Gene regulation by long non-coding RNAs and its biological functions

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Abstract | Evidence accumulated over the past decade shows that long non-coding RNAs (lncRNAs) are widely expressed and have key roles in gene regulation. Recent studies have begun to unravel how the biogenesis of lncRNAs is distinct from that of mRNAs and is linked with their specific subcellular localizations and functions. Depending on their localization and their specific interactions with DNA, RNA and proteins, lncRNAs can modulate chromatin function, regulate the assembly and function of membraneless nuclear bodies, alter the stability and translation of cytoplasmic mRNAs and interfere with signalling pathways. Many of these functions ultimately affect gene expression in diverse biological and physiopathological contexts, such as in neuronal disorders, immune responses and cancer. Tissue-specific and condition-specific expression patterns suggest that lncRNAs are potential biomarkers and provide a rationale to target them clinically. In this Review, we discuss the mechanisms of lncRNA biogenesis, localization and functions in transcriptional, post-transcriptional and other modes of gene regulation, and their potential therapeutic applications.

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Genomes are extensively transcribed and give rise to thousands of long non-coding RNAs (lncRNAs), which are defined as RNAs longer than 200 nucleotides that are not translated into functional proteins. This broad definition encompasses a large and highly heterogeneous collection of transcripts that differ in their biogenesis and genomic origin. Statistics from Human GENCODE suggest that the human genome contains more than 16,000 lncRNA genes, but other estimates exceed 100,000 human lncRNAs[1,2]. These mainly include lncRNAs transcribed by RNA polymerase II (Pol II), but also by other RNA polymerases; and lncRNAs from intergenic regions (lincRNAs) as well as sense or antisense transcripts that overlap with other genes. The resulting lncRNAs are often capped by 7-methyl guanosine (m^7G) at their 5′ ends, polyadenylated at their 3′ ends and spliced similarly to mRNAs (FIG. 1a). It is worthwhile noting that enhancer and promoter regions are also transcribed into enhancer RNAs (eRNAs) and promoter upstream transcripts, respectively[3].

The number of functional lncRNAs is still debated. Although evidence is still lacking to support the functionality of most lncRNAs, thereby rendering them transcription by-products, it is well documented that a growing number of lncRNAs have important cellular functions. The expression of a considerable number of lncRNAs is regulated and some have roles in different mechanisms of gene regulation. Several lncRNAs control the expression of nearby genes by affecting their transcription, and also affect other facets of chromatin biology, such as DNA replication or the response to DNA damage and repair. Other lncRNAs function away from their loci; their functions can be of a structural and/or regulatory nature and involve different stages of mRNA life, including splicing, turnover and translation, as well as signalling pathways. Consequently, lncRNAs affect several cellular functions that are of great physiological relevance, and alteration of their expression is inherent to numerous diseases. The specific expression patterns of these functional lncRNAs have the potential of being used as optimal disease biomarkers, and strategies are under development for their therapeutic targeting.

In this Review, we discuss emerging themes in lncRNA biology, including recent understanding of their biogenesis and their regulatory functions in *cis* and in *trans* at the transcriptional and post-transcriptional levels. We then discuss the pathological consequences of lncRNA dysregulation in neuronal disorders, haematopoiesis, immune responses and cancer. Finally, we discuss how the existing knowledge of lncRNAs allows the development of lncRNA-based therapeutic targeting.

Biogenesis of lncRNAs

Most lncRNA species are transcribed by Pol II. As such, many have 5′-end m^7G caps and 3′-end poly(A) tails, and are presumed to be transcribed and processed similarly to mRNAs. However, recent studies have begun to reveal distinct transcription, processing, export and turnover of lncRNAs, which are closely linked with their cellular fates and functions.

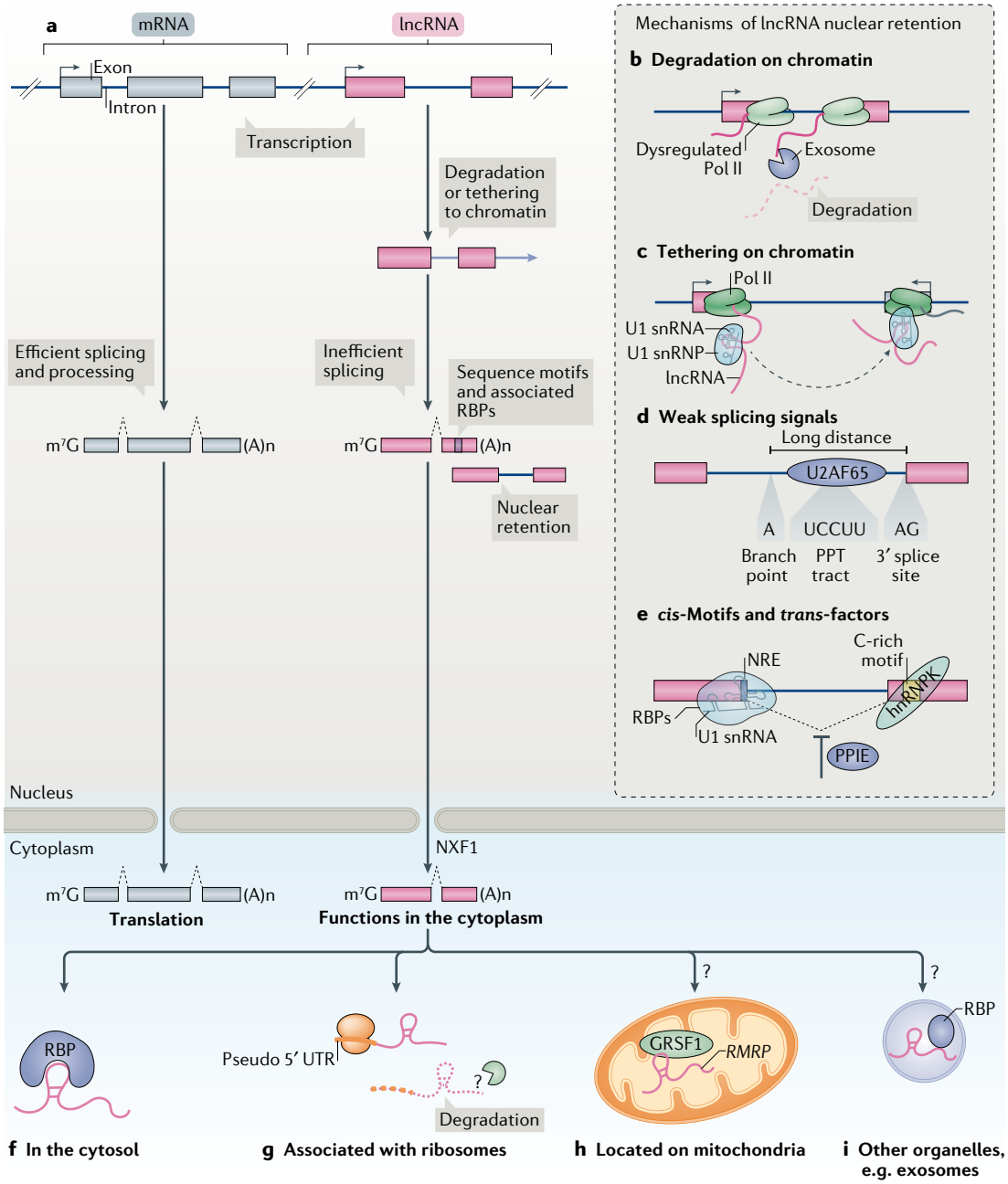


Fig. 1 | **Biogenesis and cellular fates of long non-coding RNAs. a** | Biogenesis of long non-coding RNAs (lncRNAs). Unlike mRNAs, many RNA polymerase II (Pol II)-transcribed lncRNAs are inefficiently processed[4,5,113] and are retained in the nucleus[6,10,12,13,16] (mechanisms of lncRNA nuclear retention are shown in parts **b**–**e**), whereas others are spliced and exported to the cytoplasm. The lncRNAs (and mRNAs) that contain one or only few exons are exported to the cytoplasm by nuclear RNA export factor 1 (NXF1)[23]. **b** | Some lncRNAs are transcribed by dysregulated Pol II, remain on chromatin and, subsequently, are degraded by the nuclear exosome[12]. **c** | Numerous lncRNAs with a certain U1 small nuclear RNA (U1 snRNA) binding motif can recruit the U1 small nuclear ribonucleoprotein (U1 snRNP) and through it associate with Pol II at various loci[13]. **d** | In many lncRNAs, the sequence between the 3′ splice site and the branch point is longer and contains a shorter polypyrimidine tract (PPT) than in mRNAs[10,17], which results in inefficient splicing. **e** | Sequence motifs in *cis* and factors in *trans* coordinately contribute to nuclear localization of lncRNAs. A nuclear retention element (NRE) U1 snRNA-binding site and C-rich motifs can recruit U1 snRNP[19] and heterogeneous nuclear ribonucleoprotein K (hnRNPK)[20,21], respectively, to enhance lncRNA nuclear localization. Other, differentially expressed RNA-binding proteins (RBPs), such as peptidylprolyl isomerase E (PPIE)[6], inhibit splicing of groups of lncRNAs, resulting in their nuclear retention. **f** | In the cytoplasm, lncRNAs usually interact with diverse RBPs. **g** | Many lncRNAs in the cytoplasm are associated with ribosomes through 'pseudo' 5′ untranslated regions (UTRs); ribosome-associated lncRNAs tend to have short half-lives owing to unknown mechanisms[24]. **h** | Several lncRNAs are sorted into mitochondria by unknown mechanisms[26,27]. For example, the RNA component of mitochondrial RNA-processing endoribonuclease (*RMRP*) is recruited to mitochondria and is stabilized by binding G-rich RNA sequence-binding factor 1 (GRSF1)[28]. **i** | Some lncRNAs are also found in other organelles, such as exosomes[29], probably by forming lncRNA–RBP complexes[30,31]. m^{7}G, 7-methyl guanosine 5′ cap; (A)n, poly(A) 3′ tail.

Transcription and processing of lncRNAs

Compared with mRNAs, a greater proportion of lncRNAs are localized in the nucleus[4–6], raising the fundamental question of what drives their differential localization. Dissection of the global features of lncRNAs and mRNAs suggests that lncRNA genes are less evolutionarily conserved, contain fewer exons and are less abundantly expressed[6–8]. Early studies indicated that lncRNA genes likely contain fewer exons than mRNAs[6–8]. The recently developed RNA capture long seq enabled better annotation of the full length of lncRNAs, including their 5′ ends[9,10], revealing little length difference with mRNAs, although lncRNAs contain fewer and longer exons. Single-cell sequencing found that some lncRNAs can be abundantly expressed in the human neocortex[11].

Whereas the low expression of lncRNAs is likely related to the presence of repressive histone modifications at their gene promoters[9,10], their mode of transcription may partially explain some of their other distinctive features. The phosphorylation status of the Pol II carboxy-terminal domain corresponds with different transcription stages, and a significant fraction of lncRNAs are transcribed by phosphorylation-dysregulated Pol II[12]. Such lncRNAs appear to be weakly co-transcriptionally spliced and transcription termination at these genes is independent of polyadenylation signals, leading to temporal accumulation of lncRNAs on chromatin, followed by their rapid degradation by the RNA exosome[12] (FIG. 1b). These findings provide insights into why lncRNAs are frequently nuclear, and suggest that functional lncRNAs must escape this nuclear surveillance process to accumulate at high levels in specific cell types. However, chromatin-tethered lncRNAs may not always be targeted by the nuclear surveillance process. Some chromatin-localized lncRNAs contain high levels of U1 small nuclear RNA binding sites, which recruit the U1 small nuclear ribonucleoprotein (U1 snRNP) to transcriptionally engaged Pol II, resulting in the tethering of numerous non-coding RNAs to chromatin[13] (FIG. 1c). Accumulation of certain lncRNAs on chromatin can occur when the function of the Pol II-associated elongation factor SPT6 is abolished[14,15]. The loss of SPT6 generates redistribution of histone H3 trimethylated at Lys36 (H3K36me3; a mark of active transcription) from protein-coding genes to lncRNA genes, thereby increasing their transcription. Concomitantly, SPT6 loss impairs recruitment to chromatin of the transcription termination Integrator complex, leading to accumulation of long non-coding transcripts on chromatin in the form of DNA damage-associated R-loops[15].

Overall, lncRNAs are spliced less efficiently than mRNAs[6,10,16]. They have weaker internal splicing signals and longer distances between the 3′ splice site and the branch point[10,17], which correlate with augmented nuclear retention[6,10,16] (FIG. 1d). Other factors, such as differential expression of certain splicing regulators, also contribute to the accumulation of lncRNAs in the nucleus. For example, in mouse embryonic stem cells (mESCs), the highly expressed splicing inhibitor peptidylprolyl isomerase E suppresses splicing of a subset of lncRNAs, leading to significant nuclear accumulation of many lncRNAs in mESCs[6] (FIG. 1e). Alternative polyadenylation signals within lncRNAs may also modulate their subcellular localization. For example, the *CCAT1* lncRNA gene (full names of all lncRNAs are provided in the footnote of TABLE 1) produces two isoforms: the long isoform (*CCAT1-L*) is nuclear and contains an internal polyadenylation site corresponding with the 3′ end of the short isoform (*CCAT1-S*), which is cytoplasmic[18].

In addition to these general features of lncRNA transcription and processing, lncRNAs often contain embedded sequence motifs that can recruit certain nuclear factors, which promote the nuclear localization and function of the lncRNA (FIG. 1e). For example, the lncRNA maternally expressed gene 3 (*MEG3*) contains a 356-nucleotide nuclear retention element that associates with U1 snRNP, which in turn retains *MEG3* in the nucleus[19]. Repeat elements also likely have roles in driving lncRNA nuclear retention. Recent studies using the high-throughput massively parallel RNA assays (MPRNA) have uncovered a C-rich sequence derived from *Alu* repeats that can promote the nuclear retention of lncRNAs through their association with the nuclear matrix protein heterogeneous nuclear ribonucleoprotein K (hnRNPK)[20,21] (FIG. 1e). Other repeats can also guide lncRNA nuclear localization. For instance, the lncRNA functional intergenic repeating RNA element (*FIRRE*) contains many unique repeats, ranging in length from 67 to 804 bp, termed repeating RNA domains (RRDs), which establish *FIRRE* chromatin localization by interacting with hnRNPU[22].

In summary, the nuclear localization and fate of lncRNAs are coordinately regulated at multiple layers, from transcription and processing to nuclear export through multiple sequence motifs in *cis* and factors in *trans*. In addition to being tethered to chromatin, some nuclear retained functional lncRNAs are specifically localized to membraneless nuclear domains (see below). Although the most representative lncRNAs of this type are processed by unusual biogenesis pathways (reviewed in REF.[3]), the molecular mechanism that traps such lncRNAs in specific nuclear domains still remains largely unknown. Nonetheless, given the diverse formats, sizes and functions of lncRNAs (TABLE 1), more work is warranted to dissect the distinctions and commonalities of mechanisms that control different nuclear localization patterns of lncRNAs.

Export of lncRNAs to the cytosol

A large fraction of lncRNAs are exported to the cytosol; these lncRNAs presumably share the same processing and export pathways with mRNAs. Indeed, a recent study revealed that long and A/U-rich transcripts with one or only few exons are dependent on the nuclear RNA export factor 1 (NXF1) pathway for export[23]. As lncRNAs tend to have fewer exons compared with mRNAs[10], they preferentially exploit this export pathway. Upon arrival in the cytoplasm, lncRNAs likely undergo specific sorting processes that assign different lncRNAs to specific organelles or are distributed in the cytoplasm and associate with diverse RNA-binding proteins (RBPs) (FIG. 1f). It is estimated that half the pools of 70% of cytoplasmic lncRNAs are found in polysome fractions[24]. Certain *cis* elements contribute to the localization of lncRNAs

Enhancer RNAs
Relatively short (≤2 kb) long non-coding RNAs that are bidirectionally transcribed from enhancer regions, are not consistently spliced or polyadenylated and are rapidly degraded.

Promoter upstream transcripts
Transcripts that are divergently transcribed upstream of active gene promoters and rapidly degraded by the RNA exosome complex.

RNA exosome
A complex of ribonucleases that regulates the degradation of multiple types of RNA in eukaryotes.

Integrator
A multi-protein complex associated with RNA polymerase II that modulates the fate of many nascent RNAs, including mRNAs, enhancer RNAs and promoter upstream transcripts.

R-loops
RNA–DNA hybrids formed by Watson–Crick interactions predominantly in *cis*, but also in *trans* through homologous recombination.

Table 1 | **Functions and mechanisms of long non-coding RNAs**

Function	Long non-coding RNA	Interacting with DNA; proteins	Mode of function	Sequence features	Physiopathological process
Regulation of transcription	*TARID*[79,238]	DNA (R-loops); GADD45A	Forms R-loops and recruits GADD45A to facilitate TCF21 expression	Interacts with GC-rich sequence near *TCF21* transcription start site	Demethylation of the hypermethylated *TCF21* promoter in multiple types of cancer[238]
	APOLO[80]	DNA (R-loops); LHP1	Functions as decoy for LHP1 at the R-loop; regulates expression of auxin responsive genes	Two complementary TTCTTC boxes	Involved in root development in *Arabidopsis thaliana*
	ANRIL[45,46]	PRC1, PRC2; YY1	Recruits Polycomb repressive complexes to promoters of *CDKN2A* and *CDKN2B* to inhibit their expression in *cis* and to regulate distant genes in *trans*	*Alu* retroelements	Multiple roles in diseases and cancer[239]
	LINC-PINT[44]	PRC2	Suppresses gene-expression signature of cancer cell invasion	Short, conserved motifs (CE1 and CE2)	Downregulated in multiple cancers; its overexpression reduces cell migration and proliferation in lung and colon cancers
	HOTTIP[61,62]	WDR5–MLL	Activates HOXA genes	NA	Its dysregulation is involved in leukaemogenesis
	UMLILO[60]	WDR5–MLL	Activates transcription of CXCL chemokines	NA	Primes transcription of immune genes during trained immunity
	lncPRESS1 (REF.[63])	Sirtuin 6	Functions as a decoy for sirtuin 6 in regulating gene expression	NA	Regulates ESC differentiation
	XIST[41,52,55–58]	PRC2, YY1, hnRNPK, SHARP and others[240]	Silences genes in inactive X chromosome	A-repeat containing AUCG tetraloops connected by U-rich linkers[241]; C-rich tracts for hnRNPK binding[55]; sequence features for long-range interactions[242]	Multiple roles in development and cancer[243–245]
	AIRN[41,53,96,97]	PRC2	Recruits PRC2 to distal genes *Slc22a2* and *Slc22a3*	NA	Functions in embryonic development[246]
	KCNQ1OT1 (REFS[41,54,247])	PRC2, DNMT1	Drives the spread of PRC2 over several megabases in *cis*	NA	Implicated in congenital growth disorders[248]
	ANRASSF1 (REF.[43])	DNA (R-loops); PRC2	Recruits PRC2 at R-loops for silencing of *RASSF1A*	NA	Inverse correlation between expression levels of *ANRASSF1* and its target *RASSF1* in breast and prostate cancers
	COOLAIR[91,92]	PRC2	Silences FLOWERING locus by regulating histone H3 trimethylated at Lys27	NA	Regulates flowering time within a complex gene network in response to changing temperatures
	CHASERR[95]	CHD2	Represses *Chd2* expression in *cis* and broadly regulates CHD2 targets	NA	*Chaserr* knockout mice exhibit early postnatal lethality
	NRIP1e eRNA[102]	Enhancer–promoter interactions	Recruits cohesin to promote chromatin looping and activates genes in short and long distances	NA	Together with other eRNAs, *NRIP1e* eRNA modulates transcriptional programmes in response to the activation of oestrogen receptor[102]
	CCAT1-L[18,36,139]	CTCF, hnRNPK	Induces chromatin looping by changing enhancer availability for *MYC* versus *PVT1*	NA	Cancer progression, metastasis and drug resistance; associated with multiple tumours[249]
	SWINGN[113]	SWI/SNF	Activates the proximal gene *GAS6* and distant genes in *trans* through chromatin looping	NA	*SWINGN*-depleted cells exhibit reduced cancer growth in lung cancer xenografts

Table 1 (cont.) | **Functions and mechanisms of long non-coding RNAs**

Function	Long non-coding RNA	Interacting with DNA; proteins	Mode of function	Sequence features	Physiopathological process
Post-transcriptional regulation	*PNCTR*[142]	PTBP1	Sequesters PTBP1 and suppresses PTBP1-regulated splicing	YUCUYY and YYUCUY sequences	Upregulated in cancer cells and lung cancer patients
	PNUTS[169]	miR-205	Sequesters miR-205, thereby causing upregulation of *ZEB1* and *ZEB2* mRNAs and promotes epithelial–mesenchymal transition	Seven binding sites of miR-205	Promotes epithelial–mesenchymal transition in breast cancer; promotes metastases in vivo
	TINCR[160]	STAU1	Interacts with STAU1 to promote differentiation-associated mRNA stability and expression	25-nucleotide *TINCR* box motifs	Dysregulated in multiple types of cancer, thereby modulating tumour development and progression[250]
	AS-Uchl1 (REF.[164])	*Uchl1* mRNA	Associates with *Uchl1* mRNA to activate translation	*SINEB2* retroelements	Neuroprotective function under stress through regulating *Uchl1* translation
	FAST[6]	β-TrCP	Interacts with β-TrCP to prevent β-catenin degradation, thereby activating WNT signalling	Five stem loops	Promotes pluripotency by maintaining the WNT pathway in human ESCs
	NKILA[251]	p65	Interacts with the NF-κB–IκBα complex by binding to p65 and modulates T cell activation-induced cell death by inhibiting NF-κB activity	Two stem loops	*NKILA* silencing improves adaptive immune therapy in PDX models of breast cancer
Cellular organelles	*RMRP*[28]	HuR, GRSF1	Interacts with HuR to export into cytoplasm; interacts with GRSF1 to stabilize in mitochondria	NA	Functions as an oncogene in lung cancer[252]; deletion of RMRP may impair mouse early embryo development[253]
	SAMMSON[172,173]	p32, CARF	Interacts with p32 and CARF to promote mitochondrial rRNA synthesis	NA	Highly expressed in melanoma[172,173] and promotes cell growth
Structural functions	*NEAT1* (REFS[126–131])	Paraspeckle proteins, *MALAT1* (REF.[139])	A scaffold lncRNA of paraspeckles	The long isoform contains a tRNA-like structure at the 3′ end[128]	*NEAT1* promotes breast and skin cancer growth[254,255]; *Neat1* knockout mice fail to produce functional corpus luteum and are infertile[256]
	MALAT1 (REFS[126,131,132,134–138,257,258])	SC35, SR proteins, U1 snRNA; *NEAT1*	Modulates SR protein phosphorylation in nuclear speckles	3′ end with a tRNA-like structure[258]	Aberrantly expressed in many human malignancies[259]; regulates angiogenesis[259]. Assessed for pharmacological inhibition by LNA GapmeRs that degrade it
	FIRRE[151,153]	hnRNPU	Interacts with multiple chromosomal regions in *trans* through hnRNPU and involved in gene regulation in *trans*	Repeating RNA domains	Regulates haematopoiesis in vivo[152]; its upregulation is associated with poor prognosis in B cell lymphoma[260]
	sno-lncRNAs[140]	RBFOX2	Associates with RBFOX2 and regulates mRNA splicing	snoRNA at 5′ end; UGCAU/ GCAUG sequences	Five sno-lncRNAs are missing in individuals with Prader–Willi syndrome
	SPAs[141]	hnRNPM, RBFOX2 and TDP43	Sequesters hnRNPM, RBFOX2 and TDP43 and regulates mRNA splicing	5′ snoRNA and 3′ polyadenylated; GU-rich, UGCAU/GCAUG and UG-rich sequences	Two SPAs are missing in individuals with Prader–Willi syndrome
Genome integrity	*GUARDIN*[206] (also known as long non-coding transcriptional activator of miR34a)	BRCA1 and BARD1	Stabilizes BRCA1 to promote DNA repair; a miR-23a sponge in telomere function	NA	Regulates cell survival and proliferation; its silencing inhibits colon cancer xenograft growth
	lincRNA-p21 (REFS[197,203])	hnRNPK	Recruits hnRNPK to p21 promoter and represses p53-induced gene expression in *trans*; activates p21 in *cis*	A conserved 5′ sequences	Dysregulated in multiple cancers and atherosclerosis

Table 1 (cont.) | **Functions and mechanisms of long non-coding RNAs**

Function	Long non-coding RNA	Interacting with DNA; proteins	Mode of function	Sequence features	Physiopathological process
Genome integrity (cont.)	*PANDA*[261]	NF-YA	Acts as a decoy of NF-YA and represses pro-apoptotic genes	NA	Reduces apoptosis and cell senescence
	DINO[205]	p53	The p53–DINO complex binds and actives p53 target genes	Sequences for p53 binding	Causes cell cycle arrest and induces DNA damage
	NORAD[156,157,205,262]	Pumilio proteins, RBMX	Sequesters Pumilio proteins to promote stability of mRNAs that encode proteins involved in genomic stability; assembles a complex with topoisomerase 1 to promote genomic stability	Pumilio response element (UGUANAUA)	Its dysregulation is implicated in multiple cancers[263]; deletion of *NORAD* in mice leads to a degenerative phenotype due to premature ageing[264]
	CONCR[265]	DDX11	Interacts with DDX11 to modulate cell cycle progression and DNA replication	NA	Upregulated in multiple cancers; *CONCR* knockdown impairs tumour growth in colon cancer xenografts

eRNA, enhancer RNA; ESC, embryonic stem cell; miR, microRNA; NA, not available; NF-κB, nuclear factor-κB; PDX, patient-derived xenograft; rRNA, ribosomal RNA; slc22A, solute carrier family 22 member 2; U1 snRNA, U1 small nuclear RNA. Long non-coding RNAs (lncRNAs): *AIRN*, antisense of Igf2r non-protein coding RNA; *ANRASSF1*, RNA 1 antisense RASSF1; *ANRIL*, antisense non-coding RNA gene at the INK4 locus; *APOLO*, auxin-regulated promoter loop; *AS-Uchl*, antisense to ubiquitin carboxyterminal hydrolase L1; *CCAT1-L*, long non-coding RNA colon cancer-associated transcript 1; *CHASERR*, CHD2 adjacent suppressive regulatory RNA; *COOLAIR*, cold induced long antisense intragenic RNA; *CONCR*, cohesion regulator non-coding RNA; *DINO*, damage induced non-coding; *FAST*, FOXD3 antisense transcript 1; *FIRRE*, functional intergenic repeating RNA element; *HOTTIP*, HOXA transcript at the distal tip; *KCNQ1OT1*, KCNQ1 opposite strand/antisense transcript 1; *linc-p21*, long intergenic non-coding RNA p21; *LINC-PINT*, p53 induced transcript; *lncPRESS1*, lncRNA P53 regulated and ESC associated 1; *MALAT1*, metastasis associated lung adenocarcinoma transcript 1; *NEAT1*, nuclear enriched abundant transcript 1; *NKILA*, NF-κB interacting lncRNA; *NORAD*, non-coding RNA activated by DNA damage; *NRIP1e* eRNA, nuclear receptor interacting protein 1 enhancer RNA; *PANDA*, p21-associated ncRNA DNA damage-activated; *PNCTR*, pyrimidine-rich non-coding transcript; *PNUTS*, phosphatase 1 nuclear targeting subunit lncRNA; *PVT1*, plasmacytoma variant translocation 1; *RMRP*, RNA component of mitochondrial RNA-processing endoribonuclease; *SAMMSON*, survival associated mitochondrial melanoma specific oncogenic non-coding RNA; *sno-lncRNA*, small nucleolar RNA-related lncRNA; *SPA*, 5′ small nucleolar RNA-capped and 3′-polyadenylated lncRNA; *SWINGN*, SWI/SNF interacting GAS6 enhancer non-coding RNA; *TARID*, TCF21 antisense RNA inducing demethylation; *TINCR*, terminal differentiation-induced non-coding RNA; *UMLILO*, upstream master lncRNA of the inflammatory chemokine locus; *XIST*, X-inactive specific transcript. Proteins: β-TrCP, β-transducin repeats-containing protein; BARD1, BRCA1 associated RING domain 1; BRCA1, breast cancer type 1 susceptibility protein homologue; CARF, collaborator of ARF; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; CHD2, chromodomain helicase DNA binding protein 2; CTCF, CCCTC-binding factor; DDX11, DEAD/H-box helicase 11; DNMT1, DNA methyltransferase 1; GADD45A, growth arrest and DNA damage inducible-α; GRSF1, G-rich RNA sequence-binding factor 1; hnRNPK/U, heterogeneous nuclear ribonucleoprotein K/U; HuR, human-antigen R; LHP1, like heterochromatin protein 1; NF-YA, nuclear transcription factor Y subunit-α; PRC1/2, Polycomb repressive complex 1/2; PTBP1, polypyrimidine tract-binding protein 1; RAD51, radiation sensitive 51; RBFOX2, RNA binding motif protein 9; RBMX, RNA-binding protein, X chromosome; SHARP, SMRT and HDAC associated repressor protein (also known as SPEN); SC35, splicing component, 35 kDa; SR, serine and arginine-rich; STAU1, Staufen double-stranded RNA binding protein 1; SWI/SNF, switch-sucrose non-fermenting; TCF21, transcription factor 21; TDP43, TAR DNA-binding protein 43; WDR5–MLL, WD repeat-containing protein 5–mixed lineage leukaemia; YY1, yin yang 1; ZEB1, zinc finger E-box-binding homeobox 1.

with ribosomes, such as long 'pseudo' 5′ untranslated regions, so called because they precede 'pseudo-open reading frames' in the lncRNAs[24,25] (FIG. 1g). The degradation of ribosome-associated lncRNAs may be triggered by a translation-dependent mechanism[24]. Whether the ribosome-associated lncRNAs are engaged by ribosomes for translation, have roles in translation or inertly reside in ribosomes is unknown.

Analyses of human mitochondrial transcriptomes revealed that lncRNAs exported from the nucleus can be sorted into mitochondria[26,27]. The RNA component of mitochondrial RNA-processing endoribonuclease (*RMRP*) is associated with the RBP HuR in the nucleus and exported to the cytosol by exportin 1. As soon as *RMRP* arrives at mitochondria, it is bound and stabilized by G-rich RNA sequence-binding factor 1 (GRSF1), thereby allowing its accumulation at the mitochondrial matrix[28] (FIG. 1h). RNA sequencing of human blood exosomes[29] revealed that they include many lncRNAs. It is still unknown how lncRNAs are sorted into exosomes, but the mechanism likely involves the binding of specific sequence motifs by RBPs[30,31] (FIG. 1i). Considering the growing list of cytoplasmic lncRNAs with important roles in modulating mRNA stability, translation and signalling pathways (see below), it will be important to examine how each functional lncRNA is being escorted to its site of function. Our current understanding of this aspect of lncRNA biology is still very limited.

Exosomes
Small vesicles secreted from cells that contain various RNAs and proteins.

Gene regulation by lncRNAs

Gene expression is regulated by lncRNAs at multiple levels. By interacting with DNA, RNA and proteins, lncRNAs can modulate chromatin structure and function and the transcription of neighbouring and distant genes, and affect RNA splicing, stability and translation. Furthermore, lncRNAs are involved in the formation and regulation of organelles and nuclear condensates.

Chromatin regulation

The detection of RNA–chromatin association in a genome-wide fashion[32–35], combined with chromatin conformation capture techniques, has unveiled complex lncRNA regulation of chromatin architecture and gene expression[36,37]. Although these lncRNA-mediated regulatory mechanisms should be explored individually, RNA has inherent regulatory potential. The negative charge of RNA can neutralize the positively charged

histone tails, leading to chromatin de-compaction[38], so RNA-mediated opening and closing of chromatin might function as a rapid switch of gene expression. Mechanistically, both *cis*-acting and *trans*-acting nuclear lncRNAs establish interactions with DNA to alter the chromatin environment, sometimes indirectly by virtue of their affinity for proteins that can associate with both RNA and DNA, and in other cases by binding DNA in a sequence-specific manner.

Protein–lncRNA localization and function on chromatin. Numerous lncRNAs localize on chromatin, where they can interact with proteins, facilitating or inhibiting their binding and activity at targeted DNA regions (FIG. 2a,b). Moreover, protein-assisted long-range chromatin interactions, such as CCCTC-binding factor (CTCF)-mediated chromatin interactions, can also act as facilitators of direct lncRNA transcriptional effects on target genes[18,39,40]. Although the binding of lncRNA to chromatin factors has raised considerable interest, caution is advised when evaluating such interactions, and rigorous methodologies should be applied in these studies. Moreover, the expression levels of a given lncRNA in relation to the factors it interacts with can define the extent of the effects that lncRNAs exert on the targeted chromatin[41] (Supplementary Box 1).

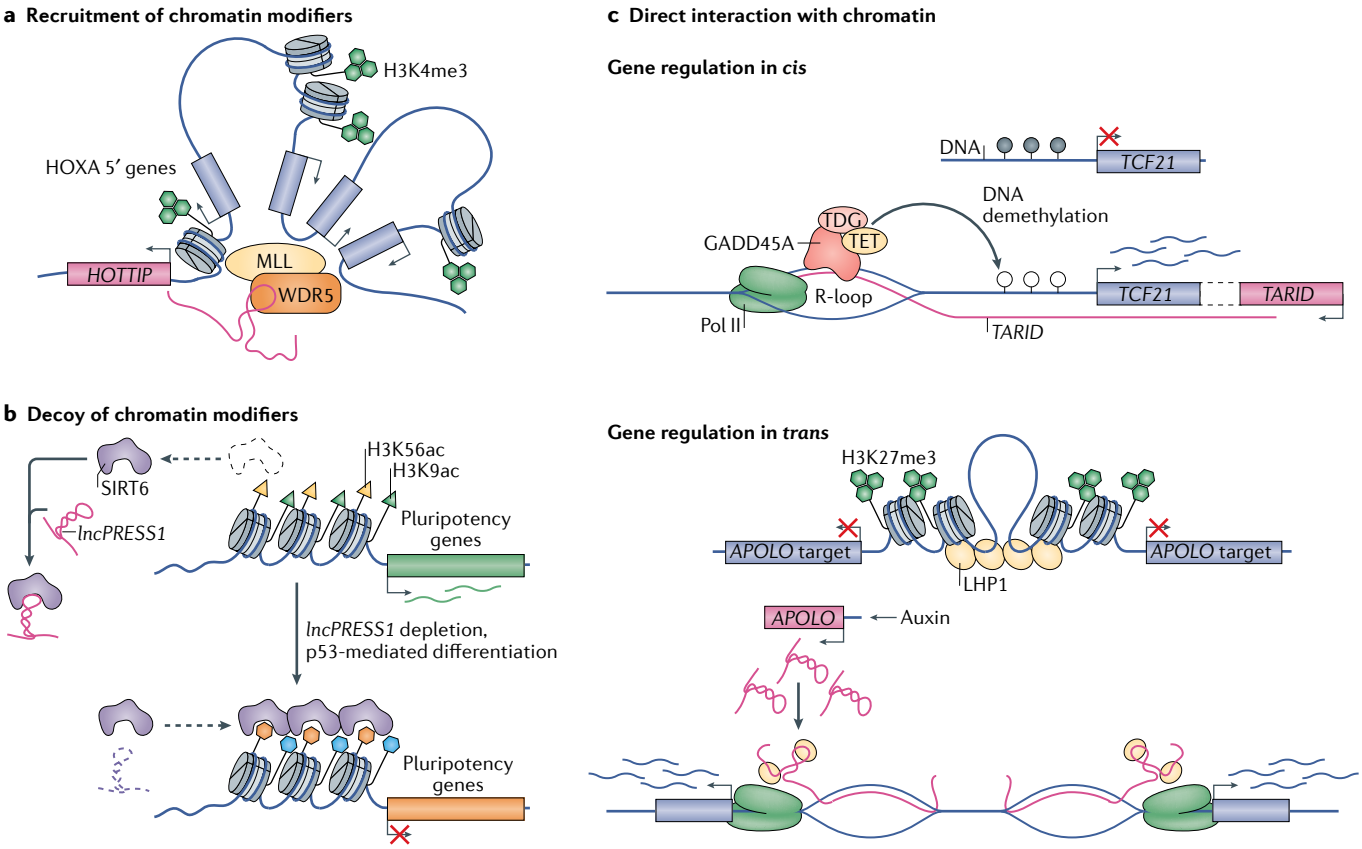


Fig. 2 | **Chromatin regulation mediated by long non-coding RNAs.** **a** | Long non-coding RNAs (lncRNAs) can interact with chromatin modifiers and recruit them to target-gene promoters in order to activate or suppress their transcription in *cis*[45,91,92], or in *trans* at distant, often multiple, loci[46]. For example, HOXA transcript at the distal tip (*HOTTIP*)[61,62] acts in *cis* at the 5′ genes of the HOXA gene cluster, with which it interacts through chromatin looping. *HOTTIP* interacts with WD repeat-containing protein 5 (WDR5), thereby targeting the complex WDR5–myeloid/lymphoid or mixed-lineage leukaemia (MLL) to the promoters of the HOXA genes and promoting histone H3 Lys4 trimethylation (H3K4me3). **b** | lncRNAs can act as decoys of specific chromatin modifiers by sequestering them from the promoters of target genes. For example, p53-regulated and embryonic stem cell-specific lncRNA (*lncPRESS1*)[63] supports the pluripotency of human embryonic stem cells by sequestering the histone deacetylase sirtuin 6 (SIRT6) from the promoters of numerous pluripotency genes. In this manner, *lncPRESS* keeps the active-gene H3 acetylated at Lys56 (H3K56ac) and Lys9 (H3K9ac) modifications as its target genes, thereby preventing the switch to activation of differentiation genes. During p53-mediated differentiation or following depletion of *lncPRESS1*, SIRT6 localizes to the chromatin and ensures the maintenance of pluripotency. **c** | lncRNAs can interact with DNA and co-transcriptionally form RNA–DNA hybrids such as R-loops, which are recognized by chromatin modifiers that activate or inhibit target-gene transcription[43,77] or by transcription factors[78]. The lncRNA TCF21 antisense RNA inducing demethylation (*TARID*)[79] forms an R-loop upstream of the promoter of its target gene transcription factor 21 (*TCF21*). The R-loop is recognized by growth arrest and DNA damage inducible-α (GADD45A), which drives the demethylation of the *TCF21* promoter DNA by interacting with thymine–DNA glycosylase (TDG) and ten–eleven translocation 1 (TET1). R-loops can also form in *trans*, with similar possible outcomes. For example, auxin-regulated promoter loop (*APOLO*)[80] is responsible for the activation of auxin responsive genes in *Arabidopsis thaliana*. *APOLO* and auxin target genes are normally silenced by H3K27me3 and the presence of chromatin loops maintained by the Polycomb factor like heterochromatin protein 1 (LHP1). Following transcriptional activation of *APOLO* in response to auxin, the lncRNA recognizes specific motifs at the promoters of its target genes, where it binds and generates R-loops that act as decoys of LHP1, thereby allowing target-gene expression[80]. Pol II, RNA polymerase II.

Triple helices
RNA–DNA structures forming when a polypurine motif of an RNA interacts with the major groove of a DNA double helix through Hoogsteen or reverse Hoogsteen base pairing; stabilized by nucleosomal organization.

Polycomb repressive complex 2 (PRC2) binding and spreading across targeted chromatin has been particularly described to be facilitated by several lncRNAs, in some cases through well-characterized sequence elements[42–44]. This type of interaction can occur in *cis* and in *trans*, as is the case of the lncRNA *ANRIL*, which mediates PRC1 and PRC2 recruitment to the promoters of its neighbouring *CDKN2A* and *CDKN2B* genes, thereby controlling their expression and regulating cell senescence[45]. Furthermore, *ANRIL* can also act in *trans* through *Alu* sequences, which drive *ANRIL* recruitment of PRC1 and PRC2 proteins to distant targets[46]. Although PRC2 requires RNA to efficiently bind to chromatin[47], the role of lncRNAs in PRC2 chromatin targeting is still under debate given the low specificity of RNA binding by PRC2 (REF.[48]). One example of many is the controversy over the *trans*-acting lncRNA HOX transcript antisense RNA (*HOTAIR*) in recruiting chromatin-modifying complexes to repress the distal HOXD genes, which has been described in detail elsewhere[49–51].

Other factors are likely involved in regulating lncRNA-mediated PRC targeting. For example, extensive studies performed in mice have shown that hnRNPK and other chromatin-associated factors interact with X-inactive specific transcript (*Xist*) and other lncRNAs at imprinted genomic loci, such as *Kcnq1ot1* and antisense of IGF2R non-protein coding RNA (*Airn*) to promote the spread of Polycomb complexes across different chromatin domains[41,52–57]. Transcription factors, such as the ubiquitously expressed YY1, are also able to target lncRNA-bound chromatin modifiers and other nascent RNAs to specific genomic loci[46,52,58,59].

In addition to gene-silencing factors, lncRNAs can recruit chromatin modifiers that promote gene activation[60,61]. The lncRNA *HOTTIP* is one of several lncRNAs that regulate the HOXA gene cluster (see below) — it binds several HOXA genes at the 5′ region of the cluster through chromatin looping, and its expression contributes to the maintenance of chromatin organization in this region. *HOTTIP* drives the WDR5–myeloid/lymphoid or mixed-lineage leukaemia (MLL; also known as KMT2A) histone methyltransferase complex to gene promoters, thereby facilitating gene expression through H3K4me3 and acting as an important regulator of mouse haematopoietic stem cells[61,62] (FIG. 2a). Finally, instead of recruiting chromatin modifiers, lncRNAs may function as decoys. The p53-regulated *lncPRESS1* is a pluripotency-associated lncRNA acting as decoy for the deacetylase sirtuin 6, which represses several pluripotency genes and, thus, promotes differentiation. In human ESCs (hESCs), *lncPRESS* interacts with sirtuin 6 and sequesters it from chromatin, thereby maintaining the transcription-permissive H3 acetylated at Lys56 (H3K56ac) and H3K9ac at pluripotency-related genes[63] (FIG. 2b).

Direct interactions between lncRNAs and DNA. An essential feature of lncRNAs is their potential to generate hybrid structures with DNA to influence chromatin accessibility. Such interactions can take the form of triple helices (triplexes) or R-loops. The actual prevalence of both of these types of structures is still unknown owing to the difficulty of detecting them in vivo. Nevertheless, the formation of triplexes and R-loops is probably widespread and essential for the regulatory activity of many lncRNAs.

RNA–DNA–DNA triplexes have been proposed as an example of non-coding RNA–DNA interplay in mediating gene silencing[64–66] or activation[66–68]. The potential to form triplexes relies primarily on the RNA sequence[69–71]. Recently, TrIP-seq (targeted RNA immunoprecipitation sequencing) has been developed to study triplex-forming sequences in vivo[72]. An example of triplex-mediated gene regulation links the function of a lncRNA with an eRNA in the activation of a neighbouring proto-oncogene, sphingosine kinase 1 (*SPHK1*). In response to cell proliferation signals, the lncRNA *KHPS1* (SPHK1 gene antisense) forms a triple helix upstream of the *SPHK1* enhancer, which helps recruit chromatin modifiers that activate the transcription of eRNA-*SPHK1* and promote the expression of *SPHK1* (REFS[71,73]). Remarkably, the role of the triplex in driving gene regulation was further shown by exchanging the *KHPS1* triplex-forming region with the *MEG3* triplex-forming region, causing *KHPS1* to switch its specificity to the *MEG3* target gene[71].

A more extensively studied mode of lncRNA interaction with chromatin occurs at R-loops, which have long been considered a threat to genome stability. However, the transient nature of R-loops makes them ideal regulatory hubs, and recent findings argue for their re-evaluation as regulators of gene expression[74–76] and as coordinators of DNA repair (BOX 1). Several lncRNAs regulate gene expression in the context of R-loops, with the aid of proteins that recognize these structures, causing a wide spectrum of outcomes[77,78] (FIG. 2a). In mESCs, the lncRNA *TARID* generates an R-loop at the CpG-rich promoter of the gene *TCF21*, which is transcribed in the opposite direction. GADD45A recognizes and binds to the R-loop at the *TCF21* promoter, recruiting the DNA demethylating factor TET1, leading to transcriptional activation of *TCF21* (REF.[79]) (FIG. 2c). Although many R-loop-forming lncRNAs act in *cis*, these R-loops can also be produced in *trans* to regulate the expression of protein-coding genes. For example, the lncRNA *APOLO* is able to form R-loops in *trans* in *Arabidopsis thaliana* as part of a widespread regulation of auxin responsive genes[80] (FIG. 2c).

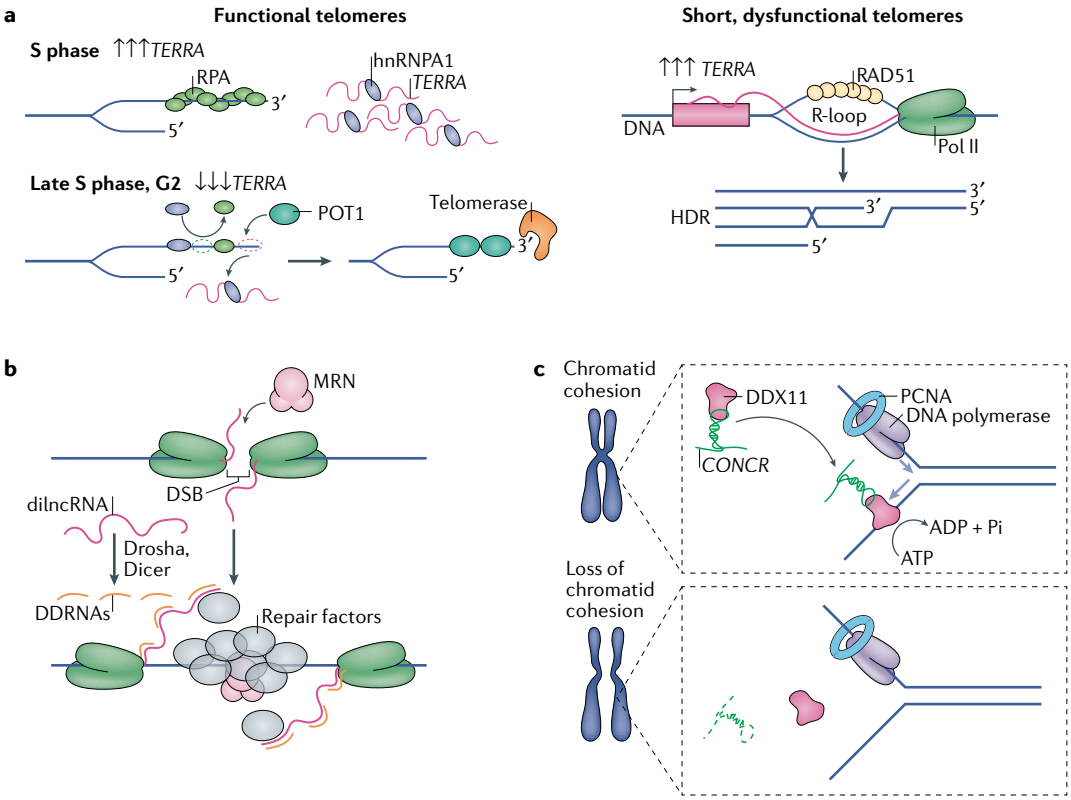
Transcription regulation

The relative position between a lncRNA and its neighbouring genes is a key determinant of their regulatory relationship. As widespread antisense and bidirectional lncRNA transcription was found to be evolutionarily conserved[81], the non-random genomic distribution of lncRNAs could represent an evolutionary adaptation of genes to regulating their own expression in a context-specific manner. For instance, the genomic arrangement of divergent lncRNAs is key for gene regulation in *cis*[82]. This regulation can be mediated by two main, non-mutually exclusive mechanisms: the lncRNA transcript can regulate neighbouring loci, and/or the act of transcription or splicing of the lncRNA can generate a chromatin state or steric impediment that

influences the expression of nearby genes. Thus, interpretation of several orthogonal loss-of-function and gain-of-function experiments is required to discern these possible modes of lncRNA functionality[83].

Gene silencing by lncRNAs. The best-known mechanisms of gene repression mediated by lncRNAs are related to gene-dosage compensation. The main representative of this functionality is the lncRNA *XIST*,

Box 1 | **Long non-coding RNAs and genome integrity**



Long non-coding RNAs (lncRNAs) have demonstrated roles in different aspects of genome regulation and homeostasis directly related to the maintenance of genome stability.

Regulation of chromatin structure and function

Heterochromatin harbours regions rich in repetitive sequences that are potentially transcribed, such as telomeres and centromeres. Functional telomeres exploit conserved regulatory RNAs to maintain their structure. Telomeric repeat-containing RNAs (*TERRA*), a heterogeneous group of lncRNAs ranging in length from 100 bp to 9 kb, are transcribed from the telomeric 5′-TTAGGG-3′ repeats[271].

At telomeres, heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) competes with replication protein A (RPA) for binding telomeric single-stranded DNA (ssDNA). By interacting with hnRNPA1, *TERRA* can regulate a switch in the binding of telomeric ssDNA from RPA to POT1 (protection of telomeres protein 1) in a cell cycle-dependent manner; POT1 recruits the telomerase complex, which maintains the length and, thus, functionality of telomeres[272] (see the figure, part **a**). *TERRA* also function as a scaffold to recruit chromatin factors, thereby facilitating heterochromatin formation at telomeres[273,274]. At short, dysfunctional telomeres, transcription of *TERRA* forms R-loops that are recognized by the recombinase RAD51, which promotes telomere-length maintenance through homology-directed repair (HDR), thereby reducing cell senescence[275,276] (see the figure, part **a**). Furthermore, *TERRA* can activate histone methylation at dysfunctional telomeres[277].

In fission yeast, centromeres encode centromeric-repeat lncRNAs (*cen*) that regulate heterochromatin formation through RNAi[278]. *cen* lncRNAs also regulate centromere and kinetochore functions in *Xenopus laevis*, where they are actively transcribed during mitosis and promote correct chromosome segregation[279,280].

Another class of chromosome stability regulators are asynchronous replication and autosomal RNAs (ASARs), which are identified at human chromosomes 6 and 15 and contain L1 retrotransposon sequences in antisense orientation[281–283]. ASARs are expressed in a mono-allelic fashion quite abundantly, so they can spread from their locus to the entire chromosome to synchronize the replication timing of each pair of autosomes. We speculate that ASARs are pervasively transcribed, and that their reciprocal interactions in regulating replication timing might be more complex than is presently known[284,285].

Roles in the DNA damage response

Endogenous ncRNAs can initiate HDR and non-homologous end joining (NHEJ) in yeast[286,287] and mammalian[288,289] cells. Interestingly, transcription of lncRNAs recruits DNA repair proteins, but it is yet to be fully determined whether RNA polymerase II (Pol II) alone can direct repair proteins to DNA damage sites or whether the nascent lncRNA has a role in such recruitment[288,290]. Damage-induced long non-coding RNAs (dilncRNAs) are produced at sites of DNA double-strand breaks following recruitment of Pol II by the MRE11–RAD50–NBS (MRN) complex, and are processed by the RNAi endoribonucleases Drosha and Dicer into small DNA damage response RNAs (DDRNAs), which pair with their nascent dilncRNA counterparts and promote the recruitment of repair proteins[290] (see the figure, part **b**).

Several lncRNAs are activated during genotoxic stress to regulate homologous recombination or NHEJ[291,292], or other DNA damage response pathways, in a p53-dependent[261,293] or p53-independent[294] manner. Modulation of specific transcriptional programmes by lncRNAs is another mechanism that regulates the DNA damage response[205,293]. For instance, non-coding RNA activated by DNA damage (*NORAD*) acts as decoy of the Pumilio RNA-binding proteins[156,157], which are involved in post-transcriptional repression of DNA replication and repair factors. Moreover, through protein binding, nuclear *NORAD* has been associated with topoisomerase 1-mediated maintenance of genomic stability[262]. A distinct function in DNA damage has been described for the lncRNA cohesion regulator non-coding RNA (*CONCR*), which regulates in *trans* the function of the DNA-dependent ATPase and helicase DEAD/H-box helicase 11 (DDX11) (REF.[295]). During DNA replication, *CONCR*, which is expressed in an MYC-dependent manner, recruits DDX11 to replication forks and facilitates its ATPase activity, which promotes replication completion and sister chromatid cohesion[265] (see the figure, part **c**). These observations reveal the crucial roles of lncRNAs in genome integrity, and suggest putative therapeutic targeting of aberrant DNA repair pathways in cancer and other diseases. DSB, double-strand break; PCNA, proliferating cell nuclear antigen; Pi, inorganic phosphate.

which is responsible for X chromosome inactivation in cells of female mammals. During embryonic development, *XIST* molecules spread over one of the two X chromosomes and cause the silencing of a large proportion of its genes[84]. *XIST* is able to silence large chromosomal regions even when it is ectopically expressed from a different chromosome[85]. A complex interplay of protein interactors contributes to *XIST*-mediated gene silencing[55–58]. In addition, a study in mESCs has revealed that the rapid coating of the X chromosome by *Xist* depends on the ability of the lncRNA to exploit the 3D chromatin organization, which allows it to spread from sites that are spatially proximal to its locus to distant loci, while it modifies the target chromatin structure through its interactions with chromatin modifiers[86]. This confers *XIST* a role in shaping the 3D architecture of the inactivated X chromosome, a process that, once initiated, has been shown to persist even in the absence of *XIST*, thereby defining the role of the lncRNA as an initiator of epigenetic memory, which is maintained during the later phases of X chromosome inactivation by the protein complexes recruited by *XIST* to chromatin[56,87,88].

At other loci, *cis*-acting lncRNAs can promote an inactive chromatin state by directly or indirectly interacting with chromatin in proximity to their site of transcription. For instance, the R-loop formed in *cis* by the lncRNA *ANRASSF1* directs PRCs to their targets to regulate gene expression[43,89,90]. In *A. thaliana*, the lncRNA *COOLAIR*, which is environmentally induced by cold at the *FLOWERING* locus, lingers at its site of transcription and coats the locus to promote PRC2-dependent H3K27me3 (REFS[91,92]).

lncRNAs can suppress gene expression by interfering with the transcription machinery, which leads to alteration of the recruitment of transcription factors or Pol II at the inhibited promoter[53], alteration of histone modifications[53,93] and reduction of chromatin accessibility[94,95]. An example of this group of regulators is the mouse imprinted *Airn* lncRNA, which determines the onset of allele-specific expression during mESC differentiation[53]. *Airn* transcription from the paternal allele causes the displacement of Pol II from the overlapping *Igf2r* promoter, resulting in transcription pausing and gene silencing[53,96,97] (FIG. 3a). Another mechanism by which a lncRNA can regulate widespread transcriptional interference is represented by the conserved lncRNA CHD2 adjacent, suppressive regulatory RNA (*Chaserr*)[95], which is located upstream of the chromatin remodeller *Chd2* gene. *Chaserr* depletion increased accessibility at the *Chd2* promoter, as well as at several other promoters, which are all regulated by CHD2. The allele specificity of *Chaserr* towards *Chd2* in *Chaserr*-mutated mouse models confirmed *Chaserr* functions strictly in *cis*. Interestingly, CHD2 binds nascent RNAs, including *Chaserr*, and promotes their expression. The reciprocal regulation of CHD2 and *Chaserr* represents a regulatory feedback loop, in which CHD2 regulates its own expression using *Chaserr* as a sensor of CHD2 levels[95].

lncRNAs transcribed at enhancers. Active enhancers can be transcribed into two major types of non-coding RNAs: eRNAs and enhancer-associated lncRNAs (elncRNAs). The main distinction between the two groups of transcripts is based on their features: eRNAs are relatively short, bidirectional capped transcripts, which are generally unspliced, non-polyadenylated and unstable[98,99]. By contrast, elncRNAs are mostly unidirectional, polyadenylated and spliced. The distinction between the two transcript types is not always clear-cut and they can be confused in the literature. Although the correlation between enhancer activity and eRNA expression is well established, whether the eRNA transcripts per se are functional is still under debate. Nevertheless, some eRNAs have been functionally linked with gene expression. In addition to functioning through pre-existing chromatin conformations (FIG. 3b), some eRNAs can facilitate or directly drive chromatin looping by interacting with scaffold proteins such as the Mediator or the structural maintenance of chromosomes complex cohesin. These interactions generate regulatory contacts between enhancers and promoters that can be located several megabases apart[100–102] (FIG. 3c).

Some enhancer loci produce elncRNAs, the expression of which is related to that of their enhancer elements[103–106]. Notably, elncRNA splicing has been positively associated with the activity of their associated enhancers and correlated with the abundance of the neighbouring protein-coding genes[107,108]. Furthermore, elncRNAs can modulate chromatin structure and topology in cooperation with chromatin-regulating proteins[40,109]. Gene-activating mechanisms described for eRNA function can also define the functions of elncRNAs (FIG. 3b,c).

Gene activation by elncRNAs often results in complex phenotypes related to human diseases[110–112]. The lncRNA *SWINGN* is located at the boundary of a topologically associating domain that includes its target gene *GAS6* (REF.[113]). *SWINGN* promotes the interaction between SWI/SNF chromatin remodelling complexes and the transcription start site of *GAS6*, but also with additional distant loci involved in malignant phenotypes, accounting for its pro-oncogenic role[113]. In addition, some lncRNAs are able to promote the formation of genomic domains comprising many inter-loci interactions, as shown for the lncRNAs ESR1 locus enhancing and activating non-coding RNAs (*ELEANORs*)[114]. Together with other similarly acting lncRNAs, these transcripts are examples of how transcription can regulate the formation of genomic compartments to drive gene expression[36,112].

As described above, it should be considered that lncRNAs can activate gene expression in a transcript-independent fashion, adding complexity to the interpretation of their gene regulatory functions. For example, functional DNA elements embedded in lncRNA loci can activate the expression of neighbouring genes[115–117]. The lncRNA Bend4-regulating effects not dependent on the RNA (*Bendr*) regulates its neighbouring gene *BEND4* in *cis* through the presence of enhancer elements in *Bendr* that are activated by its transcription. Deletion of the *Bendr* promoter, but not insertion of a premature poly(A) in the first exon of *Bendr*, suppressed occupancy of the *BEND4* promoter by Pol II[116] (FIG. 3d). Other lncRNAs have been identified with similar roles in activation of proximal enhancers[116–118].

Regulatory networks involving* cis*-acting lncRNAs. It is becoming increasingly clear that regulation in *cis* by lncRNAs is not only determined by one-on-one effects of a lncRNA on a neighbouring gene. lncRNAs are part of complex regulatory units, in which the expression of a protein-coding gene may be regulated by the coordinated activity of two or more lncRNAs and of transcript-dependent and transcript-independent

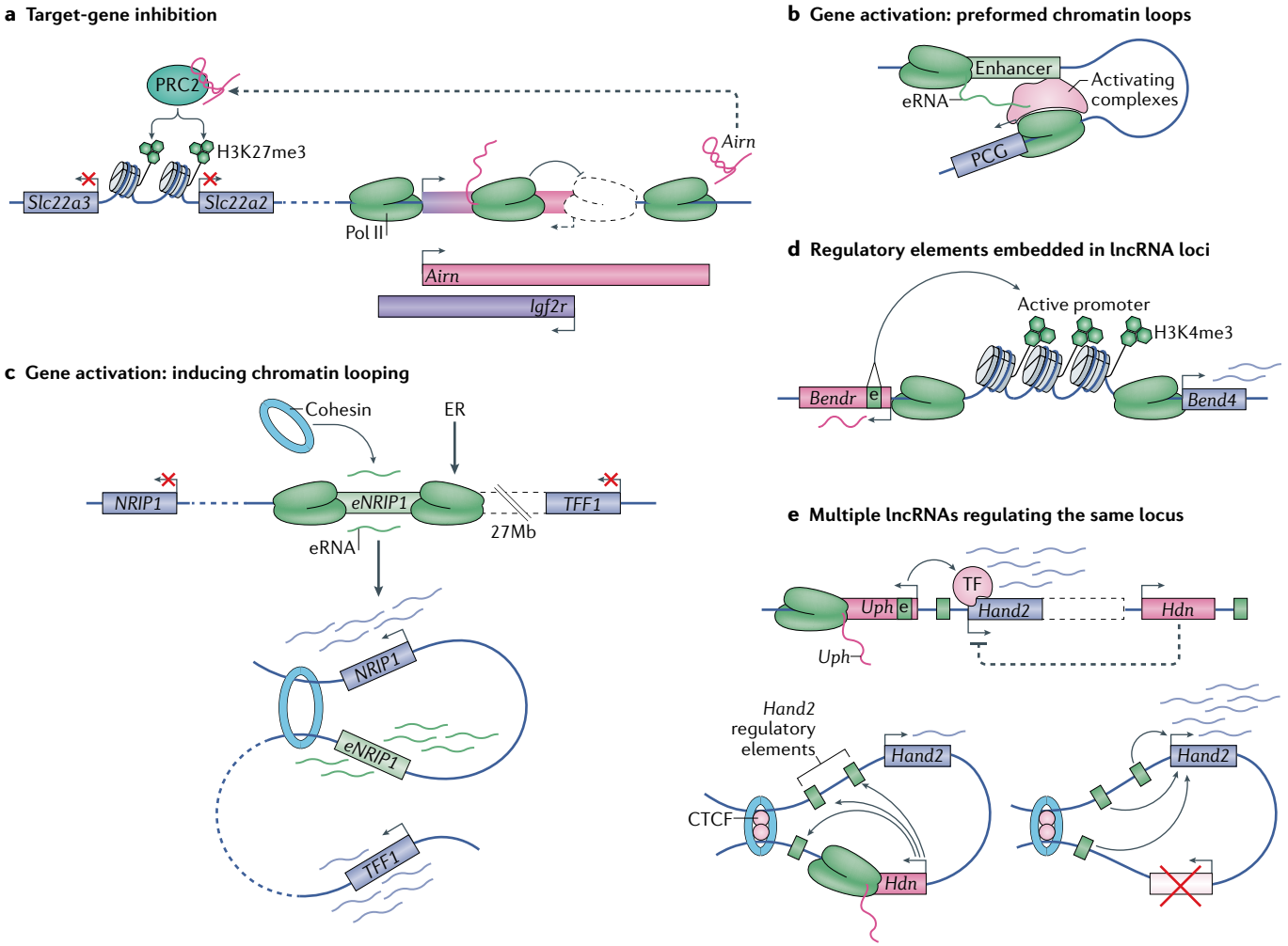


Fig. 3 | **Transcription regulation by long non-coding RNAs. a** | Long non-coding RNAs (lncRNAs) can inhibit gene expression in a transcript-dependent and/or in a transcription-dependent (that is, transcript-independent) manner. In mouse extra-embryonal tissues, antisense of IGF2R non-protein coding RNA (*Airn*) functions in *trans* as it is guided through a specific 3D chromosome conformation (not shown) to the promoters of two distal imprinted target genes, solute carrier family 22 member 2 (*Slc22a2*) and *Slc22a3*. Once there, *Airn* recruits Polycomb repressive complex 2 (PRC2), which catalyses histone H3 Lys27 trimethylation (H3K27me3) and gene silencing. *Airn* also functions in *cis*, on its overlapping protein-coding gene insulin-like growth factor 2 receptor (*Igf2r*). *Airn* transcription causes steric hindrance for RNA polymerase II (Pol II) at the transcription start site of *Igfr2r*, which is followed by promoter methylation (not shown) and *Igfr2r* silencing[53,96,97]. **b** | lncRNAs and enhancer RNAs (eRNAs) can promote the expression of protein coding genes (PCGs) that are in close proximity to their enhancers through preformed chromatin loops (for example, the eRNA P53BER (p53-bound enhancer region)[266] and the enhancer-associated lncRNA (elncRNA) *SWINGN* (SWI/SNF interacting GAS6 enhancer non-coding RNA)[113]), thereby allowing recruitment of chromatin-activating complexes to the promoters of the PCGs. **c** | An important feature of some eRNAs and elncRNAs is their ability to regulate distant genes by directly promoting chromatin looping through the recruitment of looping factors[18,36,139,267]. For example, following oestrogen receptor (ER) transcription activation, the *NRIP1* enhancer (*eNRIP*) is bi-directionally transcribed into an eRNA, which recruits cohesin to form short-range (solid line) and long-range (dashed line) chromatin loops, thereby promoting contact between the *NRIP1* enhancer and the promoters of *NRIP1* and trefoil factor 1 (*TFF1*), two of the several genes activated in response to ER activation[267]. **d** | lncRNAs can activate gene expression in a transcript-independent manner. Transcription of Bend4-regulating effects not dependent on the RNA (*Bendr*) is sufficient to activate enhancer elements (e) embedded in its locus, which promotes the formation of an active chromatin state (marked by H3K4me3) at the promoter of the proximal gene BEN domain containing protein 4 (*Bend4*)[116]. **e** | Example of a complex regulatory unit formed by the lncRNAs *Upperhand* (*Uph*) and *Handsdown* (*Hdn*) in regulating the PCG heart and neural crest derivatives expressed 2 (*Hand2*). An enhancer embedded in *Uph* activates the transcription of the proximal *Hand2* gene when the lncRNA gene is transcribed, without requiring chromatin reorganization[118]. By contrast, chromatin looping is necessary for *Hdn* function, as it puts its promoter in spatial proximity with *Hand2*-activating enhancers. When *Hdn* transcription is activated, the *Hand2* enhancers become unavailable for *Hand2* promoter activation, thereby inhibiting its expression. Removal of *Hdn* or reduction of its transcription leads to increased expression of *Hand2*. CTCF, CCCTC-binding factor; NRIP1, nuclear receptor interacting protein 1; TF, transcription factor.

mechanisms. Several of these units act on essential developmental genes or on loci with important functions in maintaining the equilibrium between normal and hyperproliferative processes.

The heart and neural crest derivatives expressed 2 (*Hand2*) gene encodes a transcription factor essential for heart development, in which dosage imbalance can cause serious malformations[119]. Two lncRNA loci are found in the vicinity of *Hand2*, which regulate its expression through different mechanisms (FIG. 3e). Deletion in mice of either of these lncRNA genes leads to embryonic lethality. One of these lncRNAs, *Upperhand* is transcribed from a bidirectional promoter, divergently from the *Hand2* promoter. A study that analysed the effects of *Upperhand* deletion on *Hand2*, by intercrossing *Hand2* and *Upperhand* knockout heterozygous mice, revealed that *Upperhand* controls *Hand2* transcription in *cis*[118]. Furthermore, the insertion of a polyadenylation signal downstream of the *Upperhand* transcription start site (thereby abolishing transcription) affected *Hand2* expression, whereas *Hand2* expression was unaffected by depletion of the mature *Upperhand* transcript, elegantly demonstrating that *Upperhand* controls *Hand2* in *cis* in a transcription-dependent but transcript-independent fashion[118]. A separate study reports partially conflicting data on the outcomes of *Upperhand* deletion, obtained from three different knockout mouse models, in which the effects on *Hand2* expression are more subtle[120]. However, in both studies, alteration of *Upperhand* expression resulted in strong cardiac abnormalities linked with *Upperhand*-mediated regulation of *Hand2*. Additional studies will be required to fully unveil the complex interaction between *Hand2* and the lncRNAs regulating it (FIG. 3e).

The lncRNA *Handsdown* is located several kilobases downstream of *Hand2*, and inhibits *Hand2* expression within a preformed chromatin loop mediated by CTCF[121]. The mechanism behind this regulation involves looping-mediated interaction between *Handsdown* promoter and regulatory elements upstream of the *Hand2* gene in mouse embryonic cardiomyocytes, which thus become unavailable for *Hand2* activation (FIG. 3e). *Upperhand* and *Handsdown* exemplify how lncRNAs can act in association to finely tune the expression of essential genes.

Another regulatory possibility is that the functions of the transcript and of the locus of a lncRNA are uncoupled and promote opposite outcomes. The locus of the lncRNA HOXA upstream non-coding transcript (*Haunt*) contains enhancers that activate the expression of HOXA genes. By contrast, the *Haunt* transcript acts as a decoy for the enhancers embedded in its own locus, thereby inhibiting the expression of HOXA genes[122]. These opposing outcomes have been implicated in preventing aberrant HOXA expression.

In conclusion, several interdependent factors emerge as crucial regulators of lncRNA function: the relative position of the lncRNA and target gene, the formation of co-transcriptional RNA–DNA and RNA–protein interactions, and whether the regulatory effect is mediated by the lncRNA transcript or by its transcription. The cell-specific co-occurrence of these factors determines the regulatory potential of individual lncRNAs.

Roles in scaffolding and condensates

Nuclear condensates are membraneless RNA–protein compartments involved in many cellular processes[123]. By virtue of their scaffolding or regulatory activities, several abundant lncRNAs are essential for the assembly and function of different nuclear condensates.

The lncRNA nuclear paraspeckle assembly transcript 1 (*NEAT1*) underlies the complex organization and functions of paraspeckles[124,125] (FIG. 4a). The *NEAT1* gene produces two isoforms that share a common 5′ end but have different 3′ ends: *NEAT1 short*, which has a poly(A) tail produced from an upstream polyadenylation signal; and *NEAT1 long*, which has a stable 3′ U–A·U triple-helix structure that is cleaved by RNase P[126–128]. *NEAT1 long*, but not *NEAT1 short*, is essential for the assembly of paraspeckles[126–128]. The middle region (8–16.6 kb) of *NEAT1 long* contains two subdomains (12–13 kb and 15.4–16.6 kb) responsible for recruiting the paraspeckle core proteins NONO and SFPQ to initiate the assembly of paraspeckles through liquid–liquid phase separation[129] (FIG. 4a). How exactly *NEAT1 long* is assembled into the core, spherical shape of paraspeckles remains unclear. Future dissection of the key structural modules of *NEAT1 long* should be helpful in gaining mechanistic insights into *NEAT1*-scaffolded condensates. As an interesting note, however, global RNA structure mapping showed that *NEAT1 long* might not contain long-range intramolecular interactions and structure[130,131]

The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is perhaps the most abundant lncRNA in most cultured cells. It is specifically localized in nuclear speckles[126,132], has important roles in pre-mRNA splicing and in transcription[133–135], and is involved in cancer progression and metastasis[136,137]. Although *MALAT1* interacts with many proteins, depletion of *MALAT1* does not affect the formation of nuclear speckles but, rather, causes defects in their composition[138]. Each nuclear speckle is a multilayered compartment, in which nuclear speckle proteins such as the splicing factors SON and SC35 (also known as SRSF2) are localized at the centre and *MALAT1* at the periphery[138] (FIG. 4b). How this unique organization of *MALAT1* facilitates the formation and function of nuclear speckles remains to be investigated. Unlike *NEAT1*, *MALAT1* forms many long-range structures[131], which are perhaps involved in its multivalent interactions with different RBPs and pre-mRNAs.

Application of the recently developed RNA in situ conformation sequencing (RIC-seq) revealed that *MALAT1* functions as an RNA hub for many highly expressed RNAs. For example, a high-confidence *NEAT1*–RNA interaction analysis suggested that the 5′ region of *NEAT1* interacts with *MALAT1* in *trans*[139]. RIC-seq also revealed multiple interaction sites between U1 small nuclear RNA and *MALAT1* (REF.[139]), which were also found using psoralen analysis of RNA interactions and structures[131] (FIG. 4b). Given the peripheral localization of *MALAT1* in nuclear speckles[138], it will be interesting to understand how the RNA hub function of *MALAT1* is achieved at the surface of nuclear speckles. These studies reveal a complicated regulatory network, which could be uncovered by further dissecting the structural modules of *MALAT1* and its functions of scaffolding different RBPs.

Paraspeckles
Nuclear condensates enriched in several proteins, a subset of mRNAs and even some primary microRNAs, which are involved in various biological processes.

Nuclear speckles
Nuclear domains in mammalian cells enriched in pre-mRNA splicing factors, located in inter-chromosomal regions.

RNA in situ conformation sequencing
(RIC-seq). A method of determining intramolecular and intermolecular RNA–RNA interactions.

Small nucleolar RNA-related lncRNAs
Unusually processed long non-coding RNAs (lncRNAs) that are derived from introns and contain small nucleolar RNAs at both ends.

5′ small nucleolar RNA-capped and 3′ polyadenylated lncRNAs
(SPAs). Unusually processed long non-coding RNAs (lncRNAs) that are derived from polycistronic transcripts, capped by small nucleolar RNAs at their 5′ end and polyadenylated at their 3′ end.

The scaffolding nature of lncRNA-mediated gene regulation is also illustrated by small nucleolar RNA-related lncRNAs and 5′ small nucleolar RNA-capped and 3′ polyadenylated lncRNAs (SPAs), which are lncRNA species produced from the Prader–Willi syndrome (PWS; a neurodevelopmental disorder) minimal deletion of chromosome 15q11–13. Whereas induced pluripotent stem cells derived from individuals with PWS

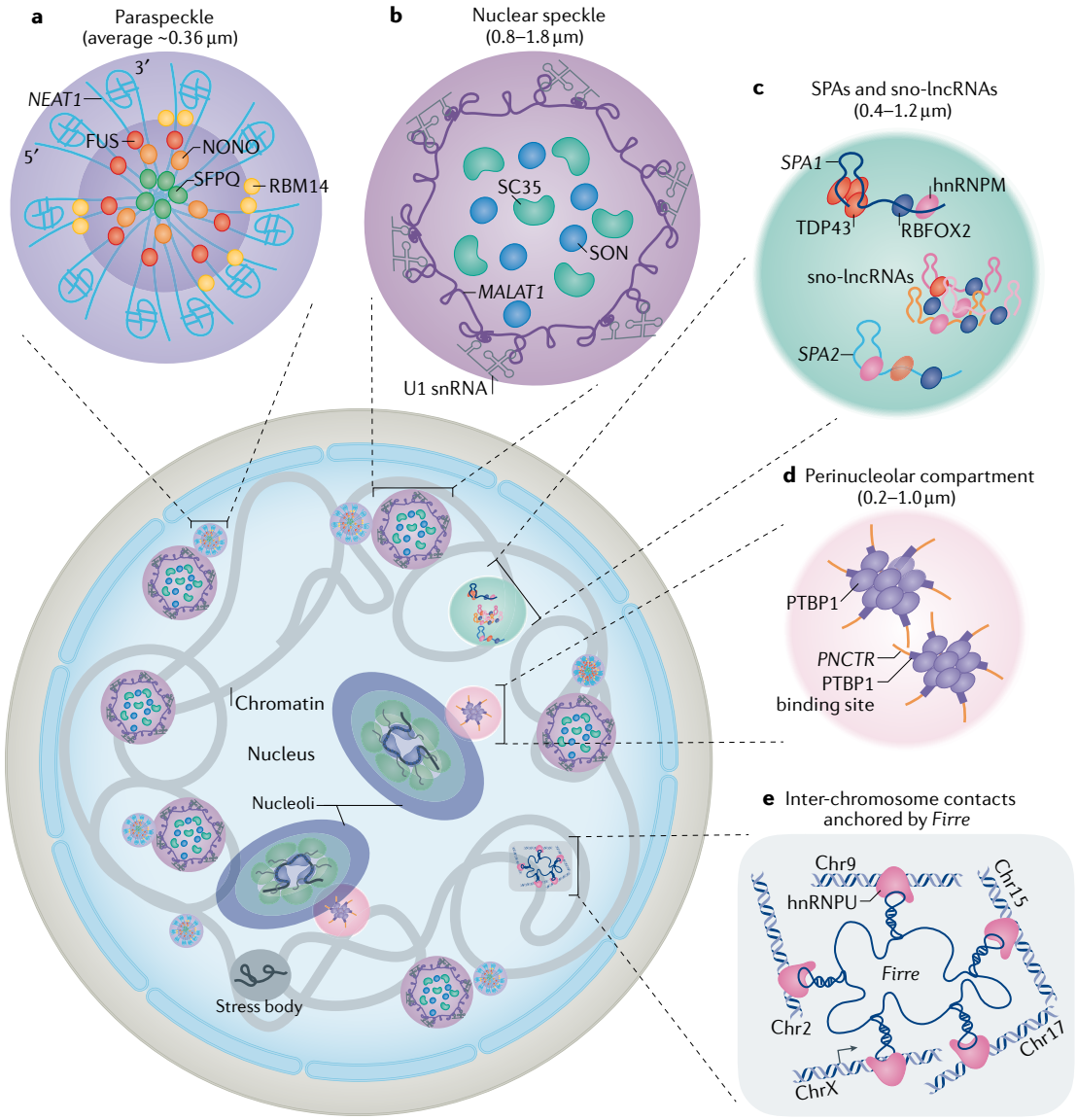


Fig. 4 | **Roles of long non-coding RNAs in nuclear organization. a** | The long non-coding RNA (lncRNA) nuclear paraspeckle assembly transcript 1 (*NEAT1*) is essential for the formation of paraspeckles[125]. *NEAT1* sequesters numerous paraspeckle proteins to form a highly organized core–shell (dark and light purple, respectively) spheroidal nuclear body[124]. The middle region of *NEAT1* is localized in the centre of paraspeckles and the 3′ and 5′ regions are localized in the periphery[124]. Different paraspeckle proteins are embedded by *NEAT1* into the spheroidal structure in the core region (non-POU domain containing octamer binding (NONO), fused in sarcoma (FUS) and splicing factor, proline- and glutamine-rich (SFPQ)) or the shell region (RNA binding motif protein 14 (RBM14))[124]. **b** | The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is localized at the periphery of nuclear speckles[126,132] and is involved in the regulation of pre-mRNA splicing[116,134,135]. At the periphery, *MALAT1* interacts with the U1 small nuclear RNA (U1 snRNA)[139], whereas proteins such as SON DNA and RNA binding protein (SON) and splicing component, 35 kDa (SC35) are localized at the centre of nuclear speckles[138]. **c** | 5′ small nucleolar RNA-capped and 3′-polyadenylated lncRNAs (SPAs)[141] and small nucleolar RNA-related lncRNAs (sno-lncRNAs)[140] accumulate at their transcription sites and interact with several splicing factors such as RNA binding protein fox-1 homologue 2 (RBFOX2), TAR DNA-binding protein 43 (TDP43) and heterogeneous nuclear ribonucleoprotein M (hnRNPM) to form a microscopically visible nuclear body that is involved in the regulation of alternative splicing[141]. **d** | The perinucleolar compartment contains the lncRNA pyrimidine-rich non-coding transcript (*PNCTR*), which sequesters pyrimidine tract-binding protein 1 (PTBP1) and, thus, suppresses PTPBP1-mediated pre-mRNA splicing elsewhere in the nucleoplasm[142]. **e** | Functional intergenic RNA repeat element (*Firre*) is transcribed from the mouse X chromosome and interacts with the nuclear matrix factor hnRNPU to tether chromosome (Chr) X, 2, 9, 15 and 17 into a nuclear domain[151,153]. The size of each type of nuclear body is indicated in parts **a**–**d**[268].

Perinucleolar compartment
A nuclear body at the periphery of the nucleolus. The perinucleolar compartment is a dynamic structure and is highly enriched in RNA-binding proteins and RNA polymerase III.

Nuclear stress bodies
Nuclear condensates that are formed under different stresses, such as heat shock and DNA damage.

Serine and arginine-rich (SR) proteins
RNA binding proteins known as regulators of constitutive and alternative splicing.

conspicuously lack these lncRNAs, they are abundantly expressed and accumulate in *cis* in normal hESCs, and sequester more than 1% of each tested splicing factor, including RBFOX2, TDP43 and hnRNPM[140,141] (FIG. 4c). Importantly, hESCs lacking PWS-associated lncRNAs exhibited altered patterns of alternative splicing and protein binding to pre-mRNAs associated with neuronal functions[141]. Similarly, gene regulation by multivalent interactions between lncRNAs and RBPs was also found for *PNCTR* in the perinucleolar compartment[142] (FIG. 4d). *PNCTR* is a short, tandem repeat-enriched RNA generated from the ribosomal DNA intergenic spacer; it is highly expressed in cancer cells and required for lung cancer cell survival. This lncRNA contains hundreds of polypyrimidine tract-binding protein 1 (PTBP1) binding motifs, and therefore sequesters PTBP1 to the perinucleolar compartment and suppresses its splicing activity elsewhere in the nucleoplasm[142]. Together, these studies show that multivalent binding between lncRNAs and RBPs is an effective mechanism of regulating disease-specific alternative splicing.

Nuclear stress bodies are another type of nuclear condensate. Their formation requires heat shock transcription factor 1 and transcription of the heterogenous lncRNAs highly repetitive satellite III (*HSATIII*) in conditions of heat and chemical stresses[143]. *HSATIII* lncRNAs accumulate at their transcription sites, sequester scaffold attachment factor B[144], Serine and arginine-rich (SR) proteins[145,146] and transcription factors[147], and assemble them into nuclear stress bodies. *HSATIII* lncRNAs were proposed to promote intron retention at hundreds of mRNAs by modulating the phosphorylation of SR proteins[148]. Similar lncRNA-induced stress bodies were also found for intergenic spacer RNAs under heat shock and other stresses[149,150].

In addition to functioning as scaffolds of proteins and RNAs at nuclear condensates, lncRNAs can bring different chromosomes into proximity in nuclear domains. *FIRRE* consists of numerous RNA variants transcribed from the X chromosome; it interacts with the nuclear matrix factor hnRNPU to maintain a nuclear domain through its scaffolding function[151] (FIG. 4e). In mice, gene-expression changes caused by *Firre* deletion can be partially rescued by expressing transgenic *Firre* RNA, indicating that it has functions in *trans*[152]. Indeed, *Firre* is localized in proximity to its locus on the X chromosome, as well as on mouse chromosomes 2, 9, 15 and 17, and functions in *trans* as a chromosome scaffold lncRNA[151,153] (FIG. 4e). It remains to be determined whether such lncRNA-anchored inter-chromosomal structures are phase separated.

Roles in post-transcriptional regulation

In addition to their roles in transcription regulation and nuclear organization, lncRNAs control several other aspects of gene expression, and some lncRNAs are even translated into functional peptides[154]. Nevertheless, as bona fide non-coding RNAs, lncRNAs mainly act through their ability to establish interactions with proteins and nucleic acids (FIG. 5). Here, we highlight a few of the many different modes of lncRNA function as post-transcriptional, translational and post-translational regulators.

Modes of direct lncRNA–protein interactions. lncRNAs are involved in post-transcriptional regulation by sequestering proteins through their binding to RNA sequence motifs or structures to form specific lncRNA–protein complexes (lncRNPs), resulting in altered mRNA splicing and turnover, and, in certain biological contexts, in the modulation of signalling pathways (FIG. 5A). Abundant lncRNAs, such as the aforementioned small nucleolar RNA-related lncRNAs[140] and SPAs[140,141] in the PWS region (FIG. 4c) and *PNCTR*[142] (FIG. 4d), contain clusters of motifs that sequester different splicing factors — including UGCAU and GCAUG motifs, which are bound by RBFOX2 (REF.[140]), UG-rich sequences bound by TDP43 (REFS[140,141]) and YUCUYY and YYUCUY motifs bound by PTBP1[142] — thereby suppressing the splicing of pre-mRNAs containing the same motifs[140–142] (FIG. 5A). Other mechanisms of lncRNA-mediated splicing regulation involve lncRNA modulating post-translational modifications of splicing factors, splicing repression through the formation of RNA–RNA hybrids with target pre-mRNAs and fine-tuning of target-gene splicing through chromatin remodelling (reviewed in REF.[155]). In the cytosol, non-coding RNA activated by DNA damage (*NORAD*) is highly expressed following DNA damage and maintains genomic stability by sequestering Pumilio proteins[156,157]. Pumilio proteins bind to a specific motif in mRNA 3′ untranslated regions and facilitates mRNA decay through deadenylation and decapping[158]. Each *NORAD* molecule contains 15 Pumilio binding motifs and, consequently, the ~500–1,000 copies of *NORAD* expressed in a single HCT116 cell can sequester ~7,500–15,000 Pumilio protein molecules, thereby sequestering most Pumilio from target mRNAs involved in maintaining genomic stability[156,157] (FIG. 5A). It should be noted, however, that although *NORAD* offers a sufficient number of Pumilio binding sites to sequester the Pumilio proteins, the number may be a small portion of the total number of Pumilio binding sites offered by all cellular transcripts.

In addition to binding to sequence motifs, lncRNAs can fold into structures that interact with proteins involved in key signalling pathways. For example, *FAST* is transcribed from the antisense strand of the *FOXD3* gene[6], is highly expressed in hESCs and is required for the maintenance of hESC pluripotency. *FAST* depletion resulted in hESC differentiation owing to impaired WNT signalling. Each *FAST* molecule forms five stem–loops, which provide a multivalent platform for interacting with and blocking the E3 ubiquitin ligase β-TrCP from binding to phosphorylated β-catenin and mediating its degradation. *FAST* therefore enables β-catenin translocation into the nucleus to activate the transcription of WNT-dependent pluripotency genes[6] (FIG. 5A). Other lncRNAs block sites of post-translational modification; for example, NF-κB interacting lncRNA (*NKILA*) forms two distinct hairpins, hairpin A (nucleotides 322–359) and hairpin B (nucleotides 395–418), which both bind to p65. Hairpin B can stabilize the association between *NKILA* and the NF-κB transcription complex and with the kinase IκB to modulate T cell activation-induced cell death by inhibiting

NF-κB activity[159]. The molecular basis of how such non-canonical RBPs proteins interact with lncRNAs remains to be explored. Nonetheless, the stoichiometric relationship between this group of lncRNAs and their interacting proteins should be carefully evaluated (Supplementary Box 1).

Pairing with other RNAs to recruit protein complexes. Some lncRNAs can directly base pair with other RNAs and subsequently recruit proteins involved in mRNA degradation. For example, Staufen-mediated mRNA decay is carried out by the double-stranded RNA-binding protein Staufen homologue 1 (STAU1), which binds

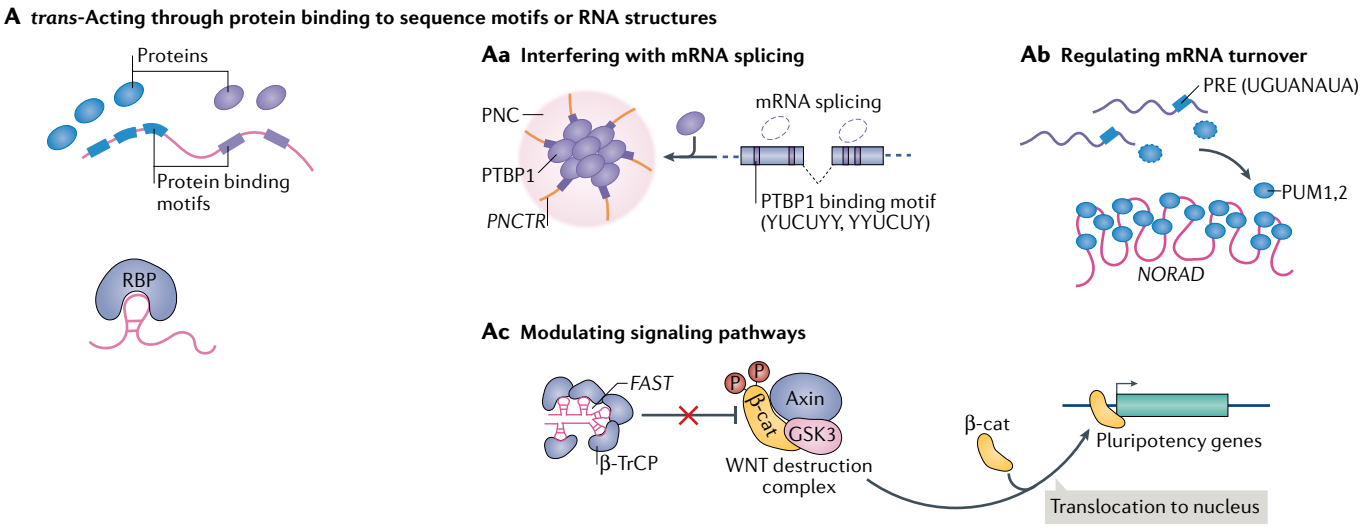


Fig. 5 | **Post-transcriptional functions of *trans*-acting long non-coding RNAs. A** | *trans*-Acting long non-coding RNAs (lncRNAs) interact with RNA-binding proteins (RBPs) through sequence motifs or by forming unique structural motifs. **Aa** | Pyrimidine-rich non-coding transcript (*PNCTR*) sequesters pyrimidine tract-binding protein 1 (PTBP1) to the perinucleolar compartment (PNC) and, thus, suppresses PTBP1-mediated mRNA splicing elsewhere in the nucleoplasm[142]. **Ab** | In the cytosol, non-coding RNA activated by DNA damage (*NORAD*) sequesters Pumilio (PUM) RBPs, which repress the stability and translation of mRNAs to which they bind[156,157,269]. **Ac** | Human FOXD3 antisense transcript 1 (*FAST*) forms several structural modules that bind the E3 ligase β-transducin repeats-containing protein (β-TrCP), thereby blocking the degradation of its substrate β-catenin (β-cat), leading to activation of WNT signalling in human embryonic stem cells[6]. **B** | *trans*-Acting lncRNAs directly interact with RNAs through base pairing. **Ba** | Terminal differentiation-induced ncRNA (*TINCR*)[160] or half-STAU1-binding site RNAs (1/2-sbsRNAs)[161] promote or suppress mRNA stability, respectively, by forming intermolecular duplexes that bind Staufen homologue 1 (STAU1), the key protein of Staufen-mediated mRNA decay[160–162]. **Bb** | The *SINEB2* repeat of mouse antisense to ubiquitin carboxyterminal hydrolase L1 (*AS-Uchl1*) complementarily binds the *Uchl1* mRNA and promotes polysome association with *Uchl1* and translation[164]. **C** | Some abundant lncRNAs affect gene expression by functioning as competitive endogenous RNAs (ce-RNAs)[165,166]. For example, *lncRNA-PNUTS* is generated by alternative splicing of the *PNUTS* pre-mRNA by heterogeneous nuclear ribonucleoprotein E1 (hnRNPE1)[169]. *lncRNA-PNUTS* contains seven miR-205 binding sites, which reduce the availability of miR-205 to bind and suppress the zinc finger E-box-binding homeobox 1 (*ZEB1*) and *ZEB2* mRNAs[169]. GSK3, glycogen synthase kinase 3; miR, microRNA; P, phosphate group; *PNUTS*, phosphatase 1 nuclear targeting subunit; PRE, Pumilio response element.

3′ untranslated regions of mRNAs undergoing translation[160–162]. lncRNAs containing *Alu* retroelements in human[161] or other short interspersed elements (SINEs) in mouse[162] can promote Staufen-mediated mRNA decay of mRNAs bearing partial or full complementarity with these repeats by recruiting STAU1. By contrast, the lncRNA *TINCR*, which is highly expressed during and required for epidermal differentiation, contains several 25-nucleotide motifs that base pair with complementary sequences in differentiation mRNAs; *TINCR* also recruits STAU1, and the *TINCR*–STAU1 complex stabilizes the differentiation mRNAs[160] (FIG. 5B). Of note, a recent study indicated that *TINCR* may code a peptide[163].

In another example, base pairing in *trans* appears to be crucial for loading mRNAs on active polyribosomes (FIG. 5B). Antisense to ubiquitin carboxy-terminal hydrolase L1 (*AS-Uchl1*) is a nuclear lncRNA containing a *SINEB2* repeat, which is involved in brain function and neurodegenerative diseases in mice[164]. Upon activation of stress signalling pathways, for example following mTORC1 inhibition by rapamycin, *AS-Uchl1* shuttles from the nucleus to the cytoplasm, where its *SINEB2* element undergoes base pairing with the 5′ end of *Uchl1* to enhance the translation of the mRNA[164].

trans-Acting lncRNAs are emerging as important post-transcriptional regulators. Future studies are warranted not only to better dissect the molecular basis of individual lncRNA–protein interactions by identifying the functional modules of the lncRNAs but also to reveal mechanistic commonalities among different lncRNPs.

Sponging microRNAs. Some abundant lncRNAs bearing microRNA (miRNA)-complementary sites can regulate gene expression as competitive endogenous RNAs or 'sponges' of miRNAs, thereby reducing miRNA availability to target mRNAs[165,166] (FIG. 5C). The stoichiometric relationship between a potential competitive endogenous lncRNA and miRNAs is important for achieving a measurable effect on target-mRNA expression[167,168] (Supplementary Box 1). In tumours, the *PNUTS* lncRNA is generated by alternative splicing of the *PNUTS* pre-mRNA, which is mediated through the binding of hnRNPE1 (REF.[169]) (FIG. 5C). The resulting *lncRNA-PNUTS* contains seven binding sites for miR-205, a well-established suppressor of the transcription repressors ZEB1 and ZEB2 and a factor required for epithelial cell maintenance. The sequestering of miR-205 by *lncRNA-PNUTS* results in the upregulation of ZEB1 and ZEB2, and consequently the promotion of epithelial–mesenchymal transition and breast cancer cell migration and invasion[169].

Regulating functions of organelles. Interestingly, numerous lncRNAs are localized to specific organelles, such as exosomes and mitochondria (FIG. 1h,i). Because exosomes are regularly released into the extracellular environment, exosome-localized lncRNAs can be secreted and end up in recipient cells, where such lncRNAs are found to be involved in epigenetic regulation, cell-type reprogramming and genomic instability (reviewed in REF.[170]). Mitochondria-localized lncRNAs can be encoded by both nuclear DNA and mitochondrial DNA, and are often associated with mitochondrial metabolism, apoptosis and the crosstalk of mitochondria with nuclei[171]. The nuclear-encoded lncRNA survival associated mitochondrial melanoma specific oncogenic non-coding RNA (*SAMMSON*) controls mitochondrial homeostasis[172], mitochondrial 16S ribosomal RNA maturation and expression of mitochondria-encoded polypeptides[173]. The three abundant mitochondria-encoded lncRNAs *lncND5*, *lncND6* and *lncCyt b* form intermolecular duplexes with mRNAs and regulate their stability and expression[26]. Discovery of other organelle-specific lncRNAs will likely provide additional mechanistic insight into the connection between lncRNA regulation and organelle homeostasis.

Physiopathological roles of lncRNAs

The various gene regulatory activities of lncRNAs affect different aspects of physiology, from cell differentiation, growth and responses to diverse stresses and stimuli, to key roles in the nervous, muscular[174,175], cardiovascular[176], adipose[177], haematopoietic and immune[178] systems and their associated pathologies. Here, we highlight some aspects and examples of the physiological roles of lncRNAs; we refer the reader to other Reviews for additional information[174–179].

Neuronal differentiation and disorders

The development of the central nervous system is a particularly intricate process that requires precise spatio-temporal gene regulation. The mammalian brain is a transcriptionally highly complex organ that expresses approximately 40% of mammalian lncRNAs[180]. Cell culture and mouse models have implicated lncRNAs in neuronal differentiation[181] and regeneration after injury[182,183]. These lncRNAs are often related to protein-coding genes with specific roles in neurogenesis. For example, the lncRNA *Silc1* and the transcription factor SOX11 are exquisitely co-expressed in cells of mouse dorsal root ganglia and co-induced following nerve injury. During response to injury, the *cis*-acting *Silc1* is necessary for activation of the SOX11 transcriptional programme and nerve regeneration. The mechanism behind *Silc1* interaction with the *Sox11* locus to promote its activation is not well understood, but is known to be allele-specific[183]. In line with their roles in neuronal differentiation, the deregulation of some lncRNAs has been associated with Parkinson disease, Huntington disease, lateral amyotrophic sclerosis or Alzheimer disease[184]. For example, *BACE1-AS*, antisense of the gene encoding β-site amyloid precursor protein cleaving enzyme 1 (BACE1; also known as β-secretase 1), promotes *BACE1* mRNA stability leading to increase in the levels of neurotoxic amyloid plates in the brain of individuals with Alzheimer disease[185]. *BACE1-AS* can be detected in the plasma of these individuals, and thereby serves as a potential disease biomarker[186].

Haematopoiesis and immune responses

The extensively investigated roles of lncRNAs in haematopoietic cell differentiation underscore the coordinated activity of differentiation-driving transcription factors and lncRNAs[187]. Thus, lncRNAs are decisive

in activating or suppressing the expression of genes encoding inflammatory molecules[178]. Interestingly, the induction of key immunity genes may depend on the expression of their regulating lncRNAs prior to the inflammatory stimulus, representing a necessary step for immune gene priming in trained immunity.

One of these immune gene-priming lncRNAs, named *UMLILO*, was characterized in monocytes, where it functions in *cis* on the promoters of several chemokine genes located within the same topologically associating domain, thereby facilitating the deposition of H3K4me3 by the WDR5–MLL1 complex following priming treatment[60]. Several other immunomodulatory lncRNAs are involved in chromatin regulation. lincRNA erythroid prosurvival (*lincRNA-EPS*), which is expressed in erythrocytes, macrophages and dendritic cells[188], and *lnc13*, which is expressed in macrophages[189], repress transcription of immunity genes. *lnc13* has been related to inflammatory disease, as SNPs that affect its expression lead to higher levels of *lnc13*-regulated genes and predispose to coeliac disease[189].

Besides those involved in adaptive immunity, mammalian lncRNAs are also related to the control of innate immunity in response to viral infection, which relies on the interferon response as one of its main axes. A signature of lncRNAs is induced by viral infection, including SARS-associated coronavirus, influenza virus, herpes simplex virus 1 and hepatitis C virus[190,191], and a significant subset of these lncRNAs is upregulated in response to interferon. The interferon-induced lncRNA negative regulator of interferon response (*NRIR*) is a negative regulator of several antiviral genes, thereby favouring hepatitis B virus replication[192]. Similarly, eosinophil granule ontogeny transcript (*EGOT*), which in liver cells is strongly upregulated by interferon-α and by influenza, hepatitis C virus and Semliki Forest virus infections, inhibits a set of interferon-response genes[193].

In summary, lncRNA activities are involved in responses to differentiation cues and stresses that trigger gene expression programmes, in which they exhibit highly specific regulatory functions that are required for correct differentiation and tissue homeostasis.

lncRNAs with cancer-relevant functions

The number of lncRNAs implicated in cancer initiation and progression is continuously growing (Supplementary Box 2), and can be found compiled in curated databases such as Lnc2Cancer[194] or the Cancer LncRNA Census[195]. lncRNAs have been implicated in the acquisition of every hallmark of cancer cells, from the intrinsic capacity of proliferation and survival, through increased metabolism, to the relationship with the tumour microenvironment. Early evidence of the involvement of lncRNAs in cancer came from their transcriptional regulation by key oncogenic or tumour-suppressive transcription factors such as p53 (REFS[196,197]), MYC[198,199], the oestrogen receptor[200] or signalling cascades such as the Notch pathway[201]. These lncRNAs contribute to the functional output of the oncogenic or tumour-suppressive responses. Some lncRNAs are activated by p53 following DNA damage. Mouse *lincRNA-p21* promotes apoptosis by contributing to p53-dependent transcription repression in *trans*[197] and to activation in *cis* in a transcript-independent manner of cyclin-dependent kinase inhibitor 1 (REFS[202,203]). Human *PANDA*[204] regulates p53-dependent apoptosis and cell cycle arrest; human *DINO* stabilizes p53 in the nucleus, thereby reinforcing its transcriptional activity[205]; *GUARDIN* preserves genomic integrity through two independent cytoplasmic and nuclear mechanisms[206] (FIG. 6a,b). Furthermore, lncRNAs such as *MEG3* participate in the p53 regulatory network without being transcriptional targets of p53. The imprinted *MEG3* is downregulated in multiple cancers[207] and contains an evolutionary conserved RNA structure that mediates p53 activation in *trans*[208].

In contrast to these p53-related functions, numerous lncRNAs are either regulated by[198,199,209] or regulate[210,211] the expression of the proto-oncogene MYC. An intricate regulatory network involving numerous non-coding genomic elements occurs around the *MYC* locus. *MYC* resides in the frequently amplified 8q24 chromosomal region, which contains several cancer-associated SNPs within enhancers that form tissue-specific, long-range chromatin interactions with the *MYC* gene[212,213]. Several lncRNAs are expressed from this region[18,210,214–216], which also span SNPs that predispose to cancer[215–217]. For example, *CCAT1-L* has a role in the transcriptional regulation of *MYC* by promoting long-range chromatin looping[18,214,215] (FIG. 6c). *PVT1* is co-amplified with *MYC* in cancer, and in mice functions as an oncogene by stabilizing the MYC protein[210,218]. Interestingly, in some human cell types, the promoter of *PVT1* limits *MYC* transcription by competing in *cis* for the use of specific enhancers and by acting as a DNA boundary element that regulates the expression of *MYC*, in a manner independent of the *PVT1* lncRNA[219] (FIG. 6c).

In summary, there is a large body of evidence indicating that cellular homeostasis is dependent on the action of lncRNAs. Although only a fraction of the thousands of lncRNAs expressed may function at some level in cancer cells, these still remain largely understudied. Relevant questions such as the role of lncRNAs in responses to chemotherapy and immunotherapy, their relationship with tumour prognosis and their effect on the tumour microenvironment warrant further investigation.

lncRNAs as therapeutic targets

lncRNAs with key roles in disease could be therapeutic targets. This possibility is supported by theoretical clinical advantages that represent several of their characteristics. The high tissue-specificity and regulation of specific facets of cellular networks suggests that lncRNAs are superior to proteins in terms of potential, undesired toxic effects associated with their targeting. Furthermore, the lack of translation, fast turnover and low expression levels may facilitate quicker effects with lower doses.

The most advanced attempts of therapeutic lncRNA targeting are currently based on the use of antisense oligonucleotides (ASOs). These molecules are essentially single-stranded DNA oligos that can be quickly designed based on sequence homology and RNA accessibility. Importantly, ASOs are suitable for downregulating lncRNAs that are retained in the nucleus: they bind to the target RNA through Watson–Crick base pairing and can induce RNase H-mediated co-transcriptional cleavage at

Antisense oligonucleotides (ASOs). Single-stranded deoxyribonucleotides complementary to a target RNA. Antisense oligonucleotides can affect RNA expression through various mechanisms, including modulation of splicing, steric blockade and cleavage by RNase H.

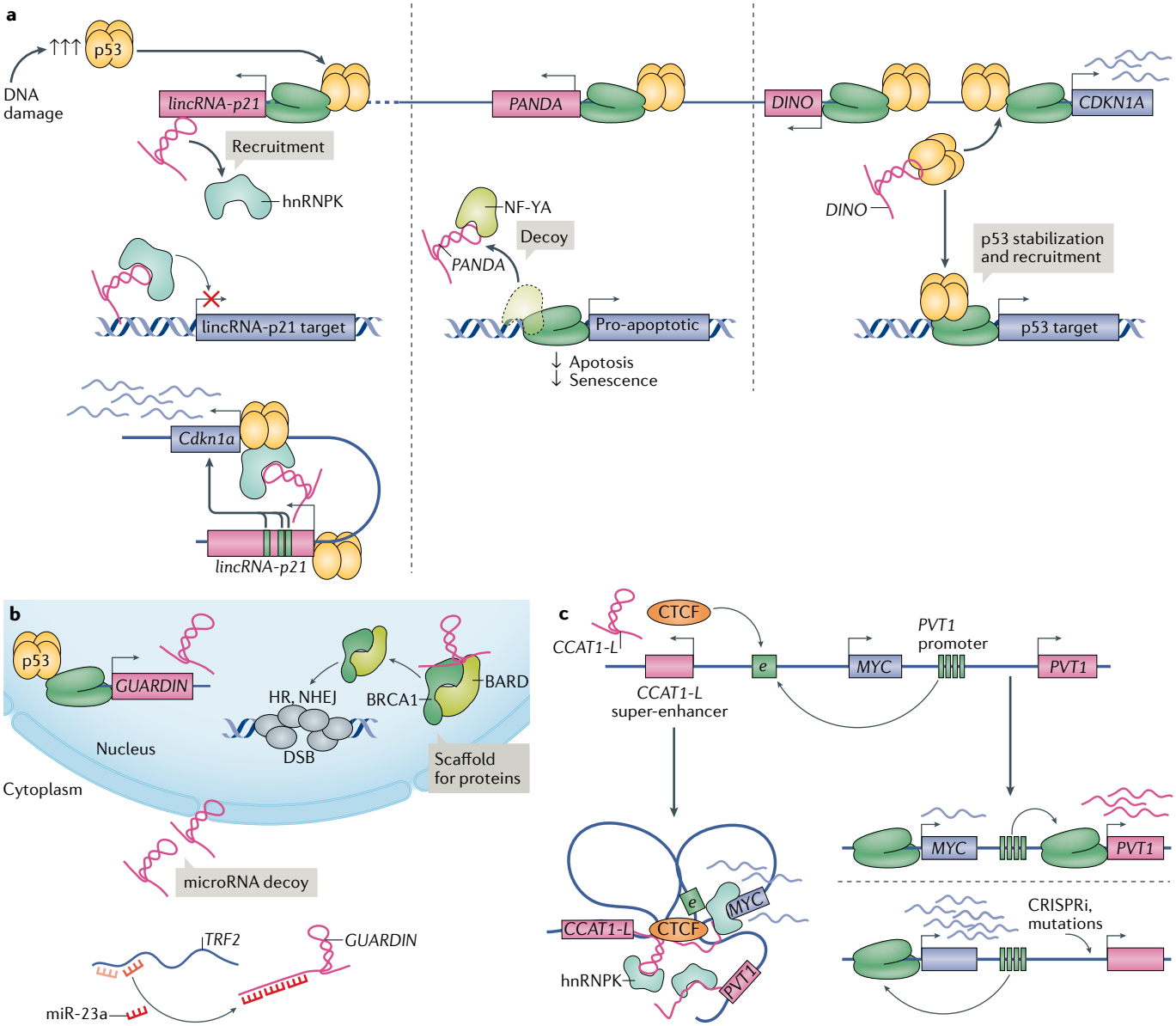


Fig. 6 | **The involvement of long non-coding RNAs in cancer. a** | Long non-coding RNAs (lncRNAs) located in the same (human or mouse) genomic region of the cyclin-dependent kinase inhibitor 1A (*CDKN1A*) gene are direct targets and effectors of p53 following DNA damage. Long intergenic non-coding RNA p21 (*lincRNA-p21*) functions in *trans* to recruit the transcription repressor heterogeneous nuclear ribonucleoprotein K (hnRNPK) to the promoter of target genes in response to p53 activation[197], or in *cis*, where it promotes activation of *Cdkn1a* in two possible ways: *lincRNA-p21* can recruit hnRNPK to the promoter of *Cdkn1a* from its site of transcription[203]; and another in vivo study has revealed the presence of multiple enhancers (green rectangles) in the *lincRNA-p21* locus, which are responsible for transcript-independent regulation in *cis* of *Cdkn1a* (REF.[202]). p21-associated ncRNA DNA damage-activated (*PANDA*) functions as a decoy for nuclear transcription factor Y subunit-α (NF-YA), thereby removing it from the promoters of its target genes and reducing apoptosis and cell senescence in a p53-dependent fashion[261]. Damage induced non-coding (*DINO*) interacts with p53 in the nucleus and promotes p53 tetramer stabilization (consequently reinforcing p53 signalling). Furthermore, *DINO* co-localizes with p53 at the promoters of several of its target genes, including *CDKN1A* (REF.[205]). **b** | *GUARDIN* (also known as long non-coding transcriptional activator of miR34a) is activated by p53 following DNA damage and contributes to genome integrity through two separate activities. Part of the *GUARDIN* pool is exported to the cytoplasm, where it acts as a sponge of miR-23a, thus preventing the destabilization of its main mRNA target, telomeric repeat-binding factor 2 (*TRF2*), which encodes a factor involved in telomere capping and stability. In the nucleus, *GUARDIN* functions as a scaffold that enables the interaction of breast cancer type 1 susceptibility protein homologue (BRCA1) and BRCA1 associated RING domain 1 (BARD1), which is important for the recruitment of DNA double-strand break (DSB) repair machinery[206]. **c** | *MYC* oncogene expression is tightly regulated by numerous non-coding RNAs, and relies on the function of several enhancers (green box labelled 'e') in the *MYC* genomic region. Among them, the super-enhancer lncRNA colon cancer associated transcript 1-long (*CCAT1-L*) promotes chromatin interactions between *MYC* enhancers and promoters through recruiting the DNA-binding protein CCCTC-binding factor (CTCF), thereby activating *Myc* expression[18,136]. Furthermore, the 5′ end of *CCAT1-L* interacts with hnRNPK, and both interact with the *MYC* promoter and with the lncRNA plasmacytoma variant translocation 1 (*PVT1*) to coordinate their expression[270]. *PVT1* competes with the *MYC* promoter for the availability of enhancers; thus, when *PVT1* is expressed, *MYC* levels are kept low. In the presence of *PVT1*-inactivating somatic mutations, which are frequent in some cancers, or when *PVT1* expression is experimentally repressed using CRISPR interference (CRISPRi), *MYC* expression is favoured[219]. HR, homologous recombination; miR, microRNA; NHEJ, non-homologous DNA end joining.

Fused aptamers
Chimeric molecules comprising at least one aptamer — a single-stranded synthetic oligonucleotide that folds into a defined architecture to specifically bind with high affinity to an RNA, a protein or another ligand — and another molecule with different biological properties.

k-mer
A short (3–8 nucleotides) sequence motif, often mediating the interaction of nucleic acids with proteins or other molecules. *k* specifies the length of the motif.

the ASO binding site, leading to premature transcription termination and reduced lncRNA levels[220,221]. ASOs have high efficacy in cells, although there are limitations to using ASOs in the clinic, mainly because of in vivo toxicity and the lack of proper delivery systems, which hampers tissue targeting by an adequate dose of therapeutic ASOs. To improve their pharmacological properties, ASOs typically are chemically modified to enhance hybridization affinity to their target RNA, thereby increasing resistance to degradation by nucleases and reducing unspecific immunostimulatory activity. These chemical variations include the GapmeR ASOs, RNA–DNA–RNA single-stranded oligonucleotide chains in which ribonucleotides may contain a 2′-*O*-methoxyethyl modified sugar backbone[222] or additional modifications such as locked nucleic acids and S-constrained ethyl residues[223]. Moreover, fused aptamers may also be used for targeted intracellular delivery of these oligo-based drugs[224]. Several mRNA-targeting ASOs have already been approved by the FDA and the European Medicines Agency[225] or have advanced to clinical trials[225], and several ASOs targeting oncogenic lncRNAs are under development and protected by patents.

Less developed is the use of small molecules for the targeting of lncRNAs. Obtaining successful molecules that bind lncRNAs with high affinity and specificity requires the identification of relevant RNA motifs with sufficient structural complexity[226]. This level of structural knowledge is so far available only for a limited number of lncRNAs, revealing that lncRNAs often fold into several modular domains potentially involved in different molecular interactions[208,227–231]. Blocking functional interactions between lncRNAs and proteins could be desirable from a therapeutic perspective. Alternatively, synthetic molecules that mimic the structure and binding properties of lncRNAs may work as decoys by competing with the lncRNA for protein binding, and therefore interfering with its function. All of these promising approaches will become more practical as the structural and molecular features of lncRNAs become better understood.

Finally, tools based on CRISPR–Cas systems are among the most versatile and promising for the precise modulation of lncRNAs. The different versions of CRISPR–Cas-engineered molecules allow the deletion (using CRISPR–Cas9)[232], inhibition (CRISRPi)[233] or activation (CRISPRa)[234] of lncRNA-encoding genes, as well as the degradation of the transcripts themselves (CRISPR–Cas13)[235]. These technologies enable relatively fast knockout, knockdown or overexpression of lncRNAs, are already widely used for research applications at single lncRNA loci and are increasingly applied to thousands of loci for high-throughput loss-of-function and gain-of-function screening in diverse experimental settings[236]. However, because of their lack of functional open reading frames, in vivo targeting of lncRNAs using CRISPR–Cas is more difficult than targeting protein-coding genes. It is therefore expected that the therapeutic application of CRISPR–Cas systems at lncRNA loci will lag behind that of protein-coding genes.

Concluding remarks

In recent years we have witnessed remarkable progress in our understanding of lncRNAs, and we now have a clearer picture of the features and functional versatility of these molecules. Nevertheless, this knowledge represents only a small fraction of the landscape of their gene regulatory potential. Several aspects of lncRNA biology still require rigorous investigation, for instance, we are still far from understanding how lncRNA sequences and structural features relate to their functions, given their non-coding nature and low sequence conservation. Interestingly, a recent study has shown that lncRNAs with similar *k*-mer content have related functions despite their lack of linear homology[237]. This study implies that short sequence elements in lncRNAs mediate interactions with proteins (and/or other molecules), and thus are key determinants of lncRNA function. However, the nature and dynamics of such interactions still need to be elucidated. It is also increasingly evident that multiple features of lncRNAs can define their functionality. These features include their sequence, expression levels, processing, cellular localization, structural organization and interactions with other molecules. The integrated knowledge of all these features will hopefully increase the identification and classification of functional lncRNAs.

How lncRNAs influence complex physiological processes and the onset of diseases are questions of great relevance. Our current knowledge indicates that lncRNAs fine-tune cell specification and disease. These functions require deeper comprehension, not only to provide a complete picture of physiopathological processes but also because lncRNAs can readably be therapeutically targeted with high specificity. Given their characteristics, disease-related lncRNAs will predictably gain greater relevance in the context of personalized medicine. Progress in this area will go hand in hand with better understanding of the gene regulation modalities of lncRNAs.

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