

Review

Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression

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A significant portion of the human genome encodes genes that transcribe long nonprotein-coding RNAs (lncRNAs). A large number of lncRNAs localize in the nucleus, either enriched on the chromatin or localized to specific subnuclear compartments. Nuclear lncRNAs participate in several biological processes, including chromatin organization, and transcriptional and post-transcriptional gene expression, and also act as structural scaffolds of nuclear domains. Here, we highlight recent studies demonstrating the role of lncRNAs in regulating gene expression and nuclear organization in mammalian cells. In addition, we update current knowledge about the involvement of the most-abundant and conserved lncRNA, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), in gene expression control.

Overview of Nuclear-Enriched lncRNAs

It is estimated that approximately 75% of the human genome is utilized for generating transcripts with no apparent protein-coding potential, and these transcripts are classified as ncRNAs [1]. lncRNAs are grouped into transcripts that are >200-nucleotides long. The human genome is estimated to contain approximately 16 000 lncRNA genes (statistics from Human GENCODE Release version 27). A significant fraction of nuclear localized lncRNAs are transcribed by RNA polymerase II (RNA Pol II), and share similar chromatin modifications on their regulatory elements with other RNA Pol II-transcribed protein-coding transcripts. Most of the lncRNAs contain normal 5'-caps and 3' poly(A) tails. However, recent studies identified lncRNAs that undergo unusual processing within their 5' and 3' ends, described in detail elsewhere [2,3] (Table 1). In this review, we discuss recent studies on RNA Pol II-transcribed nuclear lncRNAs, but direct readers to [4,5] for more information on non-RNA Pol II-transcribed nuclear lncRNAs (Box 1).

Most lncRNAs are expressed at low levels compared with protein-coding mRNAs, and show tissue or cell type-specific expression. With the rapid growth of lncRNA research, functions of many lncRNAs have been reported recently. A significant fraction of lncRNA is preferentially localized in the nucleus, and several of the well-studied nuclear-retained lncRNAs participate in vital molecular processes, including chromatin organization, transcription, and RNA processing (Table 2) [6–10]. In addition, lncRNAs have essential roles in the organization of nuclear domains [7–10].

Role of Nuclear-Retained lncRNAs in Chromatin Organization

A significant number of nuclear lncRNAs associate with chromatin and, thus, can be broadly classified as chromatin-enriched RNAs (cheRNAs) [11]. Some nuclear lncRNAs can influence chromatin architecture by interacting with chromatin-modulating proteins, such as **Switch/sucrose nonfermentable** (SWI/SNF) (see Glossary) or **Polycomb repressive complex** (PRC) subunits (Figure 1A), promoting their recruitment and/or association to chromatin, thereby controlling transcriptional activity [12–27]. For example, in hepatocarcinoma cells,

Highlights

A significant fraction of lncRNAs is retained in the nucleus, where several of them participate in vital nuclear processes, including chromatin organization, transcriptional and post-transcriptional gene expression, and nuclear structure organization.

The advent of new techniques, including chromatin isolation by RNA purification (CHIRP), capture hybridization analysis of RNA targets (CHART), RNA antisense purification (RAP), and mapping RNA genome interactions (MARGI), provides researchers more opportunity to study the chromatin-binding features of nuclear-retained lncRNAs, especially at the genomic level.

Many nuclear-retained lncRNAs are biomarkers of diagnosis and/or prognosis and/or therapeutic targets of diseases, including cancer.

The development of new computational tools and/or algorithms is required to determine the potential correlation between nuclear-retained lncRNA sequences and/or structures and their functions and/or localization.

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Table 1. Non-canonical processing of Nuclear Noncoding RNAs

Type	Feature	Refs
mRNA-like lncRNAs	5'-capping and 3' poly(A) tails can be spliced	[149,150]
Enhancer-derived ncRNAs	Transcribed from enhancer regions, normally nonpolyadenylated, that could have short half-lives	[60]
Antisense transcripts	Transcribed from divergent promoters, but Pol II does not extend on them as efficiently as on the corresponding sense genes	[151–153]
lncRNAs with noncanonical 3' end	No 3' poly(A) tails, have alternative 3' processing pathways	[154,155]

lncTCF7 facilitates the transcription of *TCF7* by recruiting the SWI/SNF complex to the *TCF7* promoter [26]. In differentiating mouse embryonic stem cells, a pseudogene-derived lncRNA, Oct4P4, is transcribed from the X-linked *Oct4* pseudogene, which induces transcriptional silencing of the ancestral *Oct4* gene by facilitating the deposition of H3K9me3 and HP1a to the *Oct4* promoter [18].

Nuclear lncRNAs can also influence gene expression by preventing the association of specific chromatin factors to specific gene loci. In such a scenario, a lncRNA acts as a 'decoy' and prevents proteins, such as histone deacetylase [28], methyl transferase [29], or chromatin-remodeling complexes [30,31], from interacting with a specific genomic locus (Figure 1B). In adult mice, Myheart (Mhrt) antisense (AS)-lncRNAs, which are transcribed from the myosin heavy chain 7 gene locus, antagonize the function of the chromatin remodeler Brg1 and, thus, protect the heart from pathological hypertrophy [30]. Mhrt uses a 'competitive inhibition mechanism' to prevent the interaction of Brg1 with chromatin, thereby repressing Brg1 activity.

Several lncRNAs influence chromatin organization and/or activity without directly interacting with chromatin or chromatin modulators; they do so through their association with other proteins (Figure 1C). For example, BCAR4 lncRNA activates the Hedgehog/GLI2 transcriptional program in breast cancer cells by influencing p300 histone acetyl transferase activity [32]. BCAR4, by associating with the RNA-binding protein (RBP) SNIP1, releases the inhibitory effect

Box 1. Introduction of Non-Pol II-Transcribed lncRNAs

RNA Pol III is also known to generate several regulatory lncRNAs. Some of the examples include the repeat-containing RNAs, such as Alu and B2 lncRNAs, and the nuclear speckle-enriched 7SK RNA. Human Alu is a repeat-containing transcript of approximately 300 base pairs, and is generated from short interspersed elements (SINEs). Alu RNA is a transcription repressor, because it competes with RNA Pol II, thereby negatively regulating the binding of RNA Pol II at gene promoters [172]. The corresponding SINE-encoded transcript in mice is B2, which is reported to display similar functions to human Alu transcripts [173]. The other Pol III-transcribed RNA 7SK (~330 nucleotides) also negatively regulates RNA Pol II-mediated transcription by affecting the activity of positive transcription elongation factor b (p-TEFb) [174]. Please see [4,175] for detailed descriptions of RNA Pol III-transcribed regulatory lncRNAs.

Promoter-associated RNA (pRNA) is a family of RNAs transcribed by RNA Pol I from the rDNA promoter region located upstream of the pre-rRNA transcription start site [176]. pRNA interacts with members of the Nucleolar chromatin-remodeling complex (NoRC), and utilizes an RNA-dependent mechanism to activate heterochromatin formation and rDNA silencing [176,177].

Plants, such as *Arabidopsis*, utilize RNA Pol V to produce noncoding transcripts from intergenic regions. These transcripts have important roles in regulating heterochromatin formation, defining the boundaries of heterochromatin and gene silencing [178,179].

Glossary

Chromatin isolation by RNA purification (CHIRP), capture hybridization analysis of RNA targets (CHART), RNA antisense purification (RAP), and mapping RNA genome interactions (MARGI): four techniques developed

to map the genomic binding sites of RNA. They are often used to discover the roles and mechanisms of lncRNAs on chromatin.

Nuclear speckle: Speckles are conserved nuclear domains that are present in the form of 10–30 irregularly shaped nuclear structures. Speckles are enriched with RNAs and proteins involved in pre-mRNA processing and mRNP export. Initial studies suggest that speckles act as storage and/or assembly sites of splicing factors, from where these proteins are recruited to active genes dispersed throughout the nucleoplasm. Recent studies further indicate that nuclear speckles act as a structural domain that controls the efficiency and integration of distinct steps in gene expression, ranging from transcription and splicing to mRNA export.

Paraspeckles: a type of subnuclear domain present in the interchromatin space of mammalian cells. They are nucleated by the lncRNA Neat1. The core paraspeckle proteins include PSF/SFPQ, P54NRB/NONO, and PSPC1.

Polycomb repressive complex (PRC): comprises two major types: PRC1 and PRC2. These complexes have important roles in chromatin compaction and transcriptional silencing.

Switch/sucrose nonfermentable (SWI/SNF): a chromatin-remodeling complex. This complex contains multiple subunits, including ATPase, which allows it to remodel nucleosomes from the energy generated through ATP hydrolysis.

X-chromosome inactivation (XCI): a phenomenon observed during mammalian female development, whereby most of the genes are inactivated (transcriptional silencing) on one of the two X chromosomes.

Table 2. Functional categorization of recently identified Nuclear Noncoding RNAs

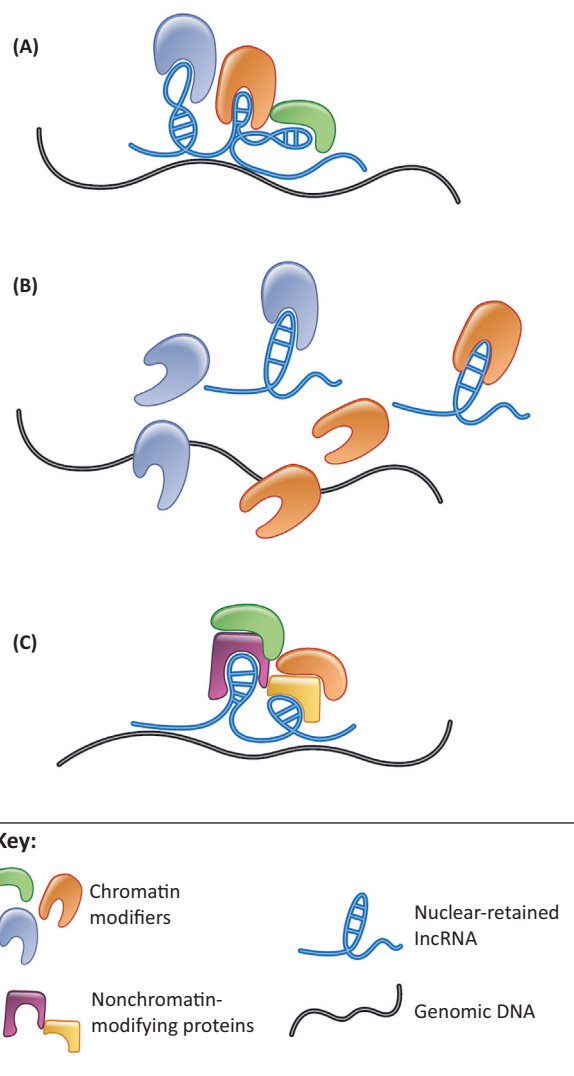
Category	Nuclear-Retained lncRNA Names and Refs
Transcription activation	PINCR [49], MANTIS [25], lncKdm2b [12], Linc-RAM [50], LncPRESS1 [28], upperhand (UPH) [63], HOTAIRM1 [13], RMRP [39], Linc-RoR [29], HoxBlinc [14], DEANR1 [156], MEG3 [23], lncTCF7 [26], myheart (mhrt) [30], BCAR4 [32], HERVH [37], THRIL [40], Dali [20], LincRNA-p21 [157], alncRNA-EC7 [158], PACER [35], Paupar [47], lincRNA-Cox2 (also repression) [53], HOXD-AS1 [54], RBM5-AS1/LUST [38], Evx1as [159], Khps1 [19], TARID [160], TERRA [34], SLERT [56]
Transcription repression	HAUNT/Gm15055 [22,44], lincRNA-EPS [51], PAPAS [17,24], PARTICLE [15], TUG1 [161], Oct4P4 [18], Evf2/Dlx6as [162], lncRNA-CD244 [163], PANDA [52], APTR [145], ANRASSF1 [164], H19 [21], SchLAP1 [31], lincRNA-Cox2 (also activation) [53], GNG12-AS1 [165], APOA1-AS [45], AS1DHRS4 [16]
Post-transcriptional regulation	HOTAIR (ubiquitylation) [166], Linc-RoR (c-myc stability [167] and p53 translation [168]), asFGFR2 (splicing) [27], Pnky (splicing) [64], MALAT1 (pre-mRNA splicing) UCA1 (p16 ^{INK} stability) [169], lincRNA-p21 (translation) [170]
Other regulations:	HAUNT/Gm15055 (DNA locus) [44], Xist (XCI) [9], Neat1 (paraspeckle and pri-miR processing) [9,80], Firre (nuclear organization) [48,144], DDSR (homologous recombination) [171], TERRA (telomere stability) [34], AluRNA (nucleolar structure) [58]

of SNIP on p300 to enhance the acetylation of H3K18 on GL12 target gene promoters. BCAR4 further recruits another RBP, PNUITS, to acetylated H3K18, ultimately resulting in the activation of GL12 target genes. In this scenario, BCAR4, by interacting with RBPs, influences signal-induced epigenetic regulation of transcription of GL12 target genes.

With the development of new techniques, such as **chromatin isolation by RNA purification** (CHIRP), **capture hybridization analysis of RNA targets** (CHART), and **RNA antisense purification** (RAP), all of which determine genome-wide chromatin association of lncRNA [33], one could anticipate that more insights into the chromatin association properties and/or chromatin-modulating functions of lncRNAs will be identified. For instance, by performing genome-wide RNA mapping analyses, a recent study identified that the telomere-associated lncRNA TERRA binds to several other sites on chromatin and modulates the transcription of genes via antagonizing the activity of the RNA helicase ATRX [34].

lncRNAs as Transcriptional Regulators

lncRNAs activate or repress transcription (summarized in Table 1) by acting locally [near the sites of their transcription (*cis*-regulation)] or distally [at sites that are located on other chromosomes (*trans*-regulation)] (Figure 2A). For instance, lncKdm2b sustains the maintenance of intestinal group 3 innate lymphoid cells (ILCs) by facilitating the transcriptional activation of a transcription factor (TF), zfp292 [12]. lncKdm2b facilitates the recruitment of chromatin organizer protein Satb1 and the nuclear remodeling factor (NURF) complex to the regulatory elements of zfp292. In another example, the low-irradiation-induced lncRNA promoter of MAT2A-antisense radiation-induced circulating lncRNA (PARTICLE) represses MAT2A expression by forming a DNA–RNA triplex at the MAT2A locus, and by recruiting transcription-repressive complex proteins G9a and SUZ12 (a subunit of PRC2) to the MAT2A promoter for methylation [15]. lncRNA PACER, which is transcribed from the antisense strand of the COX2 gene, promotes the transcription of COX2 by sequestering the repressive p50 subunit of NF- κ B away from the COX2 promoter [35]. It is not clear why p50 shows increased affinity for PACER over the COX2 promoter, although *in vitro* studies may provide more insights into this differential affinity.



Trends in Genetics

Figure 1. Role of Nuclear-Retained Long Noncoding (lnc)RNAs in Chromatin Organization. (A) Nuclear-retained lncRNAs modulate chromatin by recruiting chromatin-modulating proteins to chromatin. (B) Nuclear-retained lncRNAs can decoy chromatin-associated proteins away from chromatin. (C) Nuclear-retained lncRNAs can form indirect interactions with chromatin modulators through other kinds of protein, eventually modulating chromatin.

lncRNAs also regulate transcription by influencing TF activity. A recent study identified a role for a nuclear lncRNA in regulating the basal levels of tumor suppressor protein p53 [36]. In colorectal cancer cells, PURPL lncRNA, by associating with the p53 stabilizing protein MYBBP1A, prevents MYBBP1A-p53 complex assembly, thereby destabilizing the cellular pool of p53. In this case, PURPL modulates transcription indirectly by regulating the levels of a major TF, p53. Recent studies identified several more examples where lncRNAs regulate transcription by influencing the localization and/or activity of TFs [37,38]. It is tempting to speculate that several hundreds of the not-yet-characterized nuclear lncRNAs contribute to gene regulation by modulating the association of TFs or co-factors to chromatin in a cell- or tissue-specific manner.

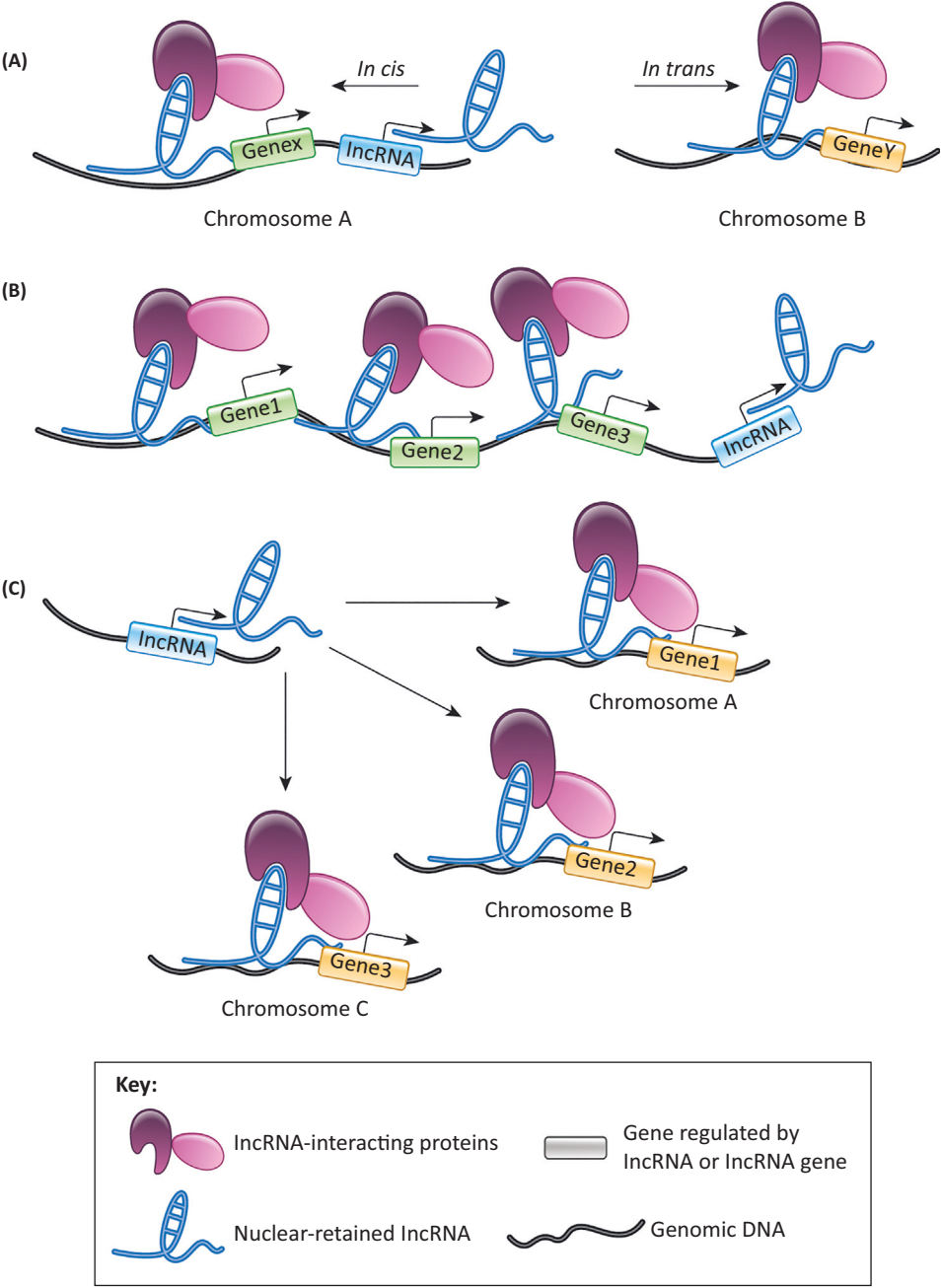


Figure 2. Nuclear-Retained Long Noncoding (lnc)RNAs Act as Transcriptional Regulators. (A) Nuclear-retained lncRNAs modulate transcription *in cis* (left) or *in trans* (right). (B) Nuclear-retained lncRNAs regulate the expression of multiple genes (gene 1, 2, and 3 in the model) that are in close proximity. (C) Nuclear-retained lncRNAs regulate the transcription of several distally located genes (gene 1, 2, and 3 on different chromosomes in the model).

RBP s constitute a class of proteins that interact with single-stranded or double-stranded RNAs and influence post-transcriptional regulation of gene expression. Interestingly, several RBPs also act as transcriptional regulators, and lncRNAs are found to modulate their activity. In T helper 17 (TH17) lymphocytes, the lncRNA RMRP interacts with the dead-box RNA helicase DDX5 and promotes the interaction between DDX5 and the RAR-related orphan nuclear receptor (ROR γ t) transcription factor [39]. DDX5 functions as a co-activator to facilitate ROR γ t-mediated transcription of TH17 genes. RMRP-depleted cells displayed reduced interaction between DDX5 and ROR γ t and defective transcription of selective TH17 genes, supporting the argument that RMRP acts as a scaffold to facilitate the interaction between an RBP and a TF. In another example, lncRNA THRIL-hnRNP L RNP complex facilitates the transcription of several immune response genes, including that encoding TNF α , in macrophages [40]. Similarly, the lncRNA-p21-hnRNP K complex promotes the transcription repression and pluripotency of genes in the p53 pathway [41,42].

In several instances, lncRNAs modulate the expression of multiple genes. Such lncRNAs can be broadly classified into two groups, based primarily on the genomic location of the genes regulated by the lncRNAs. In the first group, the lncRNA or its loci regulate the expression of multiple genes, which are located in close proximity (Figure 2B). These lncRNAs interact with chromatin, transcription factors, or RBPs, thereby increasing their local concentration near the transcription sites of lncRNAs [43]. Besides the lncRNA, these proteins also interact with genes or RNAs that are present in genomic proximity to potentially regulate their expression. In other instances, lncRNAs actively participate in the recruitment of these proteins to the nearby genomic loci [13,14,16,22,37,44,45]. Finally, transcription from the lncRNA gene locus but not the lncRNA itself could have an important role in regulating the expression of nearby genes. Genetic manipulation of a large number of gene loci, including those of lncRNA and protein-coding genes, revealed that transcription from a particular genomic locus, not necessarily involving the RNA that is synthesized from that locus, could have a crucial role in regulating the expression of nearby genes [46].

In the second group, lncRNAs can control the expression of both locally and distally located genes (Figure 2C). Paupar lncRNA, transcribed from a locus upstream of the Paired Box 6 (PAX6) gene, regulates the expression of PAX6 as well as several other genes located on distant chromosomes in a transcript-dependent manner [47]. Paupar was found to associate with approximately 3000 chromatin sites, and a significant fraction of those sites overlapped with functional elements, such as promoters. Depletion of Paupar compromised the expression of approximately 1000 genes, many of which contained Paupar-binding sites within their regulatory elements. Firre is another nuclear-enriched lncRNA that is found to act both locally and distally [48]. Firre occupies a chromatin area near its transcription site on the X chromosome and also interacts with additional domains located on four other chromosomes. It is thought to act as a scaffold to coordinate the association of these chromosomal loci, because genetic deletion of Firre leads to loss of interaction between these chromosomal loci.

lncRNAs also regulate the expression of genes belonging to specific pathways and/or biological processes [21,23,25,28,31,32,38,39,49–54]. For example, the p53-induced lncRNA *PINC*R promotes the upregulation of a subset of p53 target genes involved in G1 arrest and apoptosis [49]. *PINC*R, along with Matrin 3 and p53, associates with the enhancer region of the candidate genes and modulates the induction of prosurvival genes upon DNA damage. Another lncRNA, *MANTIS*, facilitates endothelial angiogenic function by promoting the expression of a subset of endothelial genes [25]. Mechanistically, *MANTIS* interacts with BRG1 (a subunit of the SWI/SNF chromatin remodeling complex) to collaboratively facilitate the loading

of RNA Pol II onto the endothelial gene promoters. Furthermore, MANTIS improves the ATPase activity of BRG1 by stabilizing its interaction with BAF155. Finally, Firre occupies chromatin regions containing genes involved in energy metabolism and/or adipogenesis, with loss-of-function studies revealing that Firre controls adipogenesis [48]. These studies highlight another important function of lncRNA, which is to bring together unique combinations of chromatin and/or proteins to establish gene networks that regulate a particular cellular pathway.

RNA Pol II-transcribed nuclear lncRNAs have also been shown to regulate the activity of other RNA polymerases within the cell. For instance, lncRNAs such as PAPAS and SLERT regulate the activity of RNA Pol I. Promoter and pre-rRNA antisense (PAPAS) lncRNAs comprise a group of RNA Pol II transcripts that are transcribed in the antisense orientation from a fraction of rDNA repeats [55]. PAPAS recruits suv4-20h2 histone methyl transferase to rDNA gene promoters to mediate rDNA repression by triggering H4K20me3 and chromosome compaction [17,24]. By contrast, small nucleolar (sno)RNA-ended lncRNA enhances preribosomal RNA transcription (SLERT) is synthesized from the intronic region of the *TBRG4* locus by undergoing unusual processing [56]. SLERT is a member of the recently identified family of sno-lncRNAs [57]. SLERT lacks a canonical 5' cap or 3' poly(A) tail, but contains a H/ACA type of sno-RNAs on either end, and is guided to the nucleolus by the snoRNA sequences. In the nucleolus, SLERT interacts with DDX21, and releases the inhibitory interaction between DDX21 and RNA Pol I machinery, thereby positively regulating rDNA gene transcription [56]. In addition, a recent study reported that AluRNAs that are processed from intronic Alu elements of RNA Pol II-transcribed genes modulate rDNA transcription by influencing nucleolar structure [58].

Some of the enhancer sequences within the genome are transcribed to produce nuclear RNAs, such as enhancer RNAs (eRNAs), elncRNAs, and ncRNAa [59,60]. In general, eRNAs are found to regulate the expression of nearby protein-coding genes in an RNA-dependent manner. Some eRNAs facilitate mediator- and/or cohesion-mediated enhancer-promoter interactions through chromatin looping or activate promoter-mediated transcription. For example, upon estrogen treatment, eRNAs transcribed from ER α -bound enhancers facilitate enhancer-promoter looping of several ER α target genes. Besides such *cis* regulation, eRNAs also participate in long-range intrachromosomal interactions to regulate gene expression [61,62]. Intrachromosomal interactions between TFF1 and NRIP eRNA gene loci were observed due to chromosomal looping in cells treated with 17 β -estradiol (E2), and such interactions appear to be regulated by NRIP eRNA. In the case of eRNA-mediated gene regulation, one needs to be cautious in deciphering whether eRNA transcripts or the transcription from the eRNA-bearing gene loci have vital roles in regulating gene expression. In that sense, some eRNAs could turn out to be by-products of pervasive transcription events simply due to transcriptional activity around enhancer regions. For example, transcription of Upperhand (Uph) lncRNA from a region upstream of the heart-specific TF *HAND2* gene is required to maintain the superenhancer signature and the RNA Pol II elongation through the *Hand2* enhancer locus [63]. However, only blocking of *Uph* transcription (not the depletion of the mature *Uph* transcript) inhibited *Hand2* transcription, implying that, in the case of the *Uph*-*Hand2* interaction, only the noncoding transcription is important in establishing a permissive chromatin environment to facilitate *Hand2* transcription.

lncRNAs as Post-Transcriptional Regulators

It is becoming increasingly evident that nuclear-restricted lncRNAs also regulate gene expression by influencing post-transcriptional events. The antisense-FGFR2 lncRNA promotes epithelial-specific alternative splicing of FGFR2 pre-mRNA [27]. AS-FGFR2 facilitates

the recruitment of Polycomb-group proteins and histone demethylase KDM2a to the *FGFR2* regulatory elements, thereby preventing the association and activity of a repressive-splicing adaptor complex that promotes mesenchymal-specific splicing of *FGFR2* pre-mRNA. This study uncovers the role of an lncRNA in regulating cell type-specific alternative splicing by modulating the underlying chromatin structure. The neural-specific lncRNA Pinky (Pnky) inhibits neurogenesis by modulating PTBP1-mediated alternative splicing of a set of genes that control neurogenesis [64]. Finally, several studies have identified the involvement of oncogenic nuclear lncRNA MALAT1 in alternative splicing regulation (see the section below on MALAT1 for further details). The prostate cancer-specific lncRNA PCA3, synthesized as an AS-lncRNA from the *PRUNE2* locus, negatively regulates *PRUNE2* mRNA levels in an adenosine-to-inosine (A-to-I) editing-dependent manner [65]. Recent studies documented the role of A-to-I editing in influencing the stability of RNA [66]. However, it needs to be determined how exactly A-to-I editing regulates the stability of *PRUNE2* mRNA. Finally, recently identified SPA lncRNAs, which are 5' snoRNP-ended and 3' polyadenylated transcripts, are suggested to modulate RNA processing by sequestering several RBPs, including TDP43, RBFox2, and hnRNP M [67].

Role of Nuclear-Retained lncRNAs in the Organization of Nuclear Structure

X-inactive specific transcript (Xist), one of the first functionally annotated nuclear lncRNAs, regulates dosage compensation by promoting **X-chromosome inactivation** (XCI). Xist is idealized by the scientific community as a perfect example of a nuclear lncRNA, because it coordinates several nuclear processes to achieve XCI. For example, discrete regions within Xist RNA are required for gene silencing, for the association of PRC2 to the inactive X chromosome (Xi), and for the localization of Xist to the X chromosome [68]. Xist interacts with several protein complexes, such as SHARP and PRC2, and recruits them to Xi to modulate epigenetic changes that favor X chromosome transcriptional gene silencing [69–71].

Recent studies also provided compelling data supporting the role of Xist in modulating the 3D architecture of Xi [72]. During the initial stages of XCI, the entire Xi is repositioned near the nuclear lamina (the nuclear compartment that is associated with transcriptionally inactive heterochromatic regions). Interestingly, Xist directly interacts with the Lamin B receptor (LBR), an inner nuclear membrane-associated *trans*-membrane protein that also interacts with the nuclear lamina. Depletion of LBR or disruption of the Xist–LBR interaction results in defective recruitment of Xi to the nuclear periphery. In addition, in LBR-depleted cells or cells with disrupted LBR–Xist interactions, Xist failed to associate with active genes across Xi, thus abolishing Xist-mediated gene silencing. These results imply that Xist has an active role in reshaping the chromatin structure by recruiting Xi to the nuclear lamina, and such tethering of Xi is required for the efficient association of Xist to transcriptionally active genes to exert gene silencing.

Firre, transcribed from the X chromosome, forms a punctate nuclear compartment containing several chromosomal loci, and facilitates interchromosomal interactions and activation of several genes that are transcribed from these gene loci. Firre interacts with nuclear matrix factor hnRNP U and appears to act as a 'scaffold' to modulate interchromosomal interactions [48].

Finally, lncRNAs are also known to nucleate and/or maintain specific nuclear domains (NDs). NDs are nonmembranous structures that are enriched with a unique set of proteins and RNA. Eukaryotic cells contain several NDs, including the nucleolus, **nuclear speckles**, and **paraspeckles**. Paraspeckles have been implicated in: (i) the nuclear retention of hyper-edited

RNAs: (ii) the sequestration of certain RBPs in the nucleus; and (iii) the immune response [73–75]. Neat1 lncRNA has a vital role in the nucleation and/or maintenance of paraspeckles [76–78]. Paraspeckles are initially formed at the site of Neat1 transcription, which is essential for the formation of paraspeckles. In addition, recent studies also identified the potential involvement of Neat1 in oncogene-induced cellular transformation and pri-miRNA processing [79,80].

Role of MALAT1 in Gene Regulation

MALAT1, also known as nuclear-enriched abundant transcript 2 (NEAT2), is perhaps the most-abundant (~3000 copies/cell) nuclear-retained lncRNA. MALAT1 was initially identified as a prognostic marker for stage I lung adenocarcinoma [81]. MALAT1 is highly conserved among mammalian species [81–84] and its orthologs have been identified in zebrafish, lizard, and *Xenopus* [85,86].

MALAT1 is ubiquitously expressed in all tissues, but its levels are tightly regulated during certain physiological processes, such as the cell cycle [87] and cellular differentiation [88,89], by various transcriptional and post-transcriptional mechanisms. Transcription factors, such as ER/eNOS [90], Sp1 [91], HIF-2 α [92–95], and CREB [96], recognize the MALAT1 promoter and induce *MALAT1* expression under different stress conditions. At the post-transcriptional level, the long half-life of MALAT1 in many cell lines is attributed to its unique 3'-end RNA processing [83,97,98]. The 3' end of the MALAT1 primary transcript forms a tRNA-like structure and is cleaved by RNase P, yielding a 61-nucleotide RNA called 'mascRNA' [99,100]. The rest of the 3' end sequence then folds into a triple-helix structure [101–103], which protects this end against 3'–5' exonucleases [103]. The antisense (AS) lncRNA, TALAM1, was recently shown to form an RNA duplex with the 3' end of MALAT1, which stabilizes MALAT1 by facilitating its 3' processing [104].

Molecular Function of MALAT1

MALAT1 is almost strictly retained in the nucleus [105]. A large fraction of MALAT1 is localized within nuclear speckles [82,83] and interacts with several of the speckle-enriched proteins, including splicing factors. Since MALAT1 preferentially localizes in nuclear speckles, NDs that are suggested to coordinate transcription and pre-mRNA processing, it is proposed that MALAT1 controls gene expression by modulating speckle-ascribed functions, including transcriptional and post-transcriptional gene regulation.

In the nucleus, MALAT1 is found to preferentially associate with transcriptionally active genes [106–108]. MALAT1-depleted cancer cells display reduced expression of cell cycle- or metastasis-associated genes without affecting the processing of their RNAs, implying a direct involvement of MALAT1 in transcriptional regulation [109,110]. MALAT1 might act as an 'anchorage point' for the localization of certain genes near nuclear speckles and facilitate their transcriptional activation (Figure 3A). In support of this, MALAT1-depleted cells showed defects in the: (i) association of cell cycle genes next to speckles; and (ii) expression of these cell cycle genes [111]. Such gene relocalization events partially rely on the interaction between MALAT1 and Pc2 [111]. Pc2 is a member of the PRC1 complex, and its methylation and/or demethylation play a vital role in regulating the expression of E2F target genes. Upon serum stimulation, MALAT1 interacted with unmethylated Pc2 and drove the translocation of this protein along with a group of associated cell cycle genes, from Polycomb bodies to nuclear speckles. The MALAT1-interacting pool of demethylated Pc2 then promoted the SUMOylation of E2F1, resulting in the activation of cell cycle genes [111]. In addition to Pc2, several studies showed direct interactions between MALAT1 and PRC2 components, including EZH2

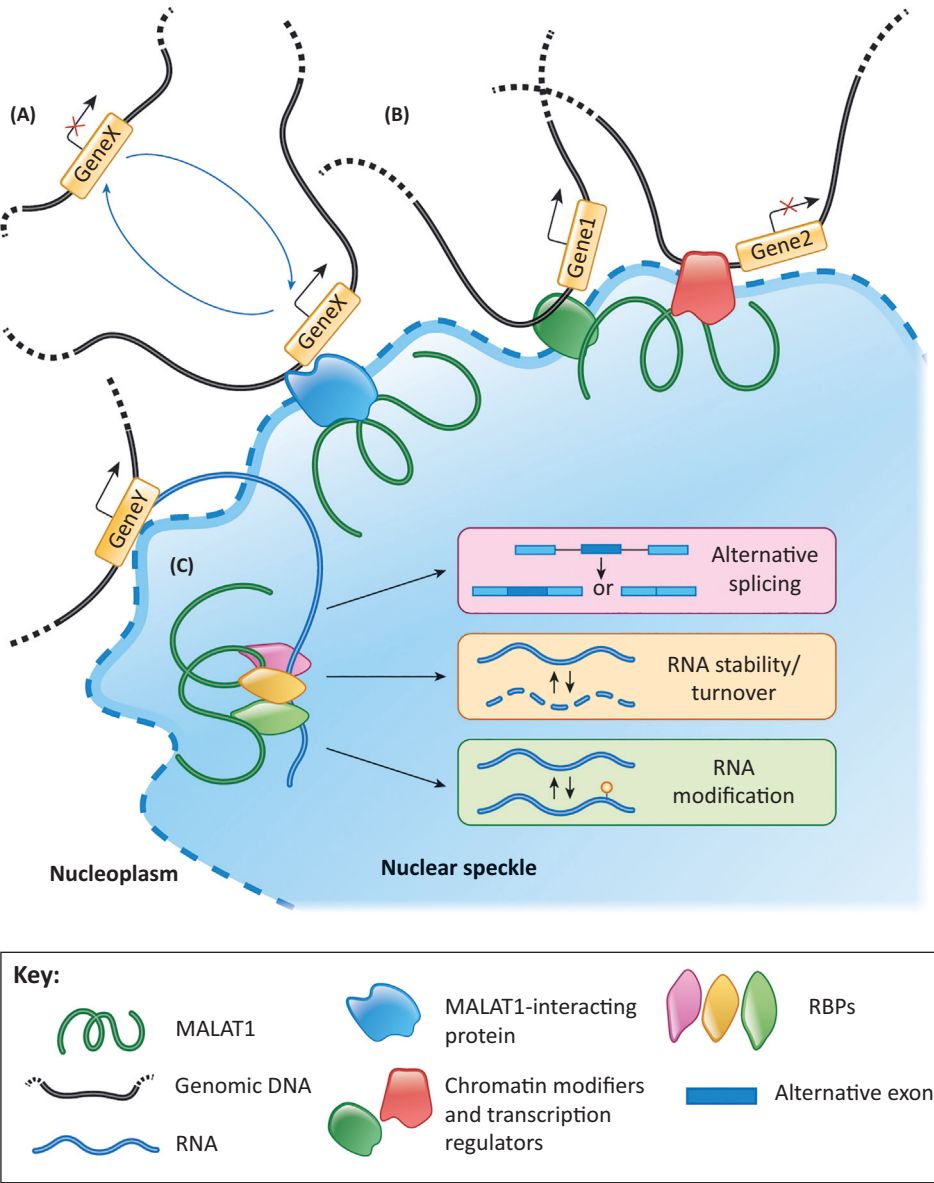


Figure 3. Model Depicting Potential Mode of Action of Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1). (A) MALAT1, along with MALAT1-interacting proteins, such as unmethylated Pc2, relocates genomic loci from the nucleoplasm or other nuclear domains to the nuclear speckle periphery and promotes the activation of corresponding genes. (B) MALAT1 interacts with multiple chromatin modifiers and transcription factors to modulate transcription. (C) MALAT1 serves as a scaffold in nuclear speckles for RNA-binding proteins (RBPs) and influences various co- and post-transcriptional processes.

[112–117] and Suz12 [116,118]. These results imply that MALAT1 promotes the activation or repression of genes by facilitating the recruitment of specific chromatin modulators to target genes in a cell type-specific manner (Figure 3B).

Recent RAP-sequencing (seq) studies revealed that MALAT1 preferentially interacts with alternatively spliced pre-mRNAs in a protein mediator-dependent manner [107]. In addition, MALAT1 interacts with a large number of RBPs, such as pre-mRNA splicing factors, hnRNPs, and RNA export factors [119–129] (Figure 3C). For example, it was shown that MALAT1 interacts with several members of the SR family of splicing factors [83,119–121,130]. SR proteins are multifunctional proteins that participate in several steps of RNP maturation, including pre-mRNA splicing, RNA export, mRNA decay, and translation [131,132]. In human cells, MALAT1 modulates the localization of SR proteins to speckles [120] and transcription sites [83]. Furthermore, MALAT1 expression-altered cells showed changes in the phosphorylation status of SR proteins and displayed defects in the interaction of SR proteins to their target pre-mRNAs [120,133], implying the involvement of MALAT1 in modulating SR protein activity. This was strengthened by the observation that MALAT1 expression-altered cells showed defects in the alternative splicing of several of the SR target pre-mRNAs [87,120,133,134]. The cancer-promoting activity of MALAT1 could also be attributed to its involvement in regulating the alternative splicing of several oncogenic and tumor-suppressor gene pre-mRNAs. For example, in breast and liver cancer cells, overexpression or depletion of MALAT1 altered the splicing of several SRSF1-target gene pre-mRNAs [133–135]. In the case of hepatocellular carcinoma, inhibition of SRSF1 abolished the oncogenic properties of cells overexpressing MALAT1 [133]. Given that SR proteins often display collaborative or sometimes redundant functions, MALAT1 may also regulate the binding kinetics of different SR proteins to fine-tune the processing of certain pre-mRNA targets.

It is still not clear exactly how MALAT1 controls the activity of speckle-localized proteins. It was observed that tethering MALAT1 to a chromatin locus facilitated the recruitment of several splicing factors [130]. Furthermore, MALAT1-depleted human cells showed disassembly of proteins from speckles [120]. By associating with several proteins, MALAT1 could act as a nucleation site to facilitate productive interactions between speckle proteins for the assembly of functional complexes. In addition, MALAT1 interacts with nascent transcripts, preferentially alternatively spliced pre-mRNA of several hundreds of transcriptionally active genes through protein intermediates. Based on these observations, we propose that MALAT1 acts as a ‘molecular scaffold’ to enhance protein–protein, protein–RNA, and protein–DNA interactions in or near speckles (Figure 3). By concentrating in speckles, MALAT1 facilitates the interaction between MALAT1-interacting RBPs, such as SRSF1, and pre-mRNAs of transcriptionally active genes, thereby influencing their post-transcriptional processing. By contrast, MALAT1 can help to reposition a group of genes to the periphery of speckles via protein mediators and influence their expression.

Most of the data ascribing a role for MALAT1 in transcription and pre-mRNA processing were observed from studies using cell lines. However, MALAT1-knockout mice did not display any abnormality in terms of development, viability, or fertility [84,136,137]. There could be several explanations for the absence of phenotype. One possible explanation is that other lncRNAs or alternate cellular pathways compensate for the loss of MALAT1. A candidate for this compensation could be the paraspeckle-localized lncRNA, NEAT1, which is transcribed from the neighboring genomic locus of MALAT1. NEAT1 has been reported to have overlapping behaviors with MALAT1 in aspects of its gene association and protein interaction [106]. Finally, similar to what has been observed for several miRNAs, MALAT1 could have essential roles under specialized conditions, such as pathological stress, and may show significant phenotypes if MALAT1-depleted cells or mice are exposed to such conditions [138]. This hypothesis is supported by the fact that MALAT1 deregulation has been found in increasing numbers of diseases and pathological conditions.

Involvement of MALAT1 in Cancer Progression and Metastasis

Ever since the initial identification of MALAT1 as a marker of metastatic lung cancer, a considerable number of studies have intimately linked MALAT1 to tumor progression and metastasis [139,140]. Elevated levels of MALAT1 are observed in a broad spectrum of cancers, and are frequently correlated with poor prognoses and chemo- or radiotherapy resistance in patients [114,134,141]. Furthermore, alterations in the levels of MALAT1 in multiple cancer cell lines and in animal tumor models significantly affect tumorigenic properties, including cell proliferation, invasion, migration, and metastasis. For example, Malat1-knockout cells or cells depleted of Malat1 by antisense oligonucleotides in a mouse MMTV-PyMT breast cancer model system showed reduced tumor growth and metastasis, accompanied with enhanced differentiation into cystic tumors [142]. It was recently demonstrated that MALAT1 facilitated cell proliferation, tumor progression, and metastasis of triple-negative breast cancer (TNBC) cells [134]. Finally, increased MALAT1 levels were associated with decreased disease-specific survival in patients with the ER-negative, lymph node-negative HER2 and TNBC molecular subtypes [134]. These results identify MALAT1 as a metastasis driver and its potential use as a prognostic marker is better suited for patients with ER-negative, lymph node-negative breast cancer, who might otherwise be classified as having low recurrence risk.

Altered expression of MALAT1 is also a factor in hepatocellular carcinoma development. In this case, MALAT1 was found to act as a proto-oncogene by promoting the oncogenic activity of splicing factor SRSF1, ultimately resulting in the induction of Wnt and mTOR signaling [133]. In the case of breast cancer and hepatocellular carcinoma, MALAT1 depletion altered the expression and pre-mRNA splicing of genes involved in cancer progression and metastasis, indicating that Malat1/MALAT1 regulates processes that are important for cancer pathogenesis [133,134].

Concluding Remarks

The human genome encodes approximately 16 000 lncRNAs, of which a significant fraction is retained in the nucleus. Nuclear lncRNAs are involved in almost all physiological and/or biological and disease-related processes. Most nuclear lncRNAs associate with chromatin and influence gene expression in a *cis* or *trans* fashion. Chromatin-associated lncRNAs control the recruitment or stabilization of various chromatin proteins or RNA-binding proteins to regulatory sequences within the gene or RNA, thereby influencing transcriptional or post-transcriptional processes. However, we still do not know how lncRNAs associate to specific chromatin regions in the genome. Some aspects of specificity could be attributed to the ability of lncRNAs to recognize DNA via forming RNA:DNA hybrids. Alternatively, lncRNA could be recruited to a chromatin site by chromatin-associated proteins. Retention of lncRNAs in the nucleus and their localization to specific nuclear compartments could be controlled by active or passive regulatory mechanisms. lncRNAs, such as Xist and eRNAs, could be retained in the nucleus via their association with chromatin. lncRNAs are also known to contain specific sequence motifs, domains, or repeat sequences within them that potentially act as nuclear retention signals [23,71,143–145]. In addition, association of nuclear proteins to lncRNAs could ensure the nuclear retention of lncRNAs [70,77,146]. Future studies on a large number of nuclear lncRNAs will help to identify the novel mechanisms utilized by lncRNAs for their nuclear localization and chromatin association (see Outstanding Questions).

Our understanding of the physiological role of lncRNAs is limited because of the lack of an adequate number of organismal studies to date. Some recent studies indicate that several lncRNAs have a vital role in embryonic and adult tissue development [147,148]. Future studies aimed at determining the molecular function of the vast number of human nuclear lncRNAs

Outstanding Questions

Several nuclear-retained lncRNAs work *in trans*. How do *trans*-acting lncRNAs transport from their sites of transcription to other gene loci where they act?

Unlike proteins whose nuclear retention signals could be predicted by the existence of a nuclear-localization signal (NLS), the nuclear localization of lncRNAs is controlled by several independent mechanisms. What type of sequence or structural elements within lncRNAs dictates their nuclear retention, DNA recognition, and protein association?

Multiple nuclear-retained lncRNAs are reported to interact with PRC2 or its subunits. However, research now identifies several instances in which PRC2 might interact promiscuously with RNA molecules. Hence, one needs to use more-stringent measures to confirm functional interactions between lncRNAs and Polycomb complex proteins.

The human genome is predicted to contain approximately 16 000 lncRNA (Gencode.org) genes. How many of these lncRNAs have important biological roles and how many are by-products of spurious transcription?

would help us to better appreciate the role of lncRNAs in gene expression and nuclear organization, as well as their potential involvement in diseases.

Acknowledgments

We thank A. Lal and S.G. Prasanth for critical reading and suggestions. We thank J. Roy, S. Sudhakar, and S. Adusumilli for proof reading the manuscript. We thank Elsevier's Illustration service (WebShop) for figure illustration. Work in the Prasanth lab is funded by NIH R01 (GM088252) and NSF EAGER (1723008) grants.

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