We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

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

Utpal Bhadra, Debabani Roy Chowdhury, Tanmoy Mondal and Manika Pal Bhadra

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/61975>

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

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

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 enhancer 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 3 α (Dnmt3a) and 3 β (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].

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

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 RevErbA α modifies the splicing of thyroid hormone receptor alpha genes (TR α) TR α 1 and TR α 2 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

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].

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.

Acknowledgements

We thank the lab members and Sohini Bose for the extensive discussion in the subjects and help in writing. This work is sponsored by the CSIR Net work grant (BSC-0121) of UB and DBT grant to M.P.B. (GAP 0362). DRC work supported by DST (GAP0432) grant.

Author details

Utpal Bhadra^{1*}, Debabani Roy Chowdhury¹, Tanmoy Mondal² and Manika Pal Bhadra²

*Address all correspondence to: utpal@ccmb.res.in

1 Functional Genomics and Gene Silencing Group, Centre for Cellular and Molecular Biology, Hyderabad, India

2 Centre for Chemical Biology, Indian Institute of Chemical Technology, Hyderabad, India

References

- [1] Britten RJ, Davidson. Gene regulation for higher cells: A theory. Science. 1969;165(3891):349–357. DOI: 10.1126/science.165.3891.349.
- [2] Jacob F, Monod J. Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol. 1961;3(3):318–356. DOI: 10.1016/S0022-2836(61)80072-7
- [3] Matzke MA, Birchler JA. RNAi-mediated pathways in the nucleus. Nat Rev Genet. 2005;6(1):24–35. DOI: 10.1038/nrg1500
- [4] Pal-Bhadra M, Bhadra U, Birchler JA. Cosuppression in *Drosophila*: Gene silencing of alcohol dehydrogenase by white-Adh transgenes is polycomb dependent. Cell. 1997;90(3):479–490. DOI: 10.1016/S0092-8674(00)80508-5

- [5] Wassenegger M. The role of the RNAi machinery in heterochromatin formation. *Cell*. 2005;122(1):13–16. DOI: 10.1016/j.cell.2005.06.034
- [6] Zaratiegui M, Irvine DV, Martienssen RA. Noncoding RNAs and gene silencing. *Cell*. 2007;128(4):763–776. DOI: 10.1016/j.cell.2007.02.016
- [7] Perkel JM. Visiting “Noncodarnia”. *Biotechniques*. 2013;54(6):301–304. DOI: 10.2144/000114037
- [8] Bassett AR, Akhtar A, Barlow DP, Bird AP, Brockdorff N, Duboule D, et al. Considerations when investigating lncRNA function *in vivo*. *Elife*. 2014;3:e03058. DOI: 10.7554/eLife.03058
- [9] Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011;12(12):861–874. DOI: 10.1038/nrg3074
- [10] Li L, Chang HY. Physiological roles of long noncoding RNAs: Insight from knockout mice. *Trends Cell Biol*. 2014;24(10):594–602. DOI: 10.1016/j.tcb.2014.06.003
- [11] Maass PG, Luft FC, Bähring S. Long non-coding RNA in health and disease. *J Mol Med (Berl)*. 2014;92(4):337–346. DOI: 10.1007/s00109-014-1131-8
- [12] Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell*. 2009;136(4):629–641. DOI: 10.1016/j.cell.2009.02.006
- [13] Wapinski O, Chang HY. Long noncoding RNAs and human disease. *Trends Cell Biol*. 2011;21(6):354–361. DOI: 10.1016/j.tcb.2011.04.001
- [14] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: Insights into functions. *Nat Rev Genet*. 2009;10(3):155–159. DOI: 10.1038/nrg2521
- [15] Quinn JJ, Ilik IA, Qu K, Georgiev P, Chu C, Akhtar A, et al. Revealing long noncoding RNA architecture and functions using domain-specific chromatin isolation by RNA purification. *Nat Biotechnol*. 2014;32(9):933–940. DOI: 10.1038/nbt.2943
- [16] Han P, Li W, Lin CH, Yang J, Shang C, Nurnberg ST, et al. A long noncoding RNA protects the heart from pathological hypertrophy. *Nature*. 2014;514(7520):102–106. DOI: 10.1038/nature13596
- [17] Han P, Chang CP. Long non-coding RNA and chromatin remodeling. *RNA Biol*. 2015;12(10):1–5. DOI: 10.1080/15476286.2015.1063770
- [18] Rinn JL. lncRNAs: Linking RNA to chromatin. *Cold Spring Harb Perspect Biol*. 2014;6(8). DOI: 10.1101/cshperspect.a018614
- [19] Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem*. 2012;81:145–166. DOI: 10.1146/annurev-biochem-051410-092902.
- [20] Devaux Y, Zangrando J, Schroen B, Creemers EE, Pedrazzini T, Chang CP, et al. Long noncoding RNAs in cardiac development and ageing. *Nat Rev Cardiol*. 2015;12(7):415–425. DOI: 10.1038/nrcardio.2015.55

- [21] Gall JG. Chromosome structure and the C-value paradox. *J Cell Biol.* 1981;91(3 Pt 2): 3s–14s. 1981;91(3):3–14.
- [22] Mirsky AE, Ris H. The deoxyribonucleic acid content of animal cells and its evolutionary significance. *J Gen Physiol.* 1951;34(4):451–462.
- [23] Thomas CA Jr. The genetic organization of chromosomes. *Annu Rev Genet.* 1971;5:237–256. DOI: 10.1146/annurev.ge.05.120171.001321
- [24] Ohno S. So much “junk” DNA in our genome. *Brookhaven Symp Biol.* 1972;23:366–370.
- [25] Pertea M, Salzberg SL. Between a chicken and a grape: Estimating the number of human genes. *Genome Biol.* 2010;11(5):206–212. DOI: 10.1186/gb-2010-11-5-206
- [26] Comings DE. The structure and function of chromatin. *Adv Hum Genet.* 1972;3:237–431.
- [27] de Koning AP, Gu W, Castoe TA, Batzer MA, Pollock DD. Repetitive elements may comprise over two-thirds of the human genome. *PLoS Genet.* 2011;7(12):e1002384. DOI: 10.1371/journal.pgen.1002384
- [28] Ricroch A, Yockteng R, Brown SC, Nadot S. Evolution of genome size across some cultivated *Allium* species. *Genome.* 2005;48(3):511–520. DOI: 10.1139/g05-017
- [29] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. *Nature.* 2012;489(7414):101–108. DOI: 10.1038/nature11233
- [30] Mercer TR, Gerhardt DJ, Dinger ME, Crawford J, Trapnell C, Jeddelloh JA, et al. Targeted RNA sequencing reveals the deep complexity of the human transcriptome. *Nat Biotechnol.* 2011;30(1):99–104. DOI: 10.1038/nbt.2024
- [31] van Bakel H, Nislow C, Blencowe BJ, Hughes TR. Most “dark matter” transcripts are associated with known genes. *PLoS Biol.* 2010;8(5):e1000371. DOI: 10.1371/journal.pbio.1000371
- [32] Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. *Mol Cell Biol.* 1990;10(1):28–36. DOI: 10.1128/MCB.10.1.28
- [33] Brockdorff N, Ashworth A, Kay GF, McCabe VM, Norris DP, Cooper PJ, et al. The product of the mouse Xist gene is a 15 kb inactive X-specific transcript containing no conserved ORF and located in the nucleus. *Cell.* 1992;71(3):515–526. DOI: 10.1016/0092-8674(92)90519-I
- [34] Brown CJ, Hendrich BD, Rupert JL, Lafreniere RG, Xing Y, Lawrence J, et al. The human XIST gene: Analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. *Cell.* 1992;71(3):527–542. DOI: 10.1016/0092-8674(92)90520-M

- [35] Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.* 2011;25(18):1915–1927. DOI: 10.1101/gad.17446611
- [36] Faghihi MA, Wahlestedt C. Regulatory roles of natural antisense transcripts. *Nat Rev Mol Cell Biol.* 2009;10(9):637–643. DOI: 10.1038/nrm2738
- [37] Lee JT, Davidow LS, Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. *Nat Genet.* 1999;21(4):400–404. DOI: 10.1038/7734
- [38] Kanduri C, Thakur N, Pandey RR. The length of the transcript encoded from the Kcnq1ot1 antisense promoter determines the degree of silencing. *EMBO J.* 2006;25(10):2096–2106. DOI: 10.1038/sj.emboj.7601090
- [39] Lyle R, Watanabe D, te Vrugte D, Lerchner W, Smrzka OW, Wutz A, et al. The imprinted antisense RNA at the Igf2r locus overlaps but does not imprint Mas1. *Nat Genet.* 2000;25(1):19–21. DOI: 10.1038/75546
- [40] Rearick D, Prakash A, McSweeney A, Shepard SS, Fedorova L, Fedorov A. Critical association of ncRNA with introns. *Nucleic Acids Res.* 2011;39(6):2357–2366. DOI: 10.1093/nar/gkq1080
- [41] Heo JB, Sung S. Vernalization-mediated epigenetic silencing by a long intronic non-coding RNA. *Science.* 2011;331(6013):76–79. DOI: 10.1126/science.1197349
- [42] Taft RJ, Kaplan CD, Simons C, Mattick JS. Evolution, biogenesis and function of promoter-associated RNAs. *Cell Cycle.* 2009;8(15):2332–2338. DOI: 10.4161/cc.8.15.9154
- [43] Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, et al. Global identification of human transcribed sequences with genome tiling arrays. *Science.* 2004;306(5705):2242–2246. DOI: 10.1126/science.1103388
- [44] Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, et al. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature.* 2002;420(6915):563–573. DOI: 10.1038/nature01266
- [45] Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, et al. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. *Genome Res.* 2012;22(9):1775–1789. DOI: 10.1101/gr.132159.111
- [46] Kong L, Zhang Y, Ye ZQ, Liu XQ, Zhao SQ, Wei L, et al. CPC: Assess the protein-coding potential of transcripts using sequence features and support vector machine. *Nucleic Acids Res.* 2007;35(Web Server issue):W345–W349. DOI: 10.1093/nar/gkm391
- [47] Lin MF, Jungreis I, Kellis M. PhyloCSF: A comparative genomics method to distinguish protein coding and non-coding regions. *Bioinformatics.* 2011;27(13):i275–i282. DOI: 10.1093/bioinformatics/btr209

- [48] Wilson BA, Masel J. Putatively noncoding transcripts show extensive association with ribosomes. *Genome Biol Evol.* 2011;3:1245–1252. DOI: 10.1093/gbe/evr099
- [49] Dinger ME, Pang KC, Mercer TR, Mattick JS. Differentiating protein-coding and non-coding RNA: Challenges and ambiguities. *PLoS Comput Biol.* 2008;4(11):e1000176. DOI: 10.1371/journal.pcbi.1000176
- [50] Campos EI, Reinberg D. Histones: Annotating chromatin. *Annu Rev Genet.* 2009;43:559–599. DOI: 10.1146/annurev.genet.032608.103928
- [51] Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A.* 2009;106(28):11667–11672. DOI: 10.1073/pnas.0904715106
- [52] Kanhere A, Viiri K, Araujo CC, Rasaiyaah J, Bouwman RD, Whyte WA, et al. Short RNAs are transcribed from repressed polycomb target genes and interact with polycomb repressive complex-2. *Mol Cell.* 2010;38(5):675–688. DOI: 10.1016/j.molcel.2010.03.019
- [53] Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, et al. Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol Cell.* 2010;40(6):939–953. DOI: 10.1016/j.molcel.2010.12.011
- [54] Guil S, Soler M, Portela A, Carrere J, Fonalleras E, Gomez A, et al. Intronic RNAs mediate EZH2 regulation of epigenetic targets. *Nat Struct Mol Biol.* 2012;19(7):664–670. DOI: 10.1038/nsmb.2315
- [55] Schwartz YB, Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet.* 2007;8(1):9–22. DOI: 10.1038/nrg1981
- [56] Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer.* 2006;6(11):846–856. DOI: 10.1038/nrc1991
- [57] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell.* 2007;129(7):1311–1323. DOI: 10.1016/j.cell.2007.05.022
- [58] Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science.* 2008;322(5902):750–756. DOI: 10.1126/science.1163045
- [59] Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science.* 2008;322(5908):1717–1720. DOI: 10.1126/science.1163802
- [60] Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, et al. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silenc-

ing through chromatin-level regulation. *Mol Cell*. 2008;32(2):232–246. DOI: 10.1016/j.molcel.2008.08.022

- [61] Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, et al. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell*. 2010;38(5):662–674. DOI: 10.1016/j.molcel.2010.03.021
- [62] Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, et al. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene*. 2011;30(16):1956–1962. DOI: 10.1038/onc.2010.568
- [63] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. *Science*. 2010;329(5992):689–693. DOI: 10.1126/science.1192002
- [64] Bertani S, Sauer S, Bolotin E, Sauer F. The noncoding RNA Mistral activates Hoxa6 and Hoxa7 expression and stem cell differentiation by recruiting MLL1 to chromatin. *Mol Cell*. 2011;43(6):1040–1046. DOI: 10.1016/j.molcel.2011.08.019
- [65] Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature*. 2011;472(7341):120–124. DOI: 10.1038/nature09819
- [66] Schuettengruber B, Martinez AM, Iovino N, Cavalli G. Trithorax group proteins: Switching genes on and keeping them active. *Nat Rev Mol Cell Biol*. 2011;12(12):799–814. DOI: 10.1038/nrm3230
- [67] Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet*. 2010;11(3):204–220. DOI: 10.1038/nrg2719
- [68] Sado T, Hoki Y, Sasaki H. Tsix defective in splicing is competent to establish Xist silencing. *Development*. 2006;133(24):4925–4931. DOI: 10.1242/dev.02670
- [69] Sun BK, Deaton AM, Lee JT. A transient heterochromatic state in Xist preempts X inactivation choice without RNA stabilization. *Mol Cell*. 2006;21(5):617–628. DOI: 10.1016/j.molcel.2006.01.028
- [70] Mohammad F, Mondal T, Guseva N, Pandey GK, Kanduri C. Kcnq1ot1 noncoding RNA mediates transcriptional gene silencing by interacting with Dnmt1. *Development*. 2010;137(15):2493–2499. DOI: 10.1242/dev.048181
- [71] McStay B, Grummt I. The epigenetics of rRNA genes: From molecular to chromosome biology. *Annu Rev Cell Dev Biol*. 2008;24:131–157. DOI: 10.1146/annurev.cell-bio.24.110707.175259

- [72] Mayer C, Schmitz KM, Li J, Grummt I, Santoro R. Intergenic transcripts regulate the epigenetic state of rRNA genes. *Mol Cell*. 2006;22(3):351–361. DOI: 10.1016/j.molcel.2006.03.028
- [73] Guetg C, Scheifele F, Rosenthal F, Hottiger MO, Santoro R. Inheritance of silent rDNA chromatin is mediated by PARP1 via noncoding RNA. *Mol Cell*. 2012;45(6):790–800. DOI: 10.1016/j.molcel.2012.01.024
- [74] Mayer C, Neubert M, Grummt I. The structure of NoRC-associated RNA is crucial for targeting the chromatin remodelling complex NoRC to the nucleolus. *EMBO Rep*. 2008;9(8):774–780. DOI: 10.1038/embor.2008.109
- [75] Schmitz KM, Mayer C, Postepska A, Grummt I. Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. *Genes Dev*. 2010;24(20):2264–2269. DOI: 10.1101/gad.590910
- [76] Guetg C, Lienemann P, Sirri V, Grummt I, Hernandez-Verdun D, Hottiger MO, et al. The NoRC complex mediates the heterochromatin formation and stability of silent rRNA genes and centromeric repeats. *EMBO J*. 2010;29(13):2135–2146. DOI: 10.1038/emboj.2010
- [77] Cam HP, Chen ES, Grewal SI. Transcriptional scaffolds for heterochromatin assembly. *Cell*. 2009;136(4):610–614. DOI: 10.1016/j.cell.2014.11.052
- [78] Moazed D. Small RNAs in transcriptional gene silencing and genome defence. *Nature*. 2009;457(7228):413–420. DOI: 10.1038/nature07756
- [79] Mao YS, Zhang B, Spector DL. Biogenesis and function of nuclear bodies. *Trends Genet*. 2011;27(8):295–306. DOI: 10.1016/j.tig.2011.05.006
- [80] Chen LL, Carmichael GG. Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: Functional role of a nuclear noncoding RNA. *Mol Cell*. 2009;35(4):467–478. DOI: 10.1016/j.molcel.2009.06.027
- [81] Sunwoo H, Dinger ME, Wilusz JE, Amaral PP, Mattick JS, Spector DL. MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. *Genome Res*. 2009;19(3):347–359. DOI: 10.1101/gr.087775.108
- [82] Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell*. 2009;33(6):717–726. DOI: 10.1016/j.molcel.2009.01.026
- [83] Sasaki YT, Ideue T, Sano M, Mituyama T, Hirose T. MENepsilon/beta noncoding RNAs are essential for structural integrity of nuclear paraspeckles. *Proc Natl Acad Sci U S A*. 2009;106(8):2525–2530. DOI: 10.1073/pnas.0807899106

- [84] Mao YS, Sunwoo H, Zhang B, Spector DL. Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs. *Nat Cell Biol.* 2011;13(1):95–101. DOI: 10.1038/ncb2140
- [85] Bernard D, Prasanth KV, Tripathi V, Colasse S, Nakamura T, Xuan Z, et al. A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. *EMBO J.* 2010;29(18):3082–3093. DOI: 10.1038/emboj.2010.199
- [86] Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell.* 2010;39(6):925–938. DOI: 10.1016/j.molcel.2010.08.011
- [87] Yang L, Lin C, Liu W, Zhang J, Ohgi KA, Grinstein JD, et al. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. *Cell.* 2011;147(4):773–778. DOI: 10.1016/j.cell.2011.08.054
- [88] Cai X, Cullen BR. The imprinted H19 noncoding RNA is a primary microRNA precursor. *RNA.* 2007;13(3):313–316. DOI: 10.1261/rna.351707
- [89] Hung T, Chang HY. Long noncoding RNA in genome regulation: Prospects and mechanisms. *RNA Biol.* 2010;7(5):582–585. DOI: 10.4161/rna.7.5.13216
- [90] Swiezewski S, Liu F, Magusin A, Dean C. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* polycomb target. *Nature.* 2009;462(7274):799–802. DOI: 10.1038/nature08618
- [91] Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, et al. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nat Gene.* 2011;43(7):621–629. DOI: 10.1038/ng.848
- [92] Yan BX, Ma JX. Promoter-associated RNAs and promoter-targeted RNAs. *Cell Mol Life Sci.* 2012;69(17):2833–2842. DOI: 10.1007/s00018-012-0953-1
- [93] Imamura T, Yamamoto S, Ohgane J, Hattori N, Tanaka S, Shiota K. Non-coding RNA directed DNA demethylation of Sphk1 CpG island. *Biochem Biophys Res Commun.* 2004;322(2):593–600. DOI: 10.1016/j.bbrc.2004.07.159
- [94] Morris KV, Chan SW, Jacobsen SE, Looney DJ. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science.* 2004;305(5688):1289–1292. DOI: 10.1126/science.1101372
- [95] Napoli S, Pastori C, Magistri M, Carbone GM, Catapano CV. Promoter-specific transcriptional interference and c-myc gene silencing by siRNAs in human cells. *EMBO J.* 2009;28(12):1708–1719. DOI: 10.1038/emboj.2009
- [96] Hawkins PG, Santoso S, Adams C, Anest V, Morris KV. Promoter targeted small RNAs induce long-term transcriptional gene silencing in human cells. *Nucleic Acids Res.* 2009;37(9):2984–2995. DOI: 10.1093/nar/gkp127

- [97] Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A*. 2008;105(5):1608–1613. DOI: 10.1073/pnas.0707594105
- [98] Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, et al. Small dsRNAs induce transcriptional activation in human cells. *Proc Natl Acad Sci U S A*. 2006;103(46):17337–17342. DOI: 10.1073/pnas.0607015103
- [99] Janowski BA, Younger ST, Hardy DB, Ram R, Huffman KE, Corey DR. Activating gene expression in mammalian cells with promoter-targeted duplex RNAs. *Nat Chem Biol*. 2007;3(3):166–173. DOI: 10.1038/nchembio860
- [100] Panganiban G, Rubenstein JL. Developmental functions of the Distal-less/Dlx homeobox genes. *Development*. 2002;129(19):4371–4386.
- [101] Hastings ML, Ingle HA, Lazar MA, Munroe SH. Post-transcriptional regulation of thyroid hormone receptor expression by *cis*-acting sequences and a naturally occurring antisense RNA. *J Biol Chem*. 2000;275(15):11507–11513. DOI: 10.1074/jbc.275.15.11507
- [102] Kretz M, Siprashvili Z, Chu C, Webster DE, Zehnder A, Qu K, et al. Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature*. 2013;493(7431):231–235. DOI: 10.1038/nature11661
- [103] Faghihi MA, Modarresi F, Khalil AM, Wood DE, Sahagan BG, Morgan TE, et al. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. *Nat Med*. 2008;14(7):723–730. DOI: 10.1038/nm1784
- [104] Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, et al. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. *Nature*. 2012;491(7424):454–457. DOI: 10.1038/nature11508
- [105] Cheetham SW, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. *Br J Cancer*. 2013;108(12):2419–2425. DOI: 10.1038/bjc.2013.233
- [106] Faraoni I, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: A typical multifunctional microRNA. *Biochim Biophys Acta*. 2009;1792(6):497–505. DOI: 10.1016/j.bbadis.2009.02.013
- [107] Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*. 2005;65(14):6029–6033. DOI: 10.1158/0008-5472.CAN-05-0137
- [108] Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nat Immunol*. 2008;9(4):405–414. DOI: 10.1038/ni1575

- [109] Concepcion CP, Bonetti C, Ventura A. The microRNA-17-92 family of microRNA clusters in development and disease. *Cancer J.* 2012;18(3):262–267. DOI: 10.1097/PPO.0b013e318258b60a
- [110] Gutschner T, Hammerle M, Eissmann M, Hsu J, Kim Y, Hung G, et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer Res.* 2013;73(3):1180–1189. DOI: 10.1158/0008-5472.CAN-12-2850
- [111] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. *Nature.* 2007;447(7148):1130–1134. DOI: 10.1038/nature05939
- [112] Gutschner T, Diederichs S. The hallmarks of cancer: A long non-coding RNA point of view. *RNA Biol.* 2012;9(6):703–719. DOI: 10.4161/rna.20481
- [113] Redon S, Reichenbach P, Lingner J. The non-coding RNA TERRA is a natural ligand and direct inhibitor of human telomerase. *Nucleic Acids Res.* 2010;38(17):5797–5806. DOI: 10.1093/nar/gkq296
- [114] Szafranski P, Dharmadhikari AV, Brosens E, Gurha P, Kolodziejska KE, Zhishuo O, et al. Small noncoding differentially methylated copy-number variants, including lncRNA genes, cause a lethal lung developmental disorder. *Genome Res.* 2013;23(1):23–33. DOI: 10.1101/gr.141887.112
- [115] Kanduri C. Long noncoding RNA and epigenomics. *Adv Exp Med Biol.* 2011;722:174–195. DOI: 10.1007/978-1-4614-0332-6_11
- [116] Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, et al. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature.* 1991;349(6304):38–44. DOI: 10.1038/349038a0
- [117] Lee JT. Epigenetic regulation by long noncoding RNAs. *Science.* 2012;338(6113):1435–1439. DOI: 10.1126/science.1231776
- [118] Caley DP, Pink RC, Trujillano D, Carter DR. Long noncoding RNAs, chromatin, and development. *Sci World J.* 2010;10:90–102. DOI: 10.1100/tsw.2010.7
- [119] Zakharova IS, Shevchenko AI, Zakian SM. Monoallelic gene expression in mammals. *Chromosoma.* 2009;118(3):279–290. DOI: 10.1007/s00412-009-0206-8
- [120] Umlauf D, Fraser P, Nagano T. The role of long non-coding RNAs in chromatin structure and gene regulation: Variations on a theme. *Biol Chem.* 2008;389(4):323–331. DOI: 10.1515/BC.2008.047
- [121] Mohammad F, Mondal T, Kanduri C. Epigenetics of imprinted long noncoding RNAs. *Epigenetics.* 2009;4(5):277–286. DOI: 10.4161/epi.4.5.9242
- [122] Lee JT, Bartolomei MS. X-inactivation, imprinting, and long noncoding RNAs in health and disease. *Cell.* 2013;152(6):1308–1323. DOI: 10.1016/j.cell.2013.02.016

- [123] Sleutels F, Barlow DP. The origins of genomic imprinting in mammals. *Adv Genet.* 2002;46:119–163. DOI: 10.1016/S0065-2660(02)46006-3
- [124] Kanduri C, Fitzpatrick G, Mukhopadhyay R, Kanduri M, Lobanenkov V, Higgins M, et al. A differentially methylated imprinting control region within the *Kcnq1* locus harbors a methylation-sensitive chromatin insulator. *J Biol Chem.* 2002;277(20):18106–18110.
- [125] Kanduri C, Holmgren C, Pilartz M, Franklin G, Kanduri M, Liu L, et al. The 5' flank of mouse H19 in an unusual chromatin conformation unidirectionally blocks enhancer-promoter communication. *Curr Biol.* 2000;10(8):449–457. DOI: 10.1016/S0960-9822(00)00442-5
- [126] Kanduri C, Pant V, Loukinov D, Pugacheva E, Qi CF, Wolffe A, et al. Functional association of CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. *Curr Biol.* 2000;10(14):853–856. DOI: 10.1016/S0960-9822(00)00597-2
- [127] Sleutels F, Zwart R, Barlow DP. The non-coding air RNA is required for silencing autosomal imprinted genes. *Nature.* 2002;415(6873):810–813. DOI: 10.1038/415810a
- [128] Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet.* 2015;47(3):199–208. DOI: 10.1038/ng.3192
- [129] Kung JTY, Colognori D, Lee JT. Long noncoding RNAs: Past, present and future. *Genetics.* 2013;193:651–659. DOI: 10.1534/genetics.112.146704
- [130] Gomes AQ, Nolasco S, Soares H. Non-coding RNAs: Multi-tasking molecules in the cell. *Int J Mol Sci.* 2013;14(8):16010–16039. DOI: 10.3390/ijms140816010