JCI The Journal of Clinical Investigation

Non-coding RNAs in muscle differentiation and musculoskeletal disease

Monica Ballarino, … , Alessandro Fatica, Irene Bozzoni

J Clin Invest. 2016;126(6):2021-2030. https://doi.org/10.1172/JCI84419.

Review

RNA is likely to be the most rediscovered macromolecule in biology. Periodically, new non-canonical functions have been ascribed to RNA, such as the ability to act as a catalytic molecule or to work independently from its coding capacity. Recent annotations show that more than half of the transcriptome encodes for RNA molecules lacking coding activity. Here we illustrate how these transcripts affect skeletal muscle differentiation and related disorders. We discuss the most recent scientific discoveries that have led to the identification of the molecular circuitries that are controlled by RNA during the differentiation process and that, when deregulated, lead to pathogenic events. These findings will provide insights that can aid in the development of new therapeutic interventions for muscle diseases.

Non-coding RNAs in muscle differentiation and musculoskeletal disease

Monica Ballarino,[1,2] Mariangela Morlando,[1] Alessandro Fatica,[1] and Irene Bozzoni[1,2,3]

[1]Department of Biology and Biotechnology Charles Darwin and [2]Institute Pasteur Fondazione Cenci-Bolognetti, Sapienza University of Rome, Rome, Italy. [3]Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy.

RNA is likely to be the most rediscovered macromolecule in biology. Periodically, new non-canonical functions have been ascribed to RNA, such as the ability to act as a catalytic molecule or to work independently from its coding capacity. Recent annotations show that more than half of the transcriptome encodes for RNA molecules lacking coding activity. Here we illustrate how these transcripts affect skeletal muscle differentiation and related disorders. We discuss the most recent scientific discoveries that have led to the identification of the molecular circuitries that are controlled by RNA during the differentiation process and that, when deregulated, lead to pathogenic events. These findings will provide insights that can aid in the development of new therapeutic interventions for muscle diseases.

Non-coding RNAs (ncRNAs) include different families of transcripts of both small (sncRNA) and large (lncRNA) sizes. The impact of these molecules in the control of cell development, differentiation, and growth has now been established, and it is clear that these molecules exert their functions in both nuclear and cytoplasmic compartments (1). Muscle differentiation has been one of the most exploited and studied processes due to the availability of suitable cellular systems that faithfully recapitulate in vivo differentiation and animal models for different muscle diseases (2–4).

Alterations in myogenesis may underlie many muscle disorders, including sarcopenia, cachexia, and muscular dystrophies, where alterations in regenerative capacity play a crucial role in disease progression and outcome. Moreover, perturbation of regulatory circuits controlling muscle homeostasis are involved in structural and functional changes that occur during muscle atrophy and hypertrophy; therefore, the identification of new components controlling muscle differentiation and regeneration could clarify the molecular pathogenic mechanisms in different diseases and potentially allow the identification of new therapeutic targets.

MicroRNAs

MicroRNAs (miRNAs) play important roles in all the steps of myogenesis. Additionally, the molecular circuits controlled by miRNAs have been largely characterized (5). During myogenesis an intricate relationship between miRNAs and myogenic factors is established, with miRNAs acting synergistically or antagonistically. miR-1, miR-133, miR-206, miR-499, and miR-208 are the so-called myomiRs because their expression is restricted to skeletal and/or cardiac muscles (6–8). Notably, the major myomiRs have an interesting evolutionary and genomic correlation: miR-1-1 and miR-1-2 are identical and differ from miR-206 by 4 nucleotides, while miR-133a-1 and miR-133a-2 are identical and differ from miR-133b by 2 nucleotides. Moreover, they are organized in duplicated clusters (miR-1-1/miR-133a-1 and miR-1-2/miR-133a-2) that in vertebrates also originated the miR-206/miR-133b locus. While mice lacking only one of the two miR-1 or miR-133 copies displayed minor defects, mainly of cardiac type, deletion of both copies resulted in lethality (9, 10). Conversely, deletion of the regenerative miR-206 in mice substantially delayed regeneration induced by cardiotoxin injury (11).

Figure 1 summarizes the most relevant circuits controlled by miRNAs in skeletal muscle cells. miRNAs can target transcription factors, either promoting or inhibiting myogenesis. Conversely, these same transcription factors can directly control expression of these miRNAs through regulatory feedback loops (9, 12, 13).

Deregulation of miRNA expression is a common feature of several skeletal muscle disorders, and rescue of their correct expression in mouse models ameliorates disease phenotypes (11, 14, 15). miRNA profiles of different skeletal muscle disorders (16–21) revealed that, despite their heterogeneity, these disorders share a common set of deregulated miRNAs, which generally includes myomiRs.

Duchenne muscular dystrophy (DMD), the most common and severe muscular disease characterized by mutations in the dystrophin gene, provides a relevant example of disease-linked miRNA activity that is involved in pathogenic circuits. Besides serving as a structural protein that protects muscle fibers from mechanical damage, dystrophin controls the switch from early to late phases of differentiation, acting as an epigenetic modulator of gene expression through a pathway involving neuronal NOS (nNOS) and histone deacetylase 2 (HDAC2) (22, 23). In normal muscle, dystrophin activates nNOS, which in turn nitrosylates HDAC2, leading to its release from the chromatin of specific target genes to drive transcriptional activation. Several miRNA genes were identified as targets of this pathway, including miRNAs involved in terminal differentiation of muscle, such as miR-1 and

Conflict of interest: The authors have declared that no conflict of interest exists.
Reference information: *J Clin Invest*. 2016;126(6):2021–2030. doi:10.1172/JCI84419.

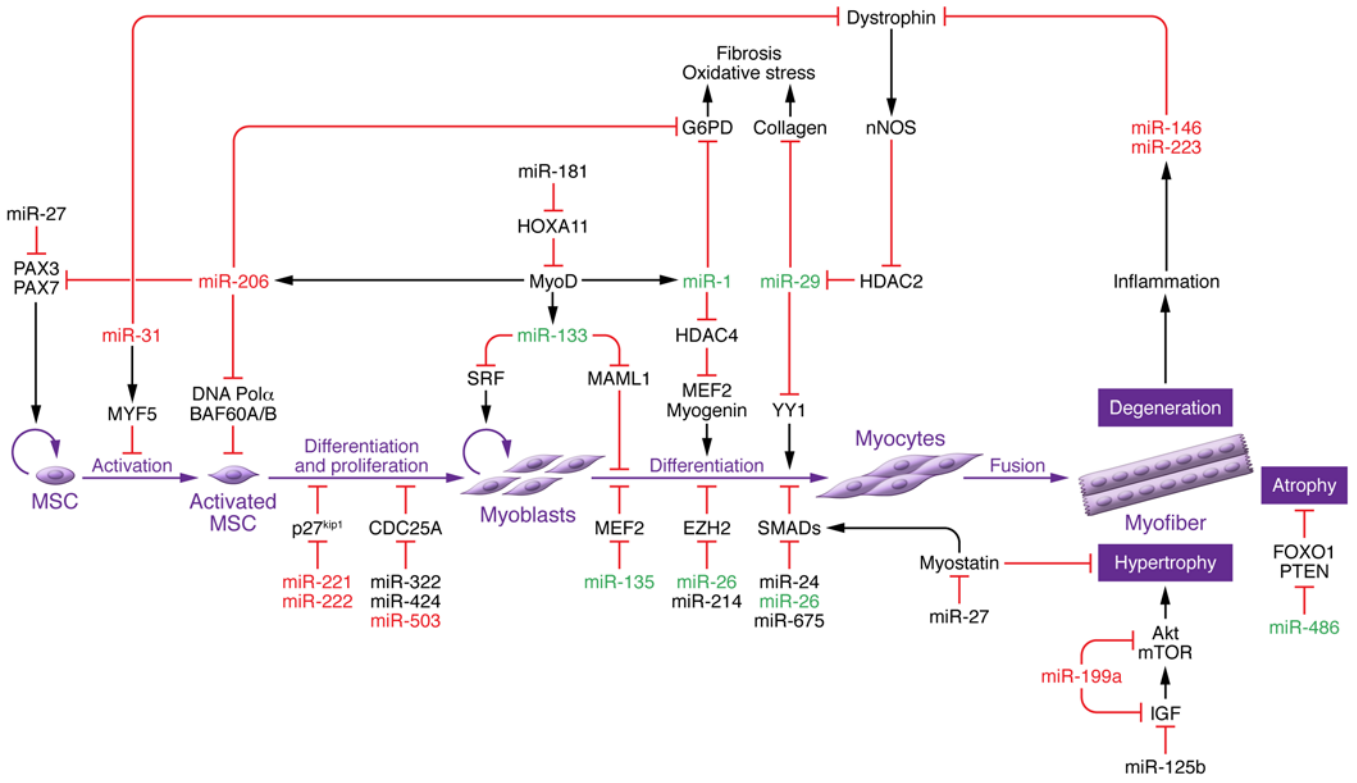

Figure 1. miRNA-mediated regulatory networks in myogenesis and skeletal muscle diseases. Schematic representation of the differentiation stages leading from progenitor muscle cells to terminally differentiated fibers. The most relevant regulatory circuits between miRNAs and protein factors are shown. miRNA species that are up- or downregulated in dystrophic cells are represented in red and green, respectively. Polα, polymerase α.

miR-133, and the more ubiquitous miR-29 and miR-30 (17). In dystrophic muscles, the absence of dystrophin disrupts such circuitry, leading to reduced levels of specific miRNAs, which favors the onset of dystrophic pathogenic traits such as oxidative damage through upregulation of the miR-1 target glucose-6-phosphate dehydrogenase (G6PD) and fibrosis through deregulation of the collagen mRNAs targeted by miR-29 (17, 24, 25) (Figure 1). Notably, both miR-1 and miR-29, which are poorly expressed in murine and human dystrophic muscles, were recovered in exon skipping–treated *mdx* mice (a murine model of DMD) and DMD myoblasts (17). Moreover, these miRNAs were downregulated in individuals with Becker muscular dystrophy (BMD), in which mutant dystrophin cannot bind nNOS (26). In line with these findings, epigenetic control mediated by HDACs was identified as a major regulatory effector in promoting muscle regeneration (22, 27) and a relevant epigenetic HDAC/myomiR network was recently shown to target the transcriptional regulators BRG1-associated factor 60A and 60B (BAF60A and BAF60B), ultimately directing promyogenic differentiation while suppressing the fibro-adipogenic phenotype (28).

miR-1 shares a seed sequence with another myomiR, miR-206; however, unlike miR-1, miR-206 is not under the control of the dystrophin/nNOS/HDAC2 pathway, but it is present at low levels in adult myofibers and is strongly upregulated in regenerating myofibers enriched in DMD muscles (17, 29). Genetic deletion of miR-206 in *mdx* mice accelerated and worsened the dystrophic phenotype (11), while sustained expression of miR-206 promoted satellite cell differentiation and fusion, suggesting that the strong activation of miR-206 in dystrophic muscles induces compensatory circuits to promote the formation of new myofibers in response to disease-induced injury. This activity is mediated at the molecular level through suppression of several negative regulators of myogenesis, including paired box protein–7 (PAX7), Notch3, and insulin-like growth factor–binding protein–5 (IGFBP5) (11, 17).

miR-206 is also linked to amyotrophic lateral sclerosis (ALS). Expression of miR-206 was dramatically increased in an ALS mouse model where pathological alterations are first detected in muscle, particularly at the neuromuscular junction (NMJ) prior to motor neuron loss (30). miR-206 was shown to be required for efficient regeneration of neuromuscular synapses after acute nerve injury, and its genetic deletion accelerated disease progression and diminished survival. Therefore, miR-206 was suggested to slow ALS progression by sensing motor neuron injury and promoting the compensatory regeneration of neuromuscular synapses (14). Two important downstream targets are likely involved in this pathway: HDAC4, which has been implicated in the control of neuromuscular gene expression (31), and the FGF signaling pathway, which promotes presynaptic differentiation at the NMJ (14). Remarkably, for both DMD and ALS, miR-206 expression was upregulated at disease onset and had beneficial effects, suggesting an important role for miR-206 as a stress-inducible suppressor of skeletal muscle disease.

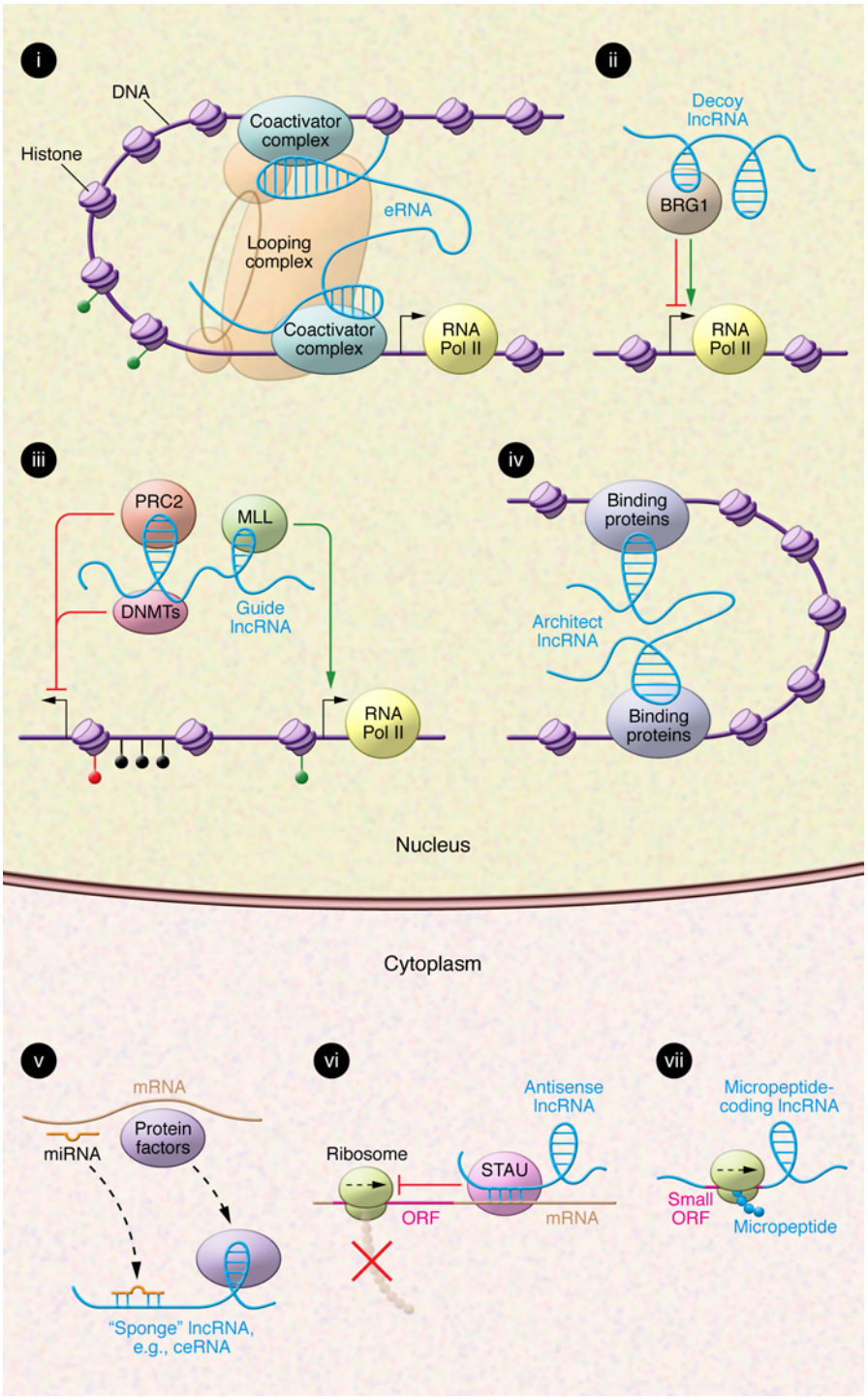

Figure 2. Models of lncRNA function in myogenesis. Nuclear lncRNAs may act as: (i) eRNAs, which regulate transcription through enhancer-like functions (such as core enhancer RNA and ^{DRR}RNA (*MUNC*) [refs. 70, 71, 87] in myogenesis); (ii) decoy lncRNAs, which act by sequestering chromatin or transcriptional regulators (such as MyHeart [ref. 82], which inhibits the chromatin remodeling factor BRG1); (iii) guide lncRNAs, which act by recruiting epigenetic regulators onto specific chromosomal loci (such as *Bvht* [ref. 104], *Fendrr* [ref. 105], *DBE-T* [80], *Dum* [ref. 108], *Meg3* [ref. 95]); (iv) architect lncRNAs, which act by modifying the three-dimensional conformation of chromatin (such as *Kcnq1ot1* lncRNA [ref. 101]). Activating (green) or repressing (red) histone modifications together with the sites of DNA methylation (black) are indicated. Additionally, cytoplasmic lncRNAs may act as (v) sponges, which compete for miRNAs (ceRNAs) or RNA binding proteins. Examples include *Linc-MD1* (110), *H19* (112), and *Malat* (116, 118) ceRNAs and LncMyoD (117); (vi) antisense lncRNAs, which base pair with mRNA to provide a binding site for specific regulators. Examples include the *1/2-sbsRNAs* that induce Staufen1-mediated (STAU-mediated) mRNA decay (119). Finally, previously defined lncRNAs can encode for short peptides (vii), such as *2310015BRik* (*linc00948*) RNA, which encodes for myoregulin (123).

miR-1 and miR-206 are both repressed in rhabdomyosarcoma (RMS), the most common pediatric soft tissue sarcoma (32, 33). RMS is also characterized by the overexpression of early myogenic markers including desmin, myogenin, and myogenic differentiation–1 (MyoD) (33), which are trapped in a nonfunctional state, thereby inhibiting terminal differentiation of myogenic progenitor cells (34). Even though the roles of miR-1 and miR-206 in RMS are still not well defined, several studies suggest that they play an important role in the pathogenesis of this cancer. For instance, the RTK oncogene MET is targeted by miR-206 and is overexpressed in RMS; therefore, MET expression is correlated with the downregulation of miR-206 in RMS (35). Additionally, the exogenous expression of miR-206 in RMS cells blocks tumor growth and promotes terminal differentiation, suggesting that reconstitution of proper miRNA levels could have potential therapeutic applications (36). Global gene expression analysis of RMS cells after miR-206 overexpression led to the identification of two modulated genes: the muscle-restricted histone methyltransferase SMYD1 and G6PD (37, 38). While SMYD1 was upregulated, likely as a consequence of MYOD activation mediated by miR-206 (39), G6PD was downregulated and has been shown to be a direct target of miR-206 (38).

miR-31 provides an interesting link between miRNAs and skeletal muscle pathologies because it is strongly enriched in dystrophic muscles and associated with delay of the muscle differentiation program (40, 41). Notably, miR-31 plays a dual role in dystrophic muscles. It controls satellite cell activation by repressing the synthesis of the myogenic determination factor MYF5 (40) and regulates fiber maturation by targeting several terminal differentiation proteins, including dystrophin (41). In exon skipping–treated human DMD myoblasts, miR-31 inhibition increased dystrophin rescue, indicating that interfering

Table 1. Functional lncRNAs in myogenesis

LncRNA	Organism	Compartment	Role in myogenesis
Braveheart (Bvht)	Mouse	Nucleus	Binds to the PRC2 component SUZ12 and assists in its recruitment to target genes (104)
DBE-T	Human	Nucleus	Activates transcription of the *FSHD* locus by binding to the TrxG/MLL component ASHL1 (80)
ceRNA	Mouse	Nucleus	Acts as an eRNA for the *MyoD* locus (70, 71)
^{DRR}RNA (MUNC)	Mouse	Nucleus	Acts as an eRNA for the myogenin locus (70, 71, 87)
Dum	Mouse	Nucleus	Represses transcription by recruiting DNA methyltransferases (108)
Fendrr	Mouse	Nucleus	Binds both PRC2 and TrxG/MLL histone-modifying complexes to assist in their recruitment to target genes (105)
Gtl2/Meg3	Mouse	Nucleus	Represses the expression of many genes from the *Dlk1-Dio3* region by interacting with the PRC2 complex (95–97)
H19	Mouse	Nucleus/cytoplasm	Promotes differentiation by acting as ceRNA for let-7 microRNA (112)
Kcnq1ot1	Mouse	Nucleus	Regulates heart-specific enhancer function by affecting the 3D conformation of chromatin (101)
Lnc-31/HG31	Mouse/human	Nucleus/cytoplasm	Promotes myoblast proliferation (43)
Linc-MD1	Mouse/Human	Cytoplasm	Promotes differentiation by acting as a ceRNA for miR-133 and miR-135 (110)
LncMyoD	Mouse	Nucleus/cytoplasm	Acts as a competitor for IMP2 protein activity (117)
Malat-1 (Neat2)	Mouse	Nucleus/cytoplasm	Promotes differentiation by acting as a ceRNA for miR-133 (116, 118)
MyHeart (Mhrt)	Mouse	Nucleus	Binds to BRG1 and prevents its remodeling activity (82)
Yam1	Mouse	Nucleus	Inhibits myogenesis by activating miR-715 transcription, which targets Wnt7b (85)
1/2-sbsRNAs	Mouse	Cytoplasm	Drives Staufen1-mediated mRNA decay (119)
2310015BRik (linc00948)	Mouse/human	Cytoplasm	Encodes MLN, a functional micropeptide that regulates muscle contractility (123)

with miR-31 activity can improve DMD therapies aimed at efficiently recovering dystrophin synthesis. Similarly, the atrial-specific upregulation of miR-31 in human atrial fibrillation (AF) was recently shown to cause atrial loss of dystrophin and nNOS, leading to the electrical phenotype induced by AF (42). This finding suggests that an anti–miR-31 therapy in atrial myocytes from patients with AF could restore dystrophin and nNOS and lead to normalization of action potential duration (APD) and APD rate dependence.

An interesting feature of the miR-31 genomic locus is the presence of an overlapping lncRNA in both mice and humans (lnc-31 and MIR31HG, respectively) (43, 44). Lnc-31 and miR-31 have similar expression profiles, with high expression in proliferating myoblasts and downregulation upon muscle differentiation. Like miR-31, lnc-31 is also enriched in *mdx* muscles, and its downregulation is less pronounced upon differentiation of dystrophic myoblasts, reinforcing the hypothesis that lnc-31 plays a crucial role in controlling myoblast proliferation and suggesting a synergistic activity of the overlapping miRNA and lncRNA transcripts (43). Moreover, human MIR31HG sustained myoblast proliferation and counteracted differentiation, indicating that, despite the poor sequence conservation, lnc-31 function is evolutionarily conserved (43).

More recently, other miRNAs (miR-146b, -223, and -374a) were found to target dystrophin mRNA and to increase in dystrophic myofibers, paralleling disease severity (45). Interestingly, miR-146b and miR-223 are activated during inflammation processes in dystrophic muscles through TNF-α–induced NF-κB signaling. Although miR-146b and miR-223 are inflammation-specific miRNAs and are not considered myomiRs, it is possible that ''crosstalk'' between immune cells and myofibers is mediated by horizontal transfer of miRNAs through exosomes or microvesicles (45). These miRNAs are also increased in a wide variety of muscle disorders, such as myositis, Miyoshi myopathy, and limb-girdle muscular dystrophy (16, 17, 46), suggesting that inflammatory miRNAs can be a common signature of muscle diseases where chronic inflammation is present. Targeting of such miRNAs could be effectively combined with other therapeutic strategies, such as the exon skipping approach in DMD (45).

Other non-muscle-specific miRNAs play important roles in myogenesis by regulating different targets (Figure 1), such as miR-26a, which regulates SMAD transcription factors (47) and the polycomb repressive complex 2 (PRC2) component EZH2 (48); miR-27, which regulates the transcription factors PAX3 (49) and myostatin (50); miR-181, which controls the transcription factor HOXA11 (51); miR-221 and miR-222, which repress the cell cycle regulator $p27^{Kip1}$ (52); and miR-322/miR-424 and miR-503, which control the expression of the cell cycle regulator Cdc25 (53).

Circulating ncRNAs as biomarkers

Extracellular miRNAs that circulate in the bloodstream are remarkably stable (54, 55). Moreover, such circulating miRNAs can be delivered to recipient cells, where they can control translation of target mRNAs (56, 57). Specific miRNA signatures have been described in a large collection of skeletal muscle diseases (58–62) and neuromuscular disorders such as ALS (63). In DMD patients and *mdx* mice in particular, many miRNAs, including several myomiRs (miR-1, miR-133, and miR-206), are released into the blood (59, 64). Measurements of the levels of these miRNAs revealed that they correlated with disease severity and decreased when correction of the phenotype and dystrophin rescue were achieved through exon skipping (59). Therefore, circulating miRNAs may be potential diagnostic markers not only for monitoring disease progression, but also for evaluating the outcomes of different thera-

pies. Moreover, the ability of exosomal and circulating miRNAs to be delivered to target cells opens new possibilities for the therapeutic regeneration of skeletal muscle (65).

Recently, lncRNAs have been found in body fluids; however, only a few studies have explored the potential use of these molecules as biomarkers for muscle pathologies. To date, alteration of lncRNA levels in plasma has been reported only in heart failure in humans and mice (66–68).

LncRNAs

Recently, several high-throughput RNA sequencing (RNAseq) experiments identified previously unannotated lncRNAs that are differentially expressed during skeletal myogenesis (43, 69–72). These findings provided a number of candidates to be functionally tested in vivo. Moreover, several transcripts appeared to be dysregulated in dystrophic versus wild-type muscles, indicating their possible link with muscle disorders (43). LncRNAs elicit vastly different effects depending on their subcellular compartmentalization, as discussed below (see Table 1).

Nuclear lncRNAs. Nuclear lncRNAs include *cis*-acting RNAs that work at neighboring genomic loci and *trans*-acting RNAs that act at a distance (Figure 2, i–iv). Recognition of the target regions by lncRNAs can occur through different mechanisms, such as bridging proteins and RNA-DNA hybrids, including triple helix formation (73–76). The ability of lncRNAs to act as scaffolds for different protein factors allows them to mediate different functions (76–79). In most cases, lncRNAs recruit chromatin remodeling and modifying complexes to activate or repress transcription (Figure 2, iii); however, they can also have indirect effects on their targets by acting as decoys for transcription factors (Figure 2, ii), modulating regulatory proteins, and controlling long-range, three-dimensional chromosomal structures (Figure 2, iv) (74, 76).

Cis-acting lncRNAs. DBE-T is one of the first examples of a nuclear lncRNA that functions in gene expression control and is involved in a severe skeletal pathology (80). In patients with facioscapulohumeral muscular dystrophy (FSHD), the presence of a reduced number of D4Z4 repeats leads to decreased polycomb complex binding to this region and consequent activation of DBE-T expression. DBE-T binds to the trithorax (TrxG) protein ASHL1 and recruits it to the FSHD locus, where it mediates the deposition of positive histone-3 lysine-36 dimethylation (H3K36me2). In turn, this chromatin modification produces derepression of FSHD candidate genes localized near the D4Z4 array to promote FSHD pathogenesis (80).

Other common forms of lncRNAs known to control gene expression in *cis* are the so-called natural antisense transcripts (NATs; ref. 81). In mice, antisense transcription from the myosin heavy chain locus (*Myh7*) produces a cluster of lncRNAs named *Myheart* (*Mhrt)*, which are interconnected in a negative feed-forward loop with the Brahma-related gene 1/HDAC/poly(ADP-ribose) polymerase chromatin remodeling complex (BRG1/HDAC/PARP complex) (82). While cardiac stress activates the BRG1/HDAC/PARP complex (83) that inhibits *Mhrt* transcription, the expression of *Mhrt* prevents BRG1 from recognizing its genomic targets, such as *Myh6* and *Myh7*, through competitive binding to the BRG1 helicase domain (82). In hypertrophic hearts, high levels of BRG1 are associated with *Mhrt* repression and decreased *Myh6* and *Myh7* transcription, whereas restoration of *Mhrt* protects against pathological hypertrophy by preventing aberrant fetal gene reactivation during cardiac stress (82). Therefore, reciprocal *Mhrt*/BRG1 inhibition constitutes a feedback circuit that is critical for maintaining cardiac function (82).

YY1-associated muscle lincRNA (Yam-1) is a *cis*-acting lncRNA that belongs to the family of Yam transcripts activated by the Yin Yang 1 (YY1) transcription factor and plays a crucial role in muscle differentiation (72). YY1 controls various processes of development and differentiation (84), is highly expressed in proliferating myoblasts, and is gradually downregulated upon initiation of differentiation. Although the mechanism is not fully understood, Yam-1 exerts its antimyogenic function in *cis* through the modulation of its neighboring *miR-715* gene, which in turn targets Wnt7b. In this way, the activation of the YY1/Yam-1/*miR-715* regulatory axis in proliferating myoblasts culminates in the repression of Wnt signaling and inhibition of myogenic differentiation (85). The downregulation of Yam-1 upon differentiation releases inhibition of skeletal differentiation and in vivo regeneration (72).

Enhancer RNAs. Enhancer RNAs (eRNAs) have been implicated in myogenesis (Figure 2, i; ref. 86). The first eRNAs identified in muscle belong to a relatively large "super-enhancer" region located upstream of *MyoD* (70, 71). The RNAs derived from the core enhancer region and from the distal regulatory region (^{DRR}RNA) (20 kb and 5 kb upstream of the *MyoD* transcriptional start site [TSS], respectively) facilitate the recruitment of the transcription machinery to proximal promoter regions. In early myogenesis core eRNAs assist in *cis* in the reorganization of the chromatin upstream of *MyoD*, while in differentiation, ^{DRR}RNA acts at a distance to activate myogenin (70). More recently, an additional contribution of the murine ^{DRR}RNA (MUNC RNA) in regeneration and repair of damaged skeletal muscles was unveiled (87). In this case, MUNC RNA knockdown had effects on myogenin as well as on *MyoD* and *Myh3* expression, and on other genes that are not known to be induced by MyoD. Notably, the transcriptional kinetics were evaluated at different time points in the two studies, possibly explaining the differential effects of ^{DRR}RNA and MUNC on *MyoD* transcription.

Expression of the imprinted locus containing *Igf2* and the lncRNA *H19* is controlled by an enhancer activity derived from the transcription of the ncRNA *Nctc1* (88). Interestingly, the alteration of *H19* and *IGF2* coexpression is associated with RMS in humans (89). In addition to eRNAs in skeletal muscle, many eRNAs have been identified in cardiac development that are deregulated in several different cardiomyopathies (90, 91). Overall, these findings place eRNAs at the top of a molecular hierarchy regulating myogenesis and predict their possible participation in muscle diseases as a result of mutations and/or altered expression (92).

Genomic imprinting is an important epigenetic mechanism that silences one of the parental copies of a gene. Imprinted regions encode different species of lncRNAs that in many cases bind to imprinted regions and are directly involved in silencing of the neighboring genomic loci (93). Many parentally imprinted genes are expressed at high levels in fetal and newborn tissues and decline during late developmental stages. Notably, different skeletal muscle diseases have been associated with defects in imprinted genes. For example, Angelman syndrome (AS) and

Prader-Willi syndrome (PWS) are imprinting disorders affecting muscle development (93). Loss of methylation on the maternally imprinted gene ε-sarcoglycan causes myoclonus dystonia, a disease characterized by uncontrolled muscle contractions and repetitive movements (94). In mice and sheep, aberrant activation of the imprinted *Dlk1-Dio3* cluster is responsible for the callipyge phenotype, an inherited skeletal muscle hypertrophy (95). Deletion of one of the lncRNAs expressed from this locus, *Gtl2* (also known as *Meg3*), led to perinatal death and skeletal muscle defects in mice (96). Interestingly, *Gtl2* interacts with the repressive PRC2 complex and is directly involved in the epigenetic silencing of many genes from the *Dlk1-Dio3* region, including *Dlk1* (95, 97).

Imprinting at lncRNA loci has been also identified in cancer and heart development. Misregulation of the imprinted locus containing *IGF2* and *H19* (discussed above) was in fact identified in RMS, a tumor that arises from skeletal muscle progenitors (98–100), while *Kcnq1ot1*, an antisense RNA produced from an intron of the *Kcnq1* gene, is a well-characterized imprinted lncRNA required for proper heart development in the mouse (101). Expression of *Kcnq1ot1* inversely correlates with that of *Kcnq1*. The altered expression is due not to the formation of repressive chromatin, but to changes in the three-dimensional conformation of chromatin that results in the inactivation of heart-specific enhancers (102). Proper *Kcnq1* levels are essential for maintaining cardiac rhythm and heart function (103).

Trans-acting lncRNAs. The best example of a nuclear lncRNA acting in *trans* is provided by Braveheart (Bvht) (104). Even if its activity is not specifically restricted to skeletal muscles (it was identified in the mesodermal germ layer, from which heart derives), it remains a paradigmatic example of a lncRNA participating in chromatin remodeling. Bvht has a defined role in the control of the progression of nascent mesoderm toward the cardiac fate. It acts in the same pathway as mesoderm posterior 1 (*Mesp1*), a master regulator of multipotent cardiovascular progenitors, and promotes the activation of the cardiac regulatory network in *trans*. Bvht was found to bind the PRC2 subunit SUZ12 to mediate epigenetic regulation of cardiac commitment (104). Whether SUZ12 competes for PRC2 binding to target sites or if it recruits PRC2 to a repressor of the cardiac program has not been determined (104). Bvht is also required for the commitment of embryonic stem cells (ESCs) toward a cardiac fate, suggesting a possible role in cardiac tissue regeneration after injury (104). Interestingly, no human homolog has been found for Bvht thus far; however, whether the lack of conservation is due to weak pressure on the primary sequence of the lncRNA has not been established yet.

Fendrr is another example of a lncRNA that promotes epigenetic modification (105). In transgenic mice, the insertion of a premature poly(A) signal to disrupt the Fendrr transcript resulted in embryonic lethality due to ventral body wall defects and hypoplastic cardiac ventricles (105). Fendrr binds in vivo to PRC2 and/or to the TrxG and MLL histone modifying complexes and aids in their recruitment to target genes. Among the identified targets, a specific interaction was found to occur with the regions upstream of forkhead box F1 (*Foxf1*) in *cis* and/or paired-like homeodomain transcription factor 2 (*Pitx2*) in *trans*; both of these genes are essential for the definition of lateral mesoderm derivatives (106, 107).

Developmental pluripotency-associated 2 upstream binding RNA (Dum) is a pro-myogenic lncRNA that regulates chromatin organization through the recruitment of the DNA methyltransferases DNMT-1, -3a, and -3b to silence its neighboring gene developmental pluripotency associated–2 (*Dppa2*) (108). The repression occurs through the formation of intrachromosomal loops between Dum and the *Dppa2* promoter that culminates with the hypermethylation of CpG islands. Beyond skeletal muscle, this mechanism might play a role in satellite cell function. In line with this hypothesis, depletion of Dum in vivo decreases PAX7 levels and impairs regeneration of injured muscles.

Cytoplasmic lncRNAs. One of the functions first attributed to cytoplasmic lncRNAs in muscle differentiation and disease is that of miRNA sponges (Figure 2, v). These molecules, called competing endogenous RNAs (ceRNAs; refs. 109, 110), act by sequestering specific miRNAs to protect corresponding target mRNAs from repression. Linc-MD1 was shown to control muscle differentiation in both mouse and human myoblasts through its ability to bind miR-133 and miR-135, alleviating repression of mastermind-like transcriptional coactivator–1 (MAML1) and myocyte enhance factor 2C (MEF2C) (110). Moreover, RNA interference targeting linc-MD1 delayed the differentiation process, whereas overexpression of linc-MD1 improved myogenesis. The identification of MEF2C as a target of linc-MD1 is instrumental in explaining the myogenic alterations observed upon its deregulation. MEF2C belongs to a family of transcription factors that activate the expression of numerous muscle-specific genes that are required for the maintenance of sarcomere integrity (111). Interestingly, in human primary myoblasts from DMD patients, linc-MD1 was strongly downregulated, in line with the notion that Duchenne cultures show a much slower progression into the differentiation process; in contrast, rescue of linc-MD1 expression corrected differentiation timing. These data highlight the importance of the deregulation of linc-MD1–dependent regulatory circuitries in DMD pathogenesis (110).

Other muscle-relevant ceRNAs have also been identified. The imprinted H19 lncRNA was described to harbor several binding sites for the microRNA let-7 and to modulate let-7 availability by acting as a ceRNA (112). Although downregulated after birth, H19 is found at high levels in adult skeletal muscle and heart and is deregulated in several diseases, such as Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS; ref. 113). H19 depletion produced accelerated muscle differentiation, a phenotype recapitulated by let-7 overexpression, suggesting that H19 might serve to inhibit let-7 activity, thereby preventing precocious differentiation. Because let-7 has been implicated in regulating glucose metabolism in muscle, it was suggested that H19 might modulate let-7 action in this organ, thereby contributing to glucose metabolism regulation (109). Low levels of H19 were found in muscles of human subjects with type 2 diabetes (T2D) and in insulin-resistant rodents (114). The increased availability of let-7 caused decreased expression of let-7 targets and impaired glucose homeostasis. Moreover, a double-negative feedback loop was described in which let-7 controls H19 stability in an insulin-dependent manner (114).

Other examples that support the important role of ceRNAs in skeletal and cardiac muscles include the lncRNAs CHRF and Malat-1. While CHRF regulates Myd88 expression and consequent cardiac hypertrophy through sponging activity targeting miR-489

(115), Malat-1 influences the interaction between miR-133 and the serum response factor (*SRF*) 3′ UTR to balance the endogenous level of SRF, and is thereby involved in myogenesis (116).

LncRNAs were recently shown to compete with RNA-binding proteins (Figure 2, v, protein sponges). LncMyoD is a lncRNA controlled by MyoD that is required for myoblast differentiation. LncMyoD was shown to interact with IGF2 mRNA–binding protein 2 (IMP2) and to perturb the translational control mediated by this factor on mRNAs involved in proliferation, such as N-Ras and c-Myc (117). Through this mechanism LncMyoD was suggested to facilitate cell-cycle exit and to promote terminal differentiation. Because LncMyoD is predominantly located in the nucleus, it is not clear where this competition with RNA-binding proteins occurs. Promiscuous partitioning between the nucleus and cytoplasm is a feature frequently exhibited by lncRNAs. Therefore, one interesting question to be addressed is whether lncRNAs mediate different activities in different subcellular compartments, as has been observed for miR-31 (43, 44), H19 (93, 112), and Malat-1 (116, 118).

Antisense-mediated mRNA regulation by lncRNAs has also been linked to myogenesis (Figure 2, vi). Staufen1-mediated decay (SMD) of mRNA was shown to occur in mouse cells, via intermolecular base pairing between short interspersed element–containing (SINE-containing) lncRNAs (m1/2-sbsRNAs) and SINE-containing mRNA 3′ UTRs (119). Notably, downregulation of three of the four m1/2-sbsRNAs altered the rate of mouse myoblast differentiation in vitro (119). In particular, downregulating one of the m1/2-sbsRNAs, which is known to trigger SMD of the E3 ubiquitin ligase mRNA (*Traf6*), promoted myogenesis, and downregulation of *Traf6* mRNA itself was found to inhibit myogenesis (119). Consistent with these findings, SMD was previously shown to increase during the differentiation of mouse myoblasts so as to augment the rate of myogenesis by degrading the mRNA encoding PAX3, which maintains myoblasts in an undifferentiated state (120).

Micropeptides. The algorithms utilized for transcriptome analysis currently discard protein-coding transcripts with ORFs that encode peptides shorter than 100 aa. However, several studies aimed at characterizing the coding potential of different transcripts (121) revealed the presence of short ORFs in previously annotated lncRNAs (122). Identification of functional ORFs in putative lncRNAs relies on sequence conservation and the presence of identifiable functional domains. While searching for new skeletal muscle genes in an unbiased bioinformatics screen, Anderson and colleagues found a highly conserved 138-bp ORF embedded in the third exon of the LINC00948 transcript in humans and the 2310015B20Rik transcript in mice, which had previously been annotated as a lncRNA (123). This short ORF encodes a micropeptide, myoregulin (MLN), which inhibits SERCA, the membrane pump that controls Ca^{2+} uptake into the sarcoplasmic reticulum and serves as a crucial regulator of muscle contractility. More recently a second micropeptide of 34 aa, DWORF, was found to be encoded by a muscle-specific lncRNA previously annotated as non-coding. Notably, this peptide also localizes to the sarcoplasmic reticulum membrane, where it activates SERCA by displacing the SERCA inhibitors phospholamban, sarcolipin, and MLN (124). These findings suggest that many functional short ORFs could be embedded in the many RNAs currently annotated as non-coding, leading to the expectation that new classes of micropeptides with relevant regulatory functions will be discovered (Figure 2, vii).

Circular RNAs

Recent breakthroughs in sequencing technology and computational analyses have revealed the widespread existence in animal cells of a novel class of covalently closed, circular RNA molecules (circRNA), which originate after a back-splicing event (125). The expression of circRNAs is generally high, dynamic, and evolutionarily conserved. Because circRNAs are strongly regulated in physiological and pathological conditions, they are expected to be involved also in muscle differentiation and disease. So far, two studies have reported that circRNAs can act as sponges for miRNAs (126, 127); however, they are likely to have other activities, including coding ability.

Conclusions

Many basic concepts of the molecular mechanisms regulating differentiation in both physiological and pathological conditions have been elucidated by studying skeletal muscle differentiation. The availability of in vitro systems that reproduce the entire differentiation process, from stem cells to mature myofibers, has made myogenesis one of the few systems where regulatory circuits have been dissected and integrated at very sophisticated levels. Moreover, the study of ncRNA function in myogenesis has provided important contributions that have generated paradigms for other organs and tissues. It is very likely that the skeletal muscle research community will continue to deliver major contributions to the understanding of organ development and regeneration and to provide additional knowledge about how transcription and posttranscriptional processes are integrated to correctly orchestrate these processes.

Acknowledgments

This work was partially supported by grants from: European Research Council (ERC-2013, AdG 340172–MUNCODD), Human Frontier Science Program (HFSP; RGP0009/2014), AFM-Téléthon (no. 17835), Epigen-Epigenomics Flagship Project, Parent Project Italia, AriSLA (full grant 2014 "ARCI"), and Fondazione Roma.

Address correspondence to: Irene Bozzoni, Sapienza University of Rome, P.le Aldo Moro, 5, Rome, UNK 00185, Italy. Phone: 39.06.49912202; E-mail: irene.bozzoni@uniroma1.it.

1. Mattick JS, Makunin IV. Non-coding RNA. *Hum Mol Genet*. 2006;15(suppl 1):R17–R29.
2. Buckingham M, Relaix F. The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. *Annu Rev Cell Dev Biol*. 2007;23:645–673.
3. Sparrow J, Hughes SM, Segalat L. Other model organisms for sarcomeric muscle diseases. *Adv Exp Med Biol*. 2008;642:192–206.
4. Nguyen MA, Hardeman EC. Mouse models for thin filament disease. *Adv Exp Med Biol*. 2008;642:66–77.
5. Kirby TJ, Chaillou T, McCarthy JJ. The role of microRNAs in skeletal muscle health and disease. *Front Biosci*. 2015;20:37–77.
6. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J Appl Physiol*.

2007;102(1):306–313.
7. Liu N, Olson EN. MicroRNA regulatory networks in cardiovascular development. *Dev Cell*. 2010;18(4):510–525.
8. Small EM, et al. Regulation of PI3-kinase/Akt signaling by muscle-enriched microRNA-486. *Proc Natl Acad Sci U S A*. 2010; 107(9):4218–4223.
9. Williams AH, Liu N, van Rooij E, Olson EN. MicroRNA control of muscle development and disease. *Curr Opin Cell Biol*. 2009;21(3):461–469.
10. Heidersbach A, et al. microRNA-1 regulates sarcomere formation and suppresses smooth muscle gene expression in the mammalian heart. *Elife*. 2013;2:e01323.
11. Liu N, et al. microRNA-206 promotes skeletal muscle regeneration and delays progression of Duchenne muscular dystrophy in mice. *J Clin Invest*. 2012;122(6):2054–2065.
12. Callis TE, Deng Z, Chen JF, Wang DZ. Muscling through the microRNA world. *Exp Biol Med*. 2008; 233(2):131–138.
13. Chen JF, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet*. 2006;38(2):228–233.
14. Williams AH, et al. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. *Science*. 2009;326(5959):1549–1554.
15. Alexander MS, et al. MicroRNA-486-dependent modulation of DOCK3/PTEN/AKT signaling pathways improves muscular dystrophy-associated symptoms. *J Clin Invest*. 2014;124(6):2651–2667.
16. Eisenberg I, et al. Distinctive patterns of microRNA expression in primary muscular disorders. *Proc Natl Acad Sci U S A*. 2007;104(43):17016–17021.
17. Cacchiarelli D, et al. microRNAs involved in molecular circuitries relevant for the Duchenne muscular dystrophy pathogenesis are controlled by the dystrophin/nNOS pathway. *Cell Metab*. 2010;12(4):341–351.
18. Dmitriev P, et al. Defective regulation of microRNA target genes in myoblasts from facioscapulohumeral dystrophy patients. *J Biol Chem*. 2013;288(49):34989–35002.
19. Georgantas RW, et al. Inhibition of myogenic microRNAs 1, 133, and 206 by inflammatory cytokines links inflammation and muscle degeneration in adult inflammatory myopathies. *Arthritis Rheumatol*. 2014;66(4):1022–1033.
20. Novak J, Vinklarek J, Bienertova-Vasku J, Slaby O. MicroRNAs involved in skeletal muscle development and their roles in rhabdomyosarcoma pathogenesis. *Pediatr Blood Cancer*. 2013;60(11):1739–1746.
21. Greco S, et al. Common micro-RNA signature in skeletal muscle damage and regeneration induced by Duchenne muscular dystrophy and acute ischemia. *FASEB J*. 2009;23(10):3335–3346.
22. Colussi C, et al. HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment. *Proc Natl Acad Sci U S A*. 2008;105(49):19183–19187.
23. Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A. *S*-nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. *Nature*. 2008;455(7211):411–415.
24. van Rooij E, et al. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. *Dev Cell*. 2009;17(5):662–673.
25. Montgomery RL, et al. MicroRNA mimicry blocks pulmonary fibrosis. *EMBO Mol Med*. 2014;6(10):1347–1356.
26. Cazzella V, et al. Exon 45 skipping through U1-snRNA antisense molecules recovers the Dys-nNOS pathway and muscle differentiation in human DMD myoblasts. *Mol Ther*. 2012;20(11):2134–2142.
27. Minetti GC, et al. Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. *Nat Med*. 2006;12(10):1147–1150.
28. Saccone V, et al. HDAC-regulated myomiRs control BAF60 variant exchange and direct the functional phenotype of fibro-adipogenic progenitors in dystrophic muscles. *Genes Dev*. 2014;28(8):841–857.
29. Muntoni F, Mateddu A, Marchei F, Clerk A, Serra G. Muscular weakness in the mdx mouse. *J Neurol Sci*. 1993;120(1):71–77.
30. Dupuis L, Loeffler JP. Neuromuscular junction destruction during amyotrophic lateral sclerosis: insights from transgenic models. *Curr Opin Pharmacol*. 2009;9(3):341–346.
31. Cohen TJ, et al. The histone deacetylase HDAC4 connects neural activity to muscle transcriptional reprogramming. *J Biol Chem*. 2007;282(46):33752–33759.
32. Missiaglia E, et al. MicroRNA-206 expression levels correlate with clinical behaviour of rhabdomyosarcomas. *Br J Cancer*. 2010;102(12):1769–1777.
33. Wachtel M, et al. Subtype and prognostic classification of rhabdomyosarcoma by immunohistochemistry. *J Clin Oncol*. 2006;24(5):816–822.
34. Keller C, Guttridge DC. Mechanisms of impaired differentiation in rhabdomyosarcoma. *FEBS J*. 2013;280(17):4323–4334.
35. Yan D, et al. MicroRNA-1/206 targets c-Met and inhibits rhabdomyosarcoma development. *J Biol Chem*. 2009;284(43):29596–29604.
36. Taulli R, et al. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. *J Clin Invest*. 2009;119(8):2366–2378.
37. Gottlieb PD, et al. Bop encodes a muscle-restricted protein containing MYND and SET domains and is essential for cardiac differentiation and morphogenesis. *Nat Genet*. 2002;31(1):25–32.
38. Coda DM, et al. SMYD1 and G6PD modulation are critical events for miR-206-mediated differentiation of rhabdomyosarcoma. *Cell Cycle*. 2015;14(9):1389–1402.
39. Macquarrie KL, Yao Z, Young JM, Cao Y, Tapscott SJ. miR-206 integrates multiple components of differentiation pathways to control the transition from growth to differentiation in rhabdomyosarcoma cells. *Skelet Muscle*. 2012;2(1):7.
40. Crist CG, Montarras D, Buckingham M. Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. *Cell Stem Cell*. 2012;11(1):118–126.
41. Cacchiarelli D, et al. miR-31 modulates dystrophin expression: new implications for Duchenne muscular dystrophy therapy. *EMBO Rep*. 2011;12(2):136–141.
42. Reilly S, et al. Evaluation of the role of miR-31-dependent reduction in dystrophin and nNOS on atrial-fibrillation-induced electrical remodelling in man. *Lancet*. 2015;385(suppl 1):S82.
43. Ballarino M, et al. Novel long noncoding RNAs (lncRNAs) in myogenesis: a miR-31 overlapping lncRNA transcript controls myoblast differentiation. *Mol Cell Biol*. 2015; 35(4):728–736.
44. Montes M, et al. The lncRNA MIR31HG regulates p16(INK4A) expression to modulate senescence. *Nat Commun*. 2015;6:6967.
45. Fiorillo AA, et al. TNF-α-induced microRNAs control dystrophin expression in Becker muscular dystrophy. *Cell Rep*. 2015;12(10):1678–1690.
46. Maciotta S, et al. Hmgb3 is regulated by microRNA-206 during muscle regeneration. *PLoS One*. 2012;7(8):e43464.
47. Dey BK, Gagan J, Yan Z, Dutta A. miR-26a is required for skeletal muscle differentiation regeneration in mice. *Genes Dev*. 2012; 26(19):2180–2191.
48. Chung FW, Tellam RL. MicroRNA-26a targets the histone methyltransferase Enhancer of Zeste homolog 2 during myogenesis. *J Biol Chem*. 2008; 283(15):9836–9843.
49. Crist CG, et al. Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression. *Proc Natl Acad Sci U S A*. 2009;106(32):13383–13387.
50. McFarlane C, et al. Negative auto- regulation of myostatin expression is mediated by Smad3 and MicroRNA-27. *PLoS One*. 2014;9(1):e87687.
51. Naguibneva I, et al. The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. *Nat Cell Biol*. 2006;8(3):278–284.
52. Cardinali B, et al. Microrna-221 and microrna-222 modulate differentiation and maturation of skeletal muscle cells. *PLoS One*. 2009;4(10):e7607.
53. Sarkar S, Dey BK, Dutta A. MiR-322/424 and -503 are induced during muscle differentiation and promote cell cycle quiescence and differentiation by down-regulation of Cdc25A. *Mol Biol Cell*. 2010;21(13):2138–2149.
54. Mitchell PS, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*. 2008;105(30):10513–10518.
55. Chen X, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res*. 2008;18(10):997–1006.
56. Xiao C, et al. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. *Cell*. 2007;131(1):146–159.
57. Zhang Y, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell*. 2010;39(1):133–144.
58. Miyachi M, et al. Circulating muscle-specific microRNA, miR-206, as a potential diagnostic marker for rhabdomyosarcoma. *Biochem Biophys Res Commun*. 2010;400(1):89–93.
59. Cacchiarelli D, et al. miRNAs as serum biomarkers for Duchenne muscular dystrophy. *EMBO Mol Med*. 2011;3(5):258–265.
60. Matsuzaka Y, et al. Three novel serum

biomarkers, miR-1, miR-133a, and miR-206 for Limb-girdle muscular dystrophy, Facioscapulohumeralmuscular dystrophy, and Becker muscular dystrophy. *Environ Health Prev Med*. 2014;19(6):452–458.
61. Jeanson-Leh L, et al. Serum profiling identifies novel muscle miRNA and cardiomyopathy-related miRNAbiomarkers in Golden Retriever muscular dystrophy dogs and Duchenne muscular dystrophy patients. *Am J Pathol*. 2014;184(11):2885–2898.
62. Vignier N, et al. Distinctive serum miRNA profile in mouse models of striated muscular pathologies. *PLoS One*. 2013;8(2):e55281.
63. Toivonen JM, Manzano R, Oliván S, Zaragoza P, García-Redondo A, Osta R. MicroRNA-206: a potential circulating biomarker candidate for amyotrophic lateral sclerosis. *PLoS One*. 2014;9(2):e89065.
64. Mizuno H, et al. Identification of muscle-specific microRNAs in serum of muscular dystrophy animal models: promising novel blood-based markers for muscular dystrophy. *PLoS One*. 2011;6(3):e18388.
65. Quattrocelli M, Sampaolesi M. The mesmiRizing complexity of microRNAs for striated muscle tissue engineering. *Adv Drug Deliv Rev*. 2015;88:37–52.
66. Li D, et al. Transcriptome analysis reveals distinct patterns of long noncoding RNAs in heart and plasma of mice with heart failure. *PLoS One*. 2013;8(10):e77938.
67. Kumarswamy R, et al. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. *Circ Res*. 2014;114(10):1569–1575.
68. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res*. 2014;115(7):668–677.
69. Trapnell C, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol*. 2010;28(5):511–515.
70. Mousavi K, et al. eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. *Mol Cell*. 2013;51(5):606–617.
71. Mousavi K, Zare H, Koulnis M, Sartorelli V. The emerging roles of eRNAs in transcriptional regulatory networks. *RNA Biol*. 2014;11(2):106–110.
72. Lu L, et al. Genome-wide survey by ChIP-seq reveals YY1 regulation of lincRNAs in skeletal myogenesis. *EMBO J*. 2013;32(19):2575–2588.
73. Schmitz KM, Mayer C, Postepska A, Grummt I. Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. *Genes Dev*. 2010;24(20):2264–2269.
74. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. *Cell*. 2013;152(6):1298–1307.
75. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem*. 2012;81:145–166.
76. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature*. 2012;482(7385):339–346.
77. Wutz A, Rasmussen TP, Jaenisch R. Chromosomal silencing and localization are mediated by different domains of Xist RNA. *Nature Genet*. 2002;30(2):167–174.
78. Zappulla DC, Cech TR. Yeast telomerase RNA: a flexible scaffold for protein subunits. *Proc Natl Acad Sci U S A*. 2004;101(27):10024–10029.
79. Tsai MC, et al. Long noncoding RNA as modular scaffold of histone modification complexes. *Science*. 2010;329(5992):689–693.
80. Cabianca DS, et al. A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. *Cell*. 2012;149(4):819–831.
81. Katayama S, et al. Antisense transcription in the mammalian transcriptome. *Science*. 2005;309(5740):1564–1566.
82. Han P, et al. A long noncoding RNA protects the heart from pathological hypertrophy. *Nature*. 2014;514(7520):102–106.
83. Hang CT, et al. Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature*. 2010;466(7302):62–67.
84. Gordon S, Akopyan G, Garban H, Bonavida B. Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. *Oncogene*. 2006;25(8):1125–1142.
85. von Maltzahn J, Chang NC, Bentzinger CF, Rudnicki MA. Wnt signaling in myogenesis. *Trends Cell Biol*. 2012;22(11):602–609.
86. Jin F, et al. A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature*. 2013;503(7475):290–294.
87. Mueller AC, et al. MUNC, a long noncoding RNA that facilitates the function of MyoD in skeletal myogenesis. *Mol Cell Biol*. 2015;35(3):498–513.
88. Eun B, et al. The Igf2/H19 muscle enhancer is an active transcriptional complex. *Nucleic Acids Res*. 2013;41(17):8126–8134.
89. Kaffer CR, Grinberg A, Pfeifer K. Regulatory mechanisms at the mouse Igf2/H19 locus. *Mol Cell Biol*. 2001;21(23):8189–8196.
90. Ounzain S, et al. Functional importance of cardiac enhancer-associated noncoding RNAs in heart development and disease. *Mol Cell Cardiol*. 2014;76:55–70.
91. Ounzain S, et al. Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. *Eur Heart J*. 2015;36(6):353–368.
92. Sakabe NJ, Savic D, Nobrega MA. Transcriptional enhancers in development and disease. *Genome Biol*. 2012;13(1):238.
93. Lee JT, Bartolomei MS. X-inactivation, imprinting, and long noncoding RNAs in health and disease. *Cell*. 2013;152(6):1308–1323.
94. Müller B, et al. Evidence that paternal expression of the epsilon-sarcoglycan gene accounts for reduced penetrance in myoclonus-dystonia. *Am J Hum Genet*. 2002;71(6):1303–1311.
95. Davis E, et al. Ectopic expression of DLK1 protein in skeletal muscle of padumnal heterozygotes causes the callipyge phenotype. *Curr Biol*. 2004;14(20):1858–1862.
96. Zhou Y, et al. Activation of paternally expressed genes and perinatal death caused by deletion of the Gtl2 gene. *Development*. 2010;137(16):2643–2652.
97. Zhao J, et al. Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol Cell*. 2010;40(6):939–953.
98. Zhan S, Shapiro DN, Helman LJ. Activation of an imprinted allele of the insulin-like growth factor II gene implicated in rhabdomyosarcoma. *J Clin Invest*. 1994;94(1):445–448.
99. Casola S, et al. Expression and parental imprinting of the H19 gene in human rhabdomyosarcoma. *Oncogene*. 1997;14(12):1503–1510.
100. Al Adhami H, et al. A systems-level approach to parental genomic imprinting: the imprinted gene network includes extracellular matrix genes and regulates cell cycle exit and differentiation. *Genome Res*. 2015;25(3):353–367.
101. Korostowski L, Sedlak N, Engel N. The Kcnq1ot1 long non-coding RNA affects chromatin conformation and expression of Kcnq1, but does not regulate its imprinting in the developing heart. *PLoS Genet*. 2012;8(9):e1002956.
102. Schultz BM, Gallicio GA, Cesaroni M, Lupey LN, Engel N. Enhancers compete with a long non-coding RNA for regulation of the Kcnq1 domain. *Nucleic Acids Res*. 2015;43(2):745–759.
103. Bokil NJ, Baisden JM, Radford DJ, Summers KM. Molecular genetics of long QT syndrome. *Mol Genet Metab*. 2010;101(1):1–8.
104. Klattenhoff CA, et al. Braveheart, a long non-coding RNA required for cardiovascular lineage commitment. *Cell*. 2013;152(3):570–583.
105. Grote P, et al. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev Cell*. 2013;24(2):206–214.
106. Mahlapuu M, Enerbäck S, Carlsson P. Haploinsufficiency of the forkhead gene Foxf1, a target for sonic hedgehog signaling, causes lung and foregut malformations. *Development*. 2001;128(12):2397–2406.
107. Kitamura K, et al. Mouse Pitx2 deficiency leads to anomalies of the ventral body wall, heart, extra- and periocular mesoderm and right pulmonary isomerism. *Development*. 1999;126(24):5749–5758.
108. Wang L, et al. LncRNA Dum interacts with Dnmts to regulate Dppa2 expression during myogenic differentiation and muscle regeneration. *Cell Res*. 2015;25(3):335–350.
109. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? *Cell*. 2011;146(3):353–358.
110. Cesana M, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell*. 2011;147(2):358–369.
111. Shen H, et al. The Notch coactivator, MAML1, functions as a novel coactivator for MEF2C-mediated transcription and is required for normal myogenesis. *Genes Dev*. 2006;20(6):675–688.
112. Kallen AN, et al. The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Mol Cell*. 2013;52(1):101–112.
113. Gabory A, Jammes H, Dandolo L. The H19 locus: role of an imprinted non-coding RNA in growth and development. *Bioessays*. 2010;32(6):473–480.
114. Gao Y, et al. The H19/let-7 double-negative feedback loop contributes to glucose metabolism in muscle cells. *Nucleic Acids Res*. 2014;42(22):13799–13811.
115. Wang K, et al. The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. *Circ Res*. 2014;114(9):1377–1388.
116. Han X, Yang F, Cao H, Liang Z. Malat1 regulates serum response factor through miR-133 as a com-

peting endogenous RNA in myogenesis. *FASEB J*. 2015;29(7):3054–3064.
117. Gong C, et al. A long non-coding RNA, LncMyoD, regulates skeletal muscle differentiation by blocking IMP2-mediated mRNA translation. *Dev Cell*. 2015;34(2):181–191.
118. Bernard D, et al. A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. *EMBO J*. 2010;29(18):3082–3093.
119. Wang J, Gong C, Maquat L. Control of myogenesis by rodent SINE-containing lncRNAs. *Genes Dev*. 2013;27(7):793–804.
120. Gong C, Kim YK, Woeller CF, Tang Y, Maquat LE. SMD and NMD are competitive pathways that contribute to myogenesis: effects on PAX3 and myogenin mRNAs. *Genes Dev*. 2009;23(1):54–66.
121. Gawron D, Gevaert K, Van Damme P. The proteome under translational control. *Proteomics*. 2014;14(23-24):2647–2662.
122. Slavoff SA, et al. Peptidomic discovery of short open reading frame-encoded peptides in human cells. *Nat Chem Biol*. 2013;9(1):59–64.
123. Anderson DM, et al. A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell*. 2015;160(4):595–606.
124. Nelson BR, et al. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science*. 2016;351(6270):271–275.
125. Ebbesen KK, Kjems J, Hansen TB. Circular RNAs: Identification, biogenesis and function. *Biochim Biophys Acta*. 2016;1859(1):163–168.
126. Memczak S, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013;495(7441):333–338.
127. Hansen TB, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495(7441):384–388.