Chapter 4

Long Noncoding RNAs are Frontier Breakthrough of RNA World and RNAi-based Gene Regulation

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Abstract

General complexities in versatile animals are not always proportional to their genome size. A notable example is that the salamander genome size is 15-fold larger than that of human, which mostly contains unfolded "junk DNA." A vast portion of this non-protein-coding unfolded DNA undergoes transcriptional regulation and produces a large number of long noncoding RNAs (lncRNAs). LncRNAs play key roles in gene expression and therapies of different human diseases. Recently, novel lncRNAs and their function on the silencing or activation of a particular gene(s) are regularly being discovered. Another important component of gene regulation is high packing of chromatin, which is composed of mainly repetitive sequences with negligible coding potential. In particular, an epigenetic marker determines the state of the gene associated with it, whether the gene will be expressed or silenced. Here, we elaborately discuss the biogenesis pathway of lncRNAs as well as their mechanism of action and role in gene silencing and regulation, including RNA interference. Moreover, several lncRNAs are the common precursors of small regulatory RNAs. It is thus becoming increasingly clear that lncRNAs can function via numerous paradigms as key regulatory molecules in different organisms.

Keywords: Transcriptional silencing, long noncoding RNA, cancer, neurological disorder, *Drosophila*

1. Introduction

Since the earliest days of molecular biology, RNA-mediated gene regulation was known to the researchers, and it was first suggested that noncoding RNA (ncRNA) might have a role in gene regulation by interacting with promoters [1, 2]. After more than four decades of research, the discovery of RNA interference (RNAi) has revolutionized our perception of the mechanism of

gene regulation, organization of chromosomes, and epigenetic regulations. Important clues to ncRNA regulatory mechanisms came from homology-dependent gene silencing in plants, which can be initiated by transgenes and recombinant viruses [3]. Studies on the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* [4], fungi mainly yeast, mammalian cells, and plants revealed transcriptional silencing mechanisms involving RNAi, chromatin, and its various modifications [3]. RNAi operates mainly posttranscriptionally; however, its components are associated with transcriptional gene silencing and heterochromatin formation, too [5].

Recent findings have made it clear that transcriptional gene silencing (TGS), posttranscriptional gene silencing (PTGS), and chromatin modifications are utilized by eukaryotic cells to bring about endogenous gene regulation, chromosome organization, and nuclear clustering. The RNA interference mechanisms mainly target the transposable elements, which are abundant and perhaps a defining component of heterochromatin. The role of ncRNA in dosage compensation, inactivation of X chromosome, genomic imprinting, polycomb silencing, and blocking of interactions between enhancers and promoters by chromatin insulators is well proven. Although the studies strongly point towards the involvement of RNAi, its role has not been demonstrated directly [6].

The non-protein-coding transcripts longer than 200 nucleotides are known as long noncoding RNAs to differentiate superficially this class of ncRNAs from microRNAs, short interfering RNAs, piwi-interacting RNAs, small nucleolar RNAs, etc [7]. LncRNAs have emerged as important regulators of cell physiology and pathology. Different studies have come up with an increasing number of lncRNAs showing tissue-specific expression; however, the exact mechanism of action of only a few lncRNAs has been elucidated *in vivo* [8–14]. The biological functions and mechanisms of action of the majority of lncRNAs still remain unknown. LncRNAs can interact with a wide range of molecules and can form RNA-RNA, RNA-DNA, or RNA-protein complexes through specific RNA functional domains [15], resulting in extensive functional diversities. Recent research focuses on lncRNAs and divulges the association of lncRNAs with epigenetic machinery to control chromatin structure, nuclear clustering, and gene expression. The studies reveal that lncRNAs may act together with many histone- and DNA-modifying enzymes to modify the histones or DNA. In addition, a recent discovery of a cardioprotective lncRNA showed a targeting mechanism through ATP-dependent chromatin remodeling factors [16], indicating an extensive role of lncRNAs in chromatin structure and regulation. The mechanisms of how lncRNAs control chromatin by covalent modifications are extensively reviewed in the literature [17–20].

The study of lncRNAs has taken the center stage for the researchers working with epigenetic regulations, and there is a report of a new lncRNA regulating a disease, or transcriptome studies come up with a new class of noncoding RNA, or we are introduced to hitherto unknown mechanisms by which an lncRNA regulates a particular gene almost on a weekly basis. These are all possible due to the introduction of many advanced, high-throughput genomic technologies such as microarrays and next-generation sequencing (NGS). There are a huge number of reported lncRNAs that are not derived from protein-coding genes, and in spite of this vast number of reports on lncRNA, we have just started getting a clear picture

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about how lncRNAs function, how many different types of lncRNAs exist, and how many of the reported lncRNAs are biologically important.

2. The C-value enigma and junk DNA

It has long been known that developmental complexity or size of an animal does not correspond with C-value or the amount of DNA in the haploid genome [21–23]. The lower animal in the evolution ladder, salamander, has a genome size 15 times larger than that of humans [21], and this discrepancy is known as the "C-value paradox" [23]. Since the introns were discovered, we started to presume that the C-value paradox was now solved [24]. We are almost sure that humans have about 25,000–35,000 protein-coding genes unlike the overestimates of 50,000–100,000 from the initial days of the Human Genome Project [25]. The remaining huge amount of noncoding DNA was termed as "junk DNA" [24, 26] due to the presence of transposons, pseudogenes, and simple repeats, which occupies about 50–70% of the human genome [27]. C-value enigma poses a discrepancy in genome size and number of protein-coding genes. Phylogenetically close genera may vary in C-value by around four- to five fold [28].

In spite of their "junk" status, scientists were always curious to study them and even realized that "being junk doesn't mean it is entirely useless" [26]. It was hypothesized that the junk DNA might be useful in chromosomal pairing, genome integrity, gene regulation, mRNA processing, and serving as a reservoir for evolutionary innovation. We are now pleasantly surprised at their foresight. In the 1970s, it was already thought that noncoding RNA products, such as rRNAs, tRNAs do not make up the whole transcribed genome.

The scale of "pervasive transcription," however, was not fully appreciated until the late 1990s and early 2000s. After the arrival of whole-genome technologies, from microarray hybridization and deep sequencing analysis techniques, it was recently shown that 70–90% of our genome is transcribed at some point during embryogenesis [29]. Some recently identified transcripts may be present at as low as 0.0006 copies per cell [30]. Another concern is that tiling microarrays can come up with false positives, low dynamic range, resolution, and low concordance between studies [31]. The existence of noncoding transcription in intergenic regions is evident from correlations with chromatin signatures, such as DNase1 hypersensitivity, and histone modifications such as H3K9ac, H3K4me3, and H3K36me3 [31]. Although these studies report novel and conserved lncRNAs, that is not enough to explain the function of 70–90% of the genome and biological functionality of the ncRNAs. In 1969, Britten and Davidson presented a model for regulation of gene expression in eukaryotic cells where ncRNAs have important roles as regulatory intermediaries to convey signals from sensory to receptor elements [1]. Some of the first examples of gene-specific regulatory roles of lncRNAs were revealed with the discovery of lncRNAs involved in epigenetic regulation, such as H19 [32] and X-inactive specific transcript (Xist) [33, 34].

3. Stand-alone lncRNAs

These lncRNAs are located as separate units and do not overlap protein-coding genes. Some of these are known as lincRNAs for large intergenic (or intervening) noncoding RNAs (lincRNAs) [35]. Many of the lincRNAs were identified through chromatin signatures for actively transcribed genes (H3K4me3 at the promoter and H3K36me3 along the transcribed length). Many of the characterized lncRNAs are transcribed by RNA Pol II, polyadenylated, and spliced and have an average length of 1 kb.

4. Natural antisense transcripts

In this study, transcription occurs in the antisense strand of annotated transcription units; about 70% of sense transcripts have reported antisense counterparts [36]. The overlap between these sense/antisense pairs can be a complete sequence, but natural antisense transcripts are mostly found to be enriched around the 5′ promoter or 3′ terminator ends of the sense transcript. The most extensively studied example of sense/antisense pairing is Xist/Tsix (lncRNA antisense to Xist), with two RNAs that control X chromosome inactivation [37]. In addition, many imprinted regions contain coding/noncoding sense/antisense pairs, such as Kcnq1 (potassium channel, voltage-gated KQT-like subfamily Q, member 1)/Kcnq1ot1 (Kcnq1 overlapping transcript 1) [38] and Igf2r (insulin-like growth factor 2 receptor)/Air (antisense Igf2r RNA) [39]. These pairs are generally less spliced or polyadenylated when compared to mRNAs or stand-alone lncRNAs.

5. Long intronic ncRNAs

Introns have long been known to contain small ncRNAs such as small nucleolar RNAs (snoRNAs) and microRNAs (miRNAs). However, by large-scale transcriptomic or computational analyses, many long transcripts have been reported to be encoded within the introns of known genes [40]. Although they have differential expression patterns and respond to the environmental stimuli differently, only a few have been extensively studied to date. One such example is cold-assisted intronic noncoding RNA (COLDAIR) that has been implicated in plant vernalization, which is located in the first intron of the flowering repressor locus FLC [41].

6. Identification of long noncoding RNAs

LncRNAs are identified by transcripts that map to genomic regions outside the boundaries of protein-coding genes. It is difficult to ascertain the function of a transcript that overlaps a protein-coding gene using targeted knockout or knockdown approaches. Thus, most experimental investigations of lncRNAs have been focused on those that are located in intron

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sequences. It is also very difficult to ascertain whether an lncRNA locus is entirely intergenic because lncRNA transcripts are often incomplete and they can originate from a protein-coding gene's promoter or enhancer on either strand [42]. Tiling microarray technique is often useful to detect intergenic transcripts [43]. However, controversial results were found here and these experiments can be ruled out [31]. Early lncRNA collections relied primarily on sequenced cDNA and EST clones [44]. More recently, RNA-Seq has come up with a number of lncRNAs derived from whole transcriptome sequencing. RNA-Seq generates millions of 35–100 nt sequences read in parallel, and it has been confirmed that a large chunk of intergenic sequences are transcribed into lncRNAs [45]. The high-throughput and impartial nature of this technique is being utilized for the detailed assessment of the contribution of lncRNAs to a variety of tissue and/or species under different conditions.

To accurately distinguish noncoding from coding transcripts, sophisticated approaches have been developed. For example, the Coding Potential Calculator [46] takes into account six features of a transcript, including the proportion of the transcript enclosed by the candidate peptide-encoding region, and the sequence similarity to known proteins. An evolutionary approach, followed in phyloCSF, predicts ncRNAs when their sequence differences among species do not show preference as to whether they disrupt or not putatively encode peptides [47].

Experimentally determined transcripts always are relied on more than predicted ones. The availability of large proteomic databases can be utilized to investigate whether a specific RNA molecule is translated into a protein. *In vitro* translation assays have been used, too, but they do not necessarily reflect *in vivo* biology. A true lncRNA should not bind with translation machinery, and this approach is also adopted in the identification of candidate lncRNA. However, a study has reported that 50% of a set of putative lncRNAs are ribosome associated [48], leaving in doubt whether this test is accurate in separating coding from noncoding transcripts. To assign an lncRNA, an experimental determination of the function of a transcript will be necessary. Nevertheless, some transcripts possess both RNA- and coding-sequence-dependent functions [49] and demarcating them will be difficult. A computational or experimental method has not yet been developed that discriminates accurately between coding and noncoding transcripts. For the time being, we can rely on *in silico* screens for the protein-coding potential of putative lncRNAs but be aware that these will contain false-positive predictions, too, especially for genes that encode short polypeptides.

Although many genomes contain a substantial number of lncRNA loci, we still do not know the proportion and number of these that are biologically functional. Because the functional mechanisms of most noncoding transcripts or transcript regions are unknown, it is difficult to design point mutation or deletion experiments and their results are difficult to interpret. Even RNAi techniques are not being helpful to assign the functionality of the ncRNAs.

7. Mechanisms of action

We do not know yet the mechanistic detail of the enormous number of reported lncRNAs. However, a few that have been thoroughly studied provide clues regarding how lncRNAs

might carry out gene regulation (Figure 1). In addition, many lncRNAs blur the line of different categories and employ several different mechanisms. The discovery of new lncRNAs and more thorough characterization of those already known will reveal additional modes of action.

It has been found that a major role of lncRNA is to recruit regulatory proteins for the regulation of chromatin states [50]. This kind of lncRNAs may act in *cis*, on adjacent or nearby genes, or they might act in *trans*, regulating genes located in distant domains or chromosomes. Polycomb repressive complex 2 (PRC2) interacts with a large number of lncRNAs [51–54]. The *Drosophila* polycomb proteins, first discovered as homeotic gene, express during development [55, 56]. These include enhance of zeste homolog 2 (Ezh2, catalytic subunit in PRC2), which is a key H3K27 methyltransferase, and the Pc/Chromobox (Cbx) family proteins in PRC1, chromodomain-containing proteins that can bind trimethylated H3K27 [55, 56]. Observed interactions of polycomb proteins with lncRNAs suggest that polycomb recruitment is RNA directed in mammals. HOX transcript antisense RNA (HOTAIR) in the homeobox (HOX) C cluster is reported to repress transcription of HOXD in *trans* through interaction with PRC2 [57]. Xist RNA-containing repeat A (RepA) has been found to recruit PRC2 [58]. RepA targets PRC2 to the Xist promoter resulting in Xist up-regulation. The interesting fact is that RepA/Xist interaction with PRC2 may be blocked by the antisense Tsix transcript, also interacting with PRC2 and competitively inhibiting the painting of Xist on inactive X chromosome [58].

Other epigenetic complexes interact with lncRNAs as well, such as the H3K9 methyltransferase G9a interacting with the imprinted lncRNA Air [59]. Kcnq1ot1 has been hypothesized to recruit both PRC2 and G9a to the promoter of Kcnq1 [60] acting as a scaffold. On the other hand, antisense ncRNA in the INK4 locus (ANRIL), associated with p15/INK4 (inhibitors of CDK4 family) B-p16/INK4A-p14/ARF tumor suppressor gene cluster, interacts with both the PRC1 component Cbx7 and the PRC2 component Suz1 [61, 62]. HOTAIR also interacts with the lysine-specific demethylase 1 (LSD1)/corepressor protein of LSD1 (CoREST)/repressor for element 1-silencing transcription factor (REST) complex in addition to PRC2 to prevent gene activation [63].

LncRNAs can also act by recruiting factors involved in gene activation. Such factors from the HOXA (homeotic gene A cluster), two lncRNAs, Mistral (Mira), and HOXA transcript at the distal tip (HOTTIP) have been involved in recruiting the mixed lineage leukemia (MLL) complex in *cis* regulation [64, 65].

An H3K4 trimethylase, myeloid/lymphoid or mixed-lineage leukemia (MLL), is a member of the Trithorax group of developmentally important gene-activating proteins in flies [66]. Using 3C or chromosome conformation capture technique, it was found that multiple loci, which are 40 kb apart in the HOXA cluster, are in close physical proximity, enabling MLL to regulate their expression. Other than histone modifications, lncRNAs also impact epigenetic regulation by modulating DNA methylation at CpG dinucleotides, which has an important role in the stable repression of genes [67]. During embryogenesis, methylation markers are first to be found on previously unmethylated DNA by the DNA (cytosine-5-)-methyltransferase 3α (Dnmt3a) and 3β (Dnmt3b) and later maintained through DNA replication by Dnmt1. Tsix might be converted to Xist by utilizing Dnmt3a activity to methylate and finally silence the Xist promoter [68, 69]. In the same way, Kcnq1ot1 may recruit Dnmt1 [70].

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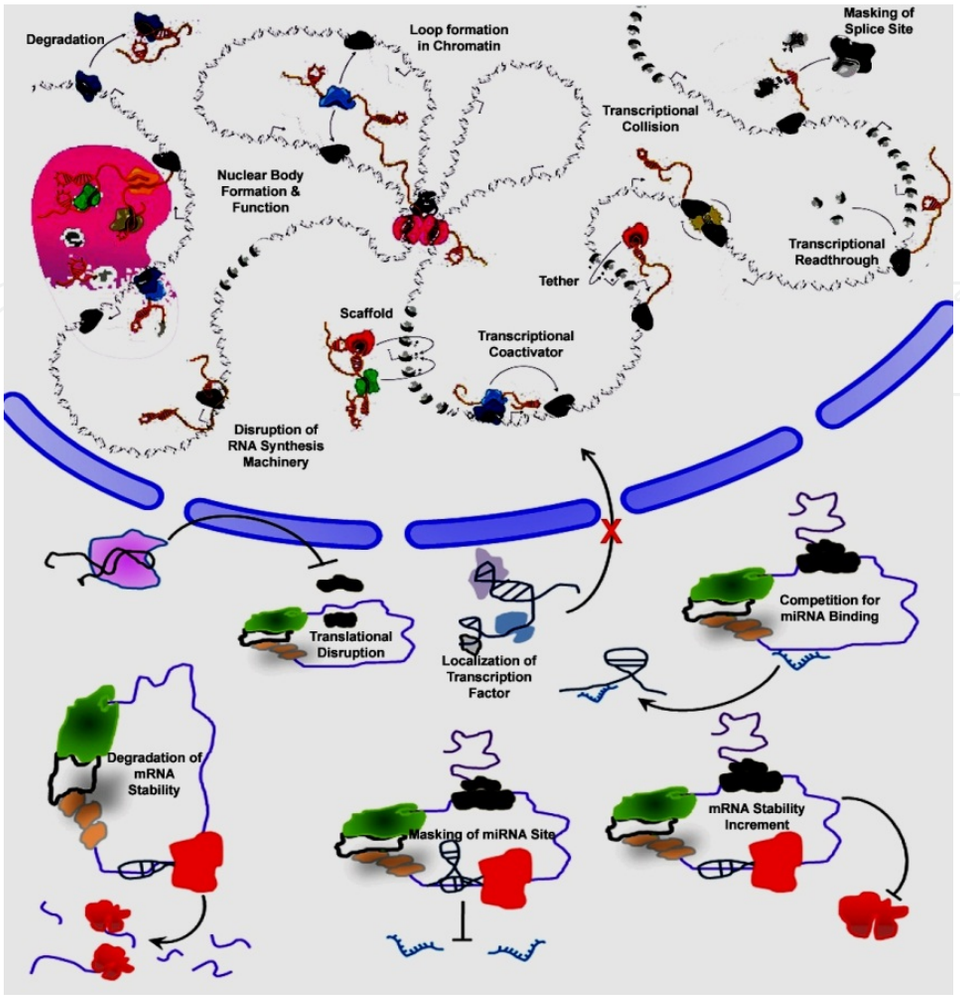

Figure 1. Mechanisms of lncRNA function (modified from Kung et al. [129]).

LncRNA-directed methylation has also been implicated in the regulation of rDNA. Ribosomal DNA exists in the genome as tandem repeat units [71]. Each unit encodes a polycistronic transcript consisting various rRNAs, and each unit is separated by intergenic spacers (IGSs) transcribed by RNA Pol I [72]. Recently, it was reported that IGS transcripts undergo processing into 150- to 300-nt fragments called promoter (p)RNAs, which act as scaffolds to recruit poly (ADP ribose)-polymerase-1 (PARP1) [73], the ATP-dependent nucleolar chromatin remodeling complex (NoRC) [74], and Dnmt3b [75]. A conserved hairpin structure is formed by pRNA that binds both PARP1 and the TIP5 subunit of NoRC, leading to TIP5 conformation change resulting in the recruitment of NoRC to the nucleolus, where rDNA is located [74, 76]. The interesting fact is that the recruitment of Dnmt3b by pRNA is dependent on DNA:RNA triplexing, possibly via Hoogsteen base pairing, between the 5′ end of pRNA and the rDNA promoter [75]. The DNA:RNA triplex formation might be a general mechanism by which lncRNAs recruit *trans* factors to specific DNA loci. LncRNAs are intrinsically bound to chromatin during transcription and transcribed from a single locus in the genome, so they have a direct allele- and locus-specific control in *cis* unlike transcription factors. The length of lncRNAs is also suitable to reach out and capture epigenetic marks. This *cis*-acting mechanism

resembles transcriptional gene silencing seen in the yeast *Schizosaccharomyces pombe* in assembling centromeric heterochromatin [77, 78].

The nucleus is always in the dynamic state and is the center for most of the essential functions of an organism [79]. Recent studies indicate that lncRNAs are the key regulators of nuclear compartments. The structure and function of several nuclear bodies seem to be controlled by RNA. One example is nuclear-enriched abundant transcript 1 (NEAT1) that maintains the stability of paraspeckles, which participate in the nuclear retention of mRNAs after adenosine-to-inosine hyperediting [80, 81]. NEAT1 interacts with paraspeckle proteins, such as p54/NONO and PSP [80–82] and recruits these proteins to form paraspeckles. This is an active process where continuous transcription of NEAT1 is required [84]. The related molecules, NEAT2 or metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), are involved in the localization of serine/arginine (SR) splicing factors to nuclear speckles where they can be stored and later modified by phosphorylation [85]. MALAT1 directs these splicing factors to sites of transcription, ultimately controlling the alternative splicing of certain mRNA precursors [86]. MALAT1 interacts with the PRC1 subunit Cbx4/Pc2 and participates in the transportation of genes between nuclear compartments for silencing and activation. Extracellular growth signals help unmethylated Cbx4 to bind MALAT1 and localize its target genes, along with Lysine (K)-specific demethylase 1A (LSD1) to interchromatin granules that usually cluster around nuclear speckles. However, Cbx4 gets methylated in the absence of extracellular signal and instead binds another lncRNA TUG1, then binds with Ezh2, and translocates to silencing compartments called polycomb bodies [87]. Although these recent observations have started to open up an avenue to understand lncRNA and their mechanisms of action, we are still way behind. The function of an overwhelming number of lncRNAs that are being discovered almost daily is unknown until now.

8. Epigenetic regulation

The two most abundant modes of action of lncRNAs are the modulation of chromatin by recruiting histone proteins and transcription factors within specific chromatin-modifying complexes. A very good example of recruitment of specific histones is X chromosome inactivation (XCI), which is caused by "Xist" as described in the earlier section [58]. A similar event is genomic imprinting, where genes are expressed from the allele of only one parent. One of the first and best studied lncRNAs is H19, which is mutually imprinted with insulin-like growth factor 2 (Igf2). This lncRNA is highly expressed, but its deletion has no phenotypic outcome, and it is anticipated to function as a microRNA precursor [88]. Other lncRNAs (e.g., Air, Kcnq1ot1, and HOTAIR) show modulatory activities both in *cis* or in *trans* and regulating gene expression through partnering with chromatin-modifying complexes [70, 89]. Specifically, HOTAIR is a *trans*-acting lncRNA that serves as a scaffold for two histone modification complexes: it binds both to PRC2 and to LSD1 [63]. In the *Arabidopsis* plant, it was found that different environmental conditions are able to induce the transcription of related NATs (i.e., COOLAIR) that eventually silence a flower repressor locus, flowering locus c (FLC) [90]. Recently, it was discovered that lncRNA, namely COLDAIR, bearing minor differences from

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COOLAIR (transcribed in the sense direction relative to FLC mRNA transcription), interacts on its own with PRC2 and targets it to FLC [41]. Other *trans*-acting lncRNAs have different functions, some of which remain incompletely defined. There are several poorly defined *trans*-acting lncRNAs, such as the p21-associated ncRNA DNA damage activated (PANDA), which is induced upon DNA damage in a p53-dependent manner and it controls the expression of proapoptotic genes [91].

9. Transcriptional regulation

The discovery and characterization of promoter-associated RNAs opened up a new understanding on how genes are regulated during transcription. These RNAs are localized within the promoter and consist of various sizes of RNA molecules [92]. The long ones are found at a single-gene level and are associated with the modification of DNA methylation and demethylation patterns [93] as mentioned earlier. Interestingly, long (antisense) pRNAs generally form double-stranded molecules that are processed into endo-siRNAs, and since they have sequence complementarity with the promoter, they induce transcriptional gene silencing [20, 94–96] or activation [97–99].

LncRNAs sometimes affect transcription by acting as coregulators or by regulating the association and activity of coregulators. One example is embryonic ventral forebrain-2 (Evf-2) that functions as a coactivator for the homeobox transcription factor distal-less homeobox 2 (Dlx2) [100].

10. Posttranscriptional regulation

lncRNAs not only have a role in transcription but also they function in splicing, mRNA stability, and translation. Antisense lncRNA sometimes bind to the sense RNA, conceal the splice sites, and thereby modify the balance between splice variants. Antisense transcript RevErbAα modifies the splicing of thyroid hormone receptor alpha genes (TRα) TRα1 and TRα2 mRNAs [101].

The terminal differentiation-induced ncRNA (TINCR) associates with Staufen 1 but not with the complex between TINCR-NA, which is a differentiation factor [102].

LncRNAs have also been implicated in translational regulation. An example is the antisense for PU1 mRNA. Its translation is inhibited by an antisense polyadenylated lncRNA with a half-life longer than the original transcript [103]. Another example is the lncRNA Uchl1, which is controlled by mammalian target of rapamycin (mTOR) pathway, shuttles from the nucleus to the cytoplasm, and controls the translation of the ubiquitin carboxy-terminal hydrolase L1 (UCHL1) mRNA by promoting its association with polysomes [104].

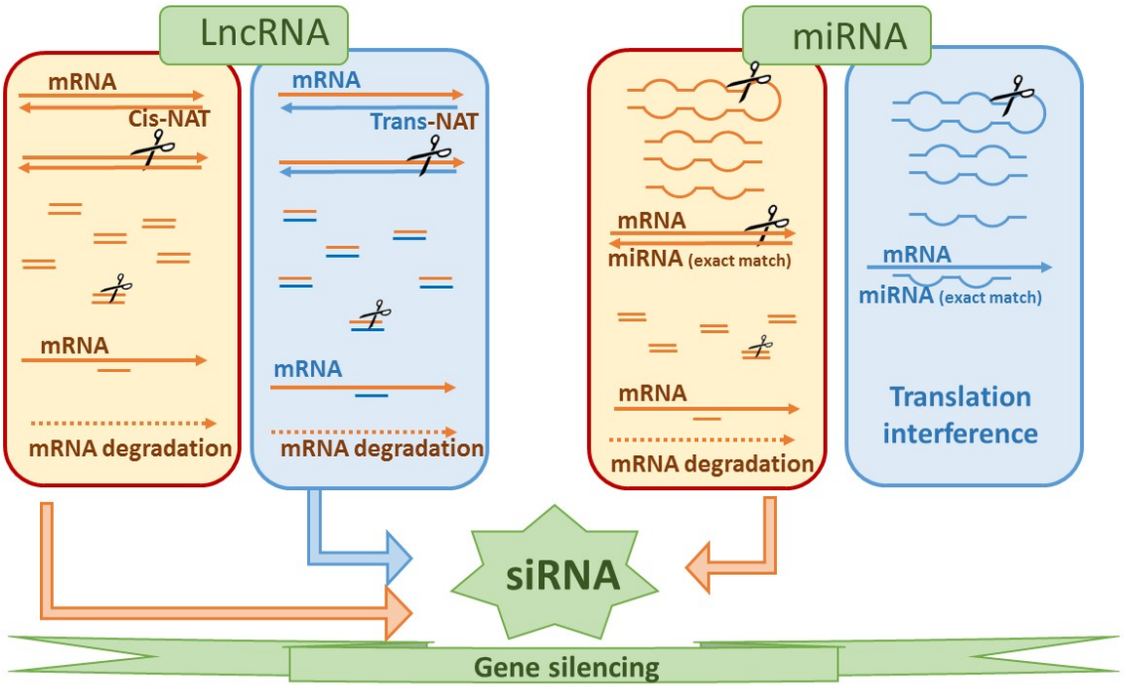

Figure 2. Posttranscriptional gene silencing by lncRNA and miRNA (adapted from Gomes et al. [130]).

11. Role of lncRNAs in cancer and other human diseases

The genome-wide association studies identify several cancer risk loci outside of protein-coding regions. Of 301 single-nucleotide polymorphisms currently linked to cancer, only 12 (3.3%) modify the amino acid sequence of the protein, and most of the loci are located in the introns (40%) or intergenic regions (44%) [105]. These facts and the observations that miRNA and lncRNAs are involved in differentiation and development point towards the fact that alterations in their expression profiles could be correlated with cancer development. Reports suggested that lncRNAs have tissue-specific expression and is found to be deregulated in distinct types of cancers. For example, overexpression of miR-155 was reported in hematopoietic, breast, lung, and colon cancers [106], whereas miR-21 is overexpressed in glioblastoma [107]. In addition, lymphoproliferative disorders were found in transgenic mice overexpressing miR-17-92 [108]. Incidences of lung, colon, and gastric cancers were found to be correlated with the overexpression of miR-17-92 cluster [109]. LncRNAs have been associated with cancer development likewise. The lncRNA MALAT1 is up-regulated in several cancer types, resulting in an increase in cell proliferation and migration in lung and colorectal cancer cells [105]. The role of MALAT1 in controlling alternative splicing of pre-mRNAs [86] can be deduced from this. A more recent study indicates that MALAT1 may also participate in the regulation of gene expression by a mechanism other than alternative splicing in lung metastasis [110].

Other studies have shown that miRNA and lncRNAs both can function as tumor suppressor genes or oncogenes. The tumor suppressor gene p53 regulates the three gene members of the

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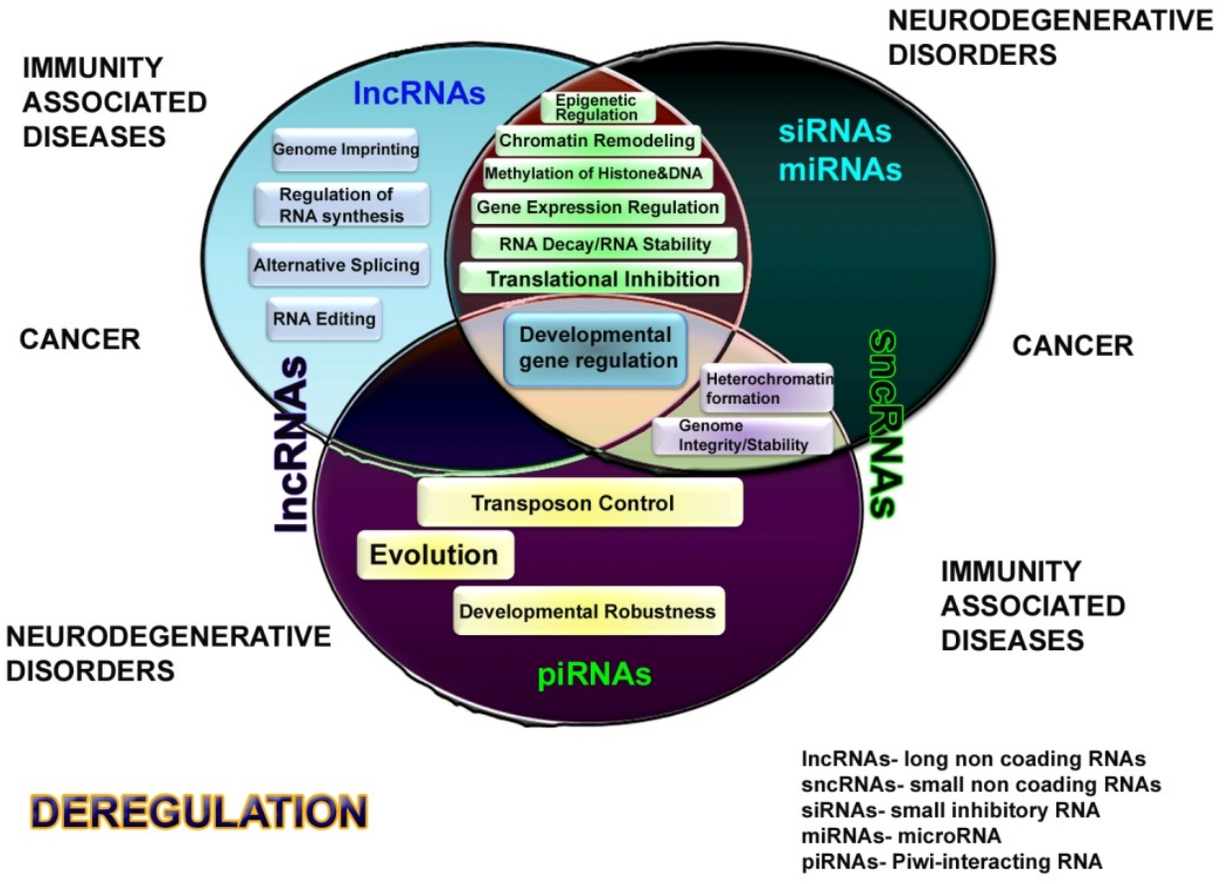

Figure 3. Relationship among various noncoding RNAs and different disorders caused by them (adapted from Gomes et al. [130].

miR-34 family. Curiously, the microRNA-34 (miR-34) activation resembles p53 activity, such as the induction of cell cycle arrest and promotion of apoptosis, and p53-mediated apoptosis becomes defective in the absence of miR-34 [111].

LncRNAs that recruit epigenetic modifiers to specific loci such as ANRIL, XIST, HOTAIR, and KCNQ1OT1 are found to have altered expression in a variety of cancers [112]. Another lncRNA called TERRA binds telomerase, inhibiting its activity *in vitro* [113], and is observed to be down-regulated in many cancer cells, linking it with the longevity of cancer cells.

Chromatin remodeling by lncRNA is linked to other diseases such as facioscapulohumeral muscular dystrophy (FSHD) [97], lethal lung developmental disorder [114], and the HELLP syndrome, a pregnancy-associated disease [114] in addition to cancer. The HELLP stands for H = hemolysis (breakdown of red blood cells), EL = elevated liver enzymes (liver function), and LP = low platelet counts (platelets help the blood clot). These examples directly link lncRNA and miRNAs in cancer biology and other human diseases and indicate the involvement of a complex interplay among their biogenesis pathways, their regulatory mechanisms, and their targets.

12. Dosage compensation and X inactivation

X chromosome inactivation (XCI) occurs in females during embryogenesis, where either the maternal or paternal X chromosome is randomly silenced. The molecular mechanisms of XCI are not yet fully understood. However, it is known that a 500-kb stretch of DNA at Xq13 known as the X-inactivation centre (XIC) is the site for initiation of X inactivation. There are several lncRNAs, including X-inactive specific transcript (Xist), its antisense transcript Tsix, X-inactivation intergenic transcription elements (Xite), Jpx transcript, and Xist activator (Jpx), and others play pivotal roles in XCI [115]. Xist was one of the first to be identified and best studied lncRNAs. It is a ~17-kb transcript (~19 kb in humans) expressed from the future inactive X chromosome (Xi) [116]. Tsix is a ~40-kb antisense transcript to Xist. It negatively regulates Xist. Recent studies indicate that Xite is a transcriptional enhancer of Tsix [115], and likewise, Jpx RNA appears to help in Xist expression [117].

When two homologous X chromosomes are brought at close proximity, Tsix and Xite initiate the inactivation process by counting, and this is associated with the presence of RNA polymerase II (RNAPII) [118, 119]. The chromatin insulator CTCF, which binds to Tsix and Xite genomic loci [120], play an important role. The transcription factor OCT4 is then hypothesized to bind with Tsix promoters of one of the X chromosomes, which then converts to active X chromosome (Xa) due to increased transcription of Tsix [120]. Thereafter, Dnmt3a is recruited to the Xa and establishes stable silencing of Xist on the Xa [115, 118].

13. LncRNA in genomic imprinting

In mammals, genomic imprinting is an epigenetic marker in a way that their expression occurs specifically in parental origin manner. This occurs during early gametogenesis in nearly 1% of protein-coding genes. To date, we have identified around 150 imprinted genes in mice. Imprinted genes are often located in clusters of size from a few kilobases to 2 to 3 Mb. LncRNAs are present in all the identified and elucidated imprinted clusters as their partners. The expression of lncRNAs is reciprocally linked with corresponding protein-coding genes [121–123].

Genomic imprinting mainly happens by chromatin insulators [124–126] and lncRNAs [38, 127]. LncRNAs repress flanking gene promoters in *cis* action (Kcnq1ot1 and Airn lncRNAs [115]). However, several reports indicate that lncRNAs function as a major force in the regulation of parent-of-origin-specific expression. Today, we know that the human genome contains more than 58,648 lncRNA expressed genes compared to only 21,313 protein-coding genes [128]. The majority of the lncRNAs act by interacting with chromatin-modifying complexes such as PRC2, G9a, hnRNPK, and SWI/SNF, recruiting them sequentially to silence genes in *cis* or *trans* action [57, 60].

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14. Perspectives

LncRNA has diversified tentacles for functions. Those include an alteration of transcriptional profiles, controlling of protein expression, complex structural or organizational roles, RNA processing or RNA editing and role of being the precursor of small RNAs. Because a very small fraction of lncRNA have been molecularly characterized to date, many more yet to be discovered that fit into this diversified functional paradigms. Future work will definitely ask many more questions about the interplay of lncRNA transcripts and whether it is sufficient to have fundamental sequence of events or not. Many lncRNAs play intermediate roles in *cis* regulation that gets represented in ectopic expression in *trans* regulation.

Most recent challenges are to identify how the molecular function of each type of lncRNA results in different diseases of the organism. LncRNA appears to expose numerous developmental events such as the generation of photoreceptor cells in retina development, control of cell surveillance, cell cycle progression of mammary gland development, and finally generation of knockout animal development. Many lncRNAs are not eliminated as transcriptional noise in the genome but are useful for normal developmental processes.

LncRNA has a tremendous impact on disease development due to its flawless miscegenation. In tumor formation, the expression of lncRNAs is very important. They function like specific markers of tumor formation. However, the exact mechanism by which tumor initiation, formation, and progression would occur is not fully understood. It is true that the interplay and significant role of lncRNA in different disease research is really an unexplored area, which is eventually determining the new therapeutic targets. Recently, it was found that lncRNA may form β-amyloid plaques in Alzheimer's disease. This possibility suggested that noncoding transcript might serve as an attractive drug target for Alzheimer's disease.

Most conventionally, genetic information may run through protein-coding sequences, but it is now found that transcription is pervasive through the nucleic acid content of eukaryotic genome, which generated a numerous number of lncRNA, which are possibly the key regulators of protein-coding sequences. We anticipate that many more surprises are yet to be explored in the coming decades. Therefore, future research might provide more pleasant but unexpected surprises in the lncRNA function.

15. Conclusion

The above description exhibits a brief survey of the current status of knowledge regarding the identification, localization, functions, and mechanisms of actions of lncRNAs related to different human diseases. A fraction of genomic nucleic acid is transcribed to protein, but an overwhelming majority of the genome sectors of the organisms contain lncRNA with unknown functional efficacy. Some are nuclear or cytoplasmic and are highly overexpressed, and others are rarely detected. Truly, it is impossible to discern the important criteria such as stability, conservation, and time of expression related to human diseases. LncRNA in the Xic is only

found in placental mammals and is not conserved in other mammals. However, this limited conservation might not be essential in other higher animals. The true test for real function lies in the mechanism, genetic pathway, and tissue-specific activity for each lncRNA. The genome of an organism is not always streamlined by the natural selection. Thus, here, we really tried to avoid the speculative statements about localization, function, and dissecting mechanism regarding long noncoding RNA. Truly, we have just begun to scratch the skin of LncRNA in the human body. The lncRNA world is so galactically vast that we have an enormous task to completely learn about it. We feel that additional discoveries of lncRNA may provide a real exciting phase in the study of RNA world.

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