Long noncoding RNAs, emerging players in muscle differentiation and disease

Maria Victoria Neguembor, Mathivanan Jothi and Davide Gabellini*

Abstract

The vast majority of the mammalian genome is transcribed giving rise to many different types of noncoding RNAs. Among them, long noncoding RNAs are the most numerous and functionally versatile class. Indeed, the lncRNA repertoire might be as rich as the proteome. LncRNAs have emerged as key regulators of gene expression at multiple levels. They play important roles in the regulation of development, differentiation and maintenance of cell identity and they also contribute to disease. In this review, we present recent advances in the biology of lncRNAs in muscle development and differentiation. We will also discuss the contribution of lncRNAs to muscle disease with a particular focus on Duchenne and facioscapulohumeral muscular dystrophies.

Keywords: Chromatin, DMD, FSHD, Muscular dystrophy, ncRNA, Repeat

Review

Long non protein-coding RNAs (lncRNAs)

In mammals, the vast majority of the transcriptional output is noncoding [1]. While 75% of the genome is transcribed, only 2% encodes for proteins [2]. Non protein-coding RNAs (ncRNAs) are operationally divided in two classes according to their size. Small ncRNAs are below 200 bp and include transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNAs (snRNA), small nucleolar RNAs (snoRNA), microRNAs, siRNAs and Piwi-interacting RNAs (piRNA) [3-7]. Long ncRNAs (lncRNAs) include all ncRNA transcripts greater than 200 bp with little or no coding potential. Although discovered relatively recently, lncRNAs are considered the most numerous and functionally diverse class of RNAs [8]. Up to 15,000 lncRNAs have been identified so far [9] and, as the number constantly increases, the lncRNA assortment might turn out to be as rich as the proteome.

LncRNAs loci are often in close association with protein-coding genes as they are encoded from exonic or intronic sequences in both sense and antisense orientation or even from gene regulatory regions [10]. LncRNAs can also arise from intergenic regions including repetitive sequences [11]. Most lncRNAs are transcribed by RNA polymerase II and may share mRNA-like features such as 5’cap, polyA tail and splicing sites [12,13]. Alternatively, non-polyadenylated lncRNAs are likely generated by RNA polymerase III [14,15].

In terms of transcriptional profile, lncRNAs are generally expressed at lower levels than protein-coding transcripts and, compared to the latter, their pattern of expression is more developmental stage- and cell type-specific [2,16]. The intrinsic nature and complex secondary structures of lncRNAs enable them to specifically interact with DNA, RNA and proteins. Since lncRNAs are localized both in the nucleus and the cytosol, they can act at virtually every level of gene expression [17,18].

LncRNA, a molecular ‘passepartout’

LncRNAs can be divided into multiple functional categories based on the site of action and the level of gene expression at which they act. However, as our knowledge of lncRNAs increases, new functional groups emerge and the distinction between classes is not always adequate. Here, we present a very brief classification to provide a framework for the examples of lncRNAs acting in muscle differentiation and disease later described.

Nuclear lncRNAs can be subdivided into cis-acting RNAs that work in proximity to their site of transcription, and trans-acting RNAs that operate at distant loci. Both cis- and trans-acting lncRNAs can activate or repress transcription through the recruitment of chromatin
remodelers and modifiers, thus shaping the chromatin status of a particular locus or even of an entire chromosome (Figure 1A) [19-24]. Besides, lncRNAs are able to recruit or prevent the binding of the transcriptional machinery and transcription factors directly impacting the transcriptional output of a region (Figure 1B) [25-28]. Among these, enhancer RNAs (eRNAs), are encoded by extragenic enhancer regions and promote transcription of surrounding genes [29-32]. LncRNAs also participate in co- and post-transcriptional regulation in the nucleus. For example, lncRNAs can interact with the splicing machinery or directly with nascent mRNAs to guide particular splicing events (Figure 1C) [33-35]. In addition to the cis versus trans distinction, lncRNAs can shape the subnuclear architecture in different ways. Certain lncRNAs regulate chromosome looping, favoring or disrupting chromosomal interactions (Figure 1D) [36,37]. Others act as structural scaffolds for the formation and regulation of

**Figure 1** Mechanisms for long noncoding RNA (lncRNA) function. (A) LncRNAs (in red) are able to recruit chromatin modifiers mediating the deposition of activatory (green dots) or repressive (red dots) histone marks. (B) LncRNAs control the recruitment of transcription factors and core components of the transcriptional machinery. (C) LncRNAs can directly bind mRNAs and modulate splicing events. (D-E) LncRNAs participate in the higher order organization of the nucleus by mediating chromatin looping (D) and as structural components for the formation and function of nuclear bodies (E). (F) LncRNAs control translation rates favoring or inhibiting polysome loading to mRNAs. (G) LncRNAs modulate mRNA decay protecting mRNA from degradation or, alternatively, mediating the recruitment of degradation machinery. (H) LncRNAs can act as miRNA sponges, thus favoring the expression of the mRNAs targeted by the sequestered miRNA.
nuclear compartments such as speckles [33], paraspeckles [38] and Polycomb bodies [39] (Figure 1E).

In the cytoplasm, lncRNAs influence translational output in different ways. Firstly, they can modulate the translational rate by regulating polysome loading to an mRNA molecule (Figure 1F) or through the control of internal ribosomal entry sites (IRES) [40-42]. Secondly, they can regulate gene expression by reducing or stimulating mRNA decay (Figure 1G) [43,44]. A particular class of cytoplasmic lncRNAs, the competing endogenous RNAs (ceRNA), regulates both the translation and the degradation rates of miRNAs by acting as molecular sponges for miRNAs, thus modulating the repressive activity of miRNA on their mRNA targets (Figure 1H) [45-49].

Altogether, lncRNAs exhibit remarkable functional flexibility and tightly regulated expression that confer on them an enormous potential as fine tuners of cell function and identity. Due to their versatility, they are able to control different aspects of cellular development, from stem cell maintenance to commitment and differentiation and we anticipate their biological role in a great variety of cell types to be uncovered in the near future [50]. In this review, we will focus on the fascinating discoveries regarding the role of lncRNAs in muscle differentiation and disease, with a particular focus on Duchenne muscular dystrophy (DMD) and facioscapulohumeral muscular dystrophy (FSHD).

LncRNAs in myogenic differentiation

Myogenesis is the process where progenitor cells give rise to myoblasts that fuse onto multi-nucleated myofibers endowed with contractile ability. This complex and tightly regulated process starts from extra/intra cellular signals impinging on the myogenic transcription program. An enormous literature provides evidence that the myogenic gene expression program is orchestrated by a transcriptional hierarchy, including the Myogenic Regulatory Factors (MyoD, Myf5, Myogenin, and MRF4) and the Myocyte Enhancer Factor-2 (MEF2A-D) families of transcription factors (reviewed in [51] and [52]). In a stage-specific manner, these factors act in coordination with other transcriptional regulators, including epigenetic factors, to execute the muscle differentiation program [53]. Still, this scenario is not complete as new players are gradually emerging. Indeed, there is increasing evidence that ncRNAs are also part of the muscle regulatory network. So far, miRNAs are the most extensively studied and characterized [54]. However, in the last years lncRNAs are emerging as critical regulators of muscle differentiation (Table 1).

| LncRNA | Site of action | Function | Effector molecule | Regulation during muscle differentiation |
|--------|----------------|----------|-------------------|-----------------------------------------|
| eRNAs (CE DR RNAs) | Nucleus | Transcriptional activation | MyoD | Up [30] |
| Gtl2/Meg3 | Nucleus | Epigenetic repression | PRC2 | Up [55,56] |
| H19 | Nucleus and cytoplasm | Epigenetic repression, miRNAs sponge | PRC2, let-7 miRNAs | Up [57] |
| Linc-MD1 | Cytoplasm | miRNAs sponge | HuR | Up [46,58] |
| Malat1 | Nucleus | Epigenetic repression, pre-mRNA splicing | Cbx4 and SR family of splicing factors | Up [59] |
| Neat1 | Nucleus | Structural integrity of nuclear paraspeckles | Various RNA-binding proteins | Up [60] |
| Nctc1 | Nucleus? | Unknown | Unknown | Up [61] |
| SRA | Nucleus | Scaffold factor | MyoD | Up [62,63] |
| SINE containing IncRNAs | Cytoplasm | miRNA decay | STAU1 and STAU2 | Up [64] |
| Yams | Nucleus | Transcriptional activation | Unknown | Up/down [65] |

Table 1 Long noncoding RNAs (lncRNAs) involved in muscle differentiation

References:
1. Neguembor et al. Skeletal Muscle 2014, 4:8
2. http://www.skeletalmusclejournal.com/content/4/1/8
the activation of gene expression in cis or in trans (Figure 1B) [29,32,36,67-70]. Recently, a key role for eRNAs in the regulation of muscle differentiation was uncovered [30]. In myotubes, ChIP-seq analyses revealed that the myogenic regulatory factors MyoD and MyoG display a very similar genome-wide binding profile and are mostly associated with extragenic regions, many of which exhibit eRNA features [30]. Several of these eRNAs are preferentially localized to the nucleus and are mainly regulated by MyoD, as MyoG silencing has a marginal effect on their expression [30]. In turn, two eRNAs generated by upstream regulatory regions of MyoD (CE and DRR) regulate the expression of MyoD and MyoG (Figure 2A and Table 1) [71,72]. Both eRNAs are involved in the activation of gene expression, but they differ in their mode of action. While the CE-eRNA functions in cis to activate expression of MyoD, DRR-RNA works in trans to promote MyoG transcription and muscle differentiation (Figure 2A). At their site of action, both eRNAs mediate increased chromatin accessibility and recruitment of RNAPII [30]. Collectively, these findings suggest that eRNAs regulate myogenesis by directing chromatin-remodeling events,

![Figure 2 Distinct roles of long noncoding RNAs (lncRNAs) in muscle differentiation.](image)

(A) Enhancer IncRNAs
(B) Scaffold IncRNAs
(C) LncRNAs recruiting epigenetic regulators
(D) IncRNAs as microRNA sponges
(E) LncRNAs regulating mRNA decay

(A) Enhancer RNAs (eRNAs) CE and DRR RNAs can induce expression of myogenic regulators MyoD and MyoG acting in cis or in trans, respectively. (B) IncRNA SRA acts as a scaffold molecule for MyoD, p68 and p72 at the promoter region of myogenic genes to activate their expression. (C) LncRNAs Malat1, H19 and Gtl-Meg3 interact with PRC1/2 complex to modulate their target genes. (D) As a molecular sponge H19 inhibits let-7 mediated mRNA degradation of myogenic negative regulators Hmga2 and Igfbp2. (E) Short interspersed element (SINE) containing lncRNAs can bind to UTR region of Cdc6 and Traf6 mRNAs and promote their decay at different stages of muscle differentiation.
controlling the hierarchy within the myogenic gene regulatory network.

The transcription factor Yin Yang 1 (YY1) is another important regulator of myogenesis at multiple levels [73-77]. By comparing YY1 ChIP-seq results in proliferating and differentiated C2C12 muscle cells, a number of IncRNAs regulated by YY1 (YY1-associated muscle IncRNAs or Yams) have been recently identified [65]. Yams display distinct expression patterns during muscle differentiation and affect myogenesis differently (Table1). For example, while Yam-2 and -3 promote C2C12 muscle differentiation, Yam-1 and -4 inhibit it [65]. The mechanism of action of Yam-1 was investigated in more detail. Yam-1 is a single exon transcript, regulated during \textit{in vivo} and \textit{in vitro} myogenesis and equally distributed in the nucleus and cytoplasm. Throughout development, in muscle regeneration and in tissue culture, Yam-1 is downregulated during terminal muscle differentiation [65]. Yam-1 knockdown promotes muscle differentiation in tissue culture and accelerates regeneration following muscle injury \textit{in vivo} [65]. Importantly, Yam-1 knockdown rescues the myogenic defects caused by YY1 overexpression indicating that Yam-1 is an important mediator of YY1 activity in muscle [65]. Silencing of Yam-1 reduces the expression of several nearby genes, suggesting that it could be a positive cis-regulator of surrounding genes similar to eRNAs (Figure 1B) [65]. One of the nearby genes co-regulated with Yam-1 encodes for \textit{miR-715}, a miRNA targeting \textit{Wnt7b}. Since Wnt signaling is a critical modulator of skeletal muscle formation [78], it is tempting to speculate that Yam-1 could function, at least in part, by activating \textit{miR-715 in cis} leading to \textit{Wnt7b} downregulation.

Besides regulating chromatin accessibility like eRNAs, nuclear IncRNAs can also control gene expression by directly affecting the activity of sequence-specific transcription factors. This is the case for \textit{steroid receptor RNA activator} (SRA) [79], the first example of IncRNA regulating myogenesis [62]. SRA likely functions as a scaffold, bringing together multiple factors that modulate gene expression [80] including the master regulator of muscle differentiation MyoD (Figure 2B and Table1) [62]. In muscle, a complex composed by SRA, MyoD and the RNA helicases p68 and p72 has been identified (Figure 2B) [62]. Knockdown and overexpression studies indicate that p68/p72 and SRA are coactivators required for the transcription of a subset of MyoD target genes and for muscle differentiation [62]. The SRA gene produces multiple transcripts through alternative splicing. While retention of intron 1 gives rise to the non-protein-coding SRA transcript, splicing of the intron creates an open reading frame that generates the SRA protein (SRAP) [81]. The ratio between the coding and noncoding SRA transcripts varies during muscle differentiation with SRAP mRNA being more abundant in myoblasts and SRA IncRNA being the predominant isoform in myotubes [63]. SRAP works at least in part by regulating SRA IncRNA coactivator function. Indeed, SRAP is an RNA-binding protein that specifically binds SRA IncRNA, thus preventing SRA-mediated regulation of MyoD transcriptional activity [63]. Hence, the correct balance between coding and noncoding SRA molecules is important for normal muscle differentiation. Intriguingly, aberrant SRA splicing is present in myotonic dystrophy patients. Whether impaired splicing of SRA contributes to the pathogenesis of muscular dystrophy remains to be elucidated [63].

\textbf{Subnuclear structure-specific IncRNAs}

A number of IncRNAs are enriched in and contribute to organize specific subnuclear domains (Figure 1E) [82-88]. The IncRNA \textit{Malat1} is enriched in nuclear speckles, abundantly expressed in cancer cells and a strong predictor of tumor metastasis [89]. \textit{Malat1} has been shown to regulate gene transcription and pre-mRNA splicing by respectively interacting with the epigenetic repressor Polycomb protein Cbx4 [39] and with the SR family of splicing factors (Figure 2C and Table 1) [33]. \textit{Malat1} is upregulated during early differentiation of C2C12 mouse myoblasts and primary human skeletal muscle cells, while its knockdown leads to suppression of myoblast proliferation by arresting cells in the G0/G1 phase [59], suggesting a role for \textit{Malat1} in the transition from the proliferative phase to the permanent cell cycle exit, as well as in the commitment to differentiation. Intriguingly, \textit{Malat1} has been recently identified as a novel downstream target of myostatin [59], an important regulator of myoblast proliferation, differentiation and skeletal muscle mass [90]. Future work will indicate how relevant \textit{Malat1} is for myostatin activity.

\textbf{Imprinted IncRNAs}

The \textit{Dlk1-Dio3} region is a very complex, imprinted locus involved in tissue growth regulation and human cancers [91]. Aberrant repression of the \textit{Dlk1-Dio3} imprinted cluster is present in most induced pluripotent stem cell (iPSC) lines and is responsible for the failure of iPSCs to form viable mice [92]. Intriguingly, postnatal aberrant expression of this locus is responsible for muscle hypertrophy in mouse and sheep [93,94]. The locus contains protein-coding RNAs, IncRNAs, miRNAs and snoRNAs expressed from either the paternal or the maternal allele. Several of the IncRNAs transcribed from the \textit{Dlk1-Dio3} region are enriched in the nucleus and have been reported to bind to Polycomb Repressive Complex 1 (PRC1), PRC2 and other epigenetic repressors [95-97]. Expression of the various \textit{Dlk1-Dio3} transcripts is reciprocally regulated. For example, the \textit{Gtl2/Meg3} IncRNA binds to PRC2 recruiting it to the \textit{Dlk1-Dio3} locus to repress the protein-coding gene \textit{Dlk1} and the IncRNA \textit{Gtl2-as} (Figure 2C and Table 1).
Consistently, GtI2/Meg3 knockdown leads to a decreased PRC2 recruitment at the Dlk1 promoter with subsequent increased expression of Dlk1 and GtI2-as [95]. During development, GtI2/Meg3 is abundantly expressed in the paraxial mesoderm suggesting a role in myogenesis [55]. Indeed, GtI2/Meg3 knockout mice develop skeletal muscle developmental defects along with perinatal death [56], promoting GtI2/Meg3 as an example of a nuclear, cis-acting LncRNA regulating muscle development.

H19 was the first LncRNA described in mammalian cells [98]. It is transcribed from the maternal allele of the H19/Igf2 locus producing a LncRNA predominantly enriched in the cytoplasm [98]. H19 is highly expressed in developing embryo and adult muscle in human and mouse [99,100], and is upregulated during myoblast differentiation and muscle regeneration [57,101]. H19 works at multiple levels. Through binding to the PRC2 epigenetic repressor complex [95], H19 can mediate the transcriptional repression of Igf2 (Figure 2C) [102,103]. Moreover, H19 can bind Igf2 mRNA binding-protein (IMP) family members to regulate Igf2 post-transcriptionally [104]. Also, H19 contains several binding sites for the let-7 family of micro-RNAs suggesting that H19 may act as a miRNA sponge for let-7 (Figure 2D and Table 1) [57]. Among let-7 targets, Hmga2 and Igfbp2 have an important role in myoblasts proliferation and myogenesis but must be downregulated to allow the formation of multinucleated myofibers (Figure 2D) [105]. Finally, H19 exon 1 encodes the conserved microRNAs miR-675-3p and miR-675-5p [106]. Recent results indicate that, by targeting Smad1, Smad5, and Cdc6, these miRNAs play an important role in the skeletal muscle differentiation and regeneration activities associated to H19 [101].

LncRNAs containing repetitive sequences controlling mRNA decay

Short interspersed elements (SINEs) are among the most abundant repetitive sequences in mammalian genomes [107]. While initially thought of as ‘junk’ DNA, SINEs can be transcribed as individual elements by RNA polymerase III or as part of longer transcripts synthesized by RNA polymerase II and they can regulate gene expression by diverse mechanisms [41,108]. It has recently been discovered that a SINE within the 3’ UTR of a protein-coding RNA can form intermolecular base pairing with a partially complementary SINE within one or more LncRNAs [43,64]. Extensive yet imperfect stretches of double-stranded RNA (dsRNA) can be bound at multiple sites by dsRNA-binding proteins, including Staufen 1 and 2 (STAU1 and STAU2). Staufen recruitment activates Staufen-mediated mRNA decay (SMD), an important mRNA degradation process in mammalian cells. Using this mechanism, LncRNAs containing SINEs regulate the stability of several mRNAs encoding for proteins with a role in muscle differentiation, including Cdc6 and Traf6 (Figure 2E and Table 1) [64]. Both STAU1 and STAU2 interact directly with the ATP-dependent RNA helicase UPF1, a key SMD factor, enhancing its helicase activity to promote effective SMD. Because both SMD and the mechanistically related nonsense-mediated mRNA decay (NMD) employ UPF1, SMD and NMD are competitive pathways. This competition plays an important role in the control of muscle differentiation. Indeed, during myogenesis, the efficiency of NMD decreases while the efficiency of SMD increases. Interestingly, Myogenin and PAX3 are differentially targeted by these two pathways of degradation and this different susceptibility contributes to their relative abundance during differentiation. PAX3 mRNA is an SMD target and its increased decay promotes myogenesis, whereas decreased degradation of the NMD target Myogenin is required for myogenesis [109]. Importantly, since close to one third of all LncRNAs contains at least one SINE [64,110], LncRNAs containing SINE sequences could be at the heart of many physiologically important processes in addition to myogenesis.

LncRNAs in muscle diseases

Growing evidence shows that the vast majority of disease-associated genetic variations occur in the noncoding portion of the genome. In fact, whereas only 7% of disease-associated SNPs localize in protein-coding exons, the remaining 93% arise in noncoding areas of the genome, of which 43% fall in intergenic regions [111]. Considering the extensive transcription of these areas, it is reasonable to predict that a significant and yet unknown number of LncRNAs are involved in a variety of human diseases. LncRNAs can either have a primary role in the pathogenesis of a disease or rather act as modulators of disease penetrance, explaining, at least in part, the inter-personal variability observed in virtually every disorder. So far, the contribution of LncRNAs to disease has mostly been investigated in cancer and neurological disorders [112-114] but the first examples of LncRNA involved in myopathies are now unveiled. Here, we focus on the recent discoveries regarding the role of LncRNAs in Duchenne muscular dystrophy and facioscapulohumeral muscular dystrophy.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the most common and severe myopathy affecting 1:3,500 males. It is inherited in an X-linked recessive manner but, in very rare cases, heterozygous females can be mildly affected [115]. DMD is characterized by severe muscle wasting from early childhood that usually arises in leg and pelvic muscles and later extends to the trunk of the body, compromising the heart and respiratory muscles. DMD is caused by a variety of out-of-frame mutations in the dystrophin (DMD) gene encoded on the X chromosome.
(Xp21.2) resulting in the lack of a functional dystrophin protein in skeletal muscle. With 79 exons and 2.4 Mb in size, DMD is the largest gene of our genome and up to 2,900 types of mutations have been reported in DMD patients so far [116,117]. Despite the mutations triggering the disease being well characterized, the regulation of dystrophin is in part unknown and appears much more complex than previously thought. Moreover, the inter-individual variability in the severity and the disease progression is only partially explained by the types of mutations and the occurrence of female patients remains enigmatic. Therefore, the regulation of DMD is an area of intense research that in the last few years has led to the characterization of miRNA and lncRNA involvement [118].

Using custom-made tiling arrays, 14 lncRNAs transcribed from intronic sequences of the DMD gene both in sense and antisense orientations were recently identified [119]. These lncRNAs are expressed concomitantly with dystrophin in at least one of the tissues that normally express it: skeletal muscle, heart and brain [119]. Ectopic expression and promoter binding assays suggest that the lncRNAs can function in trans by downregulating the expression of specific dystrophin isoforms targeting their promoters (Figure 3A). Interestingly, an inverse correlation was found between the levels of dystrophin and a subset of lncRNAs in female carriers, both symptomatic and asymptomatic [119]. It remains to be investigated if these lncRNAs act in an allele-specific manner or whether they may also modulate the expression of the wild type dystrophin allele in female carriers. Additionally, it would be interesting to define how different DMD mutations may impact the expression of the lncRNAs.

The mutations occurring in DMD patients could also deregulate the expression of lncRNAs located outside the dystrophin gene or could even give rise to new lncRNAs as a result of translocation events. This could modulate the severity of the muscle degeneration or contribute to the development of additional symptoms such as the neurological complications observed in around one third of the patients [120,121]. A single case study recently published explores this hypothesis and describes how an intrachromosomal inversion (inv(X)p21.2;q28) disrupts the novel lncRNA KUCG1 in a DMD patient with moderate mental retardation [122]. KUCG1 is a 648-bp nuclear lncRNA expressed in a tissue specific manner [122]. Since it is normally expressed in the brain, its deregulation could contribute to the neurological impairment of the patient [122] as already reported for other pathologies [114]. Although a functional characterization of this transcript has not been performed, this study underscores the pathological potential of mutations in noncoding loci that often follow genomic rearrangements.

Another lncRNA associated with DMD is long intergenic noncoding RNA-muscle differentiation 1 (linc-MD1) [46]. Linc-MD1 is a muscle-specific lncRNA required to activate late stages of the myogenic program. Linc-MD1 is a cytoplasmic ceRNA that acts as a molecular sponge for miR-133 and miRNA-135 (Figure 3B and Table 1) [46]. Through this mechanism, linc-MD1 promotes the expression of myocyte-specific enhancer factor 2C (MEF2C) and mastermind-like protein 1 (MAML1), two transcription

![Figure 3 Proposed roles for long noncoding RNAs (lncRNAs) in Duchenne muscular dystrophy. (A) In the nucleus, sense and antisense transcription from intronic sequences of the dystrophin (DMD) gene gives rise to lncRNAs that play a repressive effect at specific DMD promoters. (B) In the cytoplasm, the muscle specific lncRNA linc-MD1 acts as a competitive endogenous RNA (ceRNA) by sequestering miRNAs away from their target mRNAs. Linc-MD1 contributes to muscle differentiation by sponging miRNA-135 and -133, and thus promoting the expression of MEF2C and MAML1. Linc-MD1 is strongly reduced in muscle cells from DMD patients.](http://www.skeletalmusclejournal.com/content/4/1/8)
factors with an important role in muscle differentiation (Figure 3B) [46]. Interestingly, the levels of linc-MD1 are strongly reduced in primary myoblasts of DMD patients and its ectopic expression rescues the myogenic differentiation potential of these cells, restoring the correct expression pattern of MAML1, MEF2C, MYOG and MHC [46]. Linc-MD1 can have a double life as lncRNA or as miRNA, since its primary transcript harbors the pri-miR-133b sequence. The balance between linc-MD1 and miR-133 biogenesis is regulated by HuR, an RNA-binding protein with a crucial role in myogenesis (Table 1) [123]. Moreover, HuR facilitates the linc-MD1-miRNA interaction, enhancing its sponge activity, thus affecting this ceRNA circuitry potentially relevant for DMD [58].

Facioscapulohumeral muscular dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common muscular dystrophy (1:14,000). FSHD is transmitted in an autosomal dominant manner and affects both sexes but presents a gender bias, as males are usually more severely affected [124]. FSHD displays a more restricted pattern of muscle weakness compared to DMD, mainly confined to the facial mimic and shoulder girdle muscles but extending to abdominal and leg muscles in the most severe cases [125,126]. The genetic lesion involved in FSHD is unusual as it does not target a protein-coding gene, but rather affects the copy number of the 3.3 kb macrosatellite D4Z4 mapping at the subtelomeric region of chromosome 4 (4q35) [127]. In the general population, D4Z4 copy number is highly polymorphic, displaying 11 to more than 100 units [128,129]. On the contrary, FSHD patients carry deletions reducing D4Z4 copy number between one and ten units [129,130]. D4Z4 deletion is associated with a profound change in the epigenetic status of the 4q35 region [131]. A recently identified IncRNA plays a key role in this transition [37]. In healthy subjects, the FSHD locus is under a repressive chromatin status, with high levels of DNA methylation, histone de-acetylation and enrichment for repressive histone marks such as H2Aub1, H3K9me3 and H3K27me3. Indeed, D4Z4 has been recently identified as a novel Polycomb (PcG) target region, suggesting that the presence of a high number of D4Z4 units leads to the extensive recruitment of PcG and the consequent repression of 4q35 genes in healthy subjects (Figure 4). In FSHD patients, instead, the reduction in number of D4Z4 units under a critical threshold leads to a reduced PcG binding with decreased levels of H3K27me3, particularly in the region immediately proximal to the D4Z4 repeat array. As a result, this region becomes more prone to transcription and gives rise to the activatory IncRNA DBE-T. DBE-T is mainly produced in FSHD patients and mediates the aberrant activation of the FSHD locus [37]. DBE-T is a nuclear transcript that acts in cis as it remains associated with the chromatin of the FSHD locus (Figure 4) [37]. DBE-T directly binds to the Trithorax (TrxG) protein ASHL1 recruiting it to the FSHD locus where it mediates the accumulation of H3K36me2 [37], a histone modification that counteracts PcG repressive activity [132-134]. Consequently, this leads to altered higher

![Figure 4 Role of DBE-T long noncoding RNA (lncRNA) in facioscapulohumeral muscular dystrophy (FSHD).](http://www.skeletalmusclejournal.com/content/4/1/8)
order chromatin organization and derepression of FSHD candidate genes localized nearby the D4Z4 array and unleashing FSHD pathogenesis [37].

Conclusion

It is increasingly evident that the functional repertoire of metazoan genomes extends far beyond protein-coding genes. A growing body of genetic and biochemical work indicates that long noncoding RNAs are important members of the complex muscle regulatory network, being engaged in diverse activities crucial for myogenesis. However, there is still a substantial gap between the expanding list of muscle IncRNAs and the precise molecular tasks they fulfill in the control of muscle differentiation. Moreover, the functional characterization of IncRNAs in muscular dystrophy is still in its infancy. Nevertheless, there is little doubt that results from such studies will significantly contribute to the formulation of specific and complementary diagnostic and therapeutic strategies for muscle wasting diseases.

Abbreviations

cellNAs: competing endogenous RNA; C2orf23: core enhancer RNA; ChiP-seq: chromatin immunoprecipitation sequencing; DBD-T: D4Z4 binding element transcript; DMD: Duchenne muscular dystrophy; DMDX: dystrophin gene; DRRNA: distal regulatory region RNA; dsRNA: double-stranded RNA; eRNA: enhancer RNA; FSHD: facioscapulohumeral muscular dystrophy; G2D/Meg3: gene trap locus 2 (maternally expressed gene 3); H3K27ac: acetylated histone H3 lysine 27; H3K4me1: monomethyl histone H3 lysine 4; IM: Igf2 mRNA binding-protein; iPSC: induced pluripotent stem cell; iRES: internal ribosomal entry site; Inc-MD1: long intergenic noncoding RNA-muscle differentiation 1; IncRNA: long intergenic noncoding RNA; IncRNA: long noncoding RNA; Malat1: metastasis associated lung adenocarcinoma transcript 1; MAM1: mastermind-like protein 1; METF2: myocyte-specific enhancer factor-2; miRNA: microRNA; MRF4: myogenic regulatory factors; MyoD: myogenic differentiation; MyoG: myogenin; ncRNA: non protein-coding RNA; Nctc1: noncoding transcript 1; NMD: nonsense-mediated mRNA decay; PRC1 and PRC2: Polycomb Repressive Complex 1 and 2; RNAPII: RNA polymerase II; rRNA: ribosomal RNA; SINE: short interspersed element RNA; SRA: steroid receptor RNA activator; SRA: protein; STAU1 and 2: Staufen 1 and 2; tRNA: transfer RNA; TrxG: Trithorax group; UTR: untranslated region; Y1: Y1-associated muscle; Y1Y1: Yin Yang 1.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

MJ: manuscript writing, final approval of the manuscript. DG: manuscript writing, final approval of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We apologize to our colleagues for the vastly incomplete documentation of their contributions. We thank Dr. Arindya Dutta for sharing results before publication. The Gabellini laboratory is supported by the Association Francaise contre les Myopathies (AFM), the ERA-Net for Research on Rare Diseases (E-Rare-2), the European Research Council (ERC), the Italian Epigenomics Flagship Project, the Italian Ministry of Health and the FSHD Global Research Foundation. D Gabellini is a recipient of a Dulbecco Telethon Institute International postdoctoral programme (DTI-IMPORT) fellowship.

References

1. Liu G, Mattick JS, Taft RJ: A meta-analysis of the genomic and transcriptomic composition of complex live. Cell Cycle 2013, 12:2001–2007.
2. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Roder M, Kokocinski F, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chrabarty S, Chen X, Chrust J, Curado J, et al: Landscape of transcription in human cells. Nature 2012, 489:101–108.
3. Moss T, Langlois F, Gagnon-Kugler T, Stefanovska V: A housekeeper with power of attorney: the lncRNA genes in ribosome biogenesis. Curr Mol Life Sci 2007, 64:29–49.
4. Phizicky EM, Hopper AK: tRNA biology charges to the front. Genes Dev 2010, 24:1832–1860.
5. Kiss T: Biogenesis of small nuclear RNAs. J Cell Sci 2004, 117:5949–5951.
6. Ghildiyal M, Zamore PD: Small silencing RNAs: an expanding universe. Nat Rev Genet 2009, 10:94–108.
7. Watkins NJ, Bohsnack MT: The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA. Wiley Interdiscip Rev RNA 2012, 3:397–414.
8. Derrien T, Guigo R, Johnson R: The long non-coding RNAs: a new (p)layer in the ‘dark matter’. Front Genet 2011, 2:107.
9. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravanli L, Ruan X, Ruan Y, Lassmann T, Caminii P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R: The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012, 22:1775–1789.
10. Kapranov P, Dekhouw J, Cheng J, Long J, Helt D, Dike S, Gingeras TR: Examples of the complex architecture of the human transcriptome revealed by RACE and high-density tilting arrays. Genome Res 2005, 15:987–997.
11. Hangauer MJ, Vaughn WM, McManus MT: Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. PLoS Genet 2013, 9:e1003569.
12. Gutmann M, Amit I, Gabber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabibi MN, Jaenisch R, Mikkelson TS, Jacks T, Haoohen N, Bernstein BE, Kells M, Regev A, Rinn JL, Landor E: Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009, 458:223–227.
13. Mercer TR, Mattick JS: Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 2013, 20:300–307.
14. Dieci G, Florino G, Castelnuovo M, Telichmann M, Pagano A: The expanding RNA polymerase III transcriptome. Trends Genet 2007, 23:614–622.
15. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Williams AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dunais E, Patel S, Helt G, Ganiell M, Ghosh S, Piccolboni A, Sementchenko V, Tamarana H, Gingeras TR: RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007, 316:1484–1488.
16. Cabili MN, Trapnell C, Goff L, Kazii M, Tazon-Vega B, Regue A, Rinn JL: Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 2011, 25:1915–1927.
17. Batista PJ, Chang HY: Long noncoding RNAs: cellular address codes in development and disease. Cell 2013, 152:1298–1307.
18. van Heesch S, van Iterson M, Jacobi J, Boymans S, Essers PB, de Bruijn E, Kapranov P, Dekhouw J, Dunais E, Patel S, Helt G, Ganiell M, Ghosh S, Piccolboni A, Sementchenko V, Tamarana H, Gingeras TR: RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007, 316:1484–1488.
21. Nagano T, Mitchell JA, Saraz LA, Pauler FM, Ferguson-Smith AC, Fell R, Fraser P: The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science 2008, 322:1717–1720.

22. Chu C, Qu K, Zhong FL, Artandi SE, Chang HY: A long non-coding RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature 2012, 491:454–457.

23. Yoon JH, Abdelmohsen K, Srikant S, Yang X, Martindale JL, De S, Huarte M, Zhan M, Becker KG, Gorospe M: LinRNA-p21 suppresses target mRNA translation. Mol Cell 2012, 47:648–655.

24. Gong C, Maquat LE: IncRNAs transactivate STA1-mediated mRNA decay by duplexing with 3' UTRs via Alu elements. Nature 2011, 470:284–288.

25. Kreiz M, Siprashvili Z, Chu C, Webster DE, Zehnder A, Qiu K, Lee CS, Fleetkilt RJ, Groff AF, Chow J, Johnston D, Kim GE, Spitale RC, Flynn RA, Zheng GX, Ayer S, Raj A, Rinn JL, Chang HY, Khavari PA: Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature 2013, 493:231–235.

26. Poliseno L, Salmena L, Zhang J, Carver B, Hember DJ, Pandolfi PP: A coding-independent function of gene and pseudogene miRNAs regulates tumour biology. Nature 2010, 465:1033–1038.

27. Cesana M, Cabrachiero D, Legnini I, Santini T, Schiantar D, Chinappi M, Tramontano A, Bozzoni I: A long non-coding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 2013, 152:349–359.

28. Feng J, Li C, Clark BS, Mady R, Shah P, Kohl JT: The Evf-2 non-coding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. Genes Dev 2006, 20:1470–1484.

29. Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Mu X, Xiong J, Guo X, Liu H: Endogenous miRNA sponge IncRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 2013, 25:69–80.

30. Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ, Kjaer M: A myRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. RNA 2013, 19:4414–4422.

31. Menczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Mowecher M, Loewer A, Ziegbold U, Landthaler M, Kocks C, Le Noble F, Rajewsky N: Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013, 495:333–338.

32. Sauvageau M, Goff LA, Lodato S, Bonev B, Groff AF, Gerhardinger C, Sanchez-Gomes DE, Hacisuleyman E, Li E, Spence M, Liapis SC, Mallard W, Morgan ME, Sveder MR, D’Eclesis MI, Moore J, Liu V, Gong G, Yancopoulos GD, Frednewell D, Kellis M, Hart RP, Valenzuela DM, Arlotta P, Rinn JL: Multiple knockout mouse models reveal IncRNAs are required for life and brain development. eLife 2013, 2:e01749.

33. Braun T, Gautel M: Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev Mol Cell Biol 2011, 12:349–361.

34. Buckingham M, Rigby PWJ: Gene regulatory networks and transcriptional mechanisms that control myogenesis. Dev Cell 2014, 28:225–238.

35. Sabourin LA, Rudnicky MA: The molecular regulation of myogenesis. Clin Genet 2000, 57:16–25.

36. Luo W, Qie Z, Zhang X: MicroRNAs involved in skeletal muscle differentiation. J Genet Genomics 2013, 40:107–117.

37. Schutz-Gessler K, Billinski P, Sado T, Ferguson-Smith A, Gessler A: The mouse Gt2z gene is differentially expressed during embryonic development, encodes multiple alternatively spliced transcripts, and may act as an RNA. Dev Dyn 1998, 212:144–228.

38. Zhou Y, Cheunsochon P, Nakayama Y, Lawlor MW, Zhong Y, Rice KA, Zhang L, Zhang X, Gordon FE, Lidov HG, Bronson RT, Kilbanski A: Activation of paternally expressed genes and perinatal death caused by deletion of the Gt2z gene. Development 2010, 137:2643–2652.

39. Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, Min W, Bennett AM, Gregory RI, Ding Y, Huang Y: The imprinted H19 IncRNA antagonizes let-7 microRNAs. Nature 2013, 502:101–112.

40. Legnini I, Morlando M, Mangiafuoco A, Fatica A, Bozzoni I: A feedforward regulatory loop between HuR and the long non-coding RNA linc-MD1 controls early phases of myogenesis. Mol Cell 2014, 55:506–514.

41. Watts R, Johnson VL, Shearer J, Hittal DS: Myostatin-inhibited induction of the long non-coding RNA Malat1 is associated with decreased myogenesis. Am J Physiol Cell Physiol 2013, 304:C995–1001.

42. Sunwoo H, Dinger ME, Wilusz JE, Amoral PP, Mattick JS, Spector DL: MEN1 epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraseptaleks. Genome Res 2009, 19:347–359.

43. Eun B, Semple ML, Good AL, Gebert CM, Pfeifer K: Promoter cross-talk via a shared enhancer explains paternally biased expression of Nctc1 at the Igf2/H19/Nctc1 imprinted locus. Nucleic Acids Res 2013, 41:B176–82.
p68/p72 and the noncoding RNA SRA are coregulators of MyoD and skeletal muscle differentiation. *Dev Cell* 2006, 11:547–560.

63. Hube F, Velasco G, Rollin J, Furling D, Franckel C. Steroid receptor RNA activator protein binds to and counteracts SRA RNA-mediated activation of MyoD and muscle differentiation. *Nucleic Acids Res* 2011, 39:513–525.

64. Wang J, Gong C, Maquat LE. Control of myogenesis by rodent SINE-containing lincRNAs. *Genes Dev* 2013, 27:795–804.

65. Lu L, Sun K, Chen X, Zou Y, Wang L, Zhou L, Sun H, Wang H. Genome-wide survey by ChiP-seq reveals YY1 regulation of lincRNAs in skeletal myogenesis. *Embo J* 2013, 32:575–2588.

66. Kim TK, Hemberg M, Gray MJ, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markscoft-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 2010, 465:182–187.

67. Lai F, Orom UA, Cesaroni M, Beringer M, Taartjes DJ, Biobel GA, Shekharat TT. Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. *Nature* 2013, 494:497–501.

68. Lam MT, Cho H, Lesch HP, Gosselin D, Heinz S, Tanaka-Oldy J, Brenner C, Kalkkonen MJ, Kim AS, Kosaka M, Lee CY, Watt A, Grossman TR, Rosenfeld MG, Evans RM, Glass CK. A steroid receptor coactivator, SRA, functions as an RNA activator protein binds to and counteracts SRA RNA-mediated activation of MyoD and muscle differentiation. *Nucleic Acids Res* 2011, 39:513–525.

69. Marquez VE, Valente S, Mai A, Forcales SV, Sartorelli V, Puri PL. A novel myoblast enhancer element mediates MyoD transcription. *Mol Cell Biol* 2007, 27:4374–4387.

70. O’Malley BW. A steroid receptor coactivator, SRA, functions as an RNA-mediated enhancer of p53-dependent enhancer activity and gene transcription. *Mol Cell* 2003, 12:1992–2004.

71. Hahn N, Murakami S, Nagao A, Danko CG, Kraus WL. Enhancer transcripts mark active estrogen receptor binding sites. *Genome Res* 2013, 23:1210–1223.

72. Melo CA, Drozd J, Wijchers PJ, van de Werken H, de Wit E, Dudel Ventlink JA, Elkon R, Melo SA, Leveille N, Kalluri R, de Laat W, Agami R. eRNAs are required for p53-dependent enhancer activity and gene transcription. *Mol Cell* 2013, 49:524–535.

73. Tassopoulou SL, Lazar AB, Weinstub HA. A novel myoblast enhancer element mediates MyoD transcription. *Mol Cell Biol* 1992, 12:4994–5003.

74. Goldhammer D, Faerman A, Shani M, Consalvi S, Caretti G, Saccone V, Proserpio V, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 2010, 465:182–187.

75. Huet C, Lesesse Y, Bosch A, Oudrhiri N, Bennaceur-Griscelli A, Duret L, Rouguè C. XACT, a long noncoding transcript coating the active X chromosome in human pluripotent cells. *Nat Genet* 2013, 45:239–241.

76. Hutchison A, Cano F, Lassar AB, Weintraub H. The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. *J Cell Sci* 2007, 120:2498–2506.

77. Clemson CM, Hutchinson JN, Sara SM, Ernsminger AW, Fox AH, Chess A, Lawrence JB. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paracompactes. *Mol Cell* 2009, 35:717–725.

78. Audas TE, Jacob MD, Lee S. Immobilization of proteins in the nucleolus by ribosomal intergenic spacer noncoding RNA. *Mol Cell* 2012, 45:147–157.

79. Ding DQ, Okasama Y, Yamane M, Tsutsui C, Haraguchi T, Yamamoto M, Hiraoka Y. Meliosis-specific noncoding RNA mediates robust pairing of homologous chromosomes in meiosis. *Science* 2012, 336:332–336.

80. Vallor C, Huet C, Lesesse Y, Bosch A, Oudrhiri N, Bennaceur-Griscelli A, Duret L, Rouguè C. XACT, a long noncoding transcript coating the active X chromosome in human pluripotent cells. *Nat Genet* 2013, 45:239–241.

81. Hacisaliman E, Goff LA, Taprell C, Williams A, Henao-Mejia J, Sun L, McLellan A, Hindsgaul D, Sauvageau M, Kelley DR, Morse M, Engreitz J, Landers ES, Guttman M, Lodish HF, Flavell RA, Yang X, Rinn JL. Topological organization of multichromosomal regions by the long intergenic noncoding RNA Fire. *Nat Struct Mol Biol* 2014, 21:198–206.

82. Schmidt LD, Spieker T, Koschmieder S, Schaffers S, Humberg J, Junge D, Bulk E, Hascher A, Wittmer D, Marra A, Hilliêan L, Wiebel K, Berdel WE, Wiewrodt R, Muller-Tidow C. The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. *J Thorac Oncol* 2013, 8:1984–1992.

83. Thomas M, Langley B, Berry C, Sharma M, Kirk S, Bazz J, Kambadur R. Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *J Biol Chem* 2000, 275:40235–40243.

84. da Rocha ST, Edwards CA, Ito M, Ogata T, Ferguson-Smith AC. Genomic imprinting at the mammalian Dlk1-Dio3 domain. *Trend Genet* 2008, 24:506–515.

85. Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, Kono T, Shioda T, Hochendlinger K. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* 2010, 465:175–181.

86. Davis E, Jensen CH, Schoeder HD, Farnir F, Shay-Haddfield T, Kliem A, Cockett N, Georges M, Charlier C. Ectopic expression of DLK1 protein in skeletal muscle of padumnal heterozygotes causes the callipyge phenotype. *Curr Biol* 2004, 14:1858–1862.

87. Feuking BA, Murphy SK, Wylie AA, Rhodes SJ, Keele JW, Levykster KA, Jirtle RL, Smith TP. Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome Res* 2002, 12:1496–1506.

88. Zhao J, Ohsumi TK, Jung JT, Ogawa Y, Grau DJ, Sarma K, Song JJ, Kingston RE, Wiewrodt R, Muller-Tidow C. The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. *J Thorac Oncol* 2013, 8:1984–1992.

89. Guttman M, Donaghey J, Carey BJ, Emmert S, Marra M, Song J, Lander ES, Lodish HF, Raj A, Regev A, Rinn JL, Young G, Lucas AB, Ach R, Bruhn L, Yang X, Amit I, Meissner A, Regev A, Rinn JL, Root DE, Lander ES. lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature* 2011, 472:295–300.

90. Kaneko S, Bonasio R, Saldana-Meyer R, Yoshida T, Son J, Nishino K, Umezawa A, Reindberg D. Interactions between JARD2 and noncoding RNAs regulate PRC2 recruitment to chromatin. *Mol Cell* 2014, 53:290–300.

91. Brannan CI, Drees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. *Mol Cell Biol* 1990, 10:28–36.

92. Gabory A, Jammes H, Dandolo L. The H19 locus: role of an imprinted non-coding RNA in growth and development. *Bioessays* 2010, 32:473–480.

93. Onyango P, Feinberg AP. A nuclear protein, H19 opposite tumor suppressor (HOTS), is a tumor growth inhibitor encoded by a human imprinted H19 antisense transcript. *Proc Natl Acad Sci U S A* 2011, 108:16759–16764.

94. Ripoche MA, Kress C, Poizier F, Dandolo L. Deletion of the H19 transcription unit reveals the existence of a putative imprinting control element. Gen Dev 1997, 11:1596–1604.

95. Forne T, Oswald J, Dean W, Saam JR, Bailleul B, Dandolo L, Tilghman SM, Walter J, Reik W. Loss of the maternal H19 gene induces changes in Igf2.
methylation in both cis and trans. Proc Natl Acad Sci U S A 1997, 94:10243–10248.

104. Runge S, Nielsen FC, Nielsen J, Lykke-Andersen J, Wever UM, Christiansen J: H19 RNA binds four molecules of insulin-like growth factor II mRNA-binding protein. J Biol Chem 2007, 282:25962–25969.

105. Li Z, Gilbert JA, Zhang Y, Zhang M, Qui O, Ramakrishnan K, Sh*hakladev T, Eash JK, Scaramozza A, Godderies MM, Kirsch DG, Campbell KP, Broack AS, Glass DJ: An HMG2A-IGFBP2 axis regulates myoblast proliferation and myogenesis. Dev Cell 2012, 23:1175–1188.

106. Cai X, Cullen BR: The imprinted H19 noncoding RNA is a primary microRNA precursor. Rheo 2007, 13:313–316.

107. Casa V, Gabellini D: 

108. Cheetham SW, Gruhl F, Mattick JS, Dinger ME: 

109. Soltanzadeh P, Friez MJ, Dunn D, von Niederhausern A, Gurvich OL, Erriquez D, Perini G, Ferlini A: 

110. Tran TH, Zhang Z, Yagi M, Lee T, Awano H, Nishida A, Okinaga T, Takeshima Y, Hewitt JE, Lyle R, Clark LN, Vallely EM, Wright TJ, Wijmenga C, van Deutekom JC, Francis F, Sharpe PT, Hofker M, Frants RR, Williamson R: Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy. Hum Mol Genet 1994, 3:1287–1295.

111. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Tonini MM, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Zatz M: Competitive pathways that contribute to myogenesis: effects on PAX3 and myogenin mRNAs. Genes Dev 2009, 23:54–66.

112. 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, 23:54–66.

113. Caputa A, Kreenerberg Z, Lynch VI, Zhuo X, Ramany L, Bourque G, Yandell M, Feschotte C: Transposable elements are major contributors to the origin, diversification, and regulation of vertebrate long noncoding RNAs. PLoS Genet 2013, 9:e1003470.

114. Mallardi M, Romeo DM, Ricotti V, Ferlini A, Gualandi F, Vicari S, Bertini E, Zonana J, Kovak K, Gospe SM Jr, Gappmaier E, Taylor LE, Howard MT, Weiss Bonnemann CG, Medne L, Mendell JR, Mathews KD, Wong BL, Sussman MD, Swoboda KJ, Sampson JB, Pestronk A, Connolly AM, Florence JM, Finkel RS, Padberg GW, Frants RR: Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. Nat Genet 1992, 2:26–30.

115. de Vente Wolke C, van Tienhoven EA, Grau ME, Hewitt JE, Padberg GW, van Ommen GJ, Hofker MH, Frants RR: FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. Hum Mol Genet 1993, 2:2037–2042.

116. Leiden Open Variation Database. ([http://www.dmd.nl/nmdb2/home.php?select_d=DMD]

117. UMD-DMD French Database. ([http://www.umd.be/DMD/W_DMD/index.html]

118. Enriquez D, Perini G, Ferlini A: Non-coding RNAs in muscle dystrophies. Int J Mol Sci 2013, 14:19681–19704.

119. Bolsente M, Enriquez D, Valls E, Brioschi S, Scorton C, Neri M, Falzarano MS, Gherardi S, Fabris M, Rinesi P, Gualandi F, Perini G, Ferlini A: The DMD locus harbours multiple long non-coding RNAs which orchestrate and control transcription of muscle dystrophin mRNA isoforms. PLoS One 2012, 7:e45328.

120. Sarrazin E, Hagen MW, Schara U, von Au K, Kaindl AM: Growth and psychomotor development of patients with Duchenne muscular dystrophy. Eur J Paediatr Neurol 2014, 18:36–44.

121. Pane M, Lombardo ME, Alfieri P, D’Ambrosio A, Bianco F, Vasco G, Piccini G, Mallardi M, Romeo DM, Rocchi V, Ferlini A, Gualandi F, Vicari S, Bertini E, Berardiinni A, Mercuri E: Attention deficit hyperactivity disorder and cognitive function in Duchenne muscular dystrophy: phenotype-genotype correlation. J Pediatr 2012, 161:705–709, e701.

122. Tran TH, Zhang Z, Yagi M, Lee T, Avano H, Nozhida A, Orakneg A, Takeshima Y, Matsuo M: Molecular characterization of an Xp21.2;q28) chromosomal inversion in a Duchenne muscular dystrophy patient with mental retardation reveals a novel long non-coding gene on Xq28. J Hum Genet 2013, 58:33–39.

123. von Roretz C, Beauchamp P, Di Marco S, Gallouzi IE: HuR and myogenesis: being in the right place at the right time. Biochim Biophys Acta 2011, 1813:1663–1667.

124. Tonini MM, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Ratz M: Asymptomatic carriers and gender differences in facioscapulohumeral muscular dystrophy (FSHD). Neuromuscul Disord 2004, 14:33–38.

125. Shahrazila N, Wills AJ: Significance of Beever’s sign in facioscapulohumeral dystrophy and other neuromuscular diseases. J Neurol Neurosurg Psychiatry 2005, 76:869–870.

126. Pandya S, King WM, Tawel R: Facioscapulohumeral dystrophy. Phys Ther 2008, 88:105–113.

127. Wijmenga C, Sandkuijl LA, Moorer P, van der Boon N, Bodrug SE, Ray PN, Brouwer OF, Murray JC, van Ommen GJ, Padberg GW, Frants RR: Genotypic linkage map of facioscapulohumeral muscular dystrophy and five polymorphic loci on chromosome 4q35-qter. Am J Hum Genet 1992, 51:411–415.

128. Neguembor NV: In junk we trust: repetitive DNA, epigenetics and facioscapulohumeral muscular dystrophy. Epigenomics 2010, 2:771–787.

129. Schmitges FW, Prusty AB, Faty M, Stutzer A, Lingnaraj GM, Awazian J, Sack R, Hess D, Li L, Zhou S, Bunker RD, Wirth U, Bouwmeester T, Bauer A, Ly-Hartig N, Zhao K, Chan H, Gu J, Gut H, Fischle W, Muller J, Thoma NH: Histone methylation by PRC2 is inhibited by active chromatin marks. Mol Cell 2011, 42:330–341.

130. Yuan W, Xu M, Huang C, Liu N, Chen S, Zhu B: H3K36 methylation antagonizes PRC2-mediated H3K27 methylation. J Biol Chem 2011, 286:7983–7989.

131. Neguembor NV, Gabellini D: In junk we trust: repetitive DNA, epigenetics and facioscapulohumeral muscular dystrophy. Epigenomics 2010, 2:771–787.

132. Dorighi KM, Tamkun JW: The trithorax group proteins Kismet and ASH1 promote H3K36 dimethylation to counteract Polycymb group repression in Drosophila. Development 2013, 140:4182–4192.

doi:10.1186/2044-5040-4-8
Cite this article as: Neguembor et al.: Long noncoding RNAs, emerging players in muscle differentiation and disease. Skeletal Muscle 2014 4:8.