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# Cellular functions of long noncoding RNAs

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**A diverse catalog of long noncoding RNAs (lncRNAs), which lack protein-coding potential, are transcribed from the mammalian genome. They are emerging as important regulators in gene expression networks by controlling nuclear architecture and transcription in the nucleus and by modulating mRNA stability, translation and post-translational modifications in the cytoplasm. In this Review, we highlight recent progress in cellular functions of lncRNAs at the molecular level in mammalian cells.**

Approximately 80% of the mammalian genome is transcribed in a cell-specific manner, particularly noncoding regions[1]. Only a small portion of the mammalian genome is transcribed into protein-coding mRNAs, and the vast majority produces numerous long noncoding RNAs (lncRNAs)[2]. lncRNAs comprise various RNA species longer than 200 nucleotides (nt) that are not translated into proteins. These include mRNA-like intergenic transcripts (lincRNAs) (Fig. 1a), antisense transcripts of protein-coding genes[3] (Fig. 1b) and primary RNA polymerase II (Pol II) transcript-derived unconventional lncRNAs whose stabilization can be achieved by RNase P cleavage to generate a mature 3′ end of a U-A-U triple-helix structure[4,5] (Fig. 1c), by capping by snoRNA–protein complexes (snoRNP)[6–8] (Fig. 1d,e) or by forming covalently closed circular structures to prevent exonucleolytic degradation[9–11] (Fig. 1f,g).

Transcriptional regulatory elements such as enhancers and promoters can often initiate Pol II transcription bidirectionally, producing enhancer RNAs (eRNAs)[12] and promoter upstream transcripts (PROMPTs)[13]. Though eRNAs have been reported to have enhancer-like functions[14,15], their depletion did not suppress enhancer activity in multiple cases[12], casting doubt on their function. So far, PROMPTs lack an apparent function; instead, their rapid degradation has been associated with the choice of promoter directionality[13]. Some studies have shown that the act of transcription or the DNA elements within lncRNA loci exert regulatory effects, whereas the produced lncRNAs do not[16–18]. Furthermore, a subset of annotated lncRNAs can be translated into short polypeptides[19,20]. Despite arguments as to whether specific lncRNAs themselves are functional, recent advances in lncRNA research (Table 1) have demonstrated their involvement in different aspects of gene regulation in diverse cellular contexts and biological processes. In this Review, we discuss specific examples of how lncRNAs regulate chromatin organization and transcription in the nucleus and modulate mRNA stability, translation and post-translational modification (PTM) in the cytoplasm (Fig. 2).

## Functions of lncRNAs in the nucleus

Although most annotated lncRNAs are mRNA-like, they are less abundant and less evolutionarily conserved, and contain fewer exons compared to mRNAs[1,2,21]. lncRNAs are generally more nuclear localized than mRNAs[2], in part owing to inefficient splicing and polyadenylation and susceptibility to degradation by exosomes on chromatin[22,23]. Harbouring *cis* elements that are associated with nuclear proteins also prevents their nuclear export[24,25]. A short C-rich sequence derived from *Alu* elements, which are primate-specific short interspersed nuclear elements (SINEs), promotes lncRNA nuclear retention via association with the nuclear matrix protein HNRNPK[26,27]. In addition, lncRNAs with unconventional forms (Fig. 1c–g) accumulate in the nucleus because of their unusual biogenesis pathways and specific *cis* elements[6,7,11,28]. Once localized to the nucleus, lncRNAs can be important regulators of nuclear organization and function (Fig. 2a).

## Roles of lncRNAs in chromatin architecture

Interphase chromosomes are highly organized to achieve coordinated transcriptional regulation[29]. lncRNAs can regulate genome organization at different levels (Fig. 2b–d).

**lncRNAs can regulate chromosome architecture.** In female mammals, X-chromosome inactivation (XCI) occurs to silence one of the two X chromosomes during early embryonic development to achieve dosage compensation. The X-inactive-specific transcript (Xist), transcribed from the future inactive X chromosome (Xi), is strictly localized within the boundary of its chromosome territory across almost the entire Xi[30] and triggers a cascade of events that entails chromosome remodelling to achieve stable silencing with relatively few genes remaining active[31,32]. Accompanying these silencing cascades, the heterochromatic Barr body of Xi is formed through chromosome condensation[30], which is preferentially localized to the perinucleolar compartment and nuclear lamina[33–35]. Comprehensive in-cell Xist-interactome analyses of crosslinked RNA–protein complexes (Table 1) revealed that the lamin B receptor (LBR), an integral component of nuclear lamina, is associated with Xist[36]. In mouse cells LBR depletion or LBR–Xist interaction disruption impaired Xi recruitment to the nuclear lamina and the subsequent Xist-mediated XCI, indicating that Xist may reshape the chromatin architecture by recruiting Xi to nuclear lamina[35]. Such recruitment may constrain chromosomal mobility and enable Xist and its associated silencing complexes to spread across the Xi (Fig. 2b). However, it is worthwhile noting that re-examination[37] of the sequencing data in this study[35] raised inconsistencies regarding the role of LBR in XCI[37], suggesting that this model (Fig. 2b) still warrants further investigation.

In contrast to Xist, some highly repetitive RNAs, $C_0T$-1 RNAs, including long interspersed nuclear elements (LINEs), may act to decompact chromatin. These RNAs are associated with euchromatin and are excluded from Xist-condensed chromatin. Loss of $C_0T$-1 RNAs results in aberrant chromatin condensation, suggesting a positive role in chromatin opening[38].

**lncRNAs modulate inter- and intrachromosomal interactions.** Firre (functional intergenic repeating RNA element) is transcribed from the

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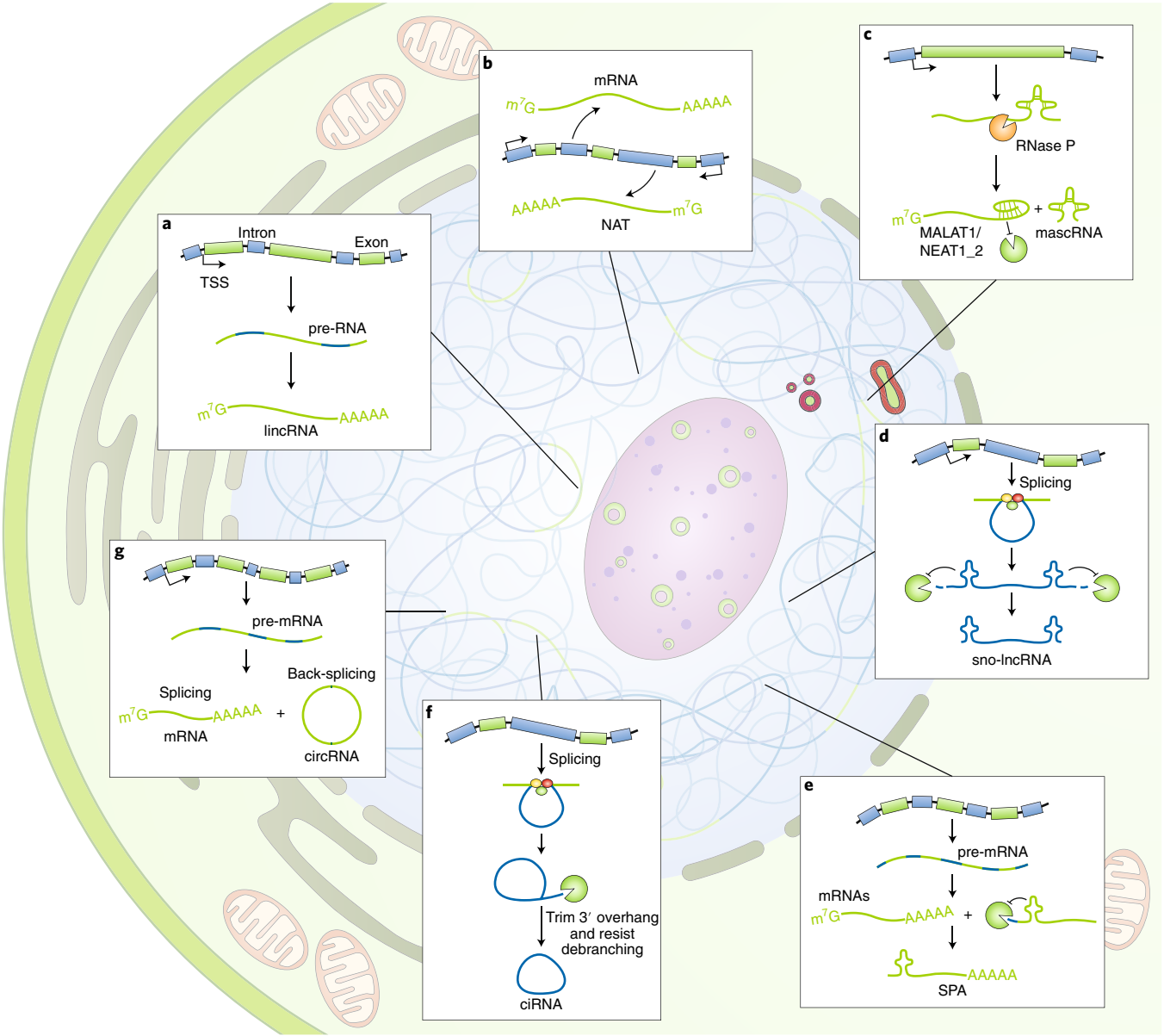


**Fig. 1 | The diversity of lncRNAs in mammalian cells. a**, Large intervening/intergenic noncoding RNAs (lincRNAs), transcribed by Pol II from intergenic regions, are presumably capped, spliced and polyadenylated. **b**, Natural antisense transcripts (NATs) are transcribed from the opposite strands of protein-coding genes by Pol II and are presumably mRNA-like lncRNAs. **c**, MALAT1 and NEAT1_2 are processed by RNase P and stabilized by U-A-U triple helix structures at their 3' ends. Their 3'-end products are further processed to form MALAT1-associated small cytoplasmic RNA (mascRNAs), which are ~60 nt in length and have unknown functions. **d**, SnoRNA-ended lncRNAs (sno-lncRNAs) are derived from excised introns. During splicing, formation of a snoRNP complex at each end protects the intronic sequences from degradation, leading to the accumulation of sno-lncRNAs flanked by snoRNAs but lacking a 5' $m^7G$ cap and 3' poly(A) tail. **e**, 5' snoRNA-ended and 3'-polyadenylated lncRNAs (SPAs) are derived from readthrough transcripts, and their 5' ends are protected by co-transcriptionally assembled snoRNPs. **f**, Circular intronic RNAs (ciRNAs) are derived from excised introns and depend on consensus RNA sequences to avoid debranching of the lariat introns. **g**, Circular RNA (circRNAs) are produced by back-splicing circularization of exons of pre-mRNAs. During splicing, pre-mRNAs can be spliced into mRNAs or back-spliced into circRNAs.

X chromosome and escapes XCI. It contains repeats of 156-nt motifs that bind to hnRNPU[24]. Aside from accumulation near its transcription site, Firre was found in five additional autosomal chromosomal loci through genome-wide mapping in mouse embryonic stem (ES) cells using RAP (RNA antisense purification)[24] followed by sequencing (Table 1). Depletion of Firre or hnRNPU abolished Firre accumulation and these transchromosomal contacts[24], suggesting that Firre functions as a scaffold to modulate interchromosomal interactions (Fig. 2c).

CCAT1-L (colorectal cancer associated transcript 1, long isoform) modulates intrachromatin loops between enhancers and promoters. Transcribed from a colorectal cancer (CRC)-specific super enhancer upstream of the human *MYC* gene, CCAT1-L promotes the transcription and oncogenic effect of MYC[39]. Mechanistically, CCAT1-L exclusively accumulates at its transcription site, interacts with CTCF and facilitates the formation of enhancer–promoter loops at the *MYC* locus[39](Fig. 2d).

## Roles of lncRNAs in chromatin remodelling

Many nuclear localized lncRNAs are associated with chromatin and involved in chromatin remodelling either *in cis* (near their

**Table 1 | Methods for studying cellular roles of lncRNAs**

| Category of the experimental strategies | | Description | Applications in understanding cellular roles of a particular lncRNA of interest |
|---|---|---|---|
| **Genetic manipulation** | Modulate gene expression | Loss of function or gain of function at either DNA or RNA levels | • Zinc-finger nuclease (ZNF)-mediated poly(A) knock-in to silence MALAT1[133] expression<br>• Transcription activator-like effector nuclease (TALEN)-mediated knock-in to activate CCAT1[39]<br>• CRISPR–Cas9-mediated knockout[7,8,17], CRISPR interference (CRISPRi[18,134]) or CRISPR activation (CRISPRa[135,136]) to interfere with gene expression at the DNA level<br>• The programmable RNA-guided RNA-targeting CRISPR–Cas13 system to knock down nuclear retained lncRNAs: HOTTIP[137], XIST[138] and MALAT1[137,138] |
| **Interaction partners** | Identify lncRNA-associated DNA, RNA and protein | Using antisense oligos of a lncRNA to capture its associated DNA, RNA and protein | • Chromatin isolation by RNA purification (CHIRP): HOTAIR[51], roX2[51], TERC[51], XIST[48]<br>• Capture hybridization analysis of RNA targets (CHART): MALAT1[109], NEAT1[109] and XIST[139]<br>• RNA antisense purification (RAP): FIRRE[24], XIST[36,140] and MALAT1[110] |
| | Identify protein-associated lncRNAs | Using antibodies of a protein of interest to identify its associated RNAs | • Crosslinking: Crosslinking and immunoprecipitation (CLIP[141]), Individual nucleotide resolution CLIP (iCLIP[7]), photoactivatable ribonucleoside-enhanced CLIP (PAR-CLIP[124]), enhanced CLIP (eCLIP[142]), etc.<br>• Non-crosslinking RNA immunoprecipitation and sequencing (RIP-seq[7]) |
| **Subcellular localization** | Fluorescence in situ hybridization (FISH) | Using antisense probes with fluorescence to visualize and quantify lncRNA localization in situ | • Regular RNA FISH: using fluorescent DNA or RNA probes to visualize lncRNAs in cells and tissues, for example, SPAs[7], XIST[40,45,140], CCAT1[39], NEAT1[82,88], MALAT1[103]<br>• Single molecule FISH (smFISH): using probes consisting of multiple fluorescent oligonucleotides (usually 25~48 oligos) to visualize and quantify individual RNAs, for example, GAS5 and PVT1, etc[143], Firre[24], NEAT1[28,80,144], and lnc-DC[131]<br>• Large-scale FISH: sequential FISH (seqFISH[145]) and multiplexed error-robust FISH (MerFISH[146]) using combinatorial labeling with encoding schemes to simultaneously measure the copy number and spatial distribution of many RNA species in single cells |
| | RNA tracking | Living cell imaging to visualize localization and dynamics of RNA | • Stem-loop labeling and fluorescence protein tagging by MS2-MCP, PP7-PCP or BoxB-λN systems to enable the visualization of RNAs in live cells, for example, NEAT1[78]<br>• Molecular beacons are hairpin shaped molecules with an internally quenched fluorophore whose fluorescence is restored when they bind to a target nucleic acid sequence. It enables the visualization of NEAT1[147] and HOTAIR[147] with minimal targeted genomic engineering<br>• Targeting RNA in living cells with CRISPR–Cas13[138] and CRISPR–dCas9[148] systems |
| | Microscopic techniques | High resolution microscopy to detect localization details | • Structured illumination microscopy (SIM) reveals a low PRC2-Xist colocalization[45], the subnucleolar localization of SLERT[8] and paraspeckles assembled by NEAT1[80,82] at 100-nm resolution<br>• Stochastic optical reconstruction microscopy (STORM) reveals Xist stoichiometry[46] and Xist cloud size[149] at 20-nm resolution |

A combination of experimental strategies including genetic manipulation, identification of lncRNA–protein complexes, and visualization of cellular localization can be used to understand cellular functions of a lncRNA of interest. See text for details.

transcription sites) or *in trans* (at sites distant from their transcription sites) (Fig. 2e,f).

**lncRNAs promote the recruitment of chromatin modifiers.** The best studied example of the 'recruitment' model is Xist-mediated XCI, extensively reviewed elsewhere[31,32]. Upon transcription, Xist localizes to and remains associated with the future Xi depending on hnRNPU[40,41] and CIZ1 (ref. [42]). A model for Xist-mediated XCI is Xist directly recruiting components of Polycomb repressive complex 2 (PRC2), leading to deposition of histone H3 lysine 27 trimethylation (H3K27me3) chromosome-wide to establish repressive chromatin across Xi[43]. However, recent findings that PRC2 interacts promiscuously with RNA[44] and that PRC2 subunits are sparsely co-localized with Xist under super-resolution microscopy[45,46] (Table 1) are difficult to reconcile with this model.

New techniques and forward genetics have provided more compelling evidence for dissecting the molecular pathway of Xist-mediated XCI. The Polycomb recruitment is likely initiated with the interaction of the noncanonical Polycomb group RING finger 3/5 (PCGF3/5)–PRC1 complex with a 4-kb region immediately downstream of *Xist* exon 1. PCGF3/5–PRC1-mediated ubiquitylation of histone H2A lysine 119 (H2AK119u1) then facilitates the recruitment of other noncanonical PRC1 complexes and PRC2. Pcgf3/5 knockout animals had defective XCI and showed female-specific embryo lethality[47]. Quantitative mass spectrometry (RAP–MS) of Xist-associated proteins captured by purification of crosslinked complexes (Table 1) revealed that Xist directly interacts with the

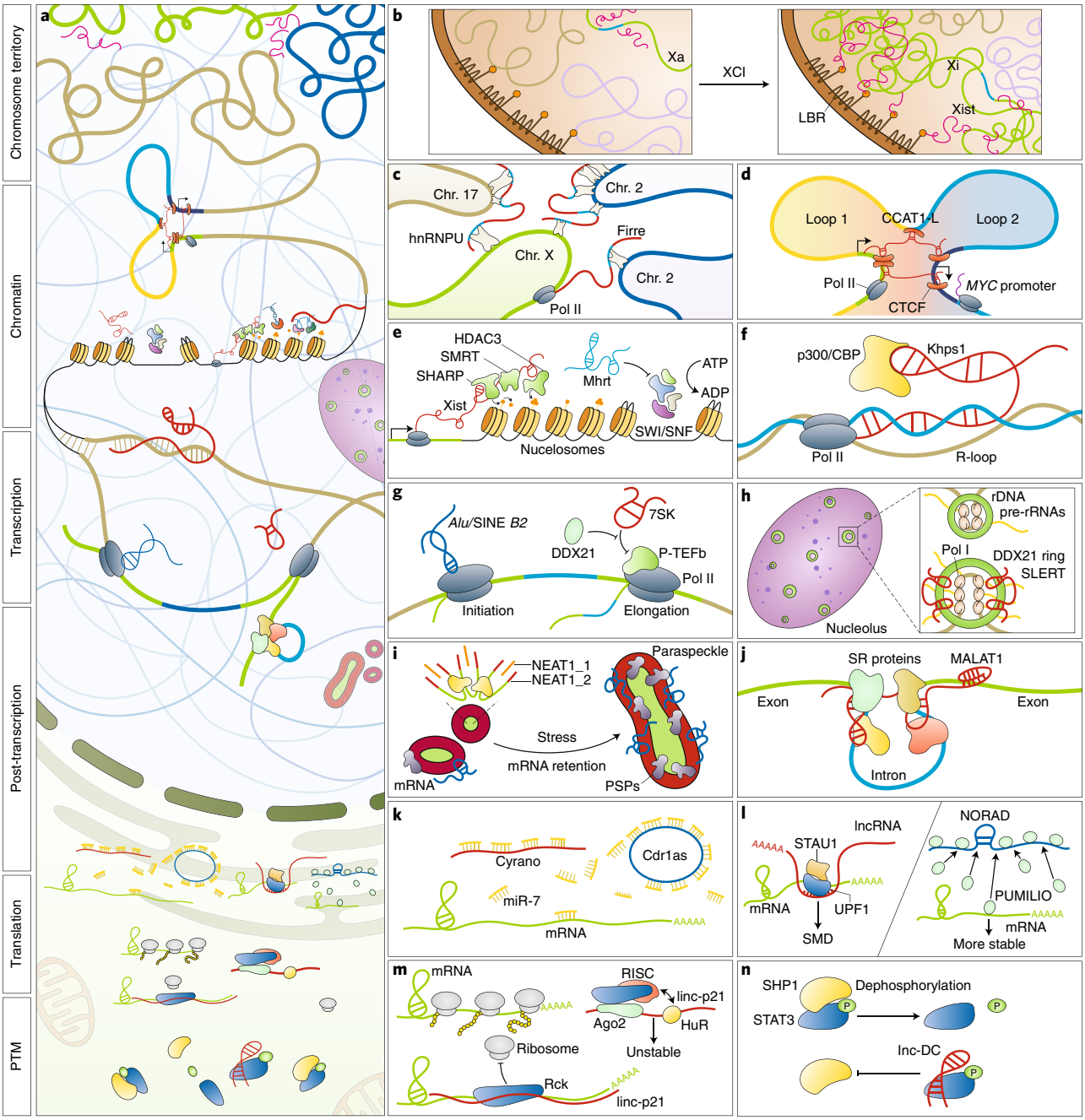


**Fig. 2 | Cellular functions of lncRNAs. a**, A snapshot of cellular functions of lncRNAs with examples shown in **b**–**n**. **b**, Xist modulates inactive X chromosome (Xi) architecture during X chromosome inactivation (XCI) by recruiting Xi to associate with the lamin B receptor (LBR) at the nuclear lamina to silence transcription. **c**, Firre transcripts localize to their transcription site and five additional autosomal chromosomal loci *in trans* to affect interactions between distant genomic regions. **d**, CCAT1-L accumulates *in cis* to modulate chromatin loops between enhancers and the promoter of *MYC*. **e**, lncRNAs regulate chromatin accessibility. Left, Xist recruits HDAC1-associated repressor protein (SHARP), silencing the mediator for retinoid and thyroid hormone receptor (SMART) and HDAC3 to silence Xi. Right, Mhrt prevents SWI/SNF binding to corresponding DNA loci. **f**, Khps1 enhances Pol II transcription by forming an R-loop that anchors Khps1-interacting p300/CBP to the *SPHK1* promoter. **g**, lncRNAs interfere with Pol II transcription machineries both at the initiation (left) and elongation (right) stages. **h**, SLERT promotes Pol I transcription by binding DDX21 to alter its conformation, thereby releasing its inhibitory effect on Pol I. **i**, NEAT1 is an architectural lncRNA that nucleates paraspeckles. Upon cellular stress, altered NEAT1 transcription and processing lead to changes of paraspeckles. PSP, paraspeckle proteins. **j**, MALAT1 interacts with SR proteins and alters their phosphorylation to impact pre-mRNA splicing in splicing speckles. **k**, A regulatory network consisting of different types of ncRNAs. Cyrano, harbouring miR-7 binding sites, targets miR-7 for degradation and prevents miR-7 from repressing its target RNAs including the circRNA Cdr1as. **l**, lncRNAs modulate mRNA stability by associating with proteins involved in mRNA degradation. Left, double-stranded RNAs formed by *Alu*-containing lncRNAs with mRNA 3′ UTRs recruit STAU1 to induce STAU1-mediated mRNA decay (SMD). Right, NORAD stabilizes PUMILIO 1/2 (PUM1/2)-targeted mRNAs via sequestering PUM1/2 from mRNAs. **m**, lncRNAs regulate translation. Association of lincRNA-p21 (linc-p21) with HuR favours the recruitment of let-7/Ago2, leading to its destabilization. In the absence of HuR, lincRNA-p21 identifies mRNA targets to repress their translation by recruiting the translation repressor Rck[129]. RISC, RNA-induced silencing complex. **n**, lncRNAs modulate post-translational modifications. Lnc-DC directly interacts with STAT3 to prevent its dephosphorylation by SHP1.

silencing mediator for retinoid and thyroid hormone receptor (SMART)/histone deacetylase 1 (HDAC1)-associated repressor protein (SHARP). SHARP in turn recruits SMART and its interacting HDAC3, leading to histone deacetylation and transcriptional repression[36,48]. This stepwise recruitment of chromatin-modulating proteins to the future Xi ultimately leads to widespread transcriptional silencing (Fig. 2e). Interestingly, SHARP knockdown had no effect on Xi recruitment to nuclear lamina[35], suggesting that XCI regulation by changes in the chromosomal territory occurs prior to local chromatin modifications.

Another example is Hotair (*Hox* antisense intergenic RNA) expressed from the *HoxC* locus in mammals. It was reported that Hotair suppressed *HoxD* gene expression *in trans* via direct recruitment of PRC2 to the *HoxD* locus[49] and that Hotair loss in mice led to homeotic transformation and derepression of genes including *HoxD*[50]. RNA immunoprecipitation (RIP) and biotinylated RNA pull-down assays (Table 1) revealed interactions of Hotair with PRC2 components[49]. Chromatin isolation by RNA purification (ChIRP) (Table 1) revealed that Hotair preferentially occupied a GA-rich DNA motif correlating with domains of PRC2 occupancy and H3K37me3 (ref. [51]), indicating that an RNA:DNA:DNA triplex may recruit the Hotair–chromatin modification complex to establish a repressed chromatin status. However, controversial discoveries were reported regarding the roles of Hotair in *Hox* gene regulation and mouse development[52–54]. Deleting Hotair[52] or Hotair deletion mutant alleles combined with *LacZ* knock-in[53] independently showed little effect on mouse embryonic development. Furthermore, deleting Hotair had no detectable effect on *HoxD* gene expression in mice[52]; HOTAIR overexpression in breast cancer cells led to subtle transcriptional changes independent of PRC2, and artificial tethering of HOTAIR to a luciferase reporter locus resulted in PRC2-independent repression[54]. These different phenotypes of Hotair knockout mouse models, together with the facts that in vitro binding and RIP experiments can yield false-positive interactions[55] and that PRC2 tends to interact with RNA nonspecifically, suggest that the challenge for the field is to understand what happens in vivo and to explore mechanisms by which Hotair, and other lncRNAs with similar features, act to modulate gene expression.

**lncRNAs prevent the recruitment of chromatin modifiers.** In addition to the 'recruitment' model, lncRNAs can act as 'decoys' to prevent the interaction of histone or chromatin modifiers to specific DNA loci. Mhrt (myosin heavy-chain-associated RNA transcripts) is a cluster of alternatively spliced, nucleus-retained lncRNAs originating from the antisense transcription of the myosin heavy chain 7 gene[56]. Mhrt protects the heart from pathological hypertrophy through antagonizing Brg1 (ref. [56]), the catalytic subunit of the BAF chromatin-remodelling complex known to promote pathological cardiac hypertrophy. Mechanistically, Mhrt sequesters Brg1 from targeting genomic loci through associating with the RNA helicase domain, which Brg1 uses for DNA binding (Fig. 2e)[56].

Another example is lncPRESS1, which is highly expressed in human ES cells and promotes pluripotency. It interacts with SIRT6, a class III HDAC that removes the acetyl group from H3K56 and H3K9, and prevents its presence at pluripotency gene promoters, resulting in high levels of H3K56 and H3K9 acetylation, activating transcription[57].

Importantly, a stoichiometric interaction between *trans*-acting lncRNAs and their interacting proteins is required for a measurable effect of these lncRNAs on the activity or abundance of associated proteins. Thus, the copy number of the lncRNA and its targeted protein(s) should be quantified to determine the feasibility of this type of mechanism.

## Roles of lncRNAs in transcriptional regulation

lncRNAs can directly regulate transcription by forming R-loop structures to recruit transcription factors (TFs) or by interfering with Pol II transcription machineries at targeted loci (Fig. 2f–h). The act of transcription and DNA elements within the lncRNA locus, rather the lncRNA itself, can also contribute.

**lncRNAs regulate transcription by forming R-loops.** Some antisense lncRNAs regulate sense mRNA transcription by forming R-loops (triple-stranded nucleic acid structures with RNA hybridized to duplex DNA). Local formation of R-loops can tether the lncRNA *in cis* and recruit transcription cofactors to corresponding promoter regions. For example, Khps1 is transcribed in the antisense orientation to the proto-oncogene *SPHK1*. Tethering Khps1 to a homopurine stretch upstream of the *SPHK1* transcription start site (TSS) leads to the formation of an R-loop that anchors Khps1-interacting histone acetyltransferase p300/CBP to the *SPHK1* promoter (Fig. 2f). Such recruitment increases local chromatin accessibility, which facilitates E2F1 binding and enhances E2F1-dependent SPHK1 expression and restriction of E2F1-induced apoptosis[58]. In another example, human *vimentin* (*VIM*), a type III intermediate filament gene associated with enhanced cell migration and invasion, harbours a head-to-head antisense transcript, VIM-AS1. VIM-AS1 forms an R-loop near *VIM* TSS. This R-loop promotes *VIM* transcription by inducing local chromatin decondensation to favour NF-κB binding to the promoter[59].

Such *in cis* regulatory mechanism by DNA–RNA base pairing is compatible with generally low levels of antisense transcription-derived lncRNAs, as only two copies of target DNA molecules are present per cell. However, not all lncRNA-mediated R-loop formation is involved in transcriptional regulation. TERRA (telomeric repeat-containing RNA), transcribed at telomeres in a conserved manner from yeast to humans, forms DNA–RNA hybrids at chromosome ends to promote homologous recombination among telomeres and sustains genome stability[60,61].

Finally, not all divergently transcribed lncRNAs act *in cis*. Divergently transcribed from the promoter region of *DDX11*, CONCR (cohesion regulator noncoding RNA) does not affect DDX11 expression, but instead directly interacts with DDX11 and enhances its enzymatic activity as an ATPase and helicase in DNA replication and sister chromatid cohesion[62].

**lncRNAs interfere with Pol II machineries.** Human *Alu* and mouse SINE *B2* RNAs, transcribed by RNA polymerase III (Pol III), can suppress transcription initiation during heat shock[63,64]. *Alu* RNA was found to incorporate into Pol II complexes at promoters and block transcription initiation in a purified Pol II transcription system and in cells (Fig. 2g). *Alu* RNA contains two loosely structured domains that each bind one Pol II molecule and are essential for transcriptional suppression. Once *Alu* is removed from pre-initiation complexes, transcription is restored.

Transcription elongation is also modulated by lncRNAs. Like *Alu* and *B2* RNAs, 7SK RNA is another abundant RNA transcribed by Pol III. It binds with the positive transcription elongation factor b (P-TEFb) and suppresses its kinase activity, which is required for Pol II elongation[65] (Fig. 2g). As part of the 7SK RNA–protein complex, the DEAD-box RNA helicase DDX21 facilitates the release of P-TEFb from this inhibitory complex in an RNA helicase-dependent manner, leading to increased phosphorylation of Ser2 of the C-terminal domain of elongating Pol II and productive elongation[66] (Fig. 2g).

RNA polymerase I (Pol I) transcription can also be regulated by lncRNAs. For example, SLERT (snoRNA-ended lncRNA enhances preribosomal RNA transcription) is translocated from its transcription site into the nucleolus depending on its snoRNA ends. In the nucleolus, SLERT interacts with DDX21, which binds to and represses Pol I in an RNA-helicase-independent manner. SLERT binding alters DDX21 conformation and releases the inhibitory interaction between DDX21 and Pol I machinery, thereby promoting rDNA transcription[8] (Fig. 2h).

**Transcription or the lncRNA locus regulate transcription.** Transcription or DNA sequences in some lncRNA loci, but not the transcripts themselves, is responsible for local gene regulation. Through genetic manipulation of 12 lncRNA-producing genomic loci in mouse ES cells, Engreitz et al. found that promoter knockouts at five of these loci up- or downregulated the expression of the nearby gene in an allele-specific manner[17]. Notably, three of these events were independent of lncRNA transcripts, but involved lncRNA production-associated processes, including the enhancer-like activity of promoters, the transcription process and the alternative splicing. For example, blocking the generation of lncRNA Blustr through promoter deletion, poly(A) site insertion, and the mutation of its first 5′ splice site all impaired its downstream Sfmbt2 expression. The extent of Blustr transcription, not the specific RNA sequence, correlated with its *cis*-activating effect. Thus, the expression of nearby gene is controlled by transcription and splicing of Blustr RNA, rather than the lncRNA product[17].

Similar findings were observed for Air, a paternally expressed antisense lncRNA of *Igf2r* required for silencing the paternal *Igf2r*[67,68], and for Upperhand (Uph), a cardiac-enriched lncRNA co-transcribed bidirectionally with the cardiac TF Hand2 (ref. [16]). Air transcriptional overlap with the *Igf2r* promoter suppresses Pol II recruitment to chromatin, thereby silencing *Igf2r*[68]. Additionally, transcription of Uph has been suggested to maintain the super-enhancer signature for local gene regulation[16]. Notably, such effects are not limited to lncRNA loci, as knockouts at protein-coding loci also led to altered expression of a neighboring gene[17].

Sequences within lncRNA loci have also been implicated as regulatory DNA elements. PVT1 (Plasmacytoma Variant Translocation 1) is transcribed downstream of *MYC*. It interacts with the MYC protein and interferes with its phosphorylation at Thr58, a modification that promotes MYC degradation[69]. A recent study to manipulate the *PVT1* locus in mice has revealed a tumour-suppressor function of the *PVT1* promoter that is independent of the PVT1 lncRNA[18]. *PVT1* and *MYC* promoters are located 55 kb apart and compete for enhancer contact *in cis*, thereby allowing the *PVT1* promoter to suppress *MYC* transcription[18].

## Roles of lncRNAs in the regulation of nuclear bodies

Nuclear bodies (NBs) are dynamic, membraneless RNA-protein complexes[70]. An increasing number of lncRNAs are found to regulate the integrity and function of NBs, altering gene expression at the post-transcriptional level (Fig. 2i,j).

**lncRNAs act as architectural RNAs.** Some lncRNAs function as the cores or scaffolds of NBs and are defined as 'architectural RNAs'[71]. One prominent example is nuclear enriched abundant transcript 1 (NEAT1)[72], responsible for the formation of paraspeckles[5,73–76]. Pol II transcription of a single exon located at human chr11 produces two isoforms, NEAT1_1 and NEAT1_2, as a consequence of alternative 3′-end processing[77] (Fig. 1c). Actions of both NEAT1_1 transcription[78] and NEAT1_2 (refs. [28,77]) are required for paraspeckle formation and maintenance. The number and morphology of paraspeckles are closely related to NEAT1 expression, especially under stress conditions[79,80]. Electron microscopy (EM)[81] and structured illumination microscopy (SIM)[80,82] (Table 1) revealed the paraspeckle as a highly ordered, spheroidal structure with the 5′ terminus of NEAT1_1 and both termini of NEAT1_2 in the outer shell and the middle region of NEAT1_2 in the inner core[80–82] (Fig. 2i).

At the cellular level, NEAT1 and paraspeckles play multiple roles in gene regulation. More than 40 proteins are localized to paraspeckles, including NONO and SFPQ[77,83]. Sequestration of SFPQ within paraspeckles prevents its binding to promoters of specific immune-related genes[79,84] or results in increased miRNA processing from certain introns[85]. mRNAs with inverted repeats, mostly SINEs in mouse and *Alu*s in human[86,87], in their 3′ UTRs can be sequestered via NONO in paraspeckles. Such sequestration undergoes dynamic changes upon cellular stresses[86,88] and during circadian rhythm[89], and involves the crosstalk with mitochondria[80] as well (Fig. 2i). Furthermore, NEAT1 is involved in the p53-mediated tumour suppressor pathway[90,91].

Though NEAT1 is highly expressed in most examined human cells[72], it is not essential for overtly normal development in mice under laboratory growth conditions[92]. Neat1 is only highly expressed in certain adult mouse tissues, including the corpus luteum and developing mammary glands. Correspondingly, mice congenitally lacking Neat1 stochastically failed to become pregnant due to defects in corpus luteum and mammary gland[93,94]. Future studies are warranted to reconcile the lack of strong phenotype in Neat1-deficient mice and emerging functions of NEAT1 and paraspeckle in human cells.

The assembly of other NBs also requires lncRNAs. Formation of nuclear stress bodies (nSBs) occurs in response to heat shock in a process initiated by transcription of satellite III (Sat III) tandem repeats[95], trapping several splicing factors by Sat III. Similar observations were found in abundant snoRNA-ended lncRNAs (sno-lncRNAs, Fig. 1d)[6] and 5′ snoRNA-ended 3′ polyadenylated lncRNAs (SPAs, Fig. 1e)[7] associated with Prader–Willi syndrome (PWS), a neurodevelopmental genetic disorder. These lncRNAs are highly expressed, accumulate near their synthesis sites and sequester splicing factors to form 1~2 μm$^3$ bodies in human ES cells[7]. In searching for additional NBs built on lncRNAs, screening using 32,651 fluorescently tagged human cDNA clones identified that localization of 32 proteins to NBs requires RNA[71,96]. These findings suggest additional unknown RNAs in the organization of RNA–protein assemblies. Such RNAs may not be classical lncRNAs. For example, intronic *Alu* elements originating from primary RNA Pol II transcripts were shown to be involved in modulating nucleolus formation[97].

**Non-architectural lncRNAs in nuclear bodies.** Metastasis-associated lung carcinoma transcript 1 (MALAT1)[72] is one of the most abundant lncRNAs and localizes to nuclear speckles, a type of NB-enriched pre-mRNA processing factor[98]. The MALAT1 primary transcript is cleaved at its 3′ end by RNase P to generate the 7,500-nt mature MALAT1, stabilized by a triple-helix structure (Fig. 1c) and a 61-nt tRNA-like mascRNA (MALAT1-associated small cytoplasmic RNA)[4].

Unlike NEAT1, knockdown of MALAT1 had little effect on the integrity of nuclear speckles[99,100]. Nuclear speckles contain SON and SC35 proteins in the central region and MALAT1 and small nuclear (sn) RNAs at the periphery[101]. MALAT1 interacts with SR proteins involved in RNA splicing and RNA export factors in nuclear speckles[102–105]. Modulating MALAT1 expression alters SR protein phosphorylation and disrupts the interaction of SR proteins with their target pre-mRNAs in cancer cells[103,106] (Fig. 2j). Additionally, MALAT1 regulates endothelial cell function[107] and mammary cancer pathogenesis[108] by influencing splicing. MALAT1 is likely to be recruited to speckles and preferentially interacts with alternatively spliced pre-mRNAs in a protein-mediator-dependent manner, as shown by genome-wide CHART-seq[109] and RAP-seq studies[110] (Table 1). On the basis of these observations, it has been proposed that MALAT1 may function as a 'scaffold' to enhance protein–protein, protein–RNA and protein–DNA interactions within or near nuclear speckles[111,112]. Moreover, MALAT1 is associated with transcriptionally active genes[109,110,113], suggesting its direct involvement in transcriptional regulation. Despite its multiple functions at the cellular level, MALAT1 is not essential for mouse development or viability[99,100].

## Functions of lncRNAs in the cytoplasm

A number of lncRNAs are exported to the cytoplasm, where they regulate mRNA stability, modulate translation and interfere with PTMs (Fig. 2k–n).

**lncRNAs regulate mRNA turnover.** lncRNAs can influence mRNA turnover in several ways. First, they can regulate mRNA stability via associated miRNAs. Competitive endogenous RNAs (ceRNAs) function as miRNA sponges by competing for miRNA binding, thereby derepressing miRNA targets[114,115]. Although this mechanism has been proposed to broadly affect miRNA availability, it remains controversial given the inadequate amount of ceRNAs in most cases[116,117]. Furthermore, the stability and abundance of a ceRNA and the copy number of miRNA binding sites on it must be considered.

A recent study has revealed a more complicated regulatory network consisting of different types of ncRNAs. A circular RNA, Cdr1as (cerebellar degeneration-related protein 1 antisense transcript) has been reported as a ceRNA to sponge miR-7 (refs. [10,118]), In an effort to illustrate how Cdr1as orchestrates miRNA activity in mammalian brain, Kleaveland et al. applied gene editing in mice to probe molecular consequences of four ncRNAs, including a lncRNA Cyrano, Cdr1as, miR-7 and miR-671 (ref. [119]). Cyrano also binds to miR-7, but instead of sequestering miR-7, this lncRNA facilitates its efficient destruction by promoting tailing and trimming of the 3′ end to induce target-RNA-directed miRNA degradation, resulting in Cdr1as accumulation in the brain. Cyrano deficiency allows miR-7 to accumulate, causing Cdr1as degradation in neurons, in part through miR-671-mediated slicing[119] (Fig. 2k). These findings show that the ceRNA theory is much more complicated than previously thought, and different types of ncRNAs may work collaboratively to establish a sophisticated regulatory network[119].

Second, lncRNAs can modulate mRNA stability by recruiting proteins to degrade mRNA. For example, a group of lncRNAs containing *Alu* can activate Staufen 1 (STAU1)-mediated mRNA decay (SMD) *in trans*. SMD-targeted mRNAs contain *Alu* elements within 3′-UTRs, which can base pair with complementary *Alu* in lncRNAs to form double-stranded RNAs (dsRNAs), a structure recognized by STAU1 (refs. [120,121]). These lncRNAs are called half-STAU1-binding site RNAs (1/2-sbsRNAs)[121] (Fig. 2l). Similarly to ceRNAs[114], an individual 1/2-sbsRNA can downregulate a subset of SMD targets, and distinct 1/2-sbsRNAs can downregulate the same SMD target[121] (Fig. 2l).

Third, lncRNAs can function as molecular decoys for RBPs involved in mRNA decay. NORAD (noncoding RNA activated by DNA damage), an abundant and conserved lncRNA in mammals, acts as a reservoir of PUMILIO 1 and PUMILIO 2 (PUM1/2) in the cytoplasm to limit their availability to target mRNAs for degradation (Fig. 2l). PUM1/2 binds to the PUMILIO response element (PRE), an 8-nt sequence at the 3′ UTR of target mRNAs[122], and stimulates mRNA deadenylation and decapping, resulting in accelerated turnover and decreased translation[123]. NORAD contains 15~17 PREs that preferentially bind to PUM1/2 in human cells[124,125]. NORAD knockout cells showed increased chromosomal instability, possibly attributable to PUM1/2 hyperactivity that leads to broad downregulation of PUMILIO target mRNAs encoding proteins involved in genome stability[124,125]. Careful examination of the stoichiometry between lncRNA/RBP binding motifs and interacting RBPs should be carried out prior to applying this strategy to particular lncRNAs. Recently, NORAD was also found in the nucleus, where it assembles into a topoisomerase complex critical for genome stability upon replication stress and DNA damage[126].

**lncRNAs regulate translation.** Although lncRNAs are not translated, ribosome profiling has identified ribosome-associated lncRNAs[127]. This is consistent with the notion that some annotated lncRNAs may be translated[19,20]. However, ribosome profiling assays may yield questionable results. For instance, some strictly nuclear located lncRNAs like MALAT1 were found to associate with ribosomes[128], suggesting a limitation of ribosome profiling rather than the translation ability of lncRNAs. Another explanation for these findings is the involvement of lncRNAs in translation regulation. For instance, lincRNA-p21 interacts with HuR, and such association favours the recruitment of let-7/Ago2 to destabilize lincRNA-p21 (ref. [129]). Upon loss of HuR, lincRNA-p21 accumulates and associates with JUNB and CTNNB1 mRNAs via base pairing to suppress their translation by recruiting the translation repressor Rck[129] (Fig. 2m). lncRNAs can also activate mRNA translation. *Uchl1* (ubiquitin carboxyterminal hydrolase L1) is a gene involved in brain function and neurodegeneration in mice. The lncRNA AS-Uchl1 (antisense to Uchl1) enhances the formation of active polysomes on Uchl1 mRNA and promotes its translation via a SINE *B2* segment complementary to a 73-nt region within the 5′ end of Uchl1 mRNA[130].

**lncRNAs interfere with PTMs.** Several lncRNAs modulate PTMs by masking sites bound by PTM enzymes or PTM sites. A cytoplasmic lncRNA exclusively expressed in human conventional dendritic cells (DCs), termed lnc-DC, regulates the phosphorylation of STAT3, a TF that controls DC differentiation[131]. Lnc-DC directly binds to STAT3 and promotes phosphorylation on Tyr705 by preventing the binding of the protein tyrosine phosphatase SHP1 (ref. [131]; Fig. 2n). In another example, NKILA (NF-κB interacting lncRNA) interacts with IκB and interferes with its phosphorylation, leading to NF-κB activation and suppression of breast cancer metastasis[132].

## Concluding remarks

Compared to other classes of ncRNAs, lncRNAs exhibit a surprisingly wide range of sizes, shapes and functions. These features have endowed them with previously underappreciated functional potentials; however, these have also presented experimental challenges for their analysis. Recent studies using robust methods (Table 1) have greatly advanced our understanding of lncRNA functions. Like proteins, lncRNAs have roles in all aspects of gene expression by different mechanisms of action. These versatile functions of lncRNAs depend on their subcellular localization and the adoption of specific structural modules with interacting partners, a process that may undergo dynamic changes in response to local environments in cells.

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## Competing interests

All authors declare no competing interests.

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