Chapter 6
Long Noncoding RNAs in Mammalian Development and Diseases

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Abstract Following analysis of sequenced genomes and transcriptome of many eukaryotes, it is evident that virtually all protein-coding genes have already been discovered. These advances have highlighted an intriguing paradox whereby the relative amount of protein-coding sequences remain constant but nonprotein-coding sequences increase consistently in parallel to increasing evolutionary complexity. It is established that differences between species map to nonprotein-coding regions of the genome that surprisingly is transcribed extensively. These transcripts regulate epigenetic processes and constitute an important layer of regulatory information essential for organismal development and play a causative role in diseases. The noncoding RNA-directed regulatory circuit controls complex characteristics. Sequence variations in noncoding RNAs influence evolution, quantitative traits, and disease susceptibility. This chapter presents an account on a class of such noncoding transcripts that are longer than 200 nucleotides (long noncoding RNA—lncRNA) in mammalian development and diseases.

Keywords lncRNAs • Evolution of complexity • Epigenetic modifications • Imprinting • Chromosome inactivation • Body patterning • Nuclear architecture • Cellular differentiation

6.1 Introduction

Recent technical advancements in high-throughput sequencing have revealed that a majority of eukaryotic genome is pervasively transcribed. Large-scale analysis (ENCODE project) has shown that ~75% of human genome is transcribed in various cell lines [1]. Why a cell spends so much of its resources on RNA production has captured the imagination of scientific community. Many of these RNAs are long

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transcripts with no apparent protein-coding potential. Initially these transcripts were discounted as artifacts and were thought to be the result of expression "noise" rather than expression "choice." But many features of these transcripts indicate a definite and important role. For example, transcription of lncRNAs is initiated from conserved promoters. Many of the transcripts are alternatively spliced and display predicted structures. They are dynamically expressed during differentiation and disease in a cell- and tissue-specific manner. However, the main argument posed against a functional role is the lack of primary sequence conservation among these transcripts. But studies have shown that lack of conservation does not necessarily mean lack of function.

lncRNA molecules are involved in diverse biological processes like genomic imprinting, dosage compensation, epigenetic and transcriptional regulation, chromosome conformation, cell cycle regulation, stem cell differentiation/reprogramming, and allosteric enzymatic activity [2]. The structure and biogenesis of lncRNAs is very similar to that of mRNAs. Like mRNAs, they are transcribed by RNA polymerase II from genomic loci in the epigenetic context similar to protein-coding genes. They are 5′-capped and spliced and commonly have a polyadenylated tail. However, unlike mRNAs, they may undergo alternative forms of processing at 3′-end. For example, an RNase P-assisted cleavage at 3′-end results in a lncRNA with stable 3′-terminal RNA triplex structure, instead of a polyadenylated tail [3]. Although lncRNAs lack coding capacity, they possess the intriguing ability to adopt a secondary/tertiary structure that may relate to their function. Depending on their position and direction of transcription in relation to protein-coding genes, lncRNAs may be classified as antisense, intergenic, intronic, bidirectional, processed, or pseudogene transcripts [4, 5]. Mechanism of action of lncRNAs is also very diverse. They may regulate genes in *cis* (i.e., in close proximity to site of transcription) or in *trans* (at a distance from transcription site) [6]. They may act as *scaffolds* to bring a group of proteins into spatial proximity, as *guides* to recruit proteins to DNA, as *decoys* to titrate away proteins, or as *enhancer RNAs* involved in chromosomal looping in enhancer-like manner [2]. Some lncRNAs are precursor to smaller regulatory RNAs, like miRNA or piwi RNAs or they may bind to complimentary RNAs to affect their turnover [7].

Mostly the lncRNAs are expressed at low levels in a highly tissue-specific manner, so much so that their expression profiles are important markers for disease or developmental state [8]. Many a times they are found next to protein-coding genes that are under tight transcriptional control, and often their expression pattern correlates with tissue differentiation, development, and disease [9]. The widespread dysregulation of lncRNA expression in human diseases and the finding that many lncRNAs are enriched for SNPs that associate with human traits/diseases have highlighted the need to understand the functional contribution of these RNAs [10, 11]. However, the study of lncRNAs using model organisms is confounded by the fact that these RNAs exhibit poor primary sequence conservation. Exons of lncRNA evolve much faster than protein-coding gene sequence and most lncRNAs are lineage specific [8, 12, 13].These RNAs rather show conservation along genomic

Table 6.1 Number of noncoding and coding transcripts in different organisms

Organism	Genome size (Mb)	lncRNAs[a]
Human	3300	141,353
Mouse	2800	117,405
D. melanogaster	120	54,819
A. thaliana	135	3853
C. elegans	100	3271
S. cerevisiae	12.5	61

[a]www.noncode.org

position (synteny), short sequence motifs, or secondary structure [14, 15]. Because of more likely structural than sequence conservation, functionality of lncRNAs could be organized into modular domains similar to proteins organized into functional motifs.

Understandably organismal complexity correlates better with the expression repertoire of lncRNA than with that of protein-coding genes (Table 6.1) [16]. This presents a pressing need to explore the functional relevance of such transcripts in the context of evolution of developmental mechanisms. In most vertebrates, exhaustive annotation of lncRNA is still not available, primarily due to incomplete genome sequences and partial annotation of protein-coding genes. Further, majority of the annotated lncRNAs remain functionally uncharacterized and only a small fraction have been explored for their biological relevance. In this chapter we give an overview of some of the characterized mammalian lncRNAs and their etiology in human diseases.

6.2 Diverse Function of lncRNAs in Mammalian Gametogenesis and Development

A large number of mammalian lncRNAs (mostly in human and mice) have been discovered in recent genome-wide expression studies. They have been found to play important role in almost all stages of mammalian development, i.e., gametogenesis, embryogenesis (during preimplantation stages as well as in placenta), body axis patterning, pre-/postnatal tissue development, and organogenesis. In diploid organisms, most genes are expressed from both alleles, but some are expressed from only one allele in a parent of origin-specific manner. Genomic imprinting and X-chromosome inactivation (XCI) are two such phenomena that lead to monoallelic expression of genes. These phenomena come into play during gametogenesis/embryonic development and have lncRNAs as a key player in the process. Similarly, spatiotemporally coordinated embryonic expression of Hox genes leads to body axis patterning in bilaterians. Epigenetic features and lncRNAs bring about this coordination of Hox gene expression. Several studies point to functional role of lncRNAs in mammalian development.

6.2.1 lncRNA in Genomic Imprinting

In mammals, some genes are epigenetically "imprinted" mainly by DNA methylation during gametogenesis by a process called "genomic imprinting." This results in allele-specific expression of either maternally or paternally inherited genes in developing embryo. The imprinting process happens during early gametogenesis and approximately 1% of mammalian protein-coding genes get imprinted. Initial cues to the phenomenon came from early experiments where nuclear transfer in mouse zygotes reconstructed from two maternal pronuclei (gynogenones) or from two paternal pronuclei (androgenones) failed to develop, while zygotes carrying one paternal pronucleus and maternal pronucleus were able to develop [17, 18]. Later, genome-wide studies and deletion and transgenic approaches led to the identification of several imprinted genes most of which are essential and have been implicated in developmental process.

To date, more than 150 imprinted genes in mouse and about half that number has been identified in humans. Most imprinted genes are organized in clusters that contain three or more genes. The size of the cluster spans from a few kilobases to several megabases on different chromosomes [19, 20] (www.mousebook.org). An imprinting control region (ICR) controls gene expression in each imprinted cluster. ICRs are rich in CpG dinucleotides and carry parental allele-specific germline-derived DNA-methylated regions (gDMR). This pattern of gDMR is maintained throughout development [21, 22]. The allele-specific expression of imprinted genes in a cluster in daughter cells after subsequent cell divisions is conferred and managed by histone modifications, insulators, and higher-order chromatin organizations [19, 20]. Surprisingly most imprinted clusters identified have one or more associated lncRNA that have been found to be inherently essential to the allele-specific expression. In general, lncRNAs show reciprocal parental allele-specific expression when compared to the imprinted genes in a cluster. ICRs are mostly located in or near the promoter of lncRNA. Further, by many overexpression and deletion experiments, it has been confirmed that lncRNA regulates imprinting of the locus in *cis* or in *trans* or both.

lncRNAs known to be involved in genomic imprinting are listed below (Table 6.2), and the mechanisms of action of two relatively better understood examples are discussed here. Although the imprinting-associated lncRNAs do not employ a common mechanism for epigenetic control, they do offer valuable insights into the biology of lncRNAs in general. Interestingly, most imprinted lncRNAs are relatively conserved at functional as well as sequence level between mice and humans. This makes genomic imprinting an attractive model system to study lncRNA-dependent epigenetic mechanisms during human development and diseases using mouse models.

6.2.1.1 H19

H19 gene encodes for a 2.3-kb lncRNA. It is among one of the first discovered and widely investigated imprinted genes in mammals. In mouse, *H19* is present along with insulin-like growth factor (*Igf2*) gene at distal segment of chromosome 7. This

Table 6.2 lncRNAs involved in mammalian genomic imprinting

Imprinted cluster	lncRNA and expression (M or P)	Type of lncRNA	*Cis*-silencing function	Genes imprinted and expression (M or P)	Refs
Igf2	*H19* (M)	Intergenic	Yes	*Igf2* (P)	[23, 24]
Kcnq1	*Kcnq1ot1*	Antisense	Yes	*Kcnq1*, *Cdkn1c*, *Slc22a18*, *Phlda2*, *Ascl2*, *Cd81*, *Tssc4*, *Tspan32*, *Osbpl5* (M)	[25]
Igf2r	*Airn*(P)	Antisense	Yes	*Igf2r*, *Slc22a2*, *Slc22a3* (M)	[26]
Pws/As	*UBE3A-ATS*	Antisense	Yes	*MAGEL2* (P), *NDN* (P), *SNRPN* (P),	[27–29]
	Ipw	Intergenic	n.d.	*SNORD115* (P),	
	Pwcr1	n.d.	Yes	*SNORD116* (P), *UBEA3A* (M)	
Dlk1	*Gtl2* (M)	Antisense	n.d.	*DLK1* (P), *DIO3* (P), *RTL1*(P)	[30–32]
	Rtl1as (M)	Antisense	Yes		
	Rian (M)	Intergenic	n.d.		
	Mirg (M)	Intergenic	n.d.		
Gnas	*Nespas*	Antisense	Yes	*Gnas* (M), *Nesp* (M)	[33, 34]
	Exon1A	Antisense	n.d.		

M maternal, *P* paternal, *n.d.* not determined

region is syntenic to the locus 11p15.5 in human [23, 35]. A differentially methylated ICR, which lies in between the two genes, regulates mutually exclusive monoallelic expression of *H19* and *Igf2* at the locus. A common enhancer located downstream of *H19* drives the expression of both the genes. The ICR and *H19* promoter are methylated in paternal allele. On the maternal allele, the un-methylated ICR binds to an architectural known as CCCTC-binding factor (CTCF) responsible for long-range chromatin interactions and chromatin looping. CTCF further triggers recruitment of cohesin to ICR, resulting in higher-order chromatin conformation that restricts the enhancer access to *Igf2* promoter. A methylated, thus unoccupied, ICR on the paternal chromosome on the other hand poses no restriction, and enhancer interacts with the *Igf2* promoter driving its expression (Fig. 6.1a) [36, 37].

Although expression of *H19* is mostly studied in relation to imprinting of *H19–Igf2* locus, studies have been carried out to understand the functions of *H19* lncRNA. *H19* knockout mice are viable and fertile with growth defects and reduced muscle regeneration capacities [35, 38]. For example, *H19* deletion on the maternally inherited chromosome led to an increase in *Igf2* expression and increased body weight that could be rescued by deletion of one *Igf2* allele. Although *H19* is highly expressed during embryogenesis, it is effective only in specific cell lineages. Apart from imprinting, deletion/overexpression of *H19* affects embryonic growth. This is because *H19* is part of an imprinted gene network (IGN), which consists of 16 co-expressing imprinted genes that include many growth regulators such as *Igf2*, *Igf2r*,

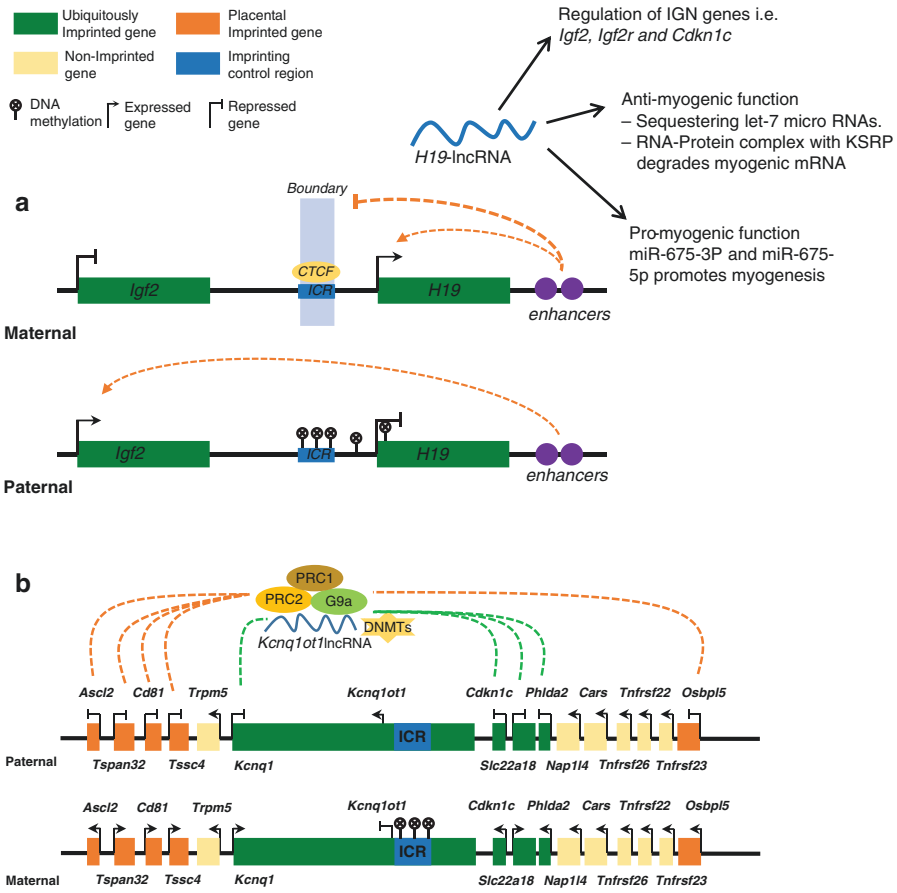


Fig. 6.1 Imprinted regulation of genes of *Igf2* and *Kcnq1* cluster by *H19* and *Kcnq1ot1* lncRNAs, respectively. (**a**) *H19* and *Igf2* show reciprocally exclusive mono-allelic expression from maternal and paternal loci, respectively. A differentially methylated ICR between the two genes and downstream enhancers regulates parent of origin-specific expression of both the genes. The ICR and *H19* promoter are methylated in paternal chromosome that represses *H19* expression, while enhancer interacts with the *Igf2* promoter driving its expression. On the maternal chromosome, the ICR is un-methylated and CTCF is bound to it. CTCF triggers recruitment of cohesin to ICR and higher-order chromatin conformation that restricts the enhancer access to *Igf2* promoter. *H19* lncRNA also involves in regulation of genes of imprinting gene networks (IGN). *H19* lncRNA shows dual function of anti-myogenic and pro-myogenic during mesenchymal stem cells (C2C12) differentiation into myocytes. (**b**) At *Kcnq1* cluster *Kcnq1ot1* expresses paternally, while all the imprinted protein-coding genes are maternally expressed. These imprinted genes are of two types—(1) placenta-specific imprinted genes (PIGs: *Ascl2*, *Cd81*, *Tssc4*, *Tspan32*, *Osbpl5*) which show imprinted silencing only in placental tissues and (2) ubiquitously imprinted genes (UIGs: *Kcnq1*, *Cdkn1c*, *Slc22a18*, *Phlda2*) which show imprinted silencing in both placental and embryonic tissues. The promoter for the *Kcnq1ot1* coincides with the differentially methylated ICR (*Kcnq1* ICR/ KVDMR1). The maternal allele-specific methylation of *Kcnq1ot1* promoter restricts the expression of lncRNA from maternal chromosome. Paternally expressed *Kcnq1ot1* lncRNA interacts with modifiers of chromatin (EZH2 and G9a) and DNA (DNMT1) that bind paternal alleles in *cis* and silence the imprinted genes by establishing higher-order chromatin compartment enriched in repressive histone and DNA modifications

and *Cdkn1c*. The noncoding RNA acts in *trans* to bring about its effect on IGN. It interacts with methyl-CpG-binding protein (MBD-1) that methylates the DMRs of IGN members like *Igf2*, *Slc38a4*, and *Peg1* via H3K9 methyltransferase [39]. This function of *H19* lncRNA that leads to establishment of H3K9me3-associated repressive chromatin occurs on both the parental alleles and is related to embryonic growth regulation.

After embryogenesis, *H19* expresses at low levels in all tissues and at a very high level in muscle. During postnatal tissue differentiation, *H19* has been implicated in contrasting pro- and anti-myogenic functions. Using mouse multipotent mesenchymal cells (C2C12 cells), it has been shown that depletion of *H19* accelerates muscle differentiation suggesting an anti-myogenic function. Two different mechanisms have been suggested for this function. In one of the studies, *H19* from human as well as mice is reported to carry conserved binding sites of Let-7 microRNAs, a pro-myogenic factor. It thus acts as a competing endogenous RNA (CeRNA), a natural sponge that sequesters Let-7 and controls its level. In another study, *H19* has been shown to interact with an RNA processing protein known as K homology-type splicing regulatory protein (KSRP). The resulting RNA–protein complex facilitates interaction between exosome and labile transcripts of protein "myogenin" promoting its degradation and eventually restricting the differentiation of C2C12 into myocytes. However, in contrast to the above findings, a pro-myogenic function of *H19* has been reported that is mediated by two microRNAs, miR-675-3p and miR-675-5p, originating from the exon1 of the *H19* transcript. *H19* along with miR-675-3p/miR-675-5p induces C2C12 differentiation into myocytes. Downregulation of *H19* or blocking the action of miR-675-3p/miR-675-5p prevents C2C12 differentiation [40–42]. The apparently contrasting functions can be reconciled with possible mechanisms that inhibit the primary role of *H19* which is to prevent myogenesis. Once its function needs to be changed, the RNA gets degraded or processed in a way that miRNAs from its exon1 are generated to eventually promote myogenesis.

In conclusion, *H19* lncRNA is an epigenetic regulator of transcription. It executes its activity by behaving as a CeRNA, miRNA precursor, or scaffold to recruit proteins. It is involved in multitude of biological processes like imprinting, growth, differentiation, and myogenesis [43].

6.2.1.2 Kcnq1ot1

Kcnq1ot1 is a 91-kb-long noncoding RNA that maps to *Kcnq1* gene in antisense orientation. The imprinted cluster approximately ~1 Mb in length and encompassing 12 genes is present at the distal end of the seventh chromosome in mouse. Its human orthologue is located on chromosome 11p15.5 [25, 44]. Promoter for the *Kcnq1ot1* gene lies in the tenth intron of *Kcnq1* host gene and coincides with the differentially methylated ICR (*Kcnq1* ICR/KVDMR1). The maternal allele-specific methylation of *Kcnq1ot1* promoter restricts the expression of lncRNA from paternal chromosome in antisense direction with respect to host gene. All the imprinted protein-coding genes are maternally expressed. These imprinted genes are of two

types—(1) placenta-specific imprinted genes (PIGs: *Ascl2*, *Cd81*, *Tssc4*, *Tspan32*, *Osbpl5*) which show imprinted silencing only in placental tissues and (2) ubiquitously imprinted genes (UIGs: *Kcnq1*, *Cdkn1c*, *Slc22a18*, *Phlda2*) which show imprinted silencing in both placental and embryonic tissues [25, 45, 46] (Fig. 6.1b).

The antisense *Kcnq1ot1* RNA is required for silencing of both UIGs and PIGs. Paternal silencing is lost when *Kcnq1ot1* promoter is deleted or a prematurely truncated RNA is produced [47, 48]. Interestingly, *Kcnq1ot1* also employs lineage-specific mechanism of action as after initiating imprinting of UIGs as well as PIGs, it is involved in the maintenance of silencing at UIGs alone [49]. To unravel the mechanism of action of the lncRNA, biochemical and genetic studies have been carried out in cells and transgenic mice models. The studies show that *Kcnq1ot1* lncRNA interacts with modifiers of chromatin (EZH2 and G9a) and DNA (DNMT1) to recruit them in *cis* to silence the imprinted genes [44, 46, 50]. The allelic silencing is achieved by establishment of higher-order chromatin compartment enriched in repressive histone modifications such as H3K27me3, H3K9me2, and H2AK119ub [50, 51]. While silencing of UIGs is controlled by repressive histone modifications and maintained by methylation of somatic DMRs, silencing of PIGs is controlled by repressive histone modification only. Recently, *Kcnq1ot1* lncRNA has been shown to mediate targeting of the entire repressed loci to distinct perinucleolar repressive compartment by virtue of a conserved 890-bp repeat-containing domain present at its 5′-end.

6.2.2 lncRNA in Dosage Compensation

In higher eukaryotes, the number of sex chromosomes differs between the two sexes. Organisms have evolved different strategies to compensate for this discrepancy by adjusting gene expression levels. To equalize transcription level of genes present on sex chromosome, the chromatin structure is modulated epigenetically. The epigenetic mechanism on one extreme leads to inactivation of one of the X chromosome in females (as observed in mammals) and on the other extreme leads to twofold higher expression of genes on the single X chromosome in males (as observed in *Drosophila*). The curiously opposite ways lead to equal level of expression of sex chromosome-associated genes in different organisms.

In female mammals, the epigenetic process of X-chromosome inactivation (XCI) regulates gene dosage of extra X chromosome. Initially the phenomena was noticed by Murray Barr in 1949 when he observed that female cat cells possess a condensed subnuclear structure which is now called as “Barr body” in his honor. Later studies demonstrated that the Barr body is nothing but a condensed X chromosome which is also transcriptionally silent [52–54]. Later a 17-kb noncoding murine transcript *Xist* was discovered that initiated the fascinating era of lncRNA biology [55]. Further discovery of its 40-kb-long antisense transcript *Tsix* highlighted the fact that untranslated RNAs dominate the regulation of XCI [56]. The process of XCI is similar to genomic imprinting as the silenced genes are clustered, are influenced by a long-distance master control region, and are associated with multiple lncRNAs.

In eutherian mammals, the process of XCI occurs in two different ways. During early embryogenesis, paternal X chromosome is inactivated in preimplanted embryos. As the embryo reaches blastula stage and gets implanted into the uterus, the outer blastular cells (future placenta) retain paternal XCI, while imprinting is erased from inner cell mass (future embryo). As these inner blastular cells (epiblasts) differentiate, either of the parental chromosome has an equal chance of inactivation (random XCI). The eutherian mammalian female is thus essentially a mosaic, with randomly active paternal/maternal X chromosome. In marsupials, however, the choice of inactivation is always fixed to paternal X chromosome.

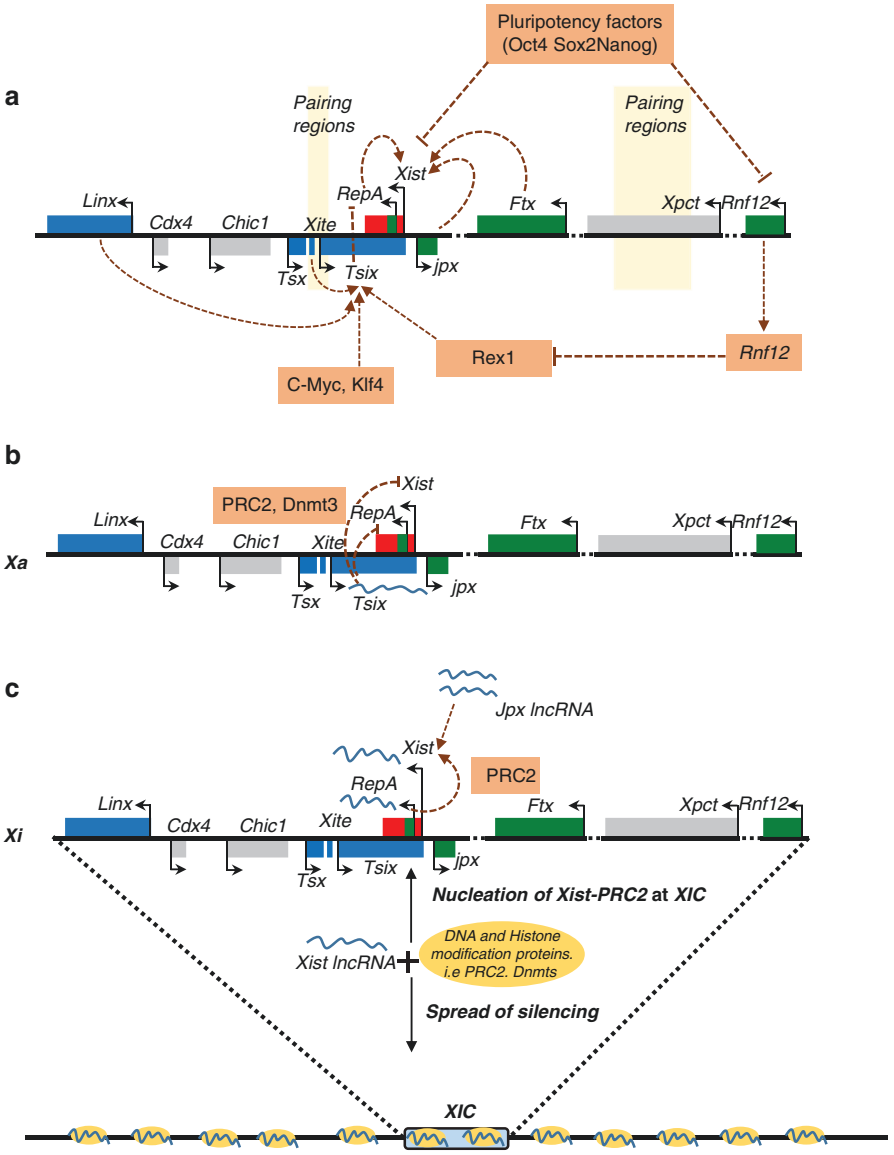
Random XCI is a coordinated stepwise process that results in silencing of ~1000 genes along the inactive X chromosome. The process is controlled by X-inactivation center (*Xic)* that codes for lncRNA with regulatory properties. The lncRNAs from *Xic* work in *cis* as well as in *trans*. In the first step which has been referred to as "counting," X chromosome-to-autosome ratio (X:A) is measured. XCI is initiated in female cells where X:A = 1 and is blocked in male cells where the ratio is 0.5 [57, 58]. Molecular details of this measurement remain elusive, but *trans*-activity of the two lncRNAs, *Tsix* and *Xite*, is implicated in the process [59]. The next step results in random "choice" of one of the X chromosome to remain active (Xa) and the other one to get inactivated (Xi). The final "sensing" is a permissive state for XCI, similar to the initial counting step but distinct from it. Eventually the Xi is epigenetically marked by repressive chromatin and DNA methylation to a transcriptionally inert state, while Xa remains open for transcription.

The *Xic* is a 100–200-kb region with at least seven lncRNA genes of which six have been shown to have specific function during XCI (Table 6.3, Fig. 6.2). Prior to initiation of XCI, the lncRNAs *Xist*, *Tsix*, and *Xite* are expressed from both the Xs at low levels. Mutually exclusive selection of Xa and Xi necessitates interchromosomal interaction and robust feedback mechanism. The 5′-end of *Tsix* gene binds to the

Table 6.3 lncRNAs involved in XCI

lncRNA	Functions	Refs
Xist	Initiation and spreading of XCI on Xi	[66]
Tsix	Negative regulation of *Xist*, dosage sensor (measurement of X:A ratio), and X-chromosome pairing for choice of Xi/Xa	[59]
Xite	Positive regulation of *Tsix*, dosage sensor (measurement of X:A ratio), and X-chromosome pairing for choice of Xi/Xa	[59, 67, 68]
DXPas34	Involve in dual function as an enhancer and a repressor of *Tsix*, counting, and X-chromosome pairing for choice of Xi/Xa	[69]
Tsx	Negatively regulates *Xist* and positively regulates *Tsix*	[70]
Linx	Co-expresses with *Tsix* and potentially involved in positive regulation of *Tsix*	[71]
RepA	Play role in upregulation of *Xist* by recruitment of PRC2 and altering the chromatin structure at *Xist* promoter on Xi	[72]
Jpx/Enox	Activates *Xist* upregulation by evicting CTCF binding to *Xist* promoter on Xi	[72, 73]
Ftx	Positive regulator of *Xist*	[74]

protein CTCF that leads to a brief transient contact of the two Xs at *Xic*. However, a role for the two lncRNA transcripts (*Tsix/Xite*) is also envisaged in the process as inhibiting Pol II activity results in abrogation of X–X pairing suggesting that the pairing requires new transcription. The contact of the two Xs results in establishment of an asymmetry and choice of Xi and Xa [60, 61]. The process of establishment of asymmetry is not clear, but it has been postulated that the proximity of Xs directs irreversible shift of proteins (Oct4 and CTCF) from one allele (future Xi) to the other (future Xa) [61–63]. Once chosen, *Tsix* is expressed in an allele-specific manner from



the Xa. As *Tsix* is antisense to *Xist*, its expression results in removal of latter from Xa in *cis*. lncRNAs *Xite* and *DXPas34* positively regulate expression of *Tsix* from Xa. Finally to seal the active state permanently, *Tsix* lncRNA directs DNA methylation (by Dnmt3a) at the *Xist* promoter resulting in stable silencing of *Xist* allele on Xa. On the other hand, removal of transcription factors (Oct4 and CTCF) from *Tsix/Xite* promoter on Xi causes a drop in transcription of these lncRNAs. In the absence of *Tsix*, *Xist* lncRNA being transcribed from Xi becomes abundant and coats it in *cis*. *Xist*-coated Xi becomes enriched for repressive chromatin marks (H3K27me3), but *Xist* transcription continues unabated in otherwise heterochromatic environment. One of the first changes that follows depletion of *Tsix* on Xi is enrichment of polycomb repressive complex (PRC2) on *Xist* promoter. This is brought about by *RepA* lncRNA that recruits PRC2 to 5′-end of *Xist*. This creates a heterochromatic patch at *Xist* promoter, essential and stimulatory for its expression [64, 65].

In conclusion, the lncRNAs coded by the *Xic* perform versatile functions to coordinate the process of XCI. They can uniquely define an address in the genome as they remain tethered to their locus of transcription and guide regulatory mechanisms in *cis*. They can also function as transcript-level regulator by RNAi (*Xist–Tsix* pair) or act as tethers and guides to recruit chromatin modifiers (*RepA* recruitment of PRC2, *Xist* recruitment of Dnmt3a).

6.2.3 *Linc-ing RNAs to Body Patterning*

Hox genes are the important group of transcriptional factors encoding genes, arranged in clusters in the bilaterian genomes. They define the body axis patterning through precisely coordinated spatiotemporal expression in the developing

Fig. 6.2 lncRNA-mediated X-chromosome inactivation. (**a**) X-inactivation center (Xic) of mouse encompasses ~500 kb regions of X chromosome that has several lncRNA loci (*Xist*, *Tsix*, *Jpx*, Ftx, *RepA*, *Xite*, *Tsx*, *Linx*) as well as protein-coding genes (*gray*). lncRNAs are involved in positive (*RepA*, *Jpx*, and *Ftx—green*) or negative (*Xite*, *Tsx*, *Linx—blue*) regulation of *Xist* by activating *Tsix*, an antisense lncRNA and negative regulator of *Xist.* Other than lncRNA at the Xic, the protein-coding *Rnf12* gene (*green*) which encodes an ubiquitin ligase is also known to promote *Xist* upregulation. Pluripotency factors (Oct4, Sox2, Nanog, C-Myc, Klf4, and Rex1) are thought to block *Xist* expression directly or indirectly through *Tsix* activation. During early embryogenesis before implantation, both the X chromosomes are active, and both express the *Tsix* lncRNA, which negatively regulates the *Xist* lncRNA. (**b**) At the onset of XCI during development or ESC differentiation, several events such as decrease in pluripotency factor levels (OCT2, NANOG, SOX2, and REX1), chromosome pairing (involves pairing region), increase in *Xist* activator expression (*jpx*, *Ftx*, and *RepA*), and induction of mono-allelic expression of *Tsix* facilitate coordinated induction of *Xist* upregulation at XIC of random chosen future Xi chromosome from one of the two X chromosomes. At the Xic of the second X chromosome (Xa), Tsix expression is maintained and proposed to be regulated by its *cis* activator lncRNAs (*DXPas34*, *Tsx*, *xite*, *Linx*) which restrict *Xist* expression from Xa. (**c**) Upregulation of *Xist* initiates XIC by coating Xi chromosome in *cis* at Xic that spread to all over the Xi chromosome. Xist coating to the entire chromosome is accompanied by recruitments of DNA and histone modifiers which direct the series of epigenetic modification that progressively silence most of the X-linked genes

embryo—a hallmark of Hox genes. While invertebrates have one set of Hox genes, vertebrates typically have four sets referred to as HoxA, HoxB, HoxC, and HoxD clusters due to the events of genome duplication during the course of evolution [75]. More recently many kinds of regulatory RNAs that are involved in Hox gene regulation have been discovered. Large body of work in the last decade has discovered and studied the noncoding RNAs in the Hox clusters, and most of them have been found to be long intergenic noncoding (linc)RNAs. The expression of these intergenic transcripts correlates with transcription of neighboring Hox genes. Very often these lincRNAs show syntenic or positional conservation between mouse and humans suggesting a common function. Here we discuss the current understanding of the role and mechanism of action of some these lincRNAs in different mammalian Hox clusters. Table 6.4 enlists various lincRNAs involved in regulation of mammalian Hox genes.

Table 6.4 lncRNAs involved in regulation of mammalian Hox genes

lncRNA	Expressed in	Transcribed from	Function	Refs
HoxA				
Halr1 and *Halr1-os*	Human, mouse ESCs	Region between *Hoxa1* and *Skap2*, ~50 kb from the 3′ end of *Hoxa1*	Retinoic acid-dependent regulation of HoxA genes	[76, 77]
Haunt (HoxA upstream noncoding transcript)	Human ESC, NPC, and NSC	40 kb upstream of HoxA cluster	Attenuates enhancer–promoter contacts acting as RA-dependent brake during ESC differentiation	[78]
HOTAIRM1 (Hox antisense intergenic RNA myeloid 1)	Human, mouse, and other mammals	Transcribed antisense to *Hoxa1* from a shared promoter between *Hoxa1* and *Hoxa2*	Specific to myeloid lineage and involved in granulocyte maturation	[79, 80]
HOXA-AS2 (HoxA cluster antisense RNA 2)	Promyelocytic leukemic cells and neutrophils	Isoforms of 339 to 2045 nucleotides from intergenic region between *Hoxa3* and *Hoxa4*	Induced by IFNγ in PMNs and TNF in NB4 cells for negative regulation of ATRA-induced TRAIL (TNF-related apoptosis-inducing ligand) during myeloid differentiation	[81]
HOXA11-AS	Human and mouse	Antisense transcript from promoter of *Hoxa11*	In gametogenesis. Knockdown results in male and female sterility due to uterine defects and failure of testes to descend from abdomen, respectively	[82]

(continued)

Table 6.4 (continued)

lncRNA	Expressed in	Transcribed from	Function	Refs
HIT18844	Human abdominal tissues like the colon, uterus, and prostate	265 bp from 5′-end of HOXA cluster, ~1.8 kb from Hoxa13	Possess ultra-conserved short stretch that results in secondary structural motif. Function unknown	[83]
HOTTIP (HoxA transcript at the distal tip)	Human and mouse	3.7-kb polyadenylated transcript starting ~330b upstream of *Hoxa13*	Spatiotemporally controls expression of 5′ HoxA genes	[84]
HoxB				
Hobbit1 (HoxB intergenic transcript)	Human and mouse	Intergenic region between *Hoxb4* and *Hoxb5*	Retinoic acid-dependent regulation of HoxB genes	[85, 86]
HoxC				
HOTAIR (Hox transcript antisense intergenic RNA)	Human	2158-bp, polyadenylated transcript from intergenic region between *Hoxc11* and *Hoxc12*	Regulates expression of HoxD genes by acting as a molecular scaffold for binding of LSD1/CoREST/REST complex	[87–89]
HoxD				
Hotdog and *Tog* (transcript from telomeric desert of HoxD cluster and twin of Hotdog)	Mouse	Telomeric region downstream of HoxD cluster	Specific to development cecum, regulation of HoxD genes	[90]
HOXD-AS1	Human	Intergenic between *Hoxd1* and *Hoxd3*	Retinoic acid-induced cell differentiation	[91]

6.2.3.1 HoxA Cluster

Heater is one of the well-studied lncRNA loci in HoxA cluster. The coding potential of this region was discovered during analysis of RNA deep sequencing data in mouse ES cells (ESCs) [76]. Heater region encodes for two lincRNAs—*halr1(linc-Hoxa1)* and *halr1os1*. Studies on the *linc-Hoxa1* (transcribed in opposite direction to *Hoxa1* and is 12 kb long) in mouse ESCs revealed that the *linc-Hoxa1* has three isoforms of which the isoform 1 is most abundant. *Hoxa1* and *linc-Hoxa1* are sensitive to retinoic acid (RA) and single transcript counting showed that their levels are antagonistic to each other. Indeed knockdown of *linc-Hoxa1* increased the level of *Hoxa1* mRNA, but the result was not reproduced when siRNA was used. To solve this mystery, the investigators checked for the levels of *linc-Hoxa1* in the nucleus and cytoplasm using RNA FISH under both experimental conditions. The number of sites of active transcription of *linc-Hoxa1* decreased by the use of antisense

oligonucleotides but not siRNAs, indicating the repression of *Hoxa1* by *linc-Hoxa1* occurs only at the site of its transcription (not by overall cellular abundance) and requires the proximity of these two genes in *cis* (neighboring *Hoxa2* levels were unaffected). Another interesting observation was that only when *linc-Hoxa1* is <10 molecules *Hoxa1* transcripts are detectable highlighting the subtlety of transcriptional regulation by these RNAs. In summary, in the absence of RA, *Hoxa1* adopts a conformation that is physically proximal to *linc-Hoxa1*. Such conformation results in repression of*Hoxa1* transcription by abrogating the binding of RA receptors to retinoic acid response elements (RAREs). When RA is present, it binds to RAREs in the *Hoxa1* promoter and pulls it out from the regulation of *linc-Hoxa1*. This finely orchestrated regulation also requires the presence of protein factor purine-rich element-binding protein B (PURB) that binds to *linc-Hoxa1* as shown in Fig. 6.3a [77]. Thus the Heater region through its multiple RAREs regulates the effect of retinoids on the noncoding transcripts that in turn fine-tunes neighboring Hox gene expression.

Another noncoding transcript known as *HOTAIRM1* was identified in HoxA cluster in human peripheral blood neutrophils of myeloid lineage, hence the name [79]. *HOTAIRM1* is not conserved across species in terms of sequence, but similarly localizing transcripts are present in other species. *HOTAIRM1* preferentially associates with CpG islands near the TSS(s) in all mammalian species. Knockdown of transcript results in lowering of expression of *Hoxa1*, *Hoxa4*, and subtly *Hoxa5*, but not *Hoxa9*, *Hoxa10*, and *Hoxa11* (*cis* effect). *HOTAIRM1* knockdown in all-trans retinoic acid (ATRA)-induced human promyelocytic leukemic cells (NB4) also showed *trans* effect by abrogating G/S cell cycle progression by interfering with CD11b, CD18, and β2 integrin signaling pathways that are involved in granulocyte maturation [80].

The discovery of *HOTTIP* was spurred by the observation that 5′-end of the HoxA cluster in anatomically distal cells (foreskin and foot fibroblast) shows broad H3K4me3 peaks and abundant chromatin interactions in contrary to the H3K27me3 marks and no long-range interactions in proximal cells (lung fibroblast). *HOTTIP* has the presence of bivalent histone marks (H3K4me3 and H3K27me3) indicating its poised regulatory function at the diametrically opposite chromatin domain at 5′-end of HoxA cluster as compared to 3′-end. Knockdown of *HOTTIP* reduced the level of transcripts from distal genes *Hoxa13*, *Hoxa11*, and in lesser severity for more proximal genes *Hoxa10–Hoxa7*. Depletion of *HOTTIP* in distal limb bud of chicken embryos resulted in bending and shortening of distal bony elements. There was an increase in H3K27me3 and overall decrease in H3K4me3 marks over the 5′-end of HoxA cluster. These observations indicate that *HOTTIP* promotes transcription of 5′ *HOXA* genes in *cis*, in a proximity-dependent fashion in distal tissues through the deposition of H3K4me3 marks. *HOTTIP* acts as a regulatory switch at the distal end of HoxA cluster by interacting with WDR5 to recruit MLL complex that activates 5′*HOXA* genes. *HOTTIP* is an elegant example of how noncoding transcript couples 3D genome organization with chromatin landscape to spatiotemporally coordinate the developmental pattern [84] Fig. 6.3b.

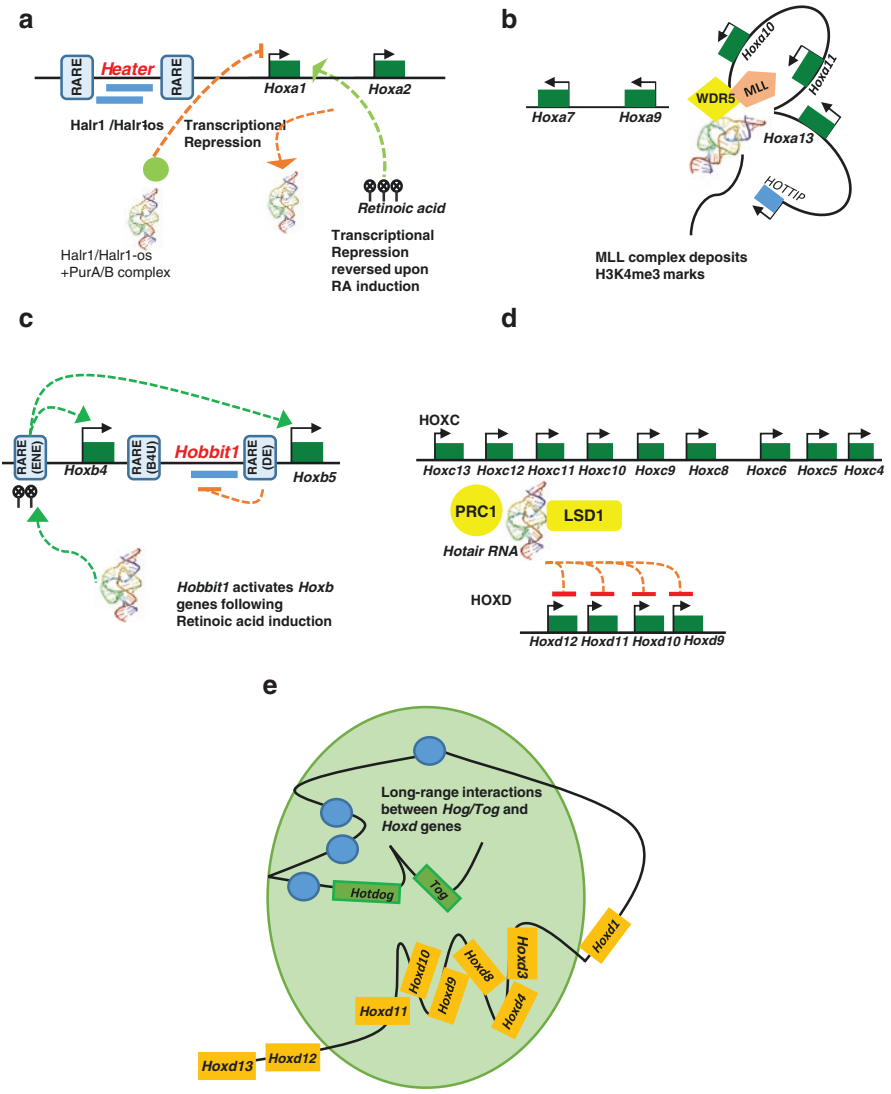


Fig. 6.3 Schematic showing the mechanism of action of Hox lincRNAs. (**a**) *Heater* region encodes two lncRNAs *halr1* and *halr1-os*. In the absence of retinoic acid, *halr1/halr1-os* binds to PURB and transcriptionally represses *Hoxa1*. When retinoic acid is present, it binds to RAREs (retinoic acid response elements) upstream of *Hoxa1* and prevents *halr1* from repressing *Hoxa1* expression. (**b**) *HOTTIP* is a regulatory RNA at the distal end of HoxA cluster that interacts with WDR5 to recruit MLL complex proteins to activate 5′ *Hoxa* genes. (**c**) *Hobbit1* regulates the expression of *Hoxb* genes in the presence of retinoic acid that acts on RAREs present in the regulatory regions. (**d**) *HOTAIR* is transcribed from HoxC cluster and regulates the expression of *Hoxd* genes by acting as a dual molecular scaffold that recruits PRC2 and LSD1/REST/coREST complexes at posterior *Hoxd* genes. (**e**) *Hotdog* and *twin of hotdog*, transcribed from the telomeric desert downstream of HoxD cluster, fold the *Hoxd3–Hoxd11* and the enhancers (*blue circles*) into an active TAD to regulate the long-range interactions necessary for proper gene expression during cecum development

6.2.3.2 HoxB Cluster

Hobbit1, the only prominent lncRNA from HoxB cluster, is a noncoding transcript that activates gene expression following RA induction. Figure 6.3c shows early neural enhancer (ENE) and distal element-retinoic acid response element (DE-RARE) enhancer modulate the expression of *Hoxb* genes in neural crest during rostral expansion maintaining the distinct domains of anterior and posterior *Hoxb* genes [85]. DE-RARE can modulate RA response of *Hobbit1* and anteriorize the 5′ *Hoxb* gene expression. This exemplifies the cross talk of *cis* regulatory DE-RARE with noncoding RNA *Hobbit1* and subsequently the neighboring *Hoxb* genes in response to the developmental cues (RA gradient) during organogenesis [86].

6.2.3.3 HoxC Cluster

HOTAIR is the most widely studied lncRNAs from Hox clusters (HoxC cluster). It was discovered in a microarray study of human Hox loci in primary fibroblast with 11 different positional identities. Knockdown of *HOTAIR* had no effect on HoxC cluster genes on Chr12 but severely affected *HoxD* cluster genes spanning 40 kb on Chr2. This was a remarkable example of noncoding transcripts acting in *trans* [87]. Chromatin immunoprecipitation studies revealed that depletion of *HOTAIR* lowered the levels of H3K27me3 and Suz12 over the HoxD cluster. Later studies showed that *HOTAIR* interacts with Ezh2 (T345) and thus recruits the PRC2 complex at the HoxD [92]. Not only this, *HOTAIR* also acts a dual molecular scaffold, as its 5′-end binds PRC2 complex, while the 3′-end binds LSD1/CoREST/REST complex. Thus it tethers the two complexes to coordinate H3K27 methylation (deposition of repressive marks) and H3K4 demethylation (removal of activation marks) as depicted in Fig. 6.3d [88].

Schoroderet and colleagues deleted the mouse *Hotair* region along with the HoxC cluster with surprisingly no discernable in vivo effects concluding that the long noncoding RNAs have evolved to perform species-specific function [89]. However, in an equally surprising report that followed this study, Li et al. showed that precise *Hotair* conditional knockout results in homeotic transformation of spine and metacarpal carpal bones. Interestingly depletion of *Hotair* also affects many other non-Hox genes including those from imprinted loci like *Dlk1-Meg3* and *Igf2-H19* [93]. Reanalysis of Schoroderet results indicated that *HoxCΔ* resulted in upregulation of all other *Hox* genes and removed genes that function antagonistic to *Hotair* leading to compensation of the deletion. The drastic difference in results of *HoxCΔ* knockout and *Hotair* knockdown on using different experimental approaches highlights the need of fine-scale experimentation to study intricate regulation of Hox lincRNAs.

HOTAIR mis-expression has also been implicated in many cancers [94–100] as it has been reported to have multiple protein partners like proteins involved in cytoskeletal and respiratory chain function [101].

6.2.3.4 HoxD Cluster

In HoxD cluster, *Hotdog* and *Tog* (*Hotdog*, lncRNA from telomeric desert of HoxD cluster; *Tog*, twin of *Hotdog* in opposite direction) are the noncoding transcripts specific to developing cecum. They arise from the telomeric region downstream of HoxD cluster. In cecum, *Hoxd9*, *Hoxd10*, and *Hoxd11* are highly expressed, while *Hoxd12* and *Hoxd13* are repressed. These two lncRNAs were discovered while trying to understand how closely spaced genes in HoxD cluster maintain a distinct chromatin/expression domain. Chromosome conformation capture and chromatin immunoprecipitation studies within the HoxD cluster showed that *Hotdog* and *Tog* fold the expressed Hox genes along with their enhancers and the telomeric desert in an active topological domain (H3K4me1/3 marked) as in Fig. 6.3e. The domain allows their precise expression during cecum budding. The repressed Hox genes remain out of the active domain. When the telomeric gene desert was separated from the HoxD cluster by chromosome inversion, the HoxD cluster genes were silenced, but the noncoding transcripts were still produced. Deletion of region from *Hoxd9* to *Hoxd11* abrogates this spatial configuration and abolishes *Hotdog/Tog* transcription as well. These results suggest a model of long-range enhancer sharing, and *Hotdog*/*Tog* are the example of how noncoding transcripts coordinate long-range interactions connecting distal regulatory elements [90].

6.2.4 lncRNA in Tissue Development and Organogenesis

Embryonic stem cells make cell-fate choices by gene regulatory programs. Terminal differentiation of cells results in patterning of tissues. The functionality of tissue throughout the life is maintained by adult stem cells. Functional studies have revealed that lncRNAs play an active role in gene regulation at virtually every stage of progression of differentiation of ESCs, namely, cell cycle, pluripotency, differentiation, cell survival, apoptosis, etc. They coordinate exit from pluripotency to terminal differentiation. After tissue differentiation, they have emerged as an important class of regulator for maintenance of adult stem cells [102].

Differentiation of skin is a regulated multistep process. Skin differentiation is well characterized at molecular level, to the extent that skin tissue can be regenerated ex vivo and grafted in vivo. It, thus, provides a robust system, where role of lncRNAs could be investigated. Two lncRNAs, *ANCR* and *TINCR*, expressed in epidermal tissue progenitor cells, play a crucial role in epidermal differentiation. They exhibit antagonistic function, where *TINCR* (terminal differentiation-induced noncoding RNA) promotes differentiation, and *ANCR* (antidifferentiation noncoding RNA) inhibits differentiation [103, 104]. *TINCR* is a cytoplasmic lncRNA expressed at low levels in epidermal progenitor cells. Its expression is induced during differentiation. A 25-nucleotide region in *TINCR (TINCR*-Box) interacts with a RNA-binding protein staufen1 (STAU1). The resulting *TINCR*-STAU1 complex mediates stabilization of many mRNAs that encode for proteins required for

differentiation of keratinocytes. Accordingly, downregulation of TINCR in human squamous cells leads to carcinoma. lncRNA *ANCR* on the other hand enforces undifferentiated cell state within the epidermis.

A few lncRNAs are responsible for maintenance of two different states. For example, lincRNA known as *TUNA* (a.k.a. *megamind* in zebrafish) is a highly conserved noncoding RNA expressed in cells of neural lineages in the adult brain, spinal cord, and eyes. It has been observed that under different cellular contexts, by virtue of its unique protein partners, *TUNA* may maintain pluripotency of ESCs or, in a contrasting role, coordinate neural lineage commitment. This is possible because *TUNA* operates through multiple molecular mechanisms at transcriptional or posttranscriptional level [105].

Recently generation of KO animal models have been used to elucidate the role of lncRNA in tissue patterning. For example, deletion of complete lncRNA *Hotair* in mouse led to skeletal deformities and homeotic transformation including abnormalities in the wrist and spine [93]. The *Mdgt* KO mice showed abnormally low body weight and slower growth. KO of *Fendrr* or *Peril* led to peri-/postnatal lethality of the animal [106]. Apart from generation of KO models, extensive characterization of ESCs has revealed the role of many lncRNAs involved in enforcement of pluripotency in these cells. The common mechanisms of action employed by these noncoding RNAs though remain the same. A vast majority of them act via interacting with chromatin modifiers including the readers, writers, and erasers of histone marks. A subset acts as competing endogenous RNA by "sponging" out miRNAs. Table 6.5 lists lncRNAs involved in mammalian tissue development and organogenesis.

6.3 lncRNA in Nuclear Architecture and Chromosome Structure Maintenance

Genomes are hierarchically folded into complex higher-order structure that gives rise to chromatin fiber, chromosomal domains, and condensed chromosomes during cell division. In interphase nuclei, chromosomes occupy distinct territory that can be defined as the nuclear space taken up by the particular chromosome. During cell division, chromatin further gets compacted into distinct X-shaped condensed chromosome, where centromere and telomere play an important role to maintain its integrity. The higher-order organization of chromatin is directly linked to gene regulation, and any defect in the organization perturbs gene expression causing diseases. Several diseases arise as a result of aberrant chromosome numbers (aneuploidy) and chromosome instability during cell division. The role of specific proteins in chromatin organization is well established and lncRNAs have now emerged as new players in this domain. lncRNAs have been found to be an integral part of this global phenomenon of higher-order chromatin organization/modulation and chromosome structure maintenance. In this section we discuss the role played by lncRNAs in nuclear organization.

Table 6.5 List of lncRNA involved in tissue development and organogenesis

lncRNA	Expressed in	Function	Refs
Skin development			
TINCR	Human	Epidermal differentiation by posttranscriptional mechanism	[104]
ANCR	Human	Suppresses differentiation pathway to maintain epidermal adult stem cell	[103]
Blood development			
LincRNA-EPS	Mouse Hematopoietic organs	Prevents apoptosis in erythroid differentiation	[107]
alncRNA-EC1	Mouse Fetal liver erythroid cell	Regulates erythropoiesis (enhancer-associated RNA)	[108]
alncRNA-EC7	Mouse Fetal liver erythroid cell	Regulates expression of *Band3* (enhancer-associated RNA)	[108]
DLEU2	Human and mouse	Regulates erythropoiesis and B cell maturation Represses of *SPRYD7* gene	[108, 109]
elncRNA-EC1	Mouse	Involved in erythroblast differentiation	[108]
lincRNA-EC9	Mouse	Involved in erythroblast differentiation	[108]
Eye development			
Tug1	Human, mouse, dog, cow, and rat Retinal cells	Involved in cone photoreceptor specification Associates with PRC2	[110]
RNCR2 (MIAT/ Gomafu)	Mouse Retinal cells	Involved in retinal cell specification	[111]
Six3	Mouse Eye and retinal cells	Involved in neural specification in ES cells of the retina and eye (promoter-associated RNA)	[112]
Vax2os	Human, other primates, and mouse Retina (outer neuroblastic layer)	Involved in retina cell specification Regulates cell cycle progression of photoreceptor progenitor cells in ventral retina	[113, 114]
Cardiac development			
aHIF	Human	Associated with cardiac pathology (Hypoxia-inducible factor 1A antisense RNA)	[115]
Kcnq1ot	Human	Involved in cardiogenesis Regulates chromatin reorganization at imprinted loci	[116]
ANRIL	Human	Involved in atherosclerosis, carcinomas, and inflammatory response Interacts with CBX7 of PRC1 complex (antisense noncoding RNA in the INK4 locus)	[117, 118]

(continued)

Table 6.5 (continued)

lncRNA	Expressed in	Function	Refs
SENCR	Human	Regulation of endothelial differentiation from pluripotent cells Controls the angiogenic capacity of human umbilical vascular endothelial cells (HUVEC) (Cytoplasmic lncRNA)	[119, 120]
LIPCAR	Human	Biomarker for myocardial infarction (Mitochondrial lncRNA)	[121]
CARL	Human	Inhibits anoxia-mediated mitochondrial fission and apoptosis Acts as mir-539 sponge (Cardiac apoptosis-related lncRNA)	[122]
Mhrt (Myheart)	Human Adult heart	Protects against cardiomyopathy A chromatin-remodeler and antagonizes Brg1 (myosin heavy-chain-associated RNA transcript)	[105]
MIAT	Human	Regulates diabetes mellitus-induced microvascular dysfunction Regulates expression of vascular endothelial growth factor and miR-150-5p (myocardial infarction-associated transcript)	[123–126]
Braveheart	Mouse Cardiac cells	Regulates cardiovascular lineage commitment Epigenetic regulator that interact with Suz12	[127]
Fendrr	Mouse	Involved in differentiation of multiple mesenchyme-derived tissues Associates with PRC2	[128, 129]
Immunological development			
TMEVPG1	Human and mouse peripheral blood lymphocytes (NK+ cells, CD4+ and CD8+ T lymphocytes)	Involved in immunity modulation	[130–132]
NeST	Mouse	Involved in immunity modulation Regulates IFNγ transcription	[133]
lncDC	Human	Controls dendritic cell differentiation Binds to STAT3	[134]
ZFAT-AS	Human CD19+ B cell	Regulates B cell function and implicated in autoimmune thyroid disease	[135, 136]

(continued)

Table 6.5 (continued)

lncRNA	Expressed in	Function	Refs
THRIL	Human	Regulates TNFα expression, immune response, and inflammation (heterogeneous nuclear ribonucleoprotein L-related immune-regulatory long noncoding RNA)	[42]
PCAER	Human	Modulates immune response Prevents binding of p50 subunit of repressive NF-κB complex to COX-2 promoter (p50-associated COX-2 extragenic RNA)	[137]
KIR-AS	Human Hematopoietic progenitors Myeloid precursors	Controls gene expression in progenitor cells (killer cell immunoglobulin-like receptor—antisense)	[138]
PRINS	Primates	Keratinocyte stress response and psoriasis pathogenesis (Psoriasis-susceptibility-related RNA gene induced by stress)	[139]
Neuronal development			
AK055040	Human	Involved in neuronal differentiation Interacts with polycomb group proteins (Promoter-associated RNA)	[140]
AK091713	Human	Involved in neurogenesis Overlaps with miRNAs like Mir125B and LET7A	[140]
AK124648	Human ES cells	Involved in promoting pluripotency and neuronal differentiation (promoter-associated RNA)	[140]
CDKN2B-AS/ ANRIL	Human Many cell types	Involved in atherosclerosis, carcinomas, and inflammatory response Interacts with CBX7 of PRC1 complex (antisense noncoding RNA in the INK4 locus/CDKN2B antisense RNA)	[117, 118]
BACE1-AS	Human and mouse	Positive regulator of BACE1 expression target for anisomycin-mediated suppression of ovarian stem cell cancer	[141]
BC1/BC200	Human Nervous system—dendrites	Regulates synaptogenesis Interacts with FMRP and translational machineries to regulate spatially restricted synaptic turnover	[142]
BDNF-AS	Human Nervous system—neurites	Controls development of neurite elaboration (Natural antisense transcript)	[142]

(continued)

Table 6.5 (continued)

lncRNA	Expressed in	Function	Refs
CDRas1	Human and zebrafish	miRNA decoy and circular RNA	[143]
Cyano	Zebrafish	miRNA (miR-7) decoy	[144]
DALI	Human Neuroblastoma cells	Controls neural differentiation by direct interaction with POU3F and DNMT1	[145]
Dlx1AS	Human	Controls neural differentiation (enhancer-associated RNA)	[146–148]
Evf2/Dlx6AS	Mouse Central nervous system	GABAergic interneuron specification Interacts with transcription factor DLX and methyl-CpG-binding protein MECP2 to epigenetically regulate expression of interneuron lineage genes	[149, 150]
GDNF-OS	Human	Transcriptional regulator of *GDNF* (promoter-associated RNA)	[142, 151]
GOMAFU	Human and mouse Dividing neural stem cells and neurons	Inhibits amacrine cell specification and Muller glia differentiation Interacts with splicing factors to regulate alternative splicing of several neuronal genes	[152, 153]
Kcna2AS	Human	Involved in causation of pain and hypersensitivity Inhibits Kcna2 expression that leads to decreased voltage-gated potassium current and increased membrane potential	[154]
Linc-Brn1a and *LincBrn1b*	Mouse Neural stem cell	Differentiation of neural stem cells and cortical neuron development Regulates basal cortical progenitor turnover	[106]
Linc00299	Human All tissues, predominantly brain	Involved in neurodevelopment, particularly brain development	[155]
Linc00237	Human	Causes MOMO (macrosomia, obesity, macrocephaly, and ocular abnormalities) syndrome	[156]
Peril	Human Brain and spinal cord	Controls the cell cycle, energy metabolism, and immune response genes Transcribed from 110 kb downstream of Sox2	[106]
MSN1PAS	Human	Involved in synapse development	[157]
MALAT1	Human and mouse Neurons	Involved in synaptogenesis and synapse formation Recruits splicing proteins to transcription sites	[158, 159]

(continued)

Table 6.5 (continued)

lncRNA	Expressed in	Function	Refs
Megamind	Human, mouse, zebrafish	Involved in brain morphogenesis and eye development	[144]
Neat1	Human and mouse	Induces of paraspeckle formation (Architectural lincRNA)	[160]
Pantr2/BRN1B	Human and mouse Brain	Regulation of differentiation of delaminating neural progenitor cells	[161]
Paupar	Neuroblastoma cells	Interacts with Pax6 to regulate cell cycle and differentiation	[162]
PNKY	Human and mouse Brain	Regulates neural stem cell turnover Balances self-renewal and differentiation of neural stem cells by regulation splicing regulator PTBP1	[163]
RMST	Mouse ESCs	Involved in neural differentiation Recruits Sox2 to neurogenesis-promoting genes	[140]
Sox2dt	Mouse Brain	Regulates Sox2 expression in neurogenic regions of the brain (enhancer-associated RNA)	[164]
SIX3OS	Human Eye	Specification of photoreceptors, bipolar cells, and Muller glia through SIX3 target genes	[112]
TUG1	Human Retinal cells	Retinal cell-type specification and proliferation	[110]
TUNA	Mouse and zebrafish	Recruits RNA-binding proteins (NCL, PTBP1, and hnRNP-K) to neural gene promoters	[165]
utNgn1	Mouse	Involved in neocortical development Regulates transcription of neurogenin (enhancer RNA transcript)	[166]
VAX2OS1	Human Retinal cells	Involved in retinal cell-type specification and proliferation	[114]
Skeletal muscle development			
Yam-1	Mouse Myoblasts	Regulator of myogenesis Muscle-associated lincRNA positively regulated by YY1 and represses muscle differentiation genes like myogenin (YY1-associated muscle lncRNA)	[167]
Linc-MD1	Human and mouse Myoblasts	Regulator of myogenesis Competing endogenous RNA that acts as a sponge for miR-133	[168]

(continued)

Table 6.5 (continued)

lncRNA	Expressed in	Function	Refs
Other organogenesis			
Mdgt	Mouse	Involved in embryonic development Transcribed from a region close to Hoxd1	[106]
Manr, *linc-Cox2*	Human and mouse Lungs	Involved in organogenesis of lung	[169]
FIRRE	Human	Controls topological organization of multiple chromosomal region Tethers inactive X chromosome to nucleolus	[170, 171]

6.3.1 Interphase Chromatin

That RNA is a significant component of nuclear architecture is known for the past four decades [172]. Early studies have conceptualized nuclear matrix (NuMat—a skeletal framework in the nucleus) as a scaffold predominantly made up of RNA and protein components. NuMat serves as a platform for virtually all nuclear processes, namely, DNA replication, repair, RNA transcription, and splicing. Moreover it is suggested that NuMat plays a fundamental role regulating gene expression [173]. NuMat is sensitive to RNase, indicating critical role of RNA in formation of the structure. Studies in recent years have shown that repeat-containing lncRNAs are involved in building up of the nucleo-skeleton. This phenomenon appears to be conserved across species. For example, *AAGAG* repeat-containing lncRNA is an important component of *Drosophila* NuMat, which when depleted leads to lethality at larval stages [174]. Similarly purine-rich *GAA* repeat-containing lncRNA was found in mammalian cells [175]. Another study provides compelling evidence that RNA transcribed from *LINE-1* interspersed repeats form a significant component of interphase chromatin in human cells. Interestingly interspersed repeat sequences, which account for almost half of human genome, were abundantly transcribed, and the repeat lncRNAs were found associated with euchromatin. Adapter protein SAF-A, by virtue of its DNA- as well as RNA-binding domains, links the *LINE-1*lncRNA to chromatin. The lncRNA stably associates with chromatin and its removal leads to aberrant chromatin distribution and condensation [176]. Thus *LINE-1* lncRNA and other lncRNA species directly associate with chromatin to add to its stability and functionality from a new class of lncRNAs known as chromatin-associated RNAs (caRNAs). However, studies on the role of the NuMat RNA in chromatin architecture and caRNAs are limited, and further explorations are needed to unravel the mechanistic details of the process.

6.3.2 Euchromatin/Heterochromatin

Based on its transcriptional property, interphase chromatin can be distinguished as euchromatin and heterochromatin. Euchromatin is loosely packed, replicates early, and is permissive to transcription, while heterochromatin is compact, replicates

later, and is refractive to transcription. These chromatin states are epigenetically marked by differentially modified histones and DNA methylation. Several lncRNAs are known that mediate these epigenetic changes by recruiting chromatin remodeling complexes to specific loci. For example, the lncRNA *HOTAIR* (described in Sect. 6.2.3) originates in HoxC but silences transcription at HoxD locus in *trans* by recruiting polycomb remodeling complex PRC2 to induce silent chromatin. Very recently, a novel lncRNA CAT7 has been identified in human neuronal cells, responsible for fine-tuning stable gene silencing by guiding PRC1 activity [177]. Other remodeling complexes like MLL and G9a methyltransferases are similarly directed by their associated lncRNAs. Thus a small repertoire of chromatin remodeling complexes with little DNA-binding specificity can be directed to a large number of genomic loci, in a spatially/temporally regulated manner, by the virtue of lncRNA molecules that act as guides. Even at constitutive heterochromatic regions (centromere and telomere), lncRNAs play a role in directing heterochromatin organization.

Apart from heterochromatin, lncRNAs regulate the functionality of euchromatin as well. It is now established that to function effectively, regulatory elements like enhancers, promoters, and boundaries are transcribed. The initial cues to the finding came from a pioneer study where using tiling microarrays the authors found that transcripts arise from beginning and end of protein-coding genes [178]. Follow-up studies confirmed bidirectional transcription from CpG-rich, nucleosome-depleted regions at gene promoters using a separate pre-initiation complex. Such transcription generates transcription start site-associated RNAs (TSSa-RNAs). These TSSa-RNAs regulate transcription initiation events [179, 180]. Such TSSa-RNAs are not only responsible for turning genes on, but at times they are responsible for causing gene-specific repression also. For example, in human cell lines, DNA damage induces the expression of lncRNA from cyclin D1 promoter, which modulates the levels of RNA-binding protein known as translocated in liposarcoma (TLS). Protein TLS in turn modulates histone acetyl-transferase activity at the loci to silence the neighborhood [181]. Similarly enhancers are transcribed in cells where they are supposed to remain active, and this strategy is used for regulation of key developmental genes [182]. According to a report, in human ESCs, ~19% of lncRNAs are enhancer RNAs (eRNAs) [183]. Interestingly, eRNAs are also often bidirectionally transcribed. Coming to another important class of regulatory elements known as boundaries/insulators, a classical example of lncRNA involved in creating a functional boundary comes from the imprinted H19/IGF2 locus, described in detail in Sect. 6.2.1. While boundaries are technically described as elements that, when present in between, prevent enhancer to promoter cross talk, insulators separate two distinct epigenetically modified chromatin domains, and when present at the junction, they may restrict the spread of heterochromatin into euchromatin. Most of mammalian boundaries and insulators are known to bind to protein CTCF [184]. CTCF further recruits cohesin complex to the loci, and this loading of cohesin is essential for insulator function. Some lncRNAs are known to act as scaffolds that stabilize interaction of CTCF along with other factors to boundaries/insulators. For example, a DEAD-box RNA helicase (p68) associates with lncRNA known as steroid receptor RNA activator (*SRA*). This complex then recruits CTCF to execute insulator function at H19/IGF2 locus [185]. Many a time, transcription of tRNA

genes (tRNAs are also lncRNAs) results in establishment of a boundary [186, 187]. In yet another example, tissue-specific transcription of a retrotransposon repeat at murine growth hormone locus leads to establishment of a boundary that blocks the influence of neighboring repressive chromatin [188]. From these examples it thus becomes evident that lncRNAs function as master regulators that control the functionality of euchromatic regulatory elements.

6.3.3 Genomic Stability

In addition to the lncRNAs mentioned above that directly interact with chromatin, there are other noncoding transcripts that are indirectly involved in maintaining the genomic stability. One such lncRNA known as *NORAD/LINC00657* (noncoding RNA activated by DNA damage) is a highly conserved and abundant transcript present in cytoplasm of human cells (more than 300–1000 copies per cell) [189]. The lncRNA was initially identified for inducing p53-mediated response to DNA damage in mouse and human cells [76]. Later investigations found that targeted inactivation of *NORAD* triggers changes in ploidy level and results in variable chromosome numbers in karyotypically stable human cells. This suggested *NORAD* to have a role in chromosomal stability. *NORAD* has conserved binding sites that sequester the PUMILIO proteins (PUM1/2). PUMILIO are RNA-binding proteins that induce chromosomal instability by repressing mitosis, DNA repair, and DNA replication factors. Interestingly, in the human brain, expression of *NORAD* decreases with increasing age. These studies indicate multiple roles of *NORAD* by alternative mechanisms that are yet to be identified. However, the discovery of *NORAD*–PUMILIO genomic stability pathway has attracted scientific community's attention to explore other unknown lncRNAs involved in genomic stability, maintenance, and their link to chromosomal abnormalities.

6.3.4 Nuclear Compartmentalization

Eukaryotic nucleus is very well compartmentalized at structural and functional level by mechanisms that are conserved across species. The mammalian nucleus contains discrete subnuclear bodies that carry out specific functions [190]. A distinguishing feature of the nuclear bodies that differentiates them from conventional cytoplasmic organelles is that a lipid membrane does not delimit them. Their structural integrity is entirely maintained by protein–RNA and protein–protein interactions. The nuclear bodies are highly dynamic as they assemble/disassemble during every cell division. They are rapidly formed as a response to specific cellular triggers [191]. Many nuclear bodies form around the site of transcription of lncRNAs. For example, nucleolus forms around site of rRNA transcription and stress bodies form around transcribing satellite III repeats. The lncRNA transcripts at these loci act as

templates to assemble RNA-binding proteins that in turn result in the formation of nuclear bodies. In addition, lncRNAs can function as architectural element away from where they are transcribed. For example, the lncRNA *NEAT1* is a polyadenylated nuclear-retained transcript, essential for the formation of paraspeckles [160]. Its causal role in the formation of paraspeckles is proven as in the absence of its transcription in human ESCs, paraspeckles are not formed [192]. Similarly, over the last decade, many noncoding RNAs like *MALAT1* (for nuclear speckles), *TUG1* (for polycomb bodies), U-snRNAs (for Cajal bodies), and U7-snRNA (for histone body locus) have been found that play an architectural role in nuclear body formation.

6.3.5 Topological Domains

Chromosome conformation capture techniques have revealed that distally located DNA elements come in close proximity in three-dimensional nuclear space. Such contacts are cell and context specific with functional consequences. These contacts define chromatin loops that provide topological framework for co-regulated genes, commonly known as topological domains (TADs) [193]. Latest developments show that lncRNAs play a vital role in chromatin looping. A remarkable example of lncRNAs involved organization of genome into TADs is that of *Firre* (functional intergenic repeating RNA element) in humans and mouse. *Firre* is expressed from a macrosatellite locus in mouse and contains several cohesin- and CTCF-binding sites required for its functionality. Repeat domains in *Firre* through its interaction with hnRNPU (a nuclear matrix component protein) localize across a 5-Mb TAD on X chromosome. By serving as a scaffold, *Firre* mediates intra-chromosomal bridges to define the TADs. Thus *Firre* plays an architectural role in organizing the X chromosome in TADs that have similar expression state [170]. This lncRNA also mediates the X-chromosome tethering to nucleolar surface where the repressive state is maintained through H3K27 methylation. Obviously and interestingly enough *Firre* itself escapes X-inactivation.

6.3.6 Centromere

Centromeres are specialized structures for proper segregation and equal partitioning of chromosomes during cell division. Centromere is functionally divided into two distinct domains, the core domain which specifies kinetochore formation and its flanking pericentric heterochromatin. DNA at the pericentric heterochromatic region is rich in α-satellite repeats. Core centromeric domain is characterized by the presence of histone H3 variant CENP-A. The pericentromeric heterochromatin contains H3K9 and DNA methylation and associates with heterochromatic protein HP1. Observations suggest that lncRNA transcribed from the satellite repeats lead to heterochromatin establishment as well as proper kinetochore assembly.

Involvement of lncRNA in organization of centromere can be seen across all phyla, from plants, yeast, and invertebrates to vertebrates [194–196]. For example, maize centromeric repeats transcribed from both strands yields 900 nucleotide long transcripts that bind to CENP-A ortholog CENH3 [197]. Frog centromeric repeat (Fcr1) noncoding RNA of ~175 nucleotides is required for normal Aurora-B (kinase) localization to centromere and kinetochore formation in *Xenopus* [198].

In mouse cell lines (MS5 and C2C12), transcripts of up to 4 kb from minor satellite repeats at centromere have been detected under normal physiological condition. These transcripts accumulate during stress or differentiation. Forced accumulation or ectopic expression of the transcripts cause impaired centromeric function and chromosome segregation defects [194]. Human centromeric α-satellite repetitive DNA is transcribed by RNA polymerase II to produce noncoding RNAs of variable size containing repetitive unit 171-bp nucleotides [195, 199]. These α-satellite transcripts have functional importance in chromosome stability and centromere regulation as the RNA is essential for localization of the proteins CENP-C and INCENP to centromere. The centromeric proteins are sequestered in nucleolus during interphase and relocated to centromere during mitosis. That RNA is responsible for proper localization of these proteins is confirmed by RNaseA treatment where RNA depletion abrogates their nucleolar and centromeric localization [195]. Further human studies have found that knockdown of α-satellite induces abnormal mitosis and formation of "grape-shaped" nuclei. The α-satellite transcripts recruit CENP-A and its chaperone HJURP into centromeric chromatin. In addition, α-satellite transcripts can also interact with Shugoshin (Sgo1)—a cohesin protein chaperone that binds and protects cohesin at inner centromere. It is an essential effector for maintaining centromeric cohesion, which if lost prematurely may result in mitotic disruption [200, 201].

Although the role of centromeric proteins in kinetochore assembly and chromosome segregation is well established, the α-satellite transcribed repeat lncRNAs have now emerged as new players in this domain. As these transcripts arise from pericentromeric heterochromatin, their abnormal accumulation reflects derepression of heterochromatin. Such a scenario is indicative of disease and stress. Higher abundance of α-satellite transcripts has been reported in pancreatic and epithelial cancers. Whether it is the cause or a consequence of global heterochromatin, derepression during cancer is a matter still under investigation [202].

6.3.7 Telomeres

Apart from centromeres, telomeres are special structures at chromosome ends that are vital for its integrity and stability during cell division. Telomeres have been termed as the cellular clocks that determine the replicative lifespan of normal somatic cells. This is because cellular senescence is associated with a gradual shortening of telomere length. Telomere shortening results because of limitations of semiconservative DNA replication machinery that cannot fully replicate the end of

a linear DNA. A specific ribonucleoprotein complex containing the enzyme telomerase (TERT) is required for DNA replication at the chromosome ends for telomere length homeostasis. TERT is a reverse transcriptase, which elongates telomeric DNA using associated RNA molecule as a template [203]. Majority of human cancer cells possesses active telomerase in contrast to normal somatic cells that have undetectable telomerase activity [204–206]. The RNA component of the telomerase complex is a lncRNA known as *TERC* (telomerase RNA component) that serves as the template for telomeric repeat synthesis and scaffold for assembly of associated factors. *TERC* knockout mice show short telomere, chromosomal instability, and premature aging suggesting its important role [207].

In addition to *TERC*, another novel lncRNA, *TERRA* (telomeric repeat-containing RNA), has been identified in mammals that are transcribed from sub-telomeric regions by RNA polymerase II and have variable lengths ranging between 100 and >9000 nucleotides [208, 209]. *TERRA* molecules play critical role in telomere maintenance as they regulate telomerase activity and heterochromatin formation at chromosome ends. One of the roles of *TERRA* is to recruit proteins including H3K9me3, HP1, and chromatin remodeling factors to promote heterochromatin formation at chromosome ends [210]. The other role envisaged for *TERRA* is in proper capping of the chromosome ends by binding to shelter in proteins (TRF1 and TRF2). Association of *TERRA* to telomeres is not only because of RNA–protein interaction, but recent evidences show that *TERRA* transcripts base pair with template DNA forming RNA–DNA hybrids known as R-loops that are important for telomere stability [211]. As *TERRA* participates in capping, it prevents activation of DNA damage response (DDR) at chromosome ends. In replicating cells lacking telomerase, telomere shortens with every cell division, eliciting a DDR that results in cellular senescence. *TERRA* actively prevents DDR by recruiting lysine-specific demethylase (LSD1) and chromatin remodeling factors to the telomere [212].

Disruption of nuclear organization correlates with diseased states and in some cases the lncRNA has been found to be the aberrant molecule. For example, in an autosomal dominant disease known as facioscapulohumeral muscular dystrophy (FSHD), loss of lncRNA *DBE-T* results in topological reorganization of the locus derepressing several genes [213].

6.4 lncRNA Etiology in Human Diseases and Disorders

As lncRNAs express in a precisely regulated pattern that is related to development/function, it makes sense that their mis-regulation or mutation would cause disease/disorder. Cancer, which poses a big challenge toward community health in the twenty-first century, is still unconquered. Most cancers arise due to somatic/germline mutations that result in loss of cellular homeostasis. Recent evidences suggest that most of these mutations lie in genomic regions that lack protein-coding capacity but express lncRNAs. Indeed genome-wide association studies (GWAS) and comparative transcriptomic studies have associated lncRNAs with cancer as well as

several other diseases. Almost half of all traits associated with SNPs in GWAS occur in intergenic sequences and only a small fraction lie in exons [214]. Another study found that lincRNAs are more than fivefold enriched for SNPs than non-expressed intergenic regions which indicates the functional significance of these lncRNAs [215].

The growing awareness of lncRNA regulatory mechanisms and the mechanism of action of lncRNA themselves offers useful therapeutic targets. It is possible to manipulate the levels of these lncRNAs in vivo, using interventions such as treatment with antisense oligonucleotides (ASO). One such example can be seen in a study where in a murine model of Angelman syndrome, ASO-based silencing of disease causing lncRNA *Ube3a-ATS* leads to activation of Ube3a. This restoration of Ube3a activity caused recovery from cognitive deficits associated with the syndrome [216]. Such use of ASO in treatment of a diseased murine model is a step forward toward use of lncRNA-based therapies in treatment of challenging diseases like cancer. To this end, exploratory results obtained using cancer cell lines, mouse models, and nonhuman primates have been very promising. Table 6.6 shows a list of lncRNA associated with various diseases and disorders. We have excluded lncRNAs associated with cancers as they are discussed in a separate chapter in the book.

Table 6.6 lncRNAs associated with human diseases/disorders/syndromes

Disease/disorder/syndrome	lncRNA	Refs
Immune system diseases and syndromes		
Systemic lupus erythematosus	*FNDC1*, *TAGP*, *SOD2*, *WTAP*, *ACAT2*	[217]
Rheumatoid arthritis	*Multiple lincRNAs*	[218]
Kawasaki disease	*THRIL*	[42]
Thyroid disease	*SAS-ZFAT*	[219]
Sézary syndrome	*SeCATs*	[220]
Skin diseases		
Psoriasis	*PRINS (psoriasis-susceptibility-related RNA)*	[139]
Melanoma	*BANCR*, *SPRY4-IT1*	[221, 222]
Developmental disorders and syndromes		
FSHD (facioscapulohumeral muscular dystrophy) syndrome	*DBE-T*	[213]
Brachydactyly Type E	*DA125492*	[223]
Immunodeficiency, centromeric region instability, facial anomalies, dyskeratosis congenital, aplastic anemia, idiopathic pulmonary fibrosis	*TERRA*	[224]
Pseudohypoparathyroidism, McCune–Albright syndrome	*NESP-AS*	[34]
Transient neonatal diabetes mellitus	*HYMAI*	[225]
Klinefelter's syndrome	*XIST*	[226]

(continued)

Table 6.6 (continued)

Disease/disorder/syndrome	lncRNA	Refs
Neurodevelopmental disorders, syndromes, and neural diseases		
Fragile X syndrome	*FRM4 (FMR1-AS1)*, *BC1*	[227, 228]
Schizophrenia	*BDNF-AS*, *Gomafu*, *DISC-2*, *Evf2*	[152, 229]
Prader–Willi syndrome	*SNORD116 (HBII-85)*, *C/D box cluster*, *ZNF127AS*	[230, 231]
Angelman syndrome	*UBE3A-AS*	[232]
Autism spectrum disorders	*ST7OT1*, *ST7OT2*, *ST7OT3*, *PTCHD1AS1*, *PTCHD1AS2*, *PTCHD1AS3*, *SHANK2-AS*, *BDNF-AS*, *MSNP1-AS*	[157, 233, 234]
Rett's syndrome	*AK087060*, *AK081227*	[235]
Microphthalmia 3 syndrome	*SOX2OT*	[164]
2p15-p16.1 microdeletion syndrome	*FLJ16341*	[236]
Down syndrome	*NRON*	[237]
Alzheimer's disease	*BACE1-AS*	[238]
Beckwith–Wiedemann syndrome	*H19 and KCNQ1OT1 l*	[239, 240]
Silver–Russell syndrome	*H19*	[239]
McCune–Albright syndrome	*NESP-AS*	[34]
Neuropathic pain	*KCNA2-AS*	[154]
Cardiac diseases and disorders		
Heart failure	*Mhrt*	[105]
Cardiac hypertrophy	*CHRF*, *Novlnc6*	[241]
Myocardial infarction	*MIAT*, *LIPCAR*	[121, 123]
Spectrum of cardiac disorders	*FENDRR*, *Braveheart*, *CARL*, *KCNQ1OT*, *MALAT1*	[105]
Blood and circulatory system disorders and syndromes		
HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome	*HELLP*	[242]
Atheromatosis and atherosclerosis	*ANRIL*	[243]

6.5 Concluding Remarks

Soon after the announcement of human genome sequence, it became clear that protein-coding region constitutes only a tiny minority of the whole genome. Nonprotein-coding DNA increases with increasing evolutionary complexity, and surprisingly most of these sequences are transcribed. Investigations have revealed that many classes of lncRNAs are transcribed from ~75% of human genome that was previously regarded as nonfunctional "selfish DNA" and part of evolutionary junk-yard. Interestingly the protein toolkit of organisms has remained the same through billion years of evolution. For example, human and mice share 99% of their protein-coding genes. The phenotypic diversity appears to have been achieved primarily by modular use of a subset of the proteome. Thus spatial and temporal control of gene

expression is instrumental in driving evolutionary diversity and lncRNAs have been found to play a key role in the process. This even challenges the central dogma where RNA was just thought to be a passive messenger between DNA and proteins and has brought regulatory role played by lncRNAs to center stage.

Deep transcriptomic analyses have begun to rediscover the RNA world and its relation with organismal complexity. Present evidences argue that evolutionary complexity results due to interactions of few and fairly similar proteins whose expression is spatially and temporally controlled by regulatory RNA network. The primary basis of higher complexity thus lies in the variation and expansion of this regulatory network. The regulatory network represented largely by lncRNAs is more plastic than the protein-coding sequences that are constrained by strict structure–function relationship. Any sequence variation in protein-coding region (mutations) can be toxic and thus deleterious, giving rise to severely compromised phenotype. But sequence variation in regulatory regions is often tolerated with mild consequences and no discernable phenotype. This "mutation" versus "variation" in nature provides the raw material for evolution.

Higher eukaryotes employ RNA-mediated regulatory mechanisms to control a plethora of molecular mechanisms. In the nucleus they regulate gene activity via chromatin remodeling, epigenetic processes, RNA transcription, splicing and processing, etc. In cytosol they can effectively control RNA translation, RNA stability, and signaling. They virtually are the primary control axis of differentiation, development, and diseases, and to find out the basis for complex human diseases, it is essential that all lncRNAs are identified, their expression pattern is unraveled, and mechanism of action is elucidated. A deeper transcriptomic analysis of different cells under physiologic and pathologic conditions may pave the way to understand complex human diseases and, thereby, help to improve the quality of human life.

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