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
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).

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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 non-functional 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
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 lncRNAs (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 p27kip1 (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 dystrophic 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-

Table 1. Functional lncRNAs in myogenesis

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>Organism</th>
<th>Compartment</th>
<th>Role in myogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braveheart (Bvht)</td>
<td>Mouse/human</td>
<td>Nucleus/cytoplasm</td>
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</tr>
<tr>
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<td>Promotes differentiation by acting as a ceRNA for miR-133 and miR-135 (110)</td>
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<td>Encodes MLN, a functional micropeptide that regulates muscle contractility (123)</td>
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<td>Acts as an eRNA for the MyoD locus (70, 71)</td>
</tr>
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<td>Ddx1</td>
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<td>Activates transcription of the FSHD locus by binding to the TrxG/MLL component ASH1 (80)</td>
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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, ii); 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 ASH1L 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 lncRNA (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 (eDNA) (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, eDNA acts at a distance to activate myogenin (70). More recently, an additional contribution of the murine eDNA (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 eDNA on 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 IncRNAs 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 Dkk1 (95, 97).

Imprinting at IncRNA 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 IncRNA 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 IncRNAs. The best example of a nuclear IncRNA 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 IncRNA 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 IncRNA has not been established yet.

Fendrr is another example of a IncRNA 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 (Pits2) 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 IncRNA 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 IncRNAs. One of the functions first attributed to cytoplasmic IncRNAs 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 enhancer 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 circuits in DMD pathogenesis (110).

Other muscle-relevant ceRNAs have also been identified. The imprinted H19 IncRNA 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 IncRNAs CHRF and Malat-1. While CHRF regulates Myd88 expression and consequent cardiac hypertrophy through sponging activity targeting miR-489
LncRNAs were recently shown to compete with RNA-binding proteins (Figure 2, v). Staufen1-mediated decay 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 LINCO00948 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 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 expression 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.

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