

# Long non-coding RNAs: definitions, functions, challenges and recommendations

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## Abstract

Genes specifying long non-coding RNAs (lncRNAs) occupy a large fraction of the genomes of complex organisms. The term ‘lncRNAs’ encompasses RNA polymerase I (Pol I), Pol II and Pol III transcribed RNAs, and RNAs from processed introns. The various functions of lncRNAs and their many isoforms and interleaved relationships with other genes make lncRNA classification and annotation difficult. Most lncRNAs evolve more rapidly than protein-coding sequences, are cell type specific and regulate many aspects of cell differentiation and development and other physiological processes. Many lncRNAs associate with chromatin-modifying complexes, are transcribed from enhancers and nucleate phase separation of nuclear condensates and domains, indicating an intimate link between lncRNA expression and the spatial control of gene expression during development. lncRNAs also have important roles in the cytoplasm and beyond, including in the regulation of translation, metabolism and signalling. lncRNAs often have a modular structure and are rich in repeats, which are increasingly being shown to be relevant to their function. In this Consensus Statement, we address the definition and nomenclature of lncRNAs and their conservation, expression, phenotypic visibility, structure and functions. We also discuss research challenges and provide recommendations to advance the understanding of the roles of lncRNAs in development, cell biology and disease.

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## Introduction

Research on long non-coding RNAs (lncRNAs), a previously unsuspected major output of genomes of complex organisms, has been dogged by uncertainty and controversy from its beginning. lncRNAs have the unfortunate distinction of being named for what they are not, rather than what they are. This loose description has its origins in the belief that the main role of RNA is to act as the intermediate between a gene and a protein, with other ‘housekeeping’ non-coding RNAs such as ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nucleolar RNAs (snoRNAs), spliceosomal RNAs and other small nuclear RNAs (snRNAs) being ancillary to this function.

Broad recognition of RNA as a regulatory molecule occurred in the early years of the first decade of the twenty-first century with the unexpected discovery of large numbers of small interfering RNAs (siRNAs), microRNAs (miRNAs) and small PIWI-interacting RNAs (piRNAs) that regulate – through Argonaute family proteins – gene expression at transcriptional, post-transcriptional and translational levels in eukaryotes<sup>1</sup>, although there were examples of other small regulatory RNAs in the literature, especially in bacteria<sup>2</sup>. A few long regulatory RNAs, notably *meiRNA* in the fission yeast *Schizosaccharomyces pombe*, *hsr $\omega$* , RNA on the X1 (*roX1*) and *roX2* in *Drosophila melanogaster*, and *H19* and X-inactive-specific transcript (*XIST*) in mammals, had also been reported in the preceding years<sup>3–7</sup>, but were regarded more as oddities than early examples of a general phenomenon. Moreover, the small regulatory RNAs did not disturb the conceptual framework that most genes encode proteins, but rather fitted comfortably into it. It was later found, however, that while some miRNAs are generated from the introns of pre-mRNAs<sup>8</sup>, non-coding primary transcripts of miRNAs and of snoRNAs can also have functions<sup>9,10</sup> and that rRNAs, tRNAs and snoRNAs are processed to generate small regulatory RNAs, including miRNAs<sup>11–14</sup>, in some cases contributing to transgenerational epigenetic inheritance<sup>15</sup>.

A bigger surprise, and challenge to the reigning understanding of genetic information, came in the early and middle years of the first decade of the twenty-first century, when global transcriptomic analyses, intended to better define the proteome, revealed that most of the genome of animals and plants is dynamically transcribed into longer RNAs that have little or no protein-coding potential<sup>16–19</sup>. This surprise was compounded by the associated finding that the number, and to a large extent the repertoire, of protein-coding genes is similar in animals of widely different developmental and cognitive complexity – the nematode worm *Caenorhabditis elegans* (comprising ~1,000 somatic cells) and humans (~30 × 10<sup>12</sup> somatic cells<sup>20</sup>) both have ~20,000 protein-coding genes – which was termed the ‘g-value paradox’<sup>21</sup>. By contrast, the extent of non-coding DNA, and consequently the transcription of non-coding RNAs, has increased with increasing developmental complexity<sup>22</sup>.

Understandably, the common initial reaction of the molecular biology community was to suspect that these unusual RNAs are transcriptional noise, because of their generally low levels of sequence conservation, low levels of expression and low visibility in genetic screens. Since then, however, there has been an explosion in the number of publications reporting the dynamic expression and biological functions of lncRNAs, aided by extensive technology development that has enabled their identification and characterization, although only a minority of lncRNAs have confident annotations and very few have mechanistic information. The realization that the genomes of plants and animals express large numbers of lncRNAs requires a framework for their classification and understanding of their functions and, more profoundly, a reassessment of the amount and type of information required to programme the development of complex organisms.

## Purpose of this Consensus Statement

In this Consensus Statement we present a current and coherent picture of the roles of lncRNAs in cell and developmental biology, identify the key issues in understanding their functions and chart the path forward. We address lncRNA definition, nomenclature, conservation, expression, phenotypic visibility, functional assays and molecular mechanisms encompassing lncRNA connections to chromatin architecture, epigenetic processes, enhancer function and biomolecular condensates, as well as the roles of lncRNAs outside the nucleus. We argue that loci expressing lncRNAs should be recognized as bona fide genes and discuss lncRNA structure–function relationships as the means to parse mechanisms and pathways. Finally, we identify the current challenges and offer recommendations for understanding the relationship of lncRNAs to genome architecture, gene regulation and cellular organization.

The authors of this Consensus Statement were suggested by recommendations of colleagues. Consensus was reached by group e-mail and discussion.

## Definition and nomenclature of lncRNAs

lncRNAs have been arbitrarily defined as non-coding transcripts of more than 200 nucleotides (200 nt), which is a convenient size cut-off in biochemical and biophysical RNA purification protocols that deplete most infrastructural RNAs, such as 5S rRNAs, tRNAs, snRNAs and snoRNAs, as well as miRNAs, siRNAs and piRNAs<sup>23</sup>. This definition also excludes some other well-known short RNAs such as the primate-specific snaRs (~80–120 nt), which associate with nuclear factor 90 (ref. 24); Y RNAs (~100 nt), which act as scaffolds for ribonucleoprotein (RNP) complexes<sup>25</sup>; vault RNAs (88–140 nt), which are involved in transferring extracellular stimuli into intracellular signals<sup>26</sup>; and promoter-associated RNAs and non-canonical small RNAs produced by post-transcriptional processing<sup>27–29</sup>. Other non-coding RNAs lie close to the 200-nt border, such as 7SK (~330 nt in vertebrates), which controls transcription pausing and termination, including at enhancers<sup>30,31</sup>, and 7SL (~300 nt), which is an integral component of the signal recognition particle that targets proteins to cell membranes<sup>32</sup> and the evolutionary ancestor of the widespread primate Alu (~280 nt) and rodent B1 (~135 nt) small interspersed nuclear elements<sup>33–35</sup>. Given this grey zone of sizes, we support the suggestion that non-coding RNAs be divided into three categories<sup>36</sup>: (1) small RNAs (less than 50 nt); (2) RNA polymerase III (Pol III) transcripts (such as tRNAs, 5S rRNA, 7SK, 7SL, and Alu, vault and Y RNAs<sup>37</sup>), Pol V transcripts in plants and small Pol II transcripts such as (most) snRNAs and intron-derived snoRNAs<sup>38,39</sup> (~50–500 nt); and (3) lncRNAs (more than 500 nt), which are mostly generated by Pol II.

Many lncRNAs are spliced and polyadenylated, which has led to their description as ‘mRNA-like’. However, other lncRNAs are not polyadenylated or 7-methylguanosine capped<sup>19,40–42</sup>, are expressed from Pol I (5.8S, 28S and 18S rRNAs) or Pol III promoters, or are processed from precursors, including from introns and repetitive elements, leading to the more agnostic descriptor ‘transcripts of unknown function’<sup>43</sup>. With respect to protein-coding genes, lncRNAs can be ‘intergenic’, antisense or intronic. They are also derived from ‘pseudogenes’, which occur commonly in metazoan genomes<sup>44</sup>, with more than 10,000 pseudogenes identified in the mouse genome<sup>45</sup> and almost 15,000 identified in the human genome<sup>46</sup>, some of which have been shown to be functional<sup>44,47</sup>. lncRNAs also include circular RNAs generated by back-splicing of coding and non-coding transcripts, also with demonstrated functions<sup>48</sup>, and *trans*-acting regulatory RNAs derived from sequences that conventionally act as the 3′ untranslated regions of mRNAs<sup>49</sup>.

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There have been many attempts at nomenclature and classification of lncRNAs, by the [HUGO Gene Nomenclature Committee](#), the [GENCODE](#) consortium and others, predominantly based on their genomic position and orientation relative to protein-coding genes<sup>46,50–53</sup>. Linking to nearby genes has been useful, as it provides context and has sometimes provided clues to lncRNA function, for example in regulating the expression of these genes, as is often the case with enhancers (see later), although enhancer activity should not be assumed to be directed to the most proximal genes.

Many early studies focused on long intergenic non-coding RNAs (lincRNAs), whose sequences do not trespass on nearby protein-coding loci, owing to the need to distinguish their function from that of proteins. However, many other lncRNAs overlap protein-coding loci or are expressed from enclosed introns. Moreover, the traditional view of genomes as linear arrangements of discrete protein-coding genes fails to accommodate the discovery that eukaryotic transcription, best characterized in human and model organisms, is a fuzzy continuum<sup>54</sup>, with ‘genes’ within genes, genes interleaved with other genes and non-coding transcripts overlapping or originating within them<sup>18,43,55</sup>, together posing a growing problem for genome annotations.

In both humans and *D. melanogaster*, for example, many protein-coding genes have 5′ exons that are incorporated into mRNA in early embryogenesis and lie hundreds of kilobases upstream of the usual first exon, bypassing many other genes in the intervening region<sup>56</sup>. Indeed, any base may be exonic, intronic or ‘intergenic’, depending on the transcriptional output of the cell at any point in its developmental trajectory or physiological state<sup>55</sup>. For this reason, unless a lncRNA is antisense to a protein-coding gene, we recommend naming lncRNAs for their own sake with allusion to a discerned characteristic or function (as has been traditional for proteins), such as *XIST*, antisense IGF2R non-protein-coding RNA<sup>57</sup> (*AIRN*), HOX antisense intergenic RNA<sup>58</sup> (*HOTAIR*), *Gomafu* (‘spotted pattern’ in Japanese; also known as *Miat*)<sup>59</sup>, *COOLAIR* (referring to plant vernalization)<sup>60</sup> and auxin-regulated promoter loop<sup>61</sup> (*APOLO*), for easy recollection, preferably accompanied by complete exon–intron structures and genomic coordinates. If no biological context is available, we recommend naming the lncRNA according to the GENCODE system<sup>46</sup>.

The wide range of functions of ‘non-coding’ RNAs precludes straightforward classification as specific RNA classes, with some acting locally and some at a distance, or both<sup>62</sup>. In the absence of more specific categorization, we recommend retention of the general descriptor ‘lncRNA’, noting that most have some type of regulatory or architectural, often related, role in cell and developmental biology, and because there are so many historical articles that use this term or variations thereof. Non-coding RNAs come in all shapes and sizes, and the territory is huge, covering most of the genome and a plethora of functions. Some RNAs have dual functions as coding and regulatory RNAs, and some, perhaps many, cytosolic lncRNAs encode small peptides<sup>63–66</sup>. Protein-coding loci also express lncRNAs through alternative splicing<sup>67–69</sup>, and, surprisingly, the major transcript produced by ~17% of human protein-coding loci is non-coding<sup>70</sup>. Indeed, both lncRNA genes and mRNA genes can produce transcripts that function following different levels of processing. Unspliced transcripts, spliced transcripts, circular RNAs, intronic RNAs and stable small RNAs generated from them can all have a function<sup>48,71,72</sup>. Any RNA can be regulatory, and any locus can encode both protein-coding and regulatory RNAs.

Well in excess of 100,000 human lncRNAs have been recorded<sup>52,73</sup>, many of which are specific to the primate lineage<sup>74</sup>. This is a vastly incomplete list due to the limited analysis of different cells at different developmental stages (see later). There are now hundreds of thousands

of catalogued lncRNAs and dozens of databases (and databases of databases) with curated information<sup>75–80</sup>. Over the past decade, there have been ~50,000 publications with ‘long non-coding RNA’ as a key term and more than 2,000 publications reporting validated lncRNA functions<sup>81</sup>, although most have yet to be followed up in any detail.

From here on, we focus on lncRNAs derived from Pol II primary transcription units (and use the term in that context), as opposed to other non-coding RNAs that are expressed from Pol I or Pol III promoters, processed from introns (which, it should be noted, constitute a major fraction of the non-coding RNA in mammals and other organisms<sup>41,82–84</sup>) or formed by back-splicing, although many of the same considerations apply.

## Conservation of lncRNAs

Most lncRNAs are less conserved among species than the mRNA sequences encoding the proteome. Initially, most of the mammalian genome (which included most lncRNA loci) was thought to be evolving neutrally, using the yardstick of the rate of divergence of common ‘ancient repeats’ (derived from transposons) between the human and mouse genomes, on the assumption that these sequences are non-functional and representative of the original distribution in the ancestor<sup>85</sup>. However, there is increasing evidence that transposable elements are widely co-opted as functional elements of gene expression and structure, forming promoters, regulatory networks, exons and splice junctions in protein-coding genes and lncRNAs<sup>86–89</sup>, and therefore cannot be used as indices of neutral evolution.

Regulatory sequences, including promoters and lncRNAs, are known to evolve rapidly due to more relaxed structure–function constraints than protein-coding sequences and due to positive selection during adaptive radiation<sup>85,90–92</sup>. Many lncRNAs are cell lineage specific. Indeed, given their association with developmental enhancers (see later), variation in the complement and sequences of lncRNAs may be a major factor in species diversity.

Loci expressing lncRNAs exhibit many of the characteristics of protein-coding genes, including promoters, multiple exons, alternative splicing, characteristic chromatin signatures, regulation by morphogens and conventional transcription factors, altered expression in cancer and other diseases<sup>74,93–98</sup>, and a range of half-lives similar to those of mRNAs<sup>99</sup>.

The promoters of lncRNAs exhibit levels of conservation comparable to those of protein-coding genes<sup>18,74</sup>. lncRNAs also have conserved exon structures, splice junctions and sequence patches<sup>18,74,93,97</sup>, and they retain orthologous functions despite rapid sequence evolution<sup>100–102</sup>. Indeed, low sequence conservation can be misleading.

The lncRNA telomerase RNA template component (*TERC*), which is required for telomere maintenance – a vital cellular function – differs widely in size and sequence, but has conserved structural topology from yeast to mammals, albeit with some variation, and a conserved catalytic core<sup>103–108</sup> (see also later). X chromosome dosage compensation in *Drosophila* spp. requires the formation of a nuclear domain through phase separation by the lncRNAs *roX1* and *roX2* interacting with the intrinsically disordered region (IDR) of a specific partner protein, male sex lethal 2 (MSL2). Replacing the IDR of the mammalian orthologue of MSL2 with that of the *D. melanogaster* protein and expression of *roX2* is sufficient to nucleate ectopic X chromosome dosage compensation in mammalian cells, showing that the *roX*–MSL2 IDR interaction is the primary determinant of compartmentalization of the X chromosome and that such interactions are preserved over vast evolutionary distances<sup>109</sup>. Similar processes are involved in the regulation

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of X chromosome dosage compensation in placental mammals by *XIST*, which performs several functions, including repulsion of euchromatic factors, scaffolding of new heterochromatic factors and reorganization of chromosome structure<sup>110–113</sup>.

## Expression

Although there are exceptions (such as metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*; also known as *NEAT2*), which is one of the most abundant Pol II transcripts in vertebrate cells<sup>114</sup>, and nuclear paraspeckle assembly transcript 1 (*NEAT1*; see later), lncRNAs generally show more restricted expression patterns than mRNAs<sup>74,115</sup>, and are often highly cell specific<sup>116</sup>, which is consistent with a role in the definition of cell state and developmental trajectory. They also have specific subcellular locations, often nuclear, although a large fraction is cytoplasmic<sup>75</sup>. Although it is sometimes asserted that there are a few hundred cell types in a human, broad classifications obscure the fact that each cell occupies a precise place in a developmental ontogeny, illustrated by the differential expression of *HOX* genes in superficially similar skin cells in different regions of the body<sup>117</sup>, and by the expression of lncRNAs in various regions of the brain<sup>118–121</sup> and at different stages of development<sup>122</sup>. lncRNAs are also dynamically expressed during differentiation of mammalian stem, muscle, mammary gland, immune and neural cells, among many others<sup>81,116</sup>, with a transition during development from broadly expressed and conserved lncRNAs towards an increasing number of lineage-specific and organ-specific lncRNAs<sup>123</sup>. lncRNA expression can also be strongly influenced by environmental factors, a feature that is especially prominent in plants<sup>124–126</sup>, which include a range of stress responses in animals and drug resistance in cancer<sup>127–133</sup>.

The restricted expression of lncRNAs in different cells at different stages of development and their generally low copy number (owing to their regulatory nature) accounts for their sparse representation in bulk-tissue RNA sequencing datasets<sup>134</sup>, whereas many lncRNAs are relatively easy to detect in particular cells<sup>118</sup>. The undersampling of lncRNAs is now being rectified by targeted capture<sup>98,135</sup>, advanced imaging<sup>136–138</sup>, spatial transcriptomics<sup>139</sup> and, in some cases, single-cell sequencing<sup>120,121,140</sup>, which make it clear that, whereas ~20,000 human lncRNA loci have been identified by GENCODE<sup>46</sup> and ~30,000 by the FANTOM consortium<sup>141</sup>, there is likely at least an order of magnitude more.

Due to the high complexity and the variation in transcription initiation and termination sites, expression levels and splicing, comprehensive characterization of transcriptomes is extremely challenging. A recent study showed that the low expression of a lncRNA can be essential for its functional role by ensuring specificity to its regulated targets, suggesting that low abundance levels may be an essential feature of how lncRNAs work<sup>142</sup>. To fully catalogue the universe of lncRNAs, and properly record their exon–intron organization and splice variants, high-depth sequencing will need to be performed on cells at all stages of differentiation and development, undergoing different neural, immunological and other physiological processes, and in various disease states. This is a huge task, but we recommend that future gene expression profiling should include full transcript analysis not just of mRNAs but also of small RNAs and lncRNAs that are intergenic, antisense and intronic to the annotated genes, and their stoichiometry<sup>143</sup>.

## Phenotypic visibility

Like miRNAs, most lncRNAs have not been identified in genetic screens. There are two reasons for this. First, most genetic screens historically focused on protein-coding mutations, which often have severe consequences that are easy to track; by contrast, regulatory mutations often

have subtle consequences that affect quantitative traits. Second, it is difficult to identify causal mutations among the many variations that occur in non-coding sequences. Indeed, most variations that influence human quantitative traits and complex disorders occur in non-coding regions, which are replete with genes expressing lncRNAs<sup>144,145</sup> that are transcribed in cell types relevant to the associated trait<sup>141,146</sup>.

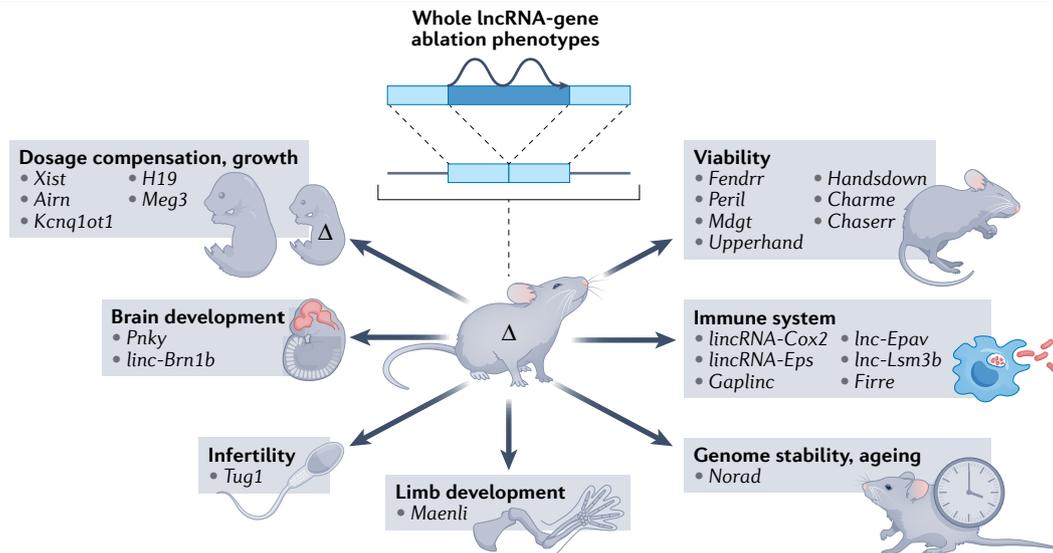
There are exceptions of lncRNAs that have been identified genetically, notably the *roX1* and *roX2* RNAs involved in X chromosome activation in male fruitflies<sup>5</sup>, mammalian parentally imprinted *H19*, *Airn* and *Kcnq1ot1* RNAs in mice<sup>6,57,147,148</sup> and others such as *Tug1* in mice<sup>149</sup>, *MAENLI* (ref. 150) and *HELLP* (named for ‘haemolysis, elevated liver enzyme levels and low platelet count’; also known as *HELLPAR*)<sup>151</sup>, which are associated with disorders or developmental processes. In *Arabidopsis thaliana*, non-coding intronic single-nucleotide polymorphisms important for flowering-time adaptation were found to alter the splicing of the lncRNA *COOLAIR*<sup>152</sup>.

Many lncRNAs have been associated with the cause and progression of cancers, through altered expression of and/or mutations (including translocation breakpoints) in lncRNAs that act as oncogenes or tumour suppressors<sup>153–155</sup>. Other lncRNAs are involved in human genetic disorders<sup>81,156,157</sup>, including DiGeorge syndrome and other neurodevelopmental and craniofacial defects<sup>158–160</sup>. Phenylketonuria, one of the first documented human genetic disorders, caused mostly by mutations in the enzyme phenylalanine hydroxylase, is caused also by mutations in a lncRNA that can be treated by modified RNA mimics<sup>161</sup>.

A route to analysing lncRNA biological function is to silence or delete, or (less commonly) ectopically express, lncRNAs that have been identified in RNA sequencing datasets, usually as being differentially expressed. There have been problems with the interpretation of such experiments, however, particularly the difficulty of disentangling the loss of lncRNA expression from the loss of DNA regulatory elements<sup>162,163</sup>, which has been addressed by strategies such as inserting polyadenylation sites for early transcription termination or transcription repression by CRISPR interference (CRISPRi), replacement of the lncRNA with a reporter gene that leaves the promoter intact or deletion of lncRNA exons (although loss of downstream regulatory elements cannot be ruled out), antisense-mediated blockade of lncRNA splice sites, CRISPR–Cas13 targeting of the lncRNA (rather than its DNA sequence) and transgene rescue<sup>163,164</sup>. There are now many studies that have demonstrated the biological roles of lncRNAs<sup>163</sup>, and high-throughput loss-of-function reverse genetic screens are increasing the search speed, identifying, for example, lncRNAs that are required for mammalian cell growth and migration, brain, skeletal, lung, muscle and heart development, immune function, epidermal homeostasis and cancer drug responses or lncRNAs that have fitness effects<sup>81,165–170</sup> (Fig. 1). CRISPR-mediated transcription repression of more than 16,000 lncRNAs in seven human cell lines identified almost 500 lncRNAs required for normal cellular proliferation, 89% of which were expressed in only one cell type<sup>167</sup>.

Phenotypic consequences of mutations in regulatory RNAs, like some protein-coding mutations, may be context dependent and not evident in laboratory conditions, and may be obscured by the robustness of biological systems<sup>171</sup>. Loss of *Malat1*, which localizes in nuclear speckles and associates with splicing factors, has no major phenotypes in mice<sup>114,172–174</sup>, however, it does affect cancer progression and synapse formation, among other physiological and pathophysiological processes<sup>175,176</sup>. *Neat1*, which is required for the assembly and function of enigmatic, mammal-specific nuclear organelles called ‘paraspeckles’<sup>177–179</sup>, does not appear to be required for normal development in mice but is important for the differentiation of reproduction-related

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**Fig. 1 | Visible phenotypes of mutations in long non-coding RNA genes in mice**<sup>163</sup>. The following long non-coding RNAs (lncRNAs) are listed in the figure underneath their associated phenotypes: *Airn*, antisense of IGF2R non-protein-coding RNA<sup>147,435</sup>; *Charme*, chromatin architect of muscle expression<sup>436</sup>; *Chaserr*, *CHD2* adjacent, suppressive regulatory RNA<sup>437</sup>; *Fendrr*, *FOXF1* adjacent non-coding developmental regulatory RNA<sup>165,438</sup>; *Firre*, functional intergenic repeating RNA element<sup>316</sup>; *Gaplinc*, gastric adenocarcinoma predictive long intergenic non-coding RNA<sup>200</sup>; *H19*, clone pH19 (ref. 439); *Handsdwn*, downstream of the protein-coding gene *Hand2* (ref. 440) *Kcnq1ot1*, *Kcnq1* overlapping antisense transcript 1 (ref. 441); *linc-Brn1b*, long intergenic non-coding RNA (lincRNA) downstream of the *Brn1* protein-coding gene<sup>165</sup>; *linc-Epav*, endogenous

retrovirus-derived lncRNA positively regulates antiviral responses<sup>442</sup>; *lincRNA-Cox2*, lncRNA downstream of the inflammation response gene *Cox2* (ref. 443); *lincRNA-Eps*, lncRNA involved in erythroid prosurvival<sup>201</sup>; *linc-Lsm3b*, interferon-inducible non-coding splice variant of the U6 small nuclear RNA-associated Sm-like protein *lsm3* gene<sup>444</sup>; *Maenli*, master activator of *engrailed1* in the limb<sup>165</sup>; *Mdgt*, midget<sup>165</sup>; *Meg3*, maternally expressed gene 3 (also known as *Gtl2*)<sup>445,446</sup>; *Norad*, non-coding RNA activated by DNA damage<sup>447</sup>; *Peril*, perinatal lethal long non-coding RNA<sup>165</sup>; *Pnky*, pinky (also known as *linc-Pou3f2*)<sup>448</sup>; *Tug1*, taurine upregulated gene 1 (refs. 165,166,449) *Upperhand*, lncRNA upstream of the *Hand2* cardiomyocyte transcription factor locus<sup>318</sup>; *Xist*, X-inactive-specific transcript<sup>450</sup>. Figure courtesy of Daniel Andergassen and John Rinn.

female tissues such as corpus luteum and mammary gland<sup>180</sup>. Deletion of brain cytoplasmic RNA 1 (*BCI*), a highly expressed brain lncRNA, is seemingly harmless in mice but results in behavioural changes that would be lethal in the wild<sup>181</sup>. So extensive phenotyping is important, especially for cognitive functions. Organoid models may help to identify phenotypes in vitro<sup>182,183</sup>.

Functional annotation of lncRNAs can also be undertaken by molecular phenotyping<sup>184</sup>. Analysis of expression patterns, lncRNA-chromatin interactions and other molecular indices following CRISPR-Cas13-mediated depletion of more than 400 lncRNAs in culture indicated that lncRNAs regulate many genes involved in development, cell cycle and cellular adhesion, among other processes<sup>185</sup>.

## Biological functions of lncRNAs

Characterized examples have indicated that RNAs participate in virtually all levels of genome organization, cell structure and gene expression, through RNA-RNA, RNA-DNA and RNA-protein interactions, often involving repeat elements<sup>88,186,187</sup>, including small interspersed nuclear elements in 3' untranslated regions<sup>188</sup>. These interactions are involved in the regulation of chromatin architecture and transcription (see later), splicing (especially by antisense lncRNAs)<sup>189-191</sup>, protein translation and localization<sup>188,192,193</sup>, and other forms of RNA processing, editing, localization and stability<sup>194,195</sup>.

Many lncRNAs are involved in the regulation of cell differentiation and development in animals and plants<sup>23,81,116,124,196</sup>. They also have roles in physiological processes such as (in mammals) the p53-mediated

response to DNA damage<sup>197</sup>, V(D)J recombination and class switch recombination in immune cells<sup>198</sup>, cytokine expression<sup>199</sup>, endotoxin shock<sup>200</sup>, inflammation and neuropathic pain<sup>201-203</sup>, cholesterol biosynthesis and homeostasis<sup>204,205</sup>, growth hormone and prolactin production<sup>206</sup>, glucose metabolism<sup>207,208</sup>, cellular signal transduction and transport pathways<sup>209-212</sup>, synapse function<sup>213,214</sup> and learning<sup>215</sup>, and have roles in the response to various biotic and abiotic stresses in plants<sup>124,125</sup>. There is also an emerging association of lncRNAs with the cell membrane<sup>216</sup> and with ribozymes<sup>217</sup>.

Presently, a growing number of lncRNAs have their own stories, and the literature is becoming replete with them. However, several convergent themes are emerging, which explain lncRNA ubiquity and importance in differentiation and development: the association of lncRNAs with chromatin-modifying proteins; the expression of lncRNAs from developmental 'enhancers'; and the formation of RNA-nucleated phase-separated coacervates.

## Control of chromatin architecture

Epigenetic modifications of chromatin supervise differentiation and development in complex organisms<sup>218</sup>. DNA methylation is known to be directed by small non-coding RNAs in plants<sup>219</sup>, and the RNAi pathway is required for heterochromatin formation and epigenetic gene silencing in fungi and animals<sup>220</sup>. The mammalian de novo DNA (cytosine 5)-methyltransferase 3A (DNMT3A) and DNMT3B, but not the maintenance DNA methylase DNMT1, bind siRNAs with high affinity<sup>221</sup>. In turn, DNMT1 (which restores methylation at hemimethylated CpG

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dinucleotides following DNA replication) binds lncRNAs to alter DNA methylation patterns at their cognate loci<sup>222–224</sup>, but this is still largely unexplored territory.

There are more than 100 different histone modifications that are differentially established by enzymes at a myriad of different positions in plant and animal genomes to control gene expression during development. The most studied are Polycomb repressive complex 1 (PRC1) and PRC2, which catalyse monoubiquitylation of histone H2A Lys119 (ref. 225) and dimethylation and trimethylation of histone H3 Lys27 (H3K27), respectively, but in mammals neither complex contains sequence-specific DNA-binding proteins<sup>218</sup>. Early studies suggested that PRC2 and/or the associated H3K9 methyltransferase G9a are recruited during mouse X chromosome inactivation by *Xist*<sup>186</sup>, and the control of parental imprinting in mice by *Airn*<sup>226</sup> and *Kcnq1ot1* (ref. 227), although these associations involve complexities and uncertainties<sup>228,229</sup>.

A subsequent survey of more than 3,300 lncRNAs in human cells showed that ~20% (but only ~2% of mRNAs) interact with PRC2, and that other lncRNAs are associated with other chromatin-modifying complexes<sup>230</sup>. Moreover, depletion of a selection of these RNAs caused derepression of genes normally silenced by PRC2 (ref. 230). PRC2 associates with many RNAs<sup>228,231,232</sup>, more than 9,000 in embryonic stem cells<sup>233</sup>. There are conflicting reports of whether these associations are nonspecific ('promiscuous')<sup>228,234</sup> or specific high-affinity interactions with different RNAs<sup>232,235</sup>, although these alternatives are not mutually exclusive<sup>229</sup>. Some recent studies have shown that RNA is required for PRC2 chromatin occupancy, PRC2 function and cell state definition<sup>236</sup>, and that the interaction of PRC2 with RNA can regulate transcription elongation<sup>232</sup>. PRC1 function also appears to be controlled by RNA<sup>237,238</sup>. However, deconvoluting RNA–protein interactions is complicated by the low affinity of many antibodies used in pulldown assays and the fact that PRC2, for example, has at least two subunits that bind RNA<sup>228</sup>. The recent development of denaturing crosslinked immunoprecipitation (dCLIP), which is based on high-affinity biotin–streptavidin pulldowns, has indicated that PRC2 interacts with G-rich RNA motifs, including RNA G-quadruplexes, to achieve specificity of RNA-mediated recruitment<sup>232,239,240</sup>.

Other lncRNAs associate with the gene-activating Trithorax complexes (which methylate H3K4), including enhancer RNAs involved in the maintenance of stem cell fates and lineage specification<sup>241–245</sup>. H3K9 dimethylation is regulated by lncRNAs during the formation of long-term memory in mice<sup>246</sup>. lncRNAs also control methylation of a number of non-histone proteins involved in animal cell signalling, gene expression and RNA processing<sup>247</sup>.

Many other proteins involved in modulating chromatin architecture, including HOX proteins, pioneer transcription factors such as NANOG, OCT4 (also known as POU5F1), SOX2 and other high mobility group (HMG) proteins, and proteins of SWI/SNF chromatin remodelling complexes, have only vague or promiscuous DNA sequence specificity<sup>248–251</sup>, which indicates that other factors are involved in determining their targets at different stages of cell differentiation and development. Moreover, binding-site selection by the zinc-finger transcription factor CTCF, which, together with cohesin complexes, anchors chromosome loops<sup>252</sup>, was shown to be controlled by the lncRNA just proximal to *Xist* (*Jpx*) during early cell differentiation, thereby regulating chromatin topology on a genome-wide scale<sup>253</sup>. CTCF binds thousands of RNAs, including *Xist*, *Jpx* and the lncRNA *Xist* antisense RNA (*Tsix*), which targets CTCF to the X inactivation centre<sup>254</sup>.

There is abundant evidence that RNA may guide chromatin remodelling complexes, although accessibility dictated by DNA and histone

modifications (which are also likely directed by regulatory RNAs) may also have a role. The *D. melanogaster* Hox protein Bicoid (which controls anterior–posterior patterning) binds RNA through its homeodomain<sup>255</sup>. SOX2 binds RNA with high affinity through its HMG domain<sup>256,257</sup>, as do other members of the HMGB family<sup>257–259</sup>.

During mouse embryogenesis, the *Sox2* locus expresses also an overlapping lncRNA<sup>260</sup>, and there are well-documented examples of lncRNAs that interact with SOX2 to regulate pluripotency, neurogenesis, neuronal differentiation and brain development<sup>257,261–264</sup>. SWI/SNF nucleosome remodelling complexes are directed to specific sites in chromatin or are antagonized by lncRNAs, including *XIST* and enhancer RNAs, in a wide range of differentiation processes and cancers<sup>251,265–270</sup>.

The lncRNA *MaTAR25*, which is overexpressed in mammary cancers, acts *in trans* to regulate the tensin1 gene through interaction with the transcription co-activator PURB<sup>271</sup>. The master transcription factor myoblast determination protein (MYOD), which can reprogramme mammalian fibroblasts into muscle cells and is central to muscle differentiation *in vivo*, is regulated by lncRNAs<sup>272–274</sup>, as are other aspects of muscle gene expression<sup>275</sup>. The pioneer transcription factor CBP also binds RNAs, including those transcribed from enhancers, to stimulate histone acetylation and consequently transcription<sup>276</sup>. Some transcription factors (OCT4, NANOG, SOX2 and SOX9) are also regulated by lncRNAs, including pseudogene-derived lncRNAs<sup>277–281</sup>, and reciprocally regulate the expression of lncRNAs<sup>282</sup>. Enhancer-derived lncRNAs also regulate the expression of the nuclear hormone receptor ESRI (ref. 283) and of CCAAT/enhancer-binding protein- $\alpha$  (CEBPA)<sup>284</sup>.

## Enhancer action

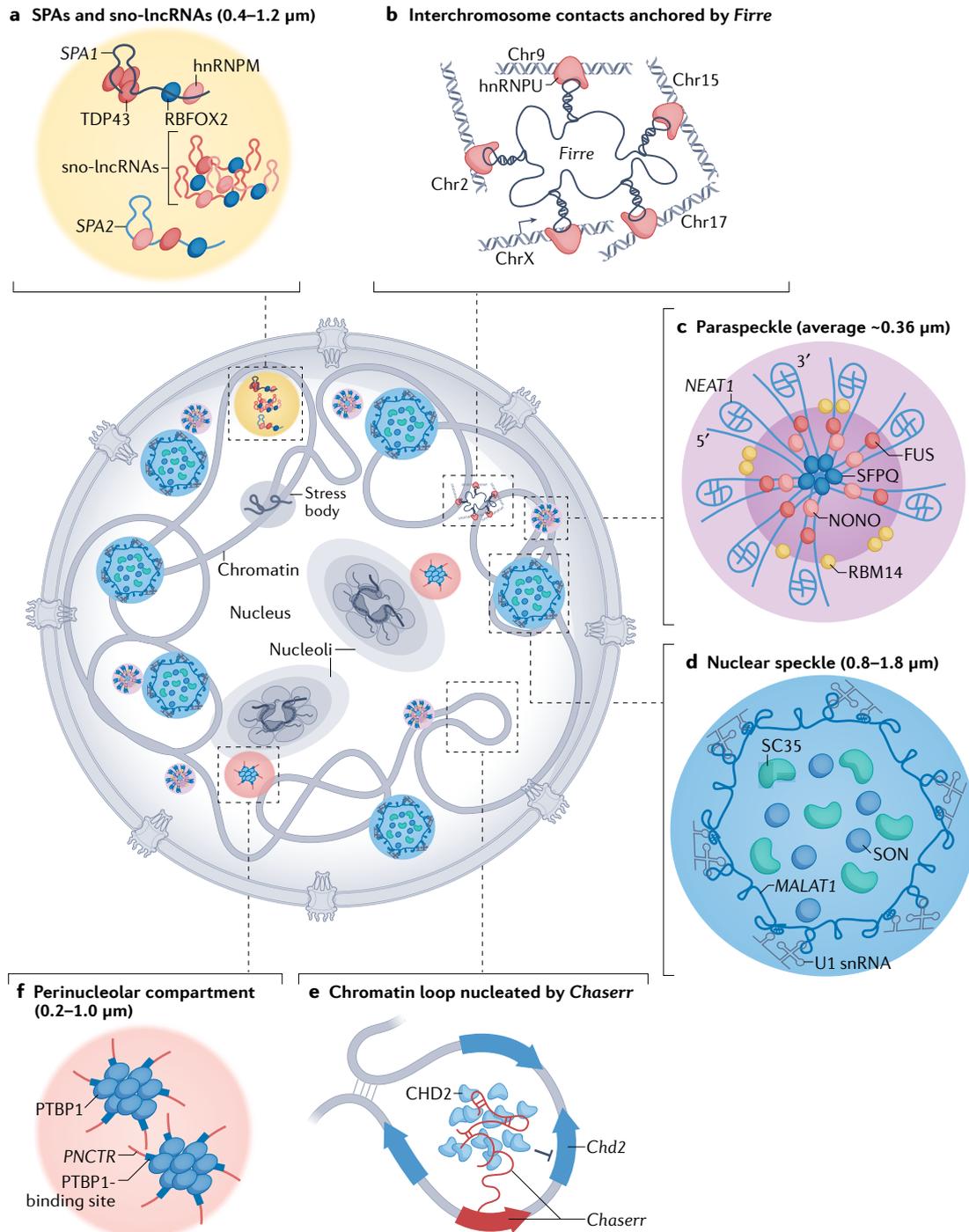
Enhancers are non-coding genomic loci that control the spatiotemporal expression of other genes during development. There appear to be ~400,000 ( $\pm 100,000$ ) enhancers in the mammalian genome<sup>285–288</sup>, sometimes clustered into 'super-enhancers' or 'enhancer jungles'<sup>288–291</sup>. Enhancers are thought to function by juxtaposing transcription factors bound at the enhancer promoters with the promoters of target genes<sup>292,293</sup>.

There is no question that enhancer action alters chromatin topology and may be responsible for the formation of chromatin-loop domains that act as local transcription and splicing hubs<sup>294,295</sup>. Enhancers are transcribed in the cells in which they are active<sup>141,289,296–299</sup>, which has led to uncertainty about whether the resulting RNAs are by-products of the binding of transcription factors or have a role in enhancer activity<sup>298</sup>.

The latter appears to be the case. The epigenetic landscape of and the features of transcription initiation at the promoters of protein-coding genes and enhancers are almost indistinguishable<sup>296–300</sup>. Enhancers express bidirectional promoter-associated short RNAs<sup>301–303</sup>, termed 'eRNAs', although such short RNAs are not specific to enhancers, as similar bidirectional transcripts are produced from the promoters of protein-coding genes<sup>304,305</sup>. Also analogously to mRNAs produced from protein-coding genes, enhancers express long (non-coding) RNAs (confusingly also referred to as 'eRNAs'<sup>298,306</sup>), and transcription is considered the best molecular indicator of enhancer activity in developmental processes<sup>296,297,306–308</sup> and cancers<sup>288</sup>. Moreover, enhancer-lncRNA splicing has been shown to modulate enhancer activity<sup>309,310</sup>.

Although the extent of congruency of combined genetic and high-depth transcriptomic data is uncertain, as their availability is still limited, the data suggest that many if not most lncRNAs are derived from enhancers<sup>141,298</sup> and that lncRNAs are required for enhancer activity<sup>163,284,311–314</sup>,

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examples including the lncRNAs *Euf2* (also known as *Dlx6os1*)<sup>315</sup>, *Firre*<sup>316</sup>, *Peril*<sup>317</sup>, *Upperhand* (also known as *Hand2os1*)<sup>318</sup> and *Maenli*<sup>319</sup> in mice. Enhancer RNA function is fertile ground for investigation, but if enhancer loci are considered bona fide ‘genes’, the g-value paradox (the perceived lack of increase in gene number with developmental complexity) is resolved. It also means that a key development in the evolution of complex organisms was the use of RNA to organize developmental trajectories<sup>319</sup>. It appears that “every cell type expresses precise lncRNA

signatures to control lineage-specific regulatory programs”<sup>270</sup>, and that cell state during ontogeny is likely directed by lncRNAs.

## Formation of biomolecular condensates

The past decade has seen the growing appreciation of the role of biomolecular condensates, or phase-separated domains (PSDs), in the organization of cells and chromatin. These condensates are highly dynamic assemblies with high local concentrations of macromolecules, a feature

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**Fig. 2 | Roles of long non-coding RNAs in nuclear organization.** **a**, 5' small nucleolar RNA-capped and 3'-polyadenylated long non-coding (lncRNAs) (SPAs)<sup>42</sup> and small nucleolar RNA-related lncRNAs (sno-lncRNAs)<sup>41</sup> accumulate at their sites of transcription 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<sup>42</sup>. **b**, The lncRNA functional intergenic repeating RNA element (*Firre*) is transcribed from the mouse X chromosome and interacts with the nuclear matrix factor hnRNPU to tether chromosome X (chrX), chr2, chr9, chr15 and chr17 into a nuclear domain<sup>451,452</sup>. **c**, The lncRNA nuclear paraspeckle assembly transcript 1 (*NEATI*) is essential for the formation of paraspeckles<sup>178</sup>. *NEATI* sequesters numerous paraspeckle proteins to form a highly organized core-shell (dark and light purple, respectively) spheroidal nuclear body<sup>453</sup>. The middle region of *NEATI* is localized in the centre of paraspeckles, and the 3'-end and 5'-end regions are localized in the periphery<sup>453</sup>. Different paraspeckle proteins are embedded by *NEATI* into the spheroidal structure in the core region (non-POU domain-containing octamer-binding protein (NONO), fused

in sarcoma (FUS) and splicing factor, proline- and glutamine-rich (SFPQ)) or in the shell region (RNA-binding motif protein 14 (RBM14))<sup>453</sup>. **d**, The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is localized at the periphery of nuclear speckles<sup>172,454</sup> and is involved in the regulation of pre-mRNA splicing<sup>339,455</sup>. *MALAT1* interacts with the U1 small nuclear RNA (U1 snRNA)<sup>428</sup>, whereas proteins such as SONDNA- and RNA-binding protein and splicing component 35 kDa (SC35) are localized at the centre of nuclear speckles<sup>456</sup>. **e**, The lncRNA *CHD2* adjacent, suppressive regulatory RNA (*Chaserr*) forms a compartment within a region of the mouse chromosome corresponding to a topologically associating domain that includes its own gene as well as the *Chd2* gene (encoding chromodomain DNA helicase protein 2 (CHD2))<sup>437</sup>. *Chaserr* limits in *cis* the expression of *Chd2*, which is important for proper regulation of many genes (not shown). **f**, The perinucleolar compartment contains the lncRNA pyrimidine-rich non-coding transcript (*PNCTR*), which sequesters pyrimidine tract-binding protein 1 (PTBP1) and thus suppresses PTBP1-mediated pre-mRNA splicing elsewhere in the nucleoplasm<sup>369</sup>. The size of nuclear bodies is indicated where relevant<sup>457</sup>. Figure adapted from ref. 80, Springer Nature. Part **e** courtesy of Inna-Marie Strazhnik and Mitch Guttman.

that promotes functional interactions. The condensates usually contain both RNA and proteins<sup>320–322</sup>, the latter having IDRs, which are the major sites of post-translational modifications<sup>323</sup>. IDRs interact with and are tunable by many partners<sup>324</sup>. The fraction of the proteome containing IDRs has expanded with cellular and developmental complexity<sup>323</sup>, and nearly all proteins involved in the regulation of development, including most transcription factors, histones, histone-modifying proteins, other chromatin-binding proteins, RNA-binding proteins, splicing factors, nuclear hormone receptors, cytoskeletal proteins and membrane receptors, contain IDRs<sup>323,325–332</sup>.

RNA is crucial for the form, composition and function of phase-separated RNA-protein condensates<sup>320–322</sup>. Specific 'architectural' lncRNAs<sup>333</sup> associate with nuclear condensates of different half-lives and functionalities, including in centrosomes<sup>334</sup>, nucleoli<sup>335</sup> (the lncRNAs *SLERT*<sup>338</sup> and *LETN*<sup>336</sup>), nuclear speckles (the lncRNA *MALAT1* (refs. 173, 337)) rich in RNA-processing factors, speckle-related condensates that contain the lncRNA *Gomafu* in mice<sup>338,339</sup> and paraspeckles (the lncRNA *NEATI* (refs. 340, 341)) (Fig. 2), in vertebrates as well as polyadenylation complexes<sup>342</sup> and other condensates in plants<sup>343</sup>. RNP condensates also include cytoplasmic membraneless organelles such as P-granules<sup>344,345</sup>, subcellular-localized translational messenger RNP assemblies<sup>346</sup> and synaptic compartments<sup>320,322,347</sup>. The mammalian cytoplasmic lncRNA *NORAD*, which is induced by DNA damage and required for genome stability, prevents aberrant mitosis by sequestering Pumilio proteins (which bind many RNAs to regulate stem cell fate, development and neurological functions) into PSDs through its repeat sequences<sup>137,348</sup>.

It has been proposed that RNAs have a central role in organizing the genome and gene expression by the formation of spatial compartments and transcriptional condensates<sup>349–353</sup>. Phase separation appears to drive chromatin long-range interactions and to be required for the action of enhancers and super-enhancers<sup>328,351,354–357</sup> as well as for transcription, transcription factors and polyadenylation complexes<sup>342,358–361</sup>, although transcription factor hubs have been reported to operate in the absence of detectable phase separation<sup>362</sup>. PSDs scaffolded by lncRNAs, including repeat-rich RNAs<sup>363,364</sup>, mediate the formation of heterochromatin<sup>353,365,366</sup>, euchromatin<sup>367</sup>, Polycomb bodies<sup>368</sup> and alternative splicing<sup>369</sup>. lncRNAs are a substantial component of rapidly renaturing, repeat-rich RNA (technically termed 'CoT-1 RNA'), and high-resolution imaging shows many repeat-containing

RNAs bound to chromatin, indicating that the collective presence of thousands of lncRNAs serves to counter chromatin condensation<sup>364</sup>. High-resolution imaging also shows the localization of many lncRNAs in compartments in the nucleus that resemble PSDs<sup>136,353</sup>. These data all suggest that there are thousands of low copy number lncRNAs involved in the organization of chromosome territories.

## lncRNA structure–function relationships

lncRNAs generally range in size from around 1 kb to longer than 100 kb (refs. 370, 371) and have a modular structure<sup>372–375</sup>. They are often multi-exonic and highly alternatively spliced (Fig. 3a), a feature that was not obvious before the advent of high-depth sequencing<sup>98</sup>. They also contain a higher proportion of GC–AG splice sites<sup>376</sup> and are therefore less efficiently spliced than protein-coding transcripts<sup>377,378</sup>, which are properties associated with alternative splicing<sup>379</sup>. Alternative splicing has, unsurprisingly, been shown to alter the function of lncRNAs<sup>42,152,380,381</sup>.

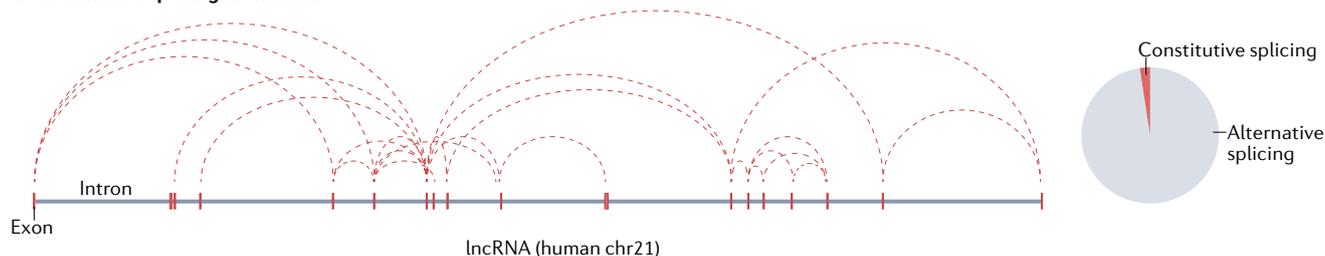
Some lncRNAs also exhibit common motifs and motif combinations<sup>101</sup>. At least 18% of the human genome is conserved among mammals at the level of predicted RNA structure<sup>382</sup>, and similar and potentially paralogous RNA structures occur at many places throughout the genome<sup>383,384</sup>. Chemical probing has shown that lncRNAs, including *Xist*, form complex multidomain structures<sup>108,385–389</sup>, with chemical data matching data predicted by evolutionary conservation of secondary structure<sup>389</sup>. Moreover, lncRNAs with similar *k*-base oligonucleotide (short motif) content have related functions despite their lack of general homology, implying that small sequence elements are also key determinants of lncRNA function<sup>390</sup>.

Many lncRNA exons are derived from transposable elements<sup>187,391</sup>. The most highly conserved sequences in *Xist*, which has been intensively studied, are its repeats<sup>7</sup>, whereas its unique sequences have evolved rapidly<sup>392</sup>, and many of its biological functions, including recruitment of gene-repressive complexes and gene silencing, are mediated through its modular repeat elements<sup>142,186,388,393–399</sup>. Transposable element-derived sequences participate in many RNA-protein interactions<sup>369,400,401</sup>, which leads to the conclusion that repeat structures are common building blocks of lncRNAs<sup>87,391,396</sup> and essential components of their function<sup>391</sup>.

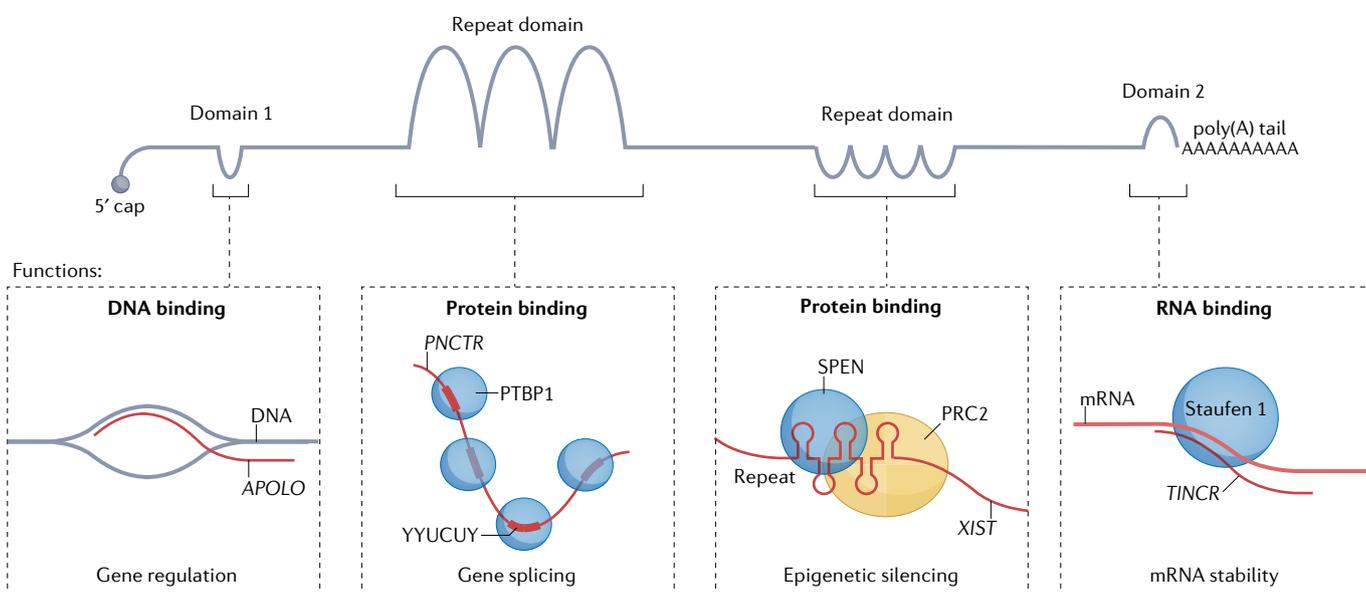
The molecular mechanisms of lncRNA action are unclear. In most well-characterized cases of RNA regulation, such as RNAi, snoRNAs,

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## a Alternative splicing of lncRNAs



## b Domain and repeat architecture of lncRNAs



**Fig. 3 | Modular structures of long non-coding RNAs.** **a**, Targeted RNA sequencing has revealed that human chromosome 21 (chr21) is pervasively transcribed into long non-coding RNAs (lncRNAs) and that lncRNA exons are almost universally (but not randomly) alternatively spliced to form diverse and complex isoforms<sup>98</sup>. The circle indicates the fraction of non-coding exons across all chr21 transcripts that are alternatively or constitutively spliced. **b**, Modular structural domains in lncRNAs that fulfil a range of functions<sup>372–375</sup>, including targeting DNA, such as in the case of auxin-regulated promoter loop (*APOLO*)<sup>61</sup>; binding other RNAs – for example, terminal differentiation-induced

non-coding RNA (*TINCR*)<sup>458</sup>, potentially involving RNA-binding proteins such as Staufen 1; and recruitment of proteins – for example, pyrimidine-rich non-coding transcript (*PNCTR*) recruiting of pyrimidine tract-binding protein 1 (PTBP1) through special RNA motifs<sup>369</sup> and X-inactive-specific transcript (*XIST*) recruiting split ends homologue (SPEN) and Polycomb repressive complex 2 (PRC2), perhaps in concert, which is the subject of active exploration and debate<sup>142,397,399,423,424,459</sup>. Modular functional domains can be repeated within a lncRNA or in multiple different lncRNAs<sup>7,87,186,369,388,391,393–401</sup>. Figure courtesy of Tim R. Mercer.

CRISPR and telomerase, RNA acts as a guide to target effector protein complexes to complementary RNA or DNA sequences. Data on selected lncRNAs (for example, *HOTAIR*, *roX1*, *roX2*, *Meg3*, *Tug1*, *PARTICLE* (also known as *PARTCL*), *PAPAS* and *KHPS1*) indicates that they form triplex structures with DNA at purine-rich GA stretches to recruit chromatin modifiers to specific loci across the genome<sup>402–408</sup>, with evidence that triplex formation by lncRNAs is a widespread phenomenon<sup>409–411</sup>. Others, especially antisense lncRNAs, appear to function through RNA–DNA hybrid formation<sup>61,412,413</sup>, but detail is presently lacking.

lncRNA RNP structure and function have been well characterized in only one instance, the telomerase complex, which has been studied for decades. Telomerase reverse transcriptase (TERT) catalyses the

addition of telomere repeats to chromosome ends, and other proteins in the complex provide nuclear localization, stability or recruitment to telomeres or to Cajal bodies. The lncRNA *TERC* provides the scaffold for assembly of the RNP and the template for DNA polymerization by TERT, and mutations in TERT and *TERC* are major contributors to the aetiology of cancer and the cause of hereditary disorders such as dyskeratosis congenita<sup>103–107,414–416</sup>.

By contrast, while we know the phenotypes caused by the loss of some lncRNAs, we know almost nothing about how most of them work, although, considering that as recently as 2010 the very existence of pervasive transcription was still a matter of contention<sup>417–419</sup> and the sheer number of lncRNAs, substantial progress has been made. It is assumed, in our view reasonably, that generally lncRNAs will engage

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## Glossary

### Cajal bodies

Nuclear structures often associated with the nucleolus that have important roles in RNA metabolism and the formation of ribonucleoproteins involved in transcription, splicing, ribosome biogenesis and telomere maintenance.

### Coacervates

Condensed liquid-like droplets formed by oppositely charged macromolecules, in vivo involving the interaction of positively charged amino acids in the intrinsically disordered regions of proteins with the negatively charged backbone of RNAs.

### GC-AG splice sites

A non-canonical variant of the major U2-type GT-AG splice junctions.

### Intrinsically disordered region

A polypeptide segment containing a high proportion of polar or charged residues and insufficient hydrophobic residues to form a stable tertiary structure.

### Modified RNA mimics

Chemically synthesized RNA molecules containing chemical modifications to increase their stability or target affinity, facilitate their action and/or bypass detection by innate immunity.

### Nuclear speckles

Irregularly shaped nuclear domains enriched in pre-mRNA splicing factors, located in the interchromatin regions of the nucleoplasm of mammalian cells.

### Pol V transcripts

Short RNAs transcribed by a specialized plant-specific RNA polymerase (Pol) which maintain the repression of transposons and genomic repeats.

### Polycomb bodies

Nuclear foci of Polycomb group proteins, within which Polycomb group protein-bound regions of DNA are localized and contact each other.

### Pioneer transcription factors

Proteins that have the unique ability to bind target sites in closed chromatin and open closed chromatin to activate gene expression and implement new cell fates.

### Primary transcription units

The long precursor RNAs transcribed from chromosomal regions ('genes') before splicing and assembly of the exons into an mRNA or long non-coding RNA and before processing of the intronic RNAs into *trans*-acting smaller RNAs.

### Quantitative traits

Phenotypic characteristics that vary continuously in natural populations, including many aspects of morphology, physiology, behaviour and disease susceptibility.

### Small interspersed nuclear elements

Repetitive non-coding sequences of 100–600 bp that are common in animal and plant genomes. They are derived from retrotransposons and are propagated through an RNA intermediate

### Super-enhancers

Enhancer-dense genomic regions found near genes that have key roles in determining cellular identity.

### Transcription factor hubs

Complex topological assemblies, in which multiple genes and regulatory factors interact with each other.

### X inactivation centre

A complex locus in the X chromosome that is required for the near-global inactivation of one of the two X chromosomes in female mammals, a spreading process initiated by the expression of *XIST*.

in multilateral interactions similarly to *TERC* and the telomerase complex<sup>108</sup>, and there is some evidence to support this assumption in cases such as *XIST* (Fig. 3b), but the assumption has not yet been rigorously tested. There are promising discoveries, such as the demonstration that conserved pseudoknots in lncRNA *Meg3* are essential for stimulation of the p53 pathway<sup>420</sup>. There is also growing evidence of discrete structural organization in lncRNAs<sup>421</sup>. Nonetheless, there is a long journey ahead to understand the structure and function of the many thousands of lncRNAs, and their splice variants, in the context of their associated RNP complexes and biomolecular condensates in both the nucleus and the cytoplasm.

## Challenges

If the complex ontogenies of animals and, to a lesser extent plants, require a large number of RNAs to guide the epigenetic decisions at each cell division, then it is not surprising that many lncRNAs have common protein-binding modules and specific targeting sequences that vary between different stages of development. The challenge is to define which lncRNAs and modules within them interact with effector proteins and which convey target (DNA or RNA) specificity. The former is complicated by the multisubunit nature of many RNP complexes, but is being addressed by technologies such as iCLIP<sup>422</sup>, RAP-MS<sup>423</sup>, ChIRP-MS<sup>388</sup> and iDRiP<sup>424</sup>. Determining target specificity is even more difficult, as specific targeting requires only short stretches of nucleotide complementarity given the strength of RNA–RNA and RNA–DNA interactions<sup>425</sup>, but it may be tackled by new methods that analyse RNA–chromatin and RNA–RNA interactions, such as GRID-seq<sup>426</sup>,

RADICL-seq<sup>427</sup>, RIC-seq<sup>428</sup> and RD-SPRITE<sup>353</sup>. Other lncRNAs are localized in cytoplasmic compartments, whose components also need to be characterized.

Understanding the roles of lncRNAs and how they function in dynamic assemblies with other macromolecules will provide a more comprehensive understanding of cell and developmental biology and of gene–environment interactions. Emerging challenges include understanding the roles of lncRNAs and RNA modifications in functional plasticity, especially in the brain, and the dysregulation of these lncRNA-mediated pathways in neurological disorders, cancer and other diseases.

## Recommendations

1. In the absence of more specific categorization, we recommend retention of the general descriptor 'lncRNA' for non-coding RNAs greater than 500 nt in length.
2. Unless a lncRNA is antisense to a protein-coding gene (in which case the designation '*gene name*-AS' should be used), we recommend naming lncRNAs for their own sake with allusion to a discerned characteristic or function (as has been traditional for proteins), preferably accompanied by complete exon–intron structures and genomic coordinates. If no biological context is available, we recommend naming the lncRNA according to the GENCODE system<sup>46</sup>.
3. We recommend that future gene expression profiling should include full transcript analysis of the isoforms and stoichiometry of mRNAs, lncRNAs and small RNAs in cells at different stages of

differentiation, and in various physiological and disease states, learning and stress conditions.

4. These efforts should be complemented by cell-based, organoid-based and in vivo studies using strategies for conditional and tissue-specific or cell type-specific gain-of-function and loss-of-function of lncRNAs.

More broadly, identifying and understanding the roles of lncRNAs and RNA regulatory networks in multicellular development, cell biology and disease will require the following:

1. The determination of the interplay between lncRNAs, chromatin modifications, proteins and the genome in the assembly of the nuclear domains essential for chromatin organization, enhancer function, transcription and splicing. This effort will require the development of antibodies with high specificity for protein–RNA complexes, and of intracellular RNA-tracking methods<sup>429</sup>.
2. The determination of lncRNA localization, structure–function relationships and interactions using a range of sequencing, chemical probing, imaging methods<sup>430–433</sup> and cryogenic electron microscopy<sup>434</sup>.
3. The identification and characterization of the many unknown nuclear and cytoplasmic compartments decorated by specific lncRNAs.
4. Harnessing the power of machine learning to interrogate large genomic, epigenomic, transcriptomic, proteomic and phenomic datasets to identify causal links and pathways.

Published online: 3 January 2023

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## Author contributions

All authors researched data for the article, made substantial contributions to discussion of the content and reviewed and/or edited the manuscript before submission. J.S.M. drafted the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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**Peer review information** *Nature Reviews Molecular Cell Biology* and the authors thank Thomas Cech, Xiangting Wang and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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