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Long non-coding RNAs as emerging regulators of differentiation, development, and disease

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Abbreviations: Bvht, braveheart; CDT, C-terminal domain; ceRNAs, competing endogenous RNAs; ciRS-7, circular RNA sponge for miR-7; DBE-T, D4Z4-binding element; DMD, Duchenne muscular dystrophy; ES, embryonic stem; Fendrr, Foxf1a called fetal-lethal non-coding developmental regulatory RNA; FSHD, facioscapulohumeral muscular dystrophy; iPS, induced pluripotent stem; lncRNAs, long non-coding RNAs; Malat1, metastasis associated lung adenocarcinoma transcript 1; MEF2, myocyte enhancer factor-2; Mesp1, mesoderm progenitor 1; MRFs, myogenic regulatory factors; ncRNAa, non-coding RNA activating; Neat2, nuclear-enriched abundant transcript 2; PRC2, polycomb group repressive complex 2; RNAP II, RNA polymerase II; SINE, short interspersed element; SR, serine arginine; SRA, steroid receptor activator; SRY, sex-determining region Y; YAM 1-4, YY1-associated muscle 1-4

A significant portion of the mammalian genome encodes numerous transcripts that are not translated into proteins, termed long non-coding RNAs. Initial studies identifying long non-coding RNAs inferred these RNA sequences were a consequence of transcriptional noise or promiscuous RNA polymerase II activity. However, the last decade has seen a revolution in the understanding of regulation and function of long non-coding RNAs. Now it has become apparent that long non-coding RNAs play critical roles in a wide variety of biological processes. In this review, we describe the current understanding of long non-coding RNA-mediated regulation of cellular processes: differentiation, development, and disease.

Introduction

Since the discovery of the structure of DNA and the genetic code, the primary paradigm for gene expression has been that of a DNA blueprint encoding RNA messengers, which are then translated into functional proteins. This paradigm became strongly embedded into the collective consciousness of molecular biology with the coining of the term "The Central Dogma of Molecular Biology"by Francis Crick, and held true across all species.[1] In the last 2 decades, however, exceptions have been emerging to the concept of proteins as the sole effectors of the genetic code in organisms. With the release of the human genome sequence, it became clear that only a small fraction of DNA encodes proteins.[2] However, a large proportion of the non-protein-coding genome is transcribed temporally and spatially in a well-regulated manner.[3] The transcribed pool of this non-coding RNA has given rise to a variety of new classes of regulatory RNA molecules, which appear to have numerous functions in cellular differentiation, development, and disease (**Table 1**).[4-7]

In retrospect, the concept of functional RNA should hardly be surprising; ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) were discovered in the 1950s as the most basic and essential components of cellular machinery of protein synthesis in all organisms.[8] The discovery of small-interfering RNAs and microRNAs established non-coding RNAs as powerful regulators of development that could alter the expression of hundreds of targets, and hold equal footing with transcription factors as powerful controllers of gene expression.[9-12] Still, the functional categories of the majority of the transcribed non-coding RNAs are difficult to predict and they have poor evolutionary sequence conservation, indicating either that a high level of transcriptional noise is present in the cell, or that numerous uncharacterized, species-specific classes of non-coding RNA exist.

Long non-coding RNAs (lncRNAs) are >200 bases long with low or no protein coding potential. These RNAs often regulate epigenetic silencing through chromatin remodeling. They are also now known to regulate splicing, recruit transcription factors, and regulate mRNA stability.[13] Intersections of ChIP-seq and RNA-seq studies have found thousands of lncRNAs in both mice and humans.[14-16] Since sequence conservation across species is poor, predicting the function of these molecules is difficult, but contemporary experimental approaches allow their genetic manipulation in vitro and in vivo and the discovery of protein- and genomic-binding partners. Such studies have established lncRNA molecules as important regulators of diverse biological functions. This review provides an update on the roles lncRNAs play during cellular differentiation, development, and disease.

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Table 1. Prominent lncRNAs and their functions in development and disease

lncRNAs	Organisms	Functions	Phenotypes/disease	References
ANRIL/CDKN2B-AS1	Human	Transcriptional regulation	Prostate cancer, leukemia	5
Airn, Kcnq1ot1	Mouse	Epigenetic regulation; Embryonic gene activation	Growth defects; breast, colon carcinoma	57,58
Malat1	Mouse, Human	Splicing, gene regulation	Tumor; myoblast differentiaiton	5,34,35,38,71
HOTAIR	Mouse, Human	Hox gene regulation; Recruitment of PRC2 and LSD1	Tumor formation; cancer metastasis	76-80
Hottip	Chicken	HoxA gene regulation	Defect in limb formation	25
Xist, Tsix	Mouse	Dosage compensation	Loss of function causes embryonic lethality	17-21
Fendrr, Braveheart	Mouse, Human	Heart development	Loss of function causes embryonic lethality	73-75
Miat, Six3os1, Tug1, Vax2os	Mouse	Retinal development	Defects in retinal specification; photoreceptor differentiation	49,50,53,54,56
Dlx1os, Dlx6os1	Mouse	Brain development	Neurological deficit	51,52
Megamind	Zebrafish	Brain and eye development	Defects in brain and eye development	55
H19	Mouse, Human	Posttranscriptional regulation by producing microRNAs	Skeletal muscle differentiation, regeneration, cancer	65,67,81
SRA	Mouse, Human	Transcriptional activity of MyoD and p53	Skeletal muscle differentiation; breast, uterus, ovary tumor	30-33
Linc-MD1	Mouse, Human	Sequestration of microRNAs	Myogenic differentiation, Duchenne Muscular Dystrophy	42
SINE-containing lncRNAs	Mouse	Staufen-mediated mRNA degradation	Myogenic differentiation	72
bII NAT	Mouse	Suppress MHC IIb transcription	Skeletal muscle development	70
CE, DRR lncRNAs	Mouse	Transcriptional regulation of MyoD	Skeletal muscle differentiation	62
YAMS	Mouse	Transcriptional regulation	Myogenic differentiation	69

Various Modes of Action of Long Non-coding RNA in Regulating Gene Expression

Many long non-coding RNAs are encoded in regions proximal to the promoters of known coding genes, or as antisense transcripts to coding genes. lncRNAs are regulated independently of adjoining genes and have their own specific histone modifications and splicing signals; in some cases, lncRNAs are located in genomic regions distant from known protein coding genes.[13] Genomic maps of long intergenic non-coding RNAs now include thousands of these RNAs in mice and humans. These non-coding genes contain histone H3K4me3 marking transcriptional start sites and H3K36me3 indicating actively transcribed regions. RNA sequencing reveals that many of these genes produce transcripts encoded by exons that are separated by introns, which are spliced out using conventional splicing signals. However, as mentioned above, sequence conservation of lncRNAs between species is much lower than that of protein-coding sequences; some of the lncRNA sequences show higher evolutionary conservation than that of non-expressed genomic sequences. In addition, many lncRNAs are regulated by a number of important developmental and homeostatic transcription factors.[14,15] We describe below several examples of how these lncRNAs regulate gene expression, highlight their diverse modes of action, and their significance in development and disease. A number of these examples are shown in **Table 1** and **Figure 1**.

X-chromosome-inactivating long non-coding RNAs

Xist is one of the earliest examples of lncRNA with a prominent role in regulating X-chromosome inactivation (**Fig. 1A**). In mammalian females, the majority of genes contained on one of the 2 X-chromosomes in each cell are silenced, accounting for the similar level of expression of these genes between females and males. During female development, the non-coding RNA *Xist* is transcribed from the X-chromosome that is destined to become inactivated in each cell.[17] *Xist* associates with the regions of the chromosome that are to be silenced, resulting in the formation of *"Xist* clouds" and recruitment of Polycomb Group Repressive complex 2 (PRC2).[18,19] PRC2 is comprised of Suz12, Eed/Ezh1/Ezh2 (H3K27 methyltransferase) and RbAp48, and represses promoters by trimethylation of H3K27. Another

lncRNA, *Tsix*, is produced from the *Xist* gene in the antisense direction on the active X chromosome. *Tsix* antagonizes *Xist* to prevent inactivation of the active X chromosome.[20] Interestingly, *Xist* was recently used as an approach for therapeutic intervention for Down syndrome, a genetic disorder caused by trisomy of chromosome 21.[21] In this work, the authors introduced an inducible *Xist* transgene into the *DYRK1A* locus on chromosome 21 of induced pluripotent stem (iPS) cells derived from Down syndrome patients and successfully inactivated the extra chromosome 21 by stable heterochromatin formation.[21] This finding raises hope that lncRNAs will have therapeutic applications, especially when considered in tandem with regenerative medicine.

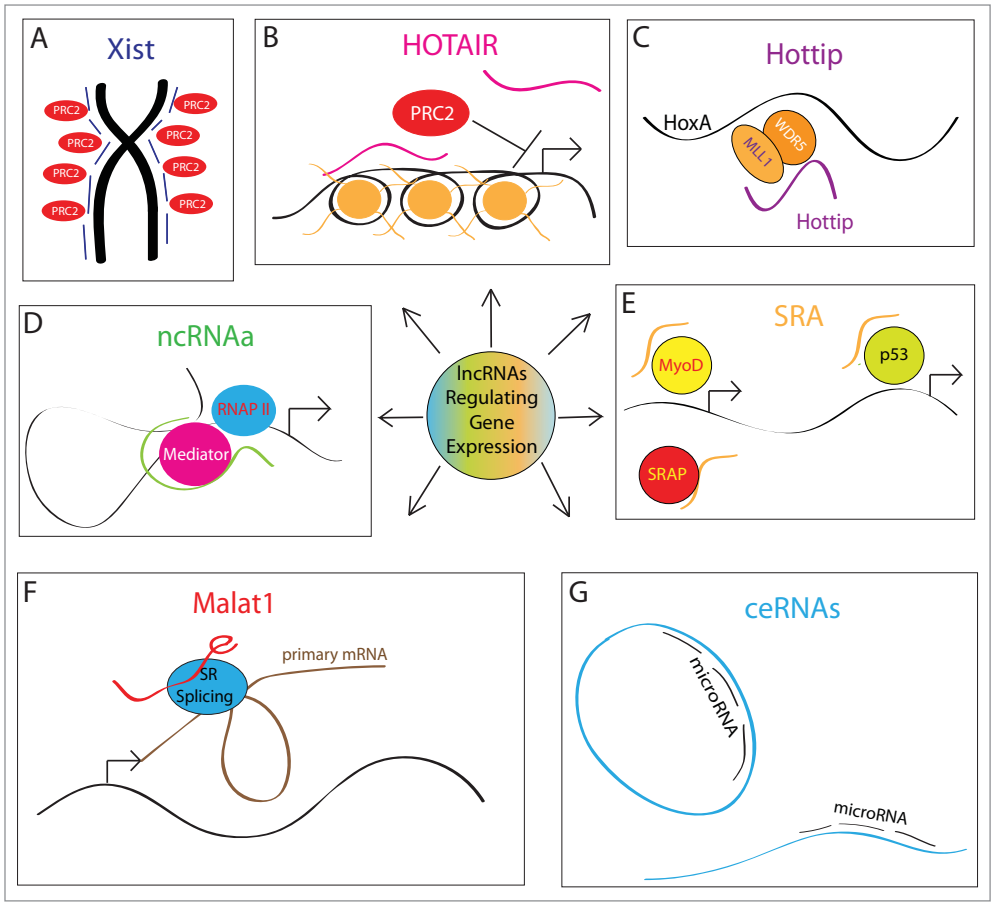

Figure 1. lncRNAs regulate gene expression using diverse modes of action.

Cis-acting long non-coding RNAs

lncRNAs have important roles in regulating transcription of protein coding genes. lncRNAs transcribed from enhancer regions of protein coding genes, called e-RNAs, often regulate the expression of adjoining protein coding genes in cis through the recruitment of transcription factors.[22-24] For example, *Hottip* lncRNA is a well-studied *cis*-acting lncRNA expressed from the *HOXA* cluster.[25] It activates transcription of nearby genes by binding the MLL-WDR5 complex and facilitating the addition of activating histone marks (H3K4me3) to the gene promoter (**Fig. 1C**). The expression of the bHLH transcription factor Neurogenin1 is dependent on the expression of an e-RNA, *utNgn1*, encoded 7 kb upstream of the *Neurogenin1* transcriptional start site. Polycomb group proteins suppress the expression of both *utNgn1* and *Neurogenin1*, and knockdown of *utNgn1* results in reduction in *Neurogenin1*, suggesting that the expression of *Neurogenin1* is positively regulated by the expression of the *utNgn1* e-RNA.[26]

A group of these cis-acting e-RNAs, termed non-coding RNA activating (ncRNAa), acts through recruitment of the transcriptional co-activator Mediator (**Fig. 1D**). Mediator physically interacts with a number of these lncRNAs, and depletion of these lncRNAs or of Mediator decreases expression of adjacent target genes. This interaction was found to facilitate chromatin looping of the adjacent genes, leading to their transcriptional activation and expression.[27] These examples suggest that many enhancer-encoded lncRNAs in the mammalian genome are essential for the cis-activating function of their corresponding enhancers, at least in part, by facilitating DNA looping so as to bring their target genes in proximity to protein factors required for transcription.

Trans-acting long non-coding RNAs

In addition to cis-acting lncRNAs, there are interesting examples of gene expression regulation by lncRNAs in trans, e.g., *7SK* and *B2* lncRNAs.[28,29] These lncRNAs impact global transcription by negatively regulating RNA polymerase II (RNAP II) activity. *7SK* lncRNA negatively regulates transcription elongation factor PTEFβ, while *B2* lncRNA represses RNAP II activity by binding RNAP II C-terminal domain (CTD) and inhibiting its phosphorylation.[28,29] Both of these lncRNAs are upregulated in response to stress signals and, thus, shut down global transcription, most likely for cellular protection.

A fascinating case of an lncRNA regulating transcription in trans is that of the *steroid receptor activator* (*SRA*) gene, which encodes both a protein (SRAP) and a functional lncRNA (*SRA*) that act as co-regulators of nuclear receptor transcriptional activity (**Fig. 1E**).[30] In addition to nuclear receptors, *SRA* facilitates the transcriptional activity of p53[31] and MyoD[32] in different cellular and developmental contexts. Overexpression of *SRA* RNA, but not SRA protein (SRAP), along with MyoD, facilitates trans-differentiation of mouse

fibroblasts to skeletal muscle. SRAP, on the other hand, inhibits muscle differentiation by binding *SRA* RNA and preventing it from activating MyoD.[33] This is a remarkable example of a gene encoding 2 products with opposing effects on transcription: a stimulatory lncRNA and an inhibitory protein.

Long non-coding RNAs in alternative splicing of pre-mRNAs

Alternative splicing of pre-mRNAs is an important event in the regulation and diversification of gene function, with a majority of multi-exonic human transcripts known to undergo alternative splicing. Several lncRNAs regulate alternative splicing. The lncRNA *Metastasis Associated Lung Adenocarcinoma Transcript 1* (*Malat1*), also known as *Nuclear-Enriched Abundant Transcript 2* (*Neat2*) is an example of such a lncRNA (**Fig. 1F**).[34] *Malat1* was originally discovered as a prognostic factor for metastasis in several human cancers, including lung cancer, but its role in cellular physiology remained elusive for many years.[35] Alternative splicing is regulated by several trans-acting protein factors including a well-characterized class of RNA-binding proteins called the Serine Arginine (SR) family proteins.[36,37] *Malat1* is now known to predominantly localize to nuclear speckles, the sites where other splicing factors are often located, and believed to regulate the alternative splicing of a large set of genes by recruiting SR splicing factors to these nuclear speckles. *Malat1* interacts with SR splicing factors and both *Malat1* and the interacting SR splicing factors are conserved among species.[34] Despite this conservation, however, alternative splicing is still seen in *Malat1* knockout mice or human knockout cell lines, suggesting the presence of unidentified molecules or pathways that can substitute for *Malat1* in alternative splicing.[38]

Competing endogenous long non-coding RNAs

microRNAs bind to the 3'UTR of their target genes and negatively regulate gene expression either by repressing translation or by promoting mRNA decay in P bodies.[39] microRNA sponging was developed as an experimental strategy in which a designed exogenous RNA with multiple target sites for a particular microRNA is expressed to "sponge up" the cellular microRNA and inhibit the repression of other cellular targets by the microRNA.[40] Interestingly, recent studies report naturally occurring microRNA sponges, termed competing endogenous RNAs (ceRNAs) (**Fig. 1G**).[41] These ceRNAs consist of a variety of RNA species that include protein-coding mRNAs, pseudogenes, lncRNAs, and circular RNAs. These ceRNAs also cross-talk and co-regulate each other by competing for binding of microRNAs for which they share common target sites.[41]

linc-MD1 is a ceRNA with a role in skeletal muscle development and disease. *linc-MD1* plays a critical role in skeletal muscle differentiation by titrating away miR-133 and miR-135 from their targets, the *MAML1* and *MEF2C* mRNAs.[42] *MAML1* and *MEF2C* mRNAs encode transcription factors that are induced during muscle differentiation and are required for proper development. Excess *linc-MD1* titrates away the microRNAs, de-represses *MAML1* and *MEF2C* and thus promotes differentiation. Conversely, differentiation is delayed upon knockdown of *linc-MD1*. The importance of *linc-MD1* in myoblast differentiation is underlined by its decrease in Duchenne muscular dystrophy (DMD), a devastating muscle degenerative disease. Myoblasts obtained from DMD patients show delay and defects in differentiation.[42] Restoration of *linc-MD1* to DMD myoblasts restored differentiation and, in particular, expression of MAML1 and MEF2C proteins.

Inhibition of microRNAs by titration by lncRNAs has also been shown to be important in embryonic stem (ES) cell renewal. OCT4, SOX2 and NANOG are essential transcription factors required for ES cell self-renewal. *linc-RoR* is abundantly expressed in human ES cells, sequesters miR-145, and thus protects OCT4, SOX2 and NANOG from miR-145-mediated repression.[43,44] Introduction of mutations in miR-145-binding sites in *linc-RoR* lncRNA abolished its ability to repress miR-145. Recently, a large number of natural circular transcripts, termed circRNAs, containing multiple target sites of the same microRNA have been reported, suggesting that circRNAs can sequester highly abundant microRNAs.[45,46] One such circRNA, *circular RNA sponge for miR-7 (ciRS-7*), contains multiple putative miR-7 target sites and is expressed in the human and mouse brain.[45,46] Overexpression of *ciRS-7* impaired brain development in zebrafish, a similar phenotype to that seen in miR-7 knockdown.[45,46] The same research group has shown that the testis-specific circRNA *sex-determining region Y* (*Sry*) sponges miR-138, indicating that the phenomenon of microRNA sponging is not an isolated example.[45] Together, these findings suggest that ceRNAs are important regulators of diverse biological functions, and that unraveling the cross-talk between these lncRNAs will provide valuable insights into a number of developmental and pathological processes.[45]

Long Non-coding RNAs in Cellular Differentiation and Development

A large number of lncRNAs are involved in cellular differentiation, maintenance of stem cell pluripotency, and development of tissues or organs. Several lncRNAs, such as *lncRNA-ES1*, *lncRNA-ES2*, and *Linc-ROR,* are associated with the maintenance of pluripotency of embryonic stem cells or iPS cells.[43,44,47] A number of lncRNAs are involved in organ development, such as brain and eye,[48-56] and growth.[57,58] We will now focus on the role of lncRNAs in skeletal and cardiac muscle development as examples of the importance of these molecules in differentiation and development.

The role of long non-coding RNAs in skeletal muscle development

Skeletal muscle differentiation and development are well coordinated and tightly regulated processes. A well characterized family of transcription factors known as Myogenic Regulatory Factors (MRFs), comprised of MyoD, Myf5, Myogenin, MRF4, and the Myocyte Enhancer Factor-2 (MEF2A-D), are known to

play key roles in these processes.[59,60] Recent studies have identified a number of lncRNAs upregulated during muscle differentiation that play important roles in regulating several of these important transcription factors, including MyoD expression and activity.

lncRNAs overlap with a number of MyoD binding sites across the genome, and are transcribed in a MyoD-dependent manner.[61] Such lncRNAs are enriched in the enhancer regions of MyoD target genes and appear to play a role in myogenesis. In a recent study, 2 eRNAs, referred to as *CE* and *DRR* lncRNAs, generated from the upstream regulatory regions of *MyoD*, were shown to regulate *MyoD* and myogenin expression by altering chromatin accessibility and recruitment of RNAP II.[62] *SRA* lncRNA is another example of a lncRNA that regulates myogenesis. As described earlier, *SRA* and its protein isoform SRAP have opposite roles in facilitating MyoD activity. The ratio of *SRA* to SRAP increases during myogenesis, which rescues *SRA* from repression by SRAP and allows *SRA* to act as a co-activator of MyoD.[33] MyoD has also been shown to regulate the lncRNA *H19*, which is located at the *Igf2* imprinted locus and expressed only from the maternal allele. *H19* expression represses transcription of the adjoining gene *Igf2*. Igf2 protein interacts with MyoD in vitro and indirectly inhibits MyoD expression.[63] Thus, MyoD de-represses its own expression by inducing H19 and thus repressing *Igf2* RNA and protein.[63]

H19 was previously called *MyoH* when identified in the same screen for inducers of myogenic differentiation that identified MyoD.[64] *H19* is abundantly expressed during embryonic development but strongly repressed in all adult tissues, except skeletal muscle. We have recently demonstrated that *H19* has a direct role in skeletal muscle differentiation and regeneration (**Fig. 2**).[65]

H19 encodes 2 conserved microRNAs, miR-675-3p and -5p. We showed that the biological function of *H19* is mediated through miR-675-3p and -5p in both muscle differentiation in vitro and muscle regeneration in vivo.[65] miR-675-3p represses the anti-myogenic bone morphogenetic protein (BMP) pathway by directly targeting the transcription factors Smad1 and Smad5.

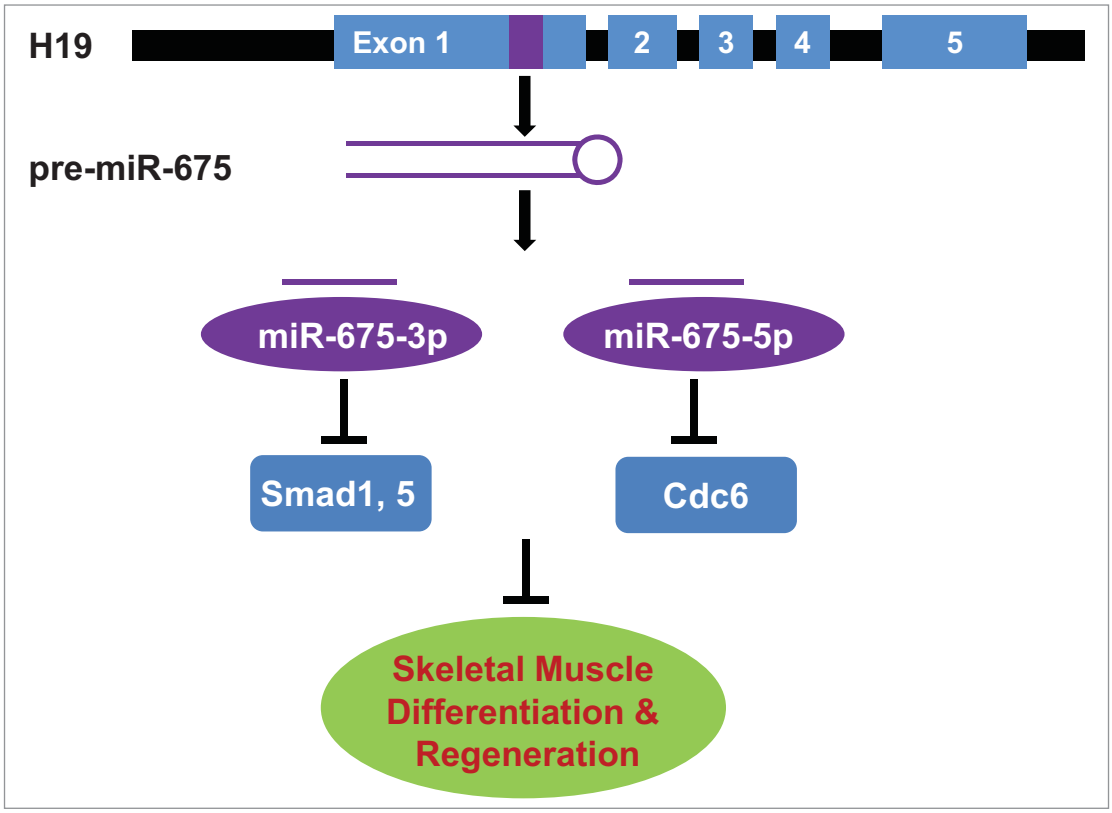

Figure 2. H19 lncRNA generates miR-675-3p and -5p and promotes skeletal muscle differentiation and regeneration by inhibiting repressors of myogenesis.

Thus, *H19* lncRNA promotes myogenesis by generating a microRNA to inhibit this negative regulator of muscle differentiation. The other microRNA, miR-675-5p, directly targets and represses the DNA replication initiation factor Cdc6. Cdc6 was shown to be activated by MyoD during the myoblast stage,[66] but the mechanism by which it is downregulated during myogenesis was previously unknown.

Paradoxically, a recent study reports that *H19* sponges let-7 in the 293T kidney cell line and suggests that *H19* inhibits C2C12 myoblast differentiation by sponging let-7.[67] However, our data strongly support a role of *H19* as a pro-myogenic factor both for myoblast cells differentiation in vitro and muscle regeneration in vivo.[65] In contrast to their finding, we did not observe a marked upregulation of let-7 in our differentiation system. These results indicate that sponging of let-7 by *H19* may not be physiologically relevant in skeletal muscle differentiation and regeneration,[65] but it does not rule out the possibility that *H19* may function by other mechanisms in different tissue types and developmental contexts. It is also possible that, in other contexts, *H19* can act as a lncRNA independently of the creation or sponging of microRNAs.

Our findings provide a new insight into how lncRNAs function through production of embedded microRNAs. Consistent with this, a genome-wide study predicted that a large number of lncRNAs encode microRNAs.[68] It will be interesting to identify how many in the rapidly expanding compendium of lncRNAS

act through microRNA pathways to regulate gene expression in different cellular contexts.

Other lncRNAs important for myogenesis are *YAM 1-4* (*YY1-associated muscle 1-4*), *Malat1*, and *bII NAT*.[69-71] *YAMs 1-4* are regulated by the myogenic transcription factor YY1. YY1 ChIP-seq data showed that YY1 binds to the regulatory elements of these lncRNAs, facilitating their expression.[69] Interestingly, *Yam-2* and *Yam -3* were shown to promote myogenic differentiation whereas *Yam-1* and *Yam-4* inhibited differentiation. *Malat1* lncRNA was originally discovered to be involved in cancer metastasis, but was later found to play a role in skeletal muscle differentiation.[71] *Malat1* expression is upregulated during C2C12 myoblast differentiation, and siRNA-mediated knockdown of this lncRNA arrests the cell cycle in G0/G1, suggesting a role in differentiation. *Malat1* is suppressed by myostatin, a well known inhibitor of myogenesis. As discussed earlier, *Malat1* is believed to be a regulator of alternative splicing, but a more detailed mechanism of the pro-myogenic function of *Malat1* remains to be elucidated. The myosin heavy chain (MHC) proteins found in skeletal muscle have multiple isoforms encoded at several locations on the genome. A natural antisense RNA (termed *bII NAT*), encoded at the MHC IIb locus, was found to suppress MHC IIb transcription, playing a role in determining which MHC isoforms are expressed during postnatal development.[70] Recently, a study illustrated the role of short interspersed element (SINE)-containing lncRNAs in regulating myogenic differentiation.[72] Briefly, a lncRNA called *m1/2-sbsRNA2(B2)* contains a B2 element that pairs with the B2 element present in the 3' UTR of mTRAF6 and promotes the degradation of mTRAF6, by Staufen-mediated degradation. mTRAF6 is a pro-myogenic factor, making *m1/2sbsRNA2(B2)* an anti-differentiation factor. Together, these studies establish that lncRNAs are important contributors to the regulation of skeletal muscle development.

The role of long non-coding RNAs in cardiac muscle development

A large number of lncRNAs are expressed during cardiomyocyte differentiation.[73-75] Two such lnRNAs, *braveheart* (*Bvht*) and a gene that is adjacent to *Foxf1a* called *Fetal-lethal non-coding developmental regulatory RNA (Fendrr),* were recently shown to be required in the development of the mammalian heart, and strongly highlighted the importance of lncRNAs in organ development.[73,74] siRNA-mediated knockdown of *Bvht* in mouse neonatal cardiomyocytes altered cardiac-specific gene expression and blocked their differentiation into mature cardiomyocytes. In cardiac progenitor cells, *Bvht* promotes expression of mesoderm progenitor 1 (Mesp1), a critical transcription factor in the network of genes that has to be activated for cardiac differentiation, by sequestering PRC2 (a writer of the repressive histone modification H3K27me3). However, *Bvht* is not conserved among species and whether the functional role of *Bvht* in humans is played by an equivalent molecule still remains to be determined.

Fendrr was also identified as a regulator of heart development.[73] Loss of function of *Fendrr* caused embryonic lethality due to defective heart morphology and function. Loss of *Fendrr* was associated with increased expression of a subset of cardiac transcription factors, including NKx2.5 and Gata6. The increase in the level of these transcripts was accompanied by a concomitant increase in H3K4me3 level in their target promoters. *Fendrr* is believed to regulate cardiac genes both in cis and in trans. Consistent with its cis regulatory function, *Fendrr* regulates its neighboring gene *Foxf1a* by interacting with and recruiting the PRC2 complex to the *Foxf1a* promoter. In addition, *Fendrr* interacts with the TrxG/MLL activating complex (a writer of the activating histone mark H3K4me3) and is important for activating cardiac-specific genes in trans. Thus, Fendrr is required for the maintenance of a fine balance between repressive and activating marks at promoters of various genes during cardiogenesis. In contrast to *Bvht*, *Fendrr* is conserved in humans, associates with PRC2, and likely plays an important role in human heart development.

Long Non-coding RNAs in Human Diseases

The list of lncRNAs implicated in human diseases is growing very fast. As lncRNAs are being discovered in key biological and developmental processes, it is likely that misregulation of lncRNAs will lead to disease. A large number of studies have implicated lncRNA expression in the pathophysiology of various cancers.[5] *HOTAIR*, a lncRNA encoded within the *HOX* gene cluster, promotes metastasis in breast, hepatocellular, nasopharyngeal, colorectal, pancreatic, and ovarian cancers.[76-80] *HOTAIR* lncRNA is overexpressed in these metastatic cancers, and alters the occupancy of PRC2 across the genome, rearranging the landscape of repressive H3K27me3 in cells (**Fig. 1B**). In particular, *HOTAIR* acts in trans to alter the target specificity of PRC2 and thus repress a number of anti-metastatic genes.[77-80]

The pro-myogenic role of *H19* is consistent with previous studies in which inactivation of *H19* was linked to the development of rhabdomyosarcoma (RMS).[81] RMS is a childhood tumor that arises from defective skeletal muscle differentiation. As miR-675-3p and -5p can promote myogenic differentiation, these 2 microRNAs might have a therapeutic potential for treatment of RMS. Since *H19* inactivation has also been linked to the development of Wilms' tumor,[81] it is worth investigating whether the tumor suppressor activity of *H19* works, in this context, via the production of the same 2 microRNAs. Several other lncRNAs involved in the pathophysiology of various cancers have been reviewed elsewhere.[5]

Apart from cancers, lncRNAs are also involved in other diseases, particularly those in which defects in differentiation are observed. For example, a few lncRNAs show altered expression in various forms of muscular dystrophies, including DMD and facioscapulohumeral muscular dystrophy (FSHD).[42,82] The expression of *linc-MD1* is reduced in myoblasts isolated from DMD patients. The abnormal kinetics of differentiation in myoblasts isolated from DMD patients was partly corrected by reintroducing exogenous *linc-MD1,* suggesting its important role in DMD.[42] As we have demonstrated a critical role of *H19* lncRNA in skeletal muscle differentiation and regeneration, we are interested in investigating whether *H19* expression, or processing into

microRNAs, is altered in differentiation-defective myoblasts from DMD patients. A third example is the lncRNA encoded by the *D4Z4-binding element* (*DBE-T*), which is selectively expressed in FSHD patient samples.[82] *DBE-T* increases H3K36me2 by recruiting the MLL1 complex and results in excessive transcriptional activation of the *FSHMD1A* locus in patients with FSHD.[82]

Conclusions and Future Perspectives

With the advent of ultra-high-throughput sequencing, the universe of non-coding RNAs is getting bigger every day. It is becoming clear that a significant part of the non-protein-coding mammalian genome could be essential for development and physiology through the expression of the various classes of lncRNAs. lncRNAs play a critical role in various aspects of biology, and many lncRNAs have now been found to direct differentiation and development, while leading to disease when misregulated. However, compared to coding genes, there are significant gaps in the current understanding of lncRNA regulation and mechanism of action. This is partly because lncRNA sequences are not evolutionarily conserved as well as protein-coding sequences are. It remains difficult to classify lncRNAs into categories beyond their genomic locations and expression patterns. Therefore, careful study of loss- or gain-of-function mutants of lncRNAs in cell lines and in appropriate animal models is essential to discern their function. However, since lncRNAs are less conserved between humans and mice than protein-coding genes are, it may not be possible to apply the findings generated with animal models to humans. Indeed, this variability in lncRNAs could explain many of the phenotypic differences in higher eukaryotes. In that scenario, advances will need to be made in computational algorithms predicting lncRNAs secondary structures, domains, and protein interactions in order to determine the best in vitro models and developmental contexts to study a given lncRNA. Importantly, lncRNAs are dysregulated in numerous biological processes and are becoming rapidly linked to numerous human diseases. The lncRNA field is still very young, but new mechanistic insights into lncRNA function are bound to emerge, and will lead to a greater understanding of many complex and devastating disorders.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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