a role for this miRNA in the regulation of hypertrophy. Instead, miR-1 was found to have arrhythmogenic potential when overexpressed in adult rat hearts (61), suggesting that miR-1 may play an essential role

in cardiac electrophysiology, in addition to its role in heart development.

Among the miRNAs with their expression altered in hypertrophy, miR-195 is up-regulated during cardiac hypertrophy and was found sufficient to induce hypertrophic growth in cultured cardiomyocytes as well as in transgenic mice (60). In contrast, transgenic mice over-expressing miR-214, a miRNA also up-regulated during hypertrophy and important for modulating Hedgehog signaling during myogenesis, caused no detectable phenotypic effect in the heart (60). Those studies indicate that some miRNAs, but not others, are sufficient to induce cardiac hypertrophy. It will be interesting to investigate whether those miRNAs are necessary for the hypertrophic response using a loss-of-function approach. In addition, how those miRNAs integrate into relevant genetic pathways to modulate the hypertrophic response warrants further investigation.

Although genetic ablation of miR-208 did not identify a critical role in the developing mouse, a striking postnatal role for miR-208 was revealed (36). Loss of miR-208 protects mice against cardiac hypertrophy and the accompanying up-regulation of  $\beta$ MHC induced by hyperthyroidism, activated calcineuron signaling and cardiac pressure-overload induced stress (36). Those results suggest that the genetic pathways coordinating cardiac hypertrophy share a common component regulated by miR-208. One of such candidate is Thyroid hormone receptor associated protein 1 (Thrap1). Thrap1 is a co-factor of the thyroid hormone nuclear receptor that can positively and negatively influence the transcription of its regulatory target genes. Expression of Thrap1 mRNA is targeted by miR-208 at its 3' UTR, therefore Thrap1 protein levels are elevated in miR-208 mutant hearts (36). Those studies suggest that miR-208 may function to modulate cardiac hypertrophy, at least in part, by regulating the expression of a thyroid hormone signaling pathway component.

miR-21, a miRNA implicated in tumor-related cell growth and apoptosis (66–68), is up-regulated in response to agonist-induced cardiac hypertrophy in cell culture experiments and in pressure-overload induced hypertrophy *in vivo* (58–60, 65). Inhibition of endogenous miR-21 was found sufficient to induce hypertrophic growth in isolated rat cardiomyocytes (59), however another study reports that inhibition of miR-21 in an agonist-induced model of hypertrophy attenuated global protein synthesis and cell growth (58). Interestingly, other reports on miR-21 function also appear contradictory: while one study documented that miR-21 inhibition provoked cell growth in HeLa cells (67), others showed that miR-21 inhibition led to activation of apoptosis and decreased cell proliferation (66, 68). Clearly, further analysis of the molecular pathways modulated by miR-21 in different biological systems is needed to better understand the biological function of this miRNA.

Collectively, these studies identify miR-1, miR-21, miR-133, miR-195, and miR-208 as a class of novel important regulators of cardiac hypertrophy. In addition, it is

speculated that the identification of the hypertrophic miRNA expression signature will unveil many hitherto unrecognized players in cardiac hypertrophy that are awaiting closer examination. Given the complexity of the cardiac remodeling occurring during hypertrophy, the identification of specific regulatory mRNA targets for those miRNAs involved in the hypertrophic response will provide more insight into the molecular mechanisms underlying this disease process.

**Future Prospects for microRNA Functions in Muscle Biology.** The budding miRNA field has expanded our understanding of gene expression by adding a novel regulatory mechanism at the post-transcriptional level. With more than one-third of human protein-coding genes predicted as subject to miRNA regulation (69), there is great potential for miRNA involvement in many aspects of muscle biology.

In a recent and intriguing example that speaks to the diversity of miRNA targets, miR-1/206 and miR-133 were found to repress the expression of mRNA splicing factor nPTB and thus regulate alternative splicing during skeletal myogenesis (70). Although much progress has been made towards establishing miRNAs as important regulators in muscle biology, few target genes have been consummately verified relative to the hundreds of predicted target genes (Table I). In addition, the role for miRNAs in smooth muscle biology has not yet been carefully addressed. Smooth muscle cells lining the arterial walls are associated with numerous cardiovascular diseases and the mechanisms by which smooth muscle cells proliferate, differentiate, as well as dedifferentiate and reenter the cell cycle, are not fully understood. Given that miRNAs were recently found aberrantly expressed in injured vascular walls (71), it is of great interest to know what processes those miRNAs might be regulating. Beyond identifying targeted genes, fundamental questions remain about the activities of miRNAs in the cell: miRNAs are generally regarded as repressors of translation normally located in the cytoplasm, however miR-206 was found within the nucleoli of skeletal muscle myoblasts and myotubes (72). What function(s) might miR-206 or other miRNAs be carrying out in the nucleolus? As the research in this field progresses, it will be interesting to see how many different ways and to what extent miRNAs are integrated into muscle biology.

1. Srivastava D. Making or breaking the heart: from lineage determination to morphogenesis. *Cell* 126:1037–48, 2006.
2. Buckingham M. Myogenic progenitor cells and skeletal myogenesis in vertebrates. *Curr Opin Genet Dev* 16:525–32, 2006.
3. Venuti JM, Cserjesi P. Molecular embryology of skeletal myogenesis. *Curr Top Dev Biol* 34:169–206, 1996.
4. Miano JM. Serum response factor: toggling between disparate programs of gene expression. *J Mol Cell Cardiol* 35:577–93, 2003.
5. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 84:767–801, 2004.

6. Berkes CA, Tapscott SJ. MyoD and the transcriptional control of myogenesis. *Semin Cell Dev Biol* 16:585–95, 2005.
7. Olson EN. Gene regulatory networks in the evolution and development of the heart. *Science* 313:1922–7, 2006.
8. Wang DZ, Olson EN. Control of smooth muscle development by the myocardin family of transcriptional coactivators. *Curr Opin Genet Dev* 14:558–66, 2004.
9. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281–97, 2004.
10. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 34:D140–4, 2006.
11. Doench JG, Sharp PA. Specificity of microRNA target selection in translational repression. *Genes Dev* 18:504–11, 2004.
12. Pillai RS, Artus CG, Filipowicz W. Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. *Rna* 10:1518–25, 2004.
13. Kiriakidou M, Tan GS, Lamprinak S, De Planell-Saguer M, Nelson PT, Mourelatos Z. An mRNA m7G cap binding-like motif within human Ago2 represses translation. *Cell* 129:1141–51, 2007.
14. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell* 122:553–63, 2005.
15. Chan SP, Slack FJ. microRNA-mediated silencing inside P-bodies. *RNA Biol* 3:97–100, 2006.
16. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol* 7:719–23, 2005.
17. Rossi JJ. RNAi and the P-body connection. *Nat Cell Biol* 7:643–4, 2005.
18. Sen GL, Blau HM. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol* 7:633–6, 2005.
19. Liu J, Rivas FV, Wohlschlegel J, Yates JR 3rd, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. *Nat Cell Biol* 7:1261–6, 2005.
20. Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. Stress-induced reversal of microRNA repression and mRNA P-body localization in human cells. *Cold Spring Harb Symp Quant Biol* 71:513–21, 2006.
21. Griffiths-Jones S. The microRNA Registry. *Nucleic Acids Res* 32: D109–11, 2004.
22. Wienholds E, Koudijs MJ, van Eeden FJ, Cuppen E, Plasterk RH. The microRNA-producing enzyme Dicer1 is essential for zebrafish development. *Nat Genet* 35:217–8, 2003.
23. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 293:834–8, 2001.
24. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. *Genes Dev* 15:2654–9, 2001.
25. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. *Nat Genet* 35:215–7, 2003.
26. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF. MicroRNAs regulate brain morphogenesis in zebrafish. *Science* 308:833–8, 2005.
27. Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. *Proc Natl Acad Sci U S A* 102: 10898–903, 2005.
28. Andl T, Murchison EP, Liu F, Zhang Y, Yunta-Gonzalez M, Tobias JW, Andl CD, Seykora JT, Hannon GJ, Millar SE. The miRNA-processing enzyme dicer is essential for the morphogenesis and maintenance of hair follicles. *Curr Biol* 16:1041–9, 2006.
29. Harris KS, Zhang Z, McManus MT, Harfe BD, Sun X. Dicer function is essential for lung epithelium morphogenesis. *Proc Natl Acad Sci U S A* 103:2208–13, 2006.
30. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1–2. *Cell* 129:303–17, 2007.
31. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38:228–33, 2006.
32. Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF. Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc Natl Acad Sci U S A* 103:8721–6, 2006.
33. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* 436:214–20, 2005.
34. Sokol NS, Ambros V. Mesodermally expressed *Drosophila* microRNA-1 is regulated by Twist and is required in muscles during larval growth. *Genes Dev* 19:2343–54, 2005.
35. Rosenberg MI, Georges SA, Asawachaicham A, Analau E, Tapscott SJ. MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206. *J Cell Biol* 175:77–85, 2006.
36. van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 316:575–9, 2007.
37. Baskerville S, Bartel DP. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *Rna* 11:241–7, 2005.
38. Kim YK, Kim VN. Processing of intronic microRNAs. *Embo J* 26: 775–83, 2007.
39. Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature* 270:725–7, 1977.
40. Kim HK, Lee YS, Sivaprasad U, Malhotra A, Dutta A. Muscle-specific microRNA miR-206 promotes muscle differentiation. *J Cell Biol* 174: 677–87, 2006.
41. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J Appl Physiol* 102: 306–13, 2007.
42. Lu J, McKinsey TA, Zhang CL, Olson EN. Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol Cell* 6:233–44, 2000.
43. Lu J, McKinsey TA, Nicol RL, Olson EN. Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proc Natl Acad Sci U S A* 97:4070–5, 2000.
44. Xu C, Lu Y, Pan Z, Chu W, Luo X, Lin H, Xiao J, Shan H, Wang Z, Yang B. 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* 120:3045–52, 2007.
45. Wang DZ. Micro or mega: how important are MicroRNAs in muscle? *Cell Cycle* 5:1015–6, 2006.
46. Anderson C, Catoe H, Werner R. MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res* 34: 5863–5871, 2006.
47. Proulx A, Merrifield PA, Naus CC. Blocking gap junctional intercellular communication in myoblasts inhibits myogenin and MRF4 expression. *Dev Genet* 20:133–44, 1997.
48. McCarthy JJ, Esser KA, Andrade FH. MicroRNA-206 is over-expressed in the diaphragm but not the hindlimb muscle of mdx mouse. *Am J Physiol Cell Physiol* 239:451–7, 2007.
49. Flynt AS, Li N, Thatcher EJ, Solnica-Krezel L, Patton JG. Zebrafish

- miR-214 modulates Hedgehog signaling to specify muscle cell fate. *Nat Genet* 39:259–63, 2007.
50. Wolff C, Roy S, Ingham PW. Multiple muscle cell identities induced by distinct levels and timing of hedgehog activity in the zebrafish embryo. *Curr Biol* 13:1169–81, 2003.
  51. Naguibneva I, Ameyar-Zazoua M, Polesskaya A, Ait-Si-Ali S, Groisman R, Souidi M, Cuvelier S, Harel-Bellan A. The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. *Nat Cell Biol* 8:278–84, 2006.
  52. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 303:83–6, 2004.
  53. Clop A, Marcq F, Takeda H, Pirottin D, Tordoir X, Bibe B, Bouix J, Caiment F, Elsen JM, Eycheenne F, Larzul C, Laville E, Meish F, Milenkovic D, Tobin J, Charlier C, Georges M. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat Genet* 38:813–8, 2006.
  54. Kwon C, Han Z, Olson EN, Srivastava D. MicroRNA1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling. *Proc Natl Acad Sci U S A* 102:18986–91, 2005.
  55. Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. *Nat Genet* 16:154–60, 1997.
  56. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 65:45–79, 2003.
  57. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol* 7:589–600, 2006.
  58. Cheng Y, Ji R, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C. MicroRNAs are aberrantly expressed in hypertrophic heart: do they play a role in cardiac hypertrophy? *Am J Pathol* 170:1831–40, 2007.
  59. Tatsuguchi M, Seok HY, Callis TE, Thomson JM, Chen JF, Newman M, Rojas M, Hammond SM, Wang DZ. Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J Mol Cell Cardiol* 42:1137–41, 2007.
  60. van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A* 103:18255–60, 2006.
  61. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G, Wang Z. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med* 13:486–91, 2007.
  62. Ikeda S, Kong SW, Lu J, Bisping E, Zhang H, Allen PD, Golub TR, Pieske B, Pu WT. Altered microRNA expression in human heart disease. *Physiol Genomics*, 2007.
  63. Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, Doevendans PA, Mummery CL, Borlak J, Haverich A, Gross C, Engelhardt S, Ertl G, Bauersachs J. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation* 116:258–67, 2007.
  64. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G. MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13:613–8, 2007.
  65. Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circ Res* 100:416–24, 2007.
  66. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 65:6029–33, 2005.
  67. Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res* 33:1290–7, 2005.
  68. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene* 26:2799–803, 2006.
  69. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120:15–20, 2005.
  70. Boutz PL, Chawla G, Stoilov P, Black DL. MicroRNAs regulate the expression of the alternative splicing factor nPTB during muscle development. *Genes Dev* 21:71–84, 2007.
  71. Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ Res* 100:1579–88, 2007.
  72. Politz JC, Zhang F, Pederson T. MicroRNA-206 colocalizes with ribosome-rich regions in both the nucleolus and cytoplasm of rat myogenic cells. *Proc Natl Acad Sci U S A* 103:18957–62, 2006.
  73. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol* 12:735–9, 2002.
  74. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133:647–58, 2007.
  75. Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J Biol Chem* 282:14328–36, 2007.
  76. Xiao J, Luo X, Lin H, Zhang Y, Lu Y, Wang N, Zhang Y, Yang B, Wang Z. MicroRNA miR-133 represses HERG K<sup>+</sup> channel expression contributing to QT prolongation in diabetic hearts. *J Biol Chem* 282:12363–7, 2007.
  77. Lagos-Quintana M, Rauhut R, Meyer J, Borkhardt A, Tuschl T. New microRNAs from mouse and human. *Rna* 9:175–9, 2003.