Mechanisms of long noncoding RNA function in development and disease

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Abstract Since decades it has been known that non-protein-coding RNAs have important cellular functions. Deep sequencing recently facilitated the discovery of thousands of novel transcripts, now classified as long non-coding RNAs (lncRNAs), in many vertebrate and invertebrate species. LncRNAs are involved in a wide range of cellular mechanisms, from almost all aspects of gene expression to protein translation and stability. Recent findings implicate lncRNAs as key players of cellular differentiation, cell lineage choice, organogenesis and tissue homeostasis. Moreover, lncRNAs are involved in pathological conditions such as cancer and cardiovascular disease, and therefore provide novel biomarkers and pharmaceutical targets. Here we discuss examples illustrating the versatility of lncRNAs in gene control, development and differentiation, as well as in human disease.

Keywords LncRNA · Differentiation · Cardiovascular disease · Cancer · Gene regulation · Chromatin · Epigenetics · Genome organization

Introduction

It has long been known that several classes of non-protein-coding RNA molecules exert important cellular functions. For instance, ribosomal RNAs (rRNAs) are essential elements of the translation machinery and small nuclear RNAs (snRNAs) are required for splicing of nascent RNA transcripts. Also, various classes of small (around 20–30 nucleotides) noncoding RNAs such as micro (mi)RNAs, small inhibitory (si)RNAs or PIWI interacting (pi)RNAs are well known as gene silencers. With the recent advent of massive parallel sequencing techniques, however, it has been observed that a tremendously high portion, approximately 70%, of the genome is transcribed in various contexts and cell types [1, 2]. A large proportion of these newly detected RNA transcripts are structurally indistinguishable from protein-coding and processed messenger RNAs (mRNAs). They tend to be expressed at a very low level and have little to no protein-coding potential. This subclass of noncoding transcripts of variable length and function is collectively referred to as long noncoding RNAs (lncRNAs).

A plethora of biological tissues, organs, pathological samples and cultured cells have been analyzed for non-coding RNA expression, and it is clear that these molecules are omnipresent. Apparently, defining noncoding RNA function has proven more challenging than detecting them, as the number of reports showing comprehensive functional data is far smaller than those describing their identification in various contexts. The flexibility of RNA transcripts and their ability to fold into complex 3D-conformations enables them to form specific interactions with proteins. They can interact with RNA or DNA molecules via base pairing, even with double-stranded DNA, and form networks with DNA, protein complexes and RNA
molecules, illustrating their large potential as an important player with many biological functions. In this review, we will discuss mechanisms of lncRNA functions with a focus on their role in development and disease (Table 1).

**Molecular and genetic structure of LncRNAs**

Like mRNAs, IncRNAs are transcribed by Polymerase II, mostly 5'-capped, polyadenylated and spliced, though on average they contain a lower number of exons than mRNAs and their expression level assessed across different tissues is lower [3, 4]. There are various algorithms calculating the coding probability based on the length of a potential open reading frame (ORF), the similarity of such an ORF to known protein-coding genes, frequency of in-frame nucleotide hexamers or other empirical sequence features [5]. In general, RNA transcripts containing short (<100 nt) non-conserved ORFs, which have no homology to known peptide sequences and do not match to peptides identified in mass spectrometry screens are considered noncoding [6]. Interestingly, the majority of IncRNAs are associated with ribosomes [7, 8], though they do not show the characteristic release of ribosomes [9] or the typical 3-nucleotide phasing corresponding to codons of an ORF [10, 11]. However, in rare instances, functional oligopeptides have been found to be translated from putative IncRNAs [12–14].

Deep-sequencing experiments revealed many examples of genes producing both protein-coding and noncoding transcripts by alternative splicing. However, so far, only very few reports demonstrate a functional role for both the noncoding RNA(s) and the protein(s) encoded by transcripts derived from the same gene [15, 16]. It is tempting to speculate that such dual usage of transcripts is more frequent than anticipated.

RNA molecules have the potential to form highly structured macromolecules by folding into double-stranded stems, single-stranded loops and bulges, which again can fold further into three-dimensional structures, allowing for the potential formation of complex shapes. So far, the structure of only a few RNAs has been experimentally determined using a combination of chemical assays, and by determining the accessibility of base-paired or single-stranded RNA by various RNases [17, 18]. However, these methods still have the limitation that they can only reveal the secondary, but not the tertiary (3D) structure. In addition, computational predictions are only beginning to provide reliable results, but program learning from experimental data might improve the predictions and more closely mirror experimental observations. This process is
accelerated by recently developed techniques combined with high-throughput sequencing, such as SHAPE-MaP and icSHAPE [19, 20].

The similarity between mRNA-encoding and lncRNA genes is furthermore reflected by the chromatin signatures at the genomic regions from where they are transcribed. Their

Fig. 2 Schematic representation of cellular mechanisms involving lncRNAs. a LncRNA transcripts evicting proteins from chromatin; here, pancRNAs prevent DNMT from methylating DNA in their promoter region, thereby ensuring mRNA transcription. b LncRNAs recruiting the Mediator complex to an enhancer region, stabilizing loop formation and transcription of the associated gene. c LncRNAs transcribed from an enhancer region interfering with enhancer-promoter contact, thereby inhibiting transcription of the protein-coding gene. d LncRNA recruiting proteins, such as chromatin-modifying complexes to specific target sites in the genome, e.g. via DNA-RNA triplex formation. e LncRNA acting as scaffold linking different proteins required for concerted action. f LncRNA binding and sequestering proteins to prevent or attenuate their action, e.g. binding to mRNAs (left); circRNA sequestering miRNAs to prevent their binding to mRNAs (right). g Example of a lncRNA changing the splicing pattern by binding to a primary RNA transcript. h LncRNA stabilizing a mRNA by recruiting proteins such as STAU1, thereby preventing degradation.
### Table 1 List of lncRNAs and their main features mentioned in this review

| Name               | Genomic category          | Neigh-boring gene | Cellular localisation | Mechanism                              | Physiological/pathological setting | References |
|--------------------|----------------------------|-------------------|-----------------------|----------------------------------------|-----------------------------------|------------|
| AIRN               | Antisense/overlapping      | IGF2R             | Nucleus               | Transcription                          | Imprinting                        | [118]      |
| ANCR (DANCR)       | LincRNA, miRNA host gene  | ERVMER34          | Nucleus               | Histone modification                   | Epidermal differentiation          | [139, 140] |
| ANRIL              | Antisense                  | CDKN2B            | Nucleus               | Histone modification                   | Different cancer types, CVD        | [58, 59, 169] |
| BCA4               | Divergent lncRNA           | RSL1D1            | Nucleus               | Histone modification                   | Breast cancer                      | [82]       |
| BRAFIP1 (Braf-1s1) | Pseudo gene                | ZDHHC15           | Cytoplasm             | Post transcriptional                   | DLBCL                             | [158]      |
| Braveheart (Bvht)  | LincRNA                    | IL17b             | Nucleus               | Histone modification                   | Cardiac differentiation            | [45]       |
| CDR1               | CircRNA                    | CDR1              | Cytoplasm             | Post transcriptional                   | Neuronal tissue                    | [26, 27]   |
| DACOR1 (TCONS_00023265) | LincRNA                | SMAD3             | Nucleus               | DNA methylation                       | Colon cancer                       | [74]       |
| DEANR1, ALIEN      | LincRNA                    | FOXA2             | Both                  | Transcription factor                   | Endoderm and cardiac differentiation | [76, 77]   |
| ecCEBPA            | Upstream lncRNA            | CEBPa             | Nucleus               | DNA methylation                       | n.d.                              | [64]       |
| Evi2 (Dlx6os)      | Divergent lncRNA/overlapping | Dlx5/6            | Nucleus               | Chromatin remodeling                   | Neuronal development               | [69, 70]   |
| FAL1               | LincRNA (2 kb)             | ECM1              | Nucleus               | Post transcriptional, histone modification | Ovarian cancer                     | [57]       |
| Fendrr             | Divergent lncRNA           | Foxf1             | Nucleus               | Histone modification                   | Development                        | [47, 123]  |
| FIRRE              | LincRNA                    | RNA5SP514         | Nucleus               | 3D genome organization                | 3D genome structure                | [103]      |
| Gtl2 (MEG3)        | LincRNA                    | RTL1              | Nucleus               | Histone modification, DNA methylation | Imprinting                         | [55, 122, 177] |
| H19                | LincRNA                    | IGF2/NCTC1        | Nucleus               | DNA methylation                       | Imprinting, muscle differentiation | [116, 117] |
| HOTAIR             | LincRNA                    | HOXC11/HOXC12     | Nucleus               | Histone modification                   | Different cancer types, skeletal development | [42–44, 152] |
| HOTAIRM1           | Divergent lncRNA           | HOXA1, HOXA2     | n.d.                  | n.d.                                  | Myeloid cancer cell lines          | [178]      |
| Hotdog             | Enhancer RNA               | HoxD              | Nucleus               | Enhancer                              | Development                        | [179]      |
| HOTTIP             | Divergent lncRNA           | HOXA13            | Nucleus               | Histone modification                   | Limb development                   | [62]       |
| Jpx                | LincRNA                    | Xist              | Nucleus               | Transcription factor                   | X-chromosome inactivation          | [83, 84]   |
| Lethe              | Pseudo gene                | Gmeb1             | Nucleus               | Transcription factor                   | Inflammation                      | [79]       |
| Line-HOX1 (Linc1547, HAUNT) | LincRNA                | HOXA1             | Nucleus               | Enhancer, histone modification         | Development                        | [99, 123]  |
| Line-P21           | LincRNA                    | p21               | Nucleus               | Transcription factor                   | Cancer, CVD                        | [123, 163, 165, 166] |
| Lnc-DC             | LincRNA                    | HEATR6            | Cytoplasm             | Transcription factor                   | Dendritic cell differentiation     | [81]       |
| LncMyoD            | LincRNA                    | Munc, MyoD1      | Both                  | Post transcriptional                   | Muscle differentiation             | [180]      |
| lncTF7 (WSPAR)     | LincRNA                    | TCF7              | Nucleus               | Chromatin remodeling                  | Hepatocellular carcinoma          | [72]       |
| LUNAR1             | Divergent lncRNA           | PGPEP1L, IGF1R   | Nucleus               | Transcription factor                   | Acute leukemia                     | [151]      |
| MALAT1 (NEAT2)     | LincRNA                    | SCVLI             | Nucleus               | Post transcriptional                   | Metastasis                         | [109, 110, 149] |
Table 1 continued

| Name | Genomic category | Neigh-boring gene | Cellular localisation | Mechanism | Physiological/pathological setting | References |
|------|------------------|-------------------|-----------------------|-----------|------------------------------------|------------|
| MiAT (Gomafu, RNCR2) | LincRNA | CRYBA4 | Nucleus | Post transcriptional | Myocardial infarction, neuronal differentiation, brain development, schizophrenia | [107, 108, 138, 167] |
| MIR31HG | LincRNA, miRNA host gene | INK4A | Nucleus | Histone modification | Senescence | [161] |
| Myheart (Mhrt) | Divergent lncRNA/overlapping | Myb67 | Nucleus | Chromatin remodeling | Myocardial infarction | [68] |
| NBAT-1 | Divergent lncRNA | CASC15 | Nucleus | Histone modification | Neuronal differentiation, different tumors | [148, 150] |
| NEAT1 | LincRNA | FRMD8 | Nucleus | n.d. | Progesterone production/corpus luteum formation | [124, 127] |
| NeST (Tmevpg1) | LincRNA | IFNg | Nucleus | Histone modification | Infections | [63] |
| NKILA | Overlapping | PMEPA1 | Cytoplasm | Transcription factor | Breast cancer | [153, 154] |
| NORAD (LINC00657) | LincRNA | CNBD2 | Cytoplasm | Post transcriptional | Genomic stability | [111] |
| PACER | Divergent lncRNA | COX2/PTGS2 | Nucleus | Transcription factor | Infection | [80] |
| pancIL17d | Divergent lncRNA | IL17b | Nucleus | DNA methylation | Preimplantation development | [66] |
| PARTICLE | Divergent lncRNA | MAT2A | Both | Histone modification | Increased in plasma from patients post-radiation | [54] |
| PCGEM1 | LincRNA | TMEF2 | Nucleus | Transcription factor, histone modification | Prostate cancer | [101, 144, 181] |
| Pint | Divergent lncRNA | 2210408F21Rik | Nucleus | Histone modification | Colorectal cancer, growth/size | [48, 123] |
| Pnky | Divergent lncRNA | Pou3f2 | Nucleus | Post transcriptional | Neuronal differentiation | [105] |
| PRNCR1 | LincRNA | CASC19 | Nucleus | Transcription factor, histone modification | Prostate cancer | [101, 181] |
| RMST | LincRNA | NEDD1 | Nucleus | Transcription factor | Neuronal differentiation | [78, 129, 133] |
| RNCR4 | Divergent lncRNA | Mirc35HG | Both | Post transcriptional | Retina development | [136] |
| SchLAP1 | LincRNA | UBE2E3 | Nucleus | Chromatin remodeling | Prostate cancer | [67] |
| TARID | Divergent lncRNA | TCF21 | Nucleus | DNA methylation | Different cancer types | [65] |
| TINCR | LincRNA | SAFb2 | Cytoplasm | Post transcriptional | Epidermal differentiation | [141, 142] |
| Tsix | LincRNA/antisense | Xist | Nucleus | Transcription factor, histone modification, chromatin remodeling | X-chromosome inactivation | [83] |
| TUNA (megamind, Linc86023) | LincRNA | TcI1 | Both | Post transcriptional | Pluripotency, Huntington | [33, 106] |
| Twin of Hotdog | Enhancer RNA | HoxD | Nucleus | Enhancer | Development | [179] |
| UCA1 | LincRNA | OR10H1 | Cytoplasm | Post transcriptional | Senescence | [162] |
| Xist | LincRNA/antisense | Tsix | Nucleus | Transcription factor, histone modification, chromatin remodeling | X-chromosome inactivation | [40, 71, 120, 121, 182, 183] |
transcriptional start site is, in most cases, marked by the H3K4me3 histone modification and the transcribed region by the H3K36me3 mark, no matter if the IncRNA originates from its own promoter or from an enhancer. However, these histone marks are less prominent than observed at mRNA coding genes, whereas H3K4me1, a characteristic mark of enhancers, tends to be more prominent at genomic regions encoding IncRNAs [2, 21–23]. As expected for transcribed regions, IncRNA promoters correspond with DnaseI hypersensitive sites, which indicates accessible chromatin [24, 25].

So far, the most commonly used categorization of the highly heterogeneous class of IncRNAs is their position in the genome relative to protein-coding genes (Fig. 1). IncRNAs can be intergenic (IncRNAs) or divergently transcribed from the same promoter as a protein-coding gene (pncRNAs). IncRNAs expressed from a promoter (with high H3K4me3 to H3K4me1 ratio) are classified as promoter-associated IncRNAs (plncRNAs). However, IncRNAs can also be transcribed from enhancers and are then termed eRNAs. They are mostly transcribed in both directions, in contrast to enhancer-associated IncRNAs (elnRNAs), which are unidirectionally transcribed from a promoter with low H3K4me3 to H3K4me1 ratio [23].

Transcription of IncRNAs can also originate from within introns, or overlap with other transcripts in sense or antisense orientation [3]. Some IncRNAs are generated by backsplicing from introns of mRNAs or other IncRNAs and are thus circular (circRNAs) [26, 27].

Genome-wide sequencing of RNA species isolated from cytosolic or nuclear fractions of cells has shown that the majority of IncRNAs tend to be localized in the nucleus or are associated with chromatin, while a considerable fraction localizes to the cytoplasm [3, 28, 29]. The subcellular localization is a good indication of the putative function of a IncRNA, since in contrast to protein-coding mRNAs, IncRNAs can already function while transcription is occurring. In fact, many nuclear and, in particular, chromatin-retained IncRNAs co-regulate transcription and/or chromatin structure at or close to their site of transcription, i.e. in cis.

**Conservation and evolutionary aspects of IncRNA**

The genomic sequences of IncRNAs are, in general, less conserved than exons, but more than introns of protein-coding genes, pointing towards a rapid evolution with moderate constraints [21, 30]. In contrast to the actual sequence, splice sites of IncRNAs have been found to be more stable during evolution [31, 32]. Despite poor RNA sequence conservation, IncRNAs have frequently been identified across species in syntenic genomic regions [33, 34]. It is therefore quite likely that evolutionary conservation of IncRNAs is embodied by a conserved 3D structure, although this is difficult to assess with current methods. A further layer of conservation is functional conservation between IncRNAs playing equivalent roles in particular biological settings [35].

A different explanation for low conservation of IncRNAs is the increasing number of such transcripts in increasingly complex species. This finding, in combination with the observed highly tissue-specific expression of many IncRNAs, suggests that IncRNAs might be key molecules promoting species-specific features and organ complexity [36–38]. Thus, IncRNAs very likely have played important roles in the evolution of complex organisms.

**LncRNAs regulate gene and genome activity**

The best-described function for IncRNAs in the nucleus is their role in regulating gene and genome activity on various levels (Fig. 2a–e). Many possible mechanisms by which IncRNAs influence chromatin modifications and chromatin structure, and thereby influence transcription or other chromatin-related functions by epigenetic mechanisms have been investigated. In the following section we will address different levels of gene and genome regulation separately.

**Histone modifications regulated by LncRNAs**

Prominent examples of histone-modifying complexes interacting with IncRNAs are the two polycomb repressive complexes, PRC1 and, in particular PRC2, which mediates methylation of lysine 27 on histone 3 (H3K27me), a histone mark associated with repressed or poised genetic loci. The first report of an interaction of PRC2 with a noncoding RNA came from studies on X-chromosome inactivation in mammals, which involves X-inactive specific transcript (Xist), a IncRNA that is highly expressed from the inactive X-chromosomes in females (Xi) and recruits PRC2 to the Xi to silence gene expression [39–41].

Another prominent example is Hotair, a conserved IncRNA that is transcribed from within the HoxC gene cluster. Hotair was shown to play a repressive role at the HoxD locus by interacting with PRC2. In addition, Hotair also interacts with the histone H3K4me1/2 demethylase LSD1 (KDM1), which removes a histone mark of active chromatin and thus reinforces the establishment of a repressive chromatin environment on target loci [42] (Fig. 2d, e). Targeted inactivation of Hotair in mice leads to de-repression of imprinted genes and of HoxD genes, which lose H3K27- methylation and gain H3K4-methylation marks, as expected from loss of the enzymatic activity of PRC2 and LSD1 at these loci. Furthermore, Hotair knockout mice show skeletal homeotic transformation phenotypes, which are typical for mutations affecting Polycomb mediated repression [43]. Interestingly, mice lacking most of the HoxC cluster
including *Hotair* do not display these phenotypes, suggesting a complex interplay of multiple genomic regions in regulating *HoxC* cluster gene expression [44].

*Braveheart* (*Bvht*) is an lncRNA that is activated during early cardiac differentiation and acts upstream of *MESP1*, a basic Helix-loop-helix transcription factor involved in early heart development. *Bvht* also exerts its function via an interaction with *PRC2* [45]. The lncRNA *Fetal-lethal developmental regulatory RNA* (*Fendrr*; or *Foxf1* adjacent noncoding developmental regulatory RNA) is likewise involved in cardiac development and heart function and interacts with *PRC2*. *Fendrr* also interacts with WDR5, which is well known for its presence in the MLL complexes that mediate H3K4 methylation, a mark that is thought to oppose H3K27me. *Fendrr* might play a role at poised genes, or is involved in tuning the balance of active and repressive marks at its target gene promoters, thus adjusting the correct expression level of its targets [46, 47]. These examples are only a small selection and many more lncRNAs seem to function in concert with the PRC2 complex; for more examples, see [48–52].

An intriguing mechanism as to how lncRNAs might recruit their protein interaction partners to specific genomic loci is DNA-RNA triplex formation [53], which has been proposed for several lncRNAs forming a complex with *PRC2* [46, 54, 55] (Fig. 2d). In this manner, lncRNAs might direct chromatin or transcriptional modulators to specific genomic sites. This could explain, to some extent, the site-specific action of chromatin-modifying complexes, which do not themselves bind to DNA in a sequence specific manner [38, 56].

An example of a lncRNA interacting with the PRC1 complex is Focally amplified lncRNA on Chromosome 1 (*FAL1*). The interaction of *FAL1* with BMI1, an essential subunit of PRC1, regulates its protein stability [57]. The knockdown of *FAL1* in an ovarian cancer cell line led to gene expression changes, slower cell cycle progression and induction of senescence, similar to the effects of the *BMI1* knockdown. This latter phenotype could be partially rescued by knockdown of the senescence-promoting factor *CDKN1A* (p21). Additional ChiP data suggested that BMI1 binds to the promoter of *CDKN1A* and, together with *FAL1*, suppresses the expression of this target and of many other genes.

Whereas *FAL1* acts in *trans* on PRC1, *ANRIL*, a lncRNA transcribed from the *INK4B-ARF-INK4A* tumor suppressor locus, acts in *cis*. The *ANRIL* transcript was discovered in a family with inherited melanoma-neural system tumors and assumed to play a role in tumorigenesis [58]. *ANRIL* recruits *CBX7* (a PRC1 component) via a POLII-dependent mechanism to its locus in order to repress the neighboring *INK4B-ARF-INK4A* genes, antagonize cellular senescence and indirectly promote cell cycle activity [58, 59].

The repressive H3K27-methylation, catalyzed by EZH2 in the PRC2 complex, is opposed by MLL complexes, which mediate methylation of H3K4me2/3, a mark associated with loci that are actively transcribed or primed for activation. WDR5 is an integral part of all MLL complexes, but also interacts with several other protein complexes [60]. WDR5 has been found to interact with more than 200 lncRNAs in mouse embryonic stem cells (mESCs) [61]. These interactions are important for WDR5 binding to chromatin since a point mutant that cannot bind RNA also fails to stably associate with chromatin. More specifically, two lncRNAs, *HOTTIP* and *NeST* have been described to recruit WDR5 to their neighboring genes and thus enhance their transcription [62, 63].

**LncRNAs modulate DNA methylation**

Whereas many lncRNAs associating with Polycomb complexes act by promoting PRC occupancy at genomic target sites, lncRNAs described in the context of DNA methylation have mostly been found to oppose this epigenetic mark. Frequently, transcription of noncoding RNAs, which interact with DNMTs, keeps a locus free of DNA methylation [54, 64–66] (Fig. 2a). Initially, it was observed that knockdown of extracoding *CEBPA* (*ecCEBPA*), a transcript starting upstream of the *CEBPA* gene, leads to down-regulation of *CEBPA* and increased DNA methylation of the locus. A subsequent global analysis of all lncRNAs bound to DNMT1 [64] revealed that loci whose RNA products interact with DNMT1 show lower levels of DNA methylation than other loci. A similar observation was made in oocytes and two-cell stage embryos [66], in which the down-regulation of several divergently transcribed promoter-associated ncRNA (pancRNA) led to lower expression of the adjacent protein-coding gene and higher DNA methylation. In the case of one particular gene, *IL17b*, the pancRNA knockdown resulted in cell death of the developing blastocyst. Supplementing the embryos with *IL17b* protein rescued the phenotype.

In the case of *Tcf21 antisense RNA reducing DNA methylation* (*TARID*), keeping the protein-coding gene (*Tcf21*) on the opposite DNA strand free of DNA methylation required GADD45A and the TET proteins [65] pointing towards a mechanism involving active DNA demethylation.

**LncRNAs regulate chromatin remodeling**

In addition to interaction with enzymatic complexes, which covalently modify chromatin, lncRNAs have also been shown to control chromatin remodeling complexes that can alter the nucleosome spacing in an energy dependent manner. In human prostate cancer, gene expression analyses showed that SWI/SNF and the lncRNA *SChlAP1* have opposing roles. *SChlAP1* interacts with the SNF5 subunit of the chromatin remodeling complex SWI/SNF,
and globally inhibits binding of SWI/SNF to chromatin, subsequently leading to genome-wide de-repression of gene activity [67]. Similarly, the lncRNA Myheart (Mhrt), a lncRNA transcribed divergently to Myh6 and overlapping with Myh7 in antisense direction, binds to BRG1, the ATPase subunit of the SWI/SNF complex, and excludes it from the Myh6/7 locus, thus preventing chromatin remodeling [68]. BRG1, in turn, down-regulates Mhrt. Cardiac stress leads to upregulation of BRG1 to a level allowing it to overcome the repulsion of the SWI/SNF complex from chromatin by Mhrt and to bind to the Myh6 locus.

The Evf2 lncRNA, transcribed from the genomic region between Dlx5 and Dlx6, promotes SWI/SNF binding to the enhancers of these genes. However, Evf2 inhibits the remodeling activity of SWI/SNF and thus interferes with upregulation of Dlx5/Dlx6. Accordingly, in Evf2 mutant mice, Dlx6 and, to a lesser extent, Dlx5 are upregulated [69, 70]. In a more recent study, it was observed that the binding of Evf2 to BRG1 can be out-competed by other RNAs of similar length and results in reduced remodeling activity, suggesting that the binding of lncRNAs to the SWI/SNF complex is promiscuous. Xist, on the other hand, binds to components of the SWI/SNF complex, but excludes the complex from the Xi, rather than recruiting it [71]. The SWI/SNF complex can also be recruited by lncRNAs and mediate gene activation, as has been found in hepatocellular carcinoma cancer stem cells. A lncRNA, lncTCF7 or WNT signaling pathway activating non-coding RNA (WSPAR), transcribed 200 kb upstream of TCF7, can recruit SWI/SNF to the TCF7 promoter and thus activate TCF7 expression and WNT signaling [72].

Is the observed binding of numerous lncRNAs to chromatin modifiers specific or promiscuous?

The specificity of lncRNA interactions with chromatin-modifying complexes has been questioned. For instance, a large number of lncRNAs have been shown to co-purify with PRC2, possibly suggesting non-specific binding [50, 52, 73]. Similarly, 15% of all lncRNAs present in a particular cellular context were shown to be bound by DNMT1 [64, 74]. Likewise, WDR5 binds to more than a thousand RNAs in mESCs, of which 20% are lncRNAs [61]. This issue of promiscuity was assessed exemplarily for PRC2 in a systematic biochemical study [75], where the authors demonstrate that PRC2 binds RNA in a promiscuous manner, but has remarkably higher affinity for specific lncRNAs than for e.g. bacterial mRNA. Furthermore, they provide evidence that the binding strength correlates with the length of the RNA. Though these studies were performed in vitro at non-physiological conditions, they suggest that the chromatin-modifying complexes can sufficiently differentiate between specific and non-specific interactors. Moreover, since most lncRNA transcripts are expressed at few copies per cell, it is likely that the number of PRC2 molecules per cell is in excess to the number of lncRNA transcripts, which contain a high affinity site for PRC2. Overall, it is not unexpected that a chromatin-modifying complex acting at numerous genomic loci binds many different individual lncRNAs, which stabilize it or recruit it to distinct genomic loci.

Interactions of lncRNAs with transcription factors

Besides chromatin modifiers, transcription factors have also been found to interact with lncRNAs. The lncRNA definitive endoderm-associated lncRNA1 (DEANR1) (also known as ALIEN), for instance, is transcribed downstream of FOXA2 and is highly induced during differentiation of ESCs towards definitive endoderm [76, 77]. Knockdown of DEANR1 inhibits endodermal differentiation due to the reduction of FOXA2 expression. This interpretation is strongly supported by the fact that endoderm differentiation is rescued in DEANR1 knockdown cells upon forced FOXA2 expression. Mechanistically, DEANR1 was proposed to interact with SMAD2/3 and to recruit it to the FOXA2 promoter.

The lncRNA rhabdomyosarcoma associated transcript (RMST) is required for neuronal differentiation by mediating binding of the transcription factor SOX2 to approximately half of its binding sites [78]. Certain lncRNAs have been linked in different ways to transcription factors of the NF-κB pathway. Lethe (after the ‘river of forgetfulness’ in Greek mythology) interacts with RelA and prevents it from binding to DNA [79], p50-associated cyclooxygenase-2 (COX-2) extragenic RNA (PACER), on the other hand, sequesters p50, which forms homodimers and is repressive at high concentration, thereby lowering its concentration and allowing it to form activating heterodimers with p65 [80]. Another lncRNA, lnc dendritic cells (lnc-DC) binds to the transcription factor STAT3 in the cytoplasm and prevents its dephosphorylation by SHP1, thereby activating STAT3 and thus dendritic cell differentiation [81].

An elaborate mechanism linking transcription factor binding and chromatin modifications has been found for breast cancer anti-estrogen resistance 4 (BCAR4). This lncRNA binds to the SMAD nuclear interacting protein 1 (SNIP1) and a phosphatase (PNUTS) involved in RNA polymerase II regulation. In response to cytokine stimulation, BCAR4 lifts the inhibitory effect of SNIP1 on p300, a histone acetyl transferase and transcriptional activator. Acetylated histones are necessary for BCAR4 mediated recruitment of PNUTS, which in turn leads to active polymerase II at GLI2 controlled genes [82].

Also CTCF, a protein with the dual function of a transcriptional regulator and a boundary factor, has been shown
to interact with numerous noncoding RNAs [83]. An example is the interplay between CTCF and the IncRNAs Jpx, Tsix and Xite, which is important for X-chromosome inactivation [83, 84]. Prior to inactivation, Tsix and Xite recruit CTCF in cis to the X-inactivation center promoting homologous X-chromosome pairing at the Xic [83]. During X-chromosome inactivation, Jpx evicts CTCF in trans from the Xist locus and thereby prevents promoter blockade by CTCF [83]. These examples illustrate the dynamic interaction between IncRNAs and transcription factors in regulating chromatin-mediated cellular functions.

Regulation of genome organization by looping of enhancers

Previously, transcripts found at enhancers had been interpreted as byproducts of transcription from promoters contacted by enhancers. Now, it is well established that RNA transcripts produced at active enhancers are functionally important [85–87]. An example of enhancer-linked transcripts, already identified two decades ago, has been described for the locus control region (LCR) of the β-globin cluster [88, 89]. To date, numerous studies have assigned functions to enhancer-associated RNAs [90] (Fig. 2b). There are different classes of RNAs observed at enhancers. The classical enhancer RNAs (eRNAs) are derived from bidirectional transcription, are uned and rather short transcripts [85, 91]. Additionally, there are also unidirectional, often spliced and processed transcripts, which are indistinguishable from canonical IncRNAs, but happen to be transcribed from an enhancer [23, 92]. These transcripts often function in cis. Finally, there are IncRNAs with enhancer-like functions (also called ncRNA-a) [93]. Whether this classification reflects different functional classes of RNAs remains to be investigated.

In general, eRNAs are co-regulated with their neighboring gene(s) [91] and presumed to be important for gene expression, possibly by supporting cohesin binding [94]. However, experimental support for the latter is rather mild. While eRNAs in some studies were shown to stabilize chromosome loops [94, 95], they appeared to be dispensable for promoter-enhancer contact in others, raising doubts about the role of eRNAs in chromosome looping [96, 97].

LncRNAs transcribed from enhancer regions can also have inhibitory effects on their target gene. The promoter deletion of the IncRNA Haunt (also known as linc-Hoxa1), for instance, has been shown to lead to upregulation of several genes of the neighboring HoxA gene cluster. However, larger deletions in the Haunt locus impair HoxA expression [98], demonstrating that the genomic locus can have opposing functions to the RNA product encoded by it. The repressive effect of Haunt was furthermore observed at the single cell level confirming that the expression of HoxA1 and Haunt are anti-correlated [99].

A similar mechanism was found for Playrr (D030025E07Rik), a IncRNA encoded upstream of the homeodomain transcription factor Pitx2 [100]. Pitx2 expression is constrained to the left side of the gut dorsal mesentery, whereas expression of Playrr is detected on the right, but spreads to both sides in Pitx2 deletion mutants. A CRISPR/Cas9 generated mutation resulting in Playrr RNA decay caused upregulation of Pitx2 expression [100]. Both experiments reveal a mutual inhibitory interaction between Pitx2 and Playrr. An enhancer involved in Pitx2 regulation in the gut overlaps the TSS of Playrr. In the cells on the left-hand side, where Pitx2 is expressed, the Pitx2 locus was found to be in closer proximity to the Playrr locus than in cells on the right side and to cause down-regulation of Playrr by an unknown mechanism. In cells on the right, on the other hand, Playrr RNA is thought to cause a separation of the Pitx2 locus from the enhancer at the Playrr locus suggesting that Playrr expression interferes with the looping of the Pitx2 promoter to its enhancer at the Playrr locus and thus with Pitx2 gene activation (Fig. 2c).

Furthermore, the IncRNAs PRNCR1 and PCGEM1 promote enhancer-promoter looping in prostate cancer cells [101]. Other IncRNAs have been suggested to assist chromosome conformation more globally. A particular situation can be observed at the inactive X-chromosome (Xi). The 3D-structure of Xi is dependent on Xist, supposedly because Xist excludes cohesin binding from Xi [71]. Deletion of Xist led to loss of the compact chromosome structure of the Xi and reorganization into chromosome domains as they are found on the active X-chromosome. Recently, another IncRNA, functional intergenic repeating RNA element (Firre), which escapes X-chromosome inactivation was suggested to stabilize the perinucleolar location of the Xi [102]. Additionally, Firre was proposed to mediate focal trans-chromosomal contacts [103]. This finding was questioned by a different study, which reported a dispersed nuclear distribution of Firre and a role in the control of factors involved in RNA processing [104]. These examples show that IncRNAs can play important roles in controlling local intra- and interchromosomal genomic structure and interactions.

Regulation at the posttranscriptional level

LncRNAs primarily found in the cytosol are thought to be involved in gene regulation on the posttranscriptional level (Fig. 2e–h). Accordingly, several IncRNAs have been shown to influence splicing patterns of either specific genes or globally by interacting with splicing factors. The IncRNA Pnky (divergent to Pou3f1) binds to the splicing
factor PTBP1 and thereby regulates the splicing patterns of a subset of genes involved in neurogenesis [105]. Whether TUNA (megamind), which interacts with PTBP1 as well, functions via the same mechanism remains to be investigated [33, 106]. In addition, myocardial infarction associated transcript (MIAT) has been found to influence splicing in different systems. In embryonic neurogenesis, MIAT is involved in controlling the splicing pattern of Wnt7b [107]. In iPS cells and mouse primary neurons, MIAT binds to splicing factors, and in post-mortem brains of schizophrenia patients, it was found to be down-regulated, suggesting that it might contribute to this pathological phenotype [108]. MALAT1, a highly expressed lncRNA enriched in nuclear speckles, has been suggested to be a general component of the splicing machinery [109]. However, this view has been challenged since the MALAT1 knockout did not show disintegration of splicing speckles [110]. A very specific role in the cytoplasm was found for the lncRNA non-coding RNA activated by DNA damage (NORAD) [111]. NORAD sequesters PUMILIO proteins and thereby promotes genomic stability (Fig. 2f). Ablation of NORAD leads to hyperactivity of PUMILIO causing chromosome instability and aneuploidy due to repression of PUMILIO target mRNAs that function in DNA replication, mitosis and DNA damage repair.

The most recently described class of lncRNAs that came into focus are the circRNAs. Due to their circular structure, they are resistant to degradation by exonucleases and are therefore thought to be highly stable within a cell. The brain specific circRNA CDR1as was shown to act as a sponge for the miRNA let-7 [26, 27] (Fig. 2f). Accordingly, overexpression of CDR1as in zebrafish caused impaired midbrain development, similarly to the effect of a let-7 inhibitor. It was also shown that small polypeptides can be derived from some circRNAs, extending the functional repertoire of these stable RNA molecules [112]. Most likely, circRNAs do not only interact with other transcripts, but also with proteins and thus may function in a wide range of different processes.

Functions of lncRNAs in development and differentiation

In the following section, we will focus on the physiological effects of lncRNAs in the organism, during embryonic development and in in vitro differentiation systems. There are still few known examples of lncRNAs shown to play important roles in vivo, and thus we will revisit some of the lncRNAs already mentioned above.

Maternal transcripts present in oocytes and zygotes contribute to early differentiation processes. In a single-cell sequencing study examining oocytes, zygotes and cells from the first cell divisions of the early mouse embryo, lncRNA transcripts were found at all stages examined [113]. One subclass of lncRNAs, the pancRNAs, was compared between murine two-cell embryos and oocytes [66]. A subset of pancRNAs found to be expressed in two-cell embryos but not in oocytes was found to be important for counteracting DNA methylation at the adjacent promoters thereby ensuring expression of the neighboring gene.

The mechanisms first found to be linked to lncRNAs were imprinting and X-chromosome inactivation. Accordingly, the first lncRNA that was genetically targeted and deleted was H19, an imprinted gene that was already suspected to be a lncRNA 25 years ago [114]. H19 is exclusively expressed from the maternal allele and is inhibitory for the adjacent gene, Insulin-like growth factor 2 (Igf2) [114–116]. An H19 deletion on the maternally inherited chromosome led to an increase in Igf2 expression and thus to increased bodyweight. This phenotype could be rescued by deletion of one Igf2 allele. H19 knockout mice are viable and fertile [116], but have reduced muscle regeneration capacities due to loss of two miRNAs encoded within the H19 transcript [117]. The imprinted Igf2r gene is silenced by the lncRNA Airn, which is transcribed from the opposite strand, overlapping the 5′-prime region of Igf2r. Here, silencing is not achieved by the lncRNA transcripts as such, but by transcription through the Igf2r promoter, which interferes with RNA PolII recruitment [118]. Several further lncRNAs with a well-established role in imprinting have been reported. For details we would like to refer to a recent review [119].

Insight into the developmental function of Xist was obtained two decades ago when Xist knockout mice were generated and analyzed [120, 121]. Male mice lacking Xist are unaffected, whereas females die during the first half of gestation. Interestingly, knockout females with a single X-chromosome (XO) lacking Xist are healthy, strongly suggesting that it is not the lack of Xist, but the failure to adjust the X-chromosome gene dosage that causes embryonic lethality.

In a more recent example, the deletion of another imprinted lncRNA gene in the mouse, Gtl2 (Meg3, maternally expressed 3), showed clear cis-acting effects on neighboring genes resulting in perinatal lethality [122].

Many other mouse models in which lncRNAs have been removed by deletion of genomic fragments did not show overt phenotypes, but after detailed analyses, some specific defects have been observed [110, 123–126]. For instance, deletion of NEAT1 resulted in viable and fertile mice, however, knockout females showed about 50% reduced fertility due to reduced progesterone production and corpus luteum formation [127]. Ovary transplantation or progesterone administration rescued offspring rates, confirming
the cause of the phenotype, though the molecular mechanism remains to be determined.

**Fendrr** is one of few rare examples of lncRNAs demonstrated to play an essential role in organ development and embryo survival. **Fendrr**, which is divergently transcribed from the **Foxf1** promoter, was analyzed by two different strategies [47, 123]. In one study, the insertion of a transcriptional stop cassette replacing the first exon of **Fendrr** leaving most of the genomic context undisturbed resulted in impaired heart function, omphalocele (defects in body wall development), and embryonic death [47]. **Fendrr** interacts with chromatin-modifying complexes and with DNA, possibly through triplex formation, and thereby changes the chromatin landscape of specific target promoters in **trans**, in particular those of mesodermal transcription factors [46]. A BAC-transgene expressing a single dose of **Fendrr** rescued the developmental defects caused by the loss of **Fendrr**, strongly suggesting that the observed phenotype is caused by lack of **Fendrr** RNA, and not by disruption of genomic sites or promoter elements of the adjacent **Foxf1** gene. This conclusion was supported by a different knockout strategy, in which the first exon was left intact and which resulted in perinatal death [123]. In the early embryo, **Fendrr** is only transiently expressed in nascent lateral mesoderm [47]. However, later during development it is expressed in the lungs, a derivative of endoderm and lateral mesoderm, where it again might play an essential role [123, 128].

More lncRNAs involved in mesoderm and endoderm development have been reported. For instance **DEANR1** (also known as **ALIEN**) is expressed downstream of the endoderm master regulator **FOXA2** [76, 77]. **DEANR1** depletion results in reduced expression of **FOXA2** and accordingly to reduced differentiation of hESCs towards definitive endoderm. Interestingly, this defect can be rescued by restoring normal levels of **FOXA2** expression, suggesting that **DEANR1** functions mainly in **cis**. Another study described the same lncRNA, here called **ALIEN**, in cardiovascular progenitor cells in mouse and zebrafish. Depletion of **ALIEN** interfered with cardiac development, although the functional mechanism remains to be investigated further. **DEANR1** was described to be localized in the nucleus, whereas **ALIEN** was seen in the perinuclear space and in the cytoplasm, even though both were analyzed in differentiating human stem cells. The discrepancy between the two studies might be a consequence of modifications in the differentiation method [76, 77].

Also, the transcription factor **Pitx2**, which is essential for the development of many different organs, especially during the establishment of left–right asymmetry, is regulated by a lncRNA in the embryonic gut [100]. In this case, the lncRNA **Playrr** is transcribed from a distant known enhancer region. The expression patterns of **Pitx2** and **Playrr** are negatively correlated due to interference of **Playrr** with **Pitx2** activation (for details see above).

LncRNAs expressed during neuronal and brain development have attracted particular attention. As described earlier, many lncRNAs are highly specifically expressed in distinct cell types, as illustratively shown in a targeted LacZ reporter screen [37] and in expression screens [36, 107, 129, 130]. A number of lncRNAs located close to the **Pou3f** genes **Pou3f3** and **Pou3f2** (**Brn1** and **Brn2**) encoding important neuronal transcription factors, were also found to play roles in neuronal development [37, 105, 123, 131]. The lncRNA **Dali** (or **Dalir**) (DNMT1-Associated Long Intergenic RNA) is transcribed 50 kb downstream of **Pou3f3**. The depletion of **Dali** leads to a reduction of **Pou3f3** expression and to impaired neuronal differentiation [131]. Gene expression analysis upon knockdown and genome-wide localization (CHART) revealed **Pou3f3** dependent and independent targets of **Dali**. Furthermore, **Dali** physically interacts not only with various chromatin modifiers, but also with **Pou3f3** itself suggesting a multilayered association between **Dali** and **Pou3f3**. The replacement of **Pantr1** (**POU domain, class 3, transcription factor 3 adjacent noncoding transcript**), which is divergently transcribed to **Pou3f3**, by insertion of the lacZ gene did not influence **Pou3f3** transcription itself, but modulated other **Pou3f** transcription factors in **trans** [37]. **Pantr2** (or **Linc-Brn1b**), expressed convergent with **Pou3f3**, did not have this effect on **Pou3f** factors, and upon deletion led to fewer intermediate progenitors in the developing cortex. Thus, each of these three different lncRNAs, which are all transcribed near the **Pou3f3** locus, affect neuronal development yet through distinct mechanisms.

Furthermore, another lncRNA located in a region important for neural development is **Evf2** in the **Dlx5/6** locus [69]. **Evf2** was targeted by inserting a polyadenylation signal close to the promoter without deleting genomic DNA. **Evf2**−/− embryos have less GABAergic neurons than wild type controls. This mutant effect could be rescued by in utero electroporation of **Evf2** RNA, confirming that the phenotype is indeed dependent on this lncRNA.

**RMST**, also termed **non-coding rhabdomyosarcoma** (**NCRMS**) in humans, is a noncoding transcript expressed in rhabdomyosarcomas [132] and in developing dopaminergic neurons during murine development [133]. **RMST** is induced during neuronal differentiation of hESCs, and its depletion interferes with neuronal differentiation via its binding partner **SOX2** (see above) [78, 107, 129]. Interestingly, **RMST** was found associated with obesity in a GWAS study, possibly pointing to a role of **RMST** in a neuronal regulatory network influencing energy balance [134].

Two more lncRNAs involved in nervous system development are the **retinal noncoding RNA2** and 4 (**RNCR2** and...
They were first found to be expressed in the developing retina and later also assigned a functional role in this process [135]. RNCR4 is part of a network including the RNA helicase DDX3X and the miR-183/96/182 that is essential for retinal architecture [136]. RNCR2, also known as MIA or Gomafu, interferes with retinal cell differentiation when overexpressed or depleted [135, 137]. Furthermore MIA is expressed earlier in the developing brain, where it localizes to the nuclear matrix [138]. Purifying distinct subpopulations of the developing brain at E14.5 revealed that MIA is highly abundant in differentiating progenitors, where it is involved in corticogenesis by regulating the splicing pattern of Wnt7b [107] (Fig. 2g).

Another ectodermal derivative, the developing epidermis, has also been shown to be regulated by lncRNAs. During epidermal differentiation and maintenance, the nuclear lncRNA ANCR (anti-differentiation ncRNA) is essential for maintaining the proliferating progenitor pool by repressing differentiation factors, amongst them the transcription factors MAF and MAFB, probably in a PRC2 linked manner [139–141]. Depletion of ANCR leads to differentiation and loss of progenitor cells. Its differentiation promoting counterpart is a cytosolic lncRNA, terminal differentiation induced ncRNA (TINCR) that interacts with STAU1 and as a protein-RNA complex stabilizes mRNAs of pro-differentiation genes including MAF and MAFB [141, 142] (Fig. 2h). Ectopic expression of MAF:MAFB in TINCR depleted cells restored the expression of the differentiation gene signature showing that TINCR is crucial for ensuring appropriate protein levels in particular of MAF and MAFB.

Functions of lncRNAs in pathological settings

LncRNAs as a “new” class of molecules have also attracted attention in pathological settings. Here, we will highlight two very prominent areas, cancer and cardiovascular disease.

Cancer

Approximately one third of publications currently listed in PubMed linked with the keyword “lncRNA” also contain the keyword “cancer”. Even before the era of next generation sequencing, a few studies found lncRNAs to be dysregulated in tumors [35, 143, 144]. To date, numerous primary tumor samples, metastatic biopsies, the adjacent normal tissue and cancer cell lines have been profiled for their RNA expression and the results recapitulate the high degree of specificity found for noncoding RNAs in normal tissue compared to protein-coding genes [25, 67, 145–147]. Clustering of lncRNA expression patterns identifies subclasses of tumor types with high significance, opening the possibility to define tumor subtypes with a high chance to respond to targeted therapy. A study that considered samples from more than 5000 tumors from the cancer genome atlas (TCGA) project, demonstrated that, while approximately the same number of protein coding and lncRNA genes were dysregulated, 60% of lncRNAs showed specificity for only one tumor type [146].

In addition, somatic copy number alterations (SCNA) found in cancer tissue often include lncRNA loci [57, 146, 147]. For example, the lncRNA FAL1, a lncRNA that was found amplified in a number of different tumor types, was shown to be important for the tumorigenic effects of different cell lines via a BMI1-mediated mechanism (see above), and its expression level and copy number significantly correlated with patient survival [57]. Furthermore, several lncRNAs have been shown to be prognostic for overall or disease-free survival of patients [67, 82, 148–150], information that is integrated into therapeutic decisions. Depletion of selected lncRNAs led to reduced malignancy, assayed as tumor size or metastatic potential in xenograft experiments [67, 72, 82, 101, 148, 149, 151–154], although these experiments were not able to distinguish between effects on tumor formation versus tumor progression. Further refinement was achieved when RNA knockdown was induced after tumor formation, mimicking therapeutic conditions. Reduced tumor growth was, for instance, observed upon FAL1 depletion [57]. In a different study, the inhibition of MALAT1 after tumor induction reduced metastasis formation in a xenograft lung cancer model and in a metastasizing breast cancer model [149, 155]. MALAT1 knockout mice do not show overt phenotypes [110, 156, 157] suggesting that down-regulation of MALAT1 in humans might also not be harmful for normal human cells, which makes it an attractive target for systemic application in patients.

A special case is the noncoding pseudo gene BRAFP1, which has been shown to be either mutated or aberrantly expressed in cancers, like its coding homologue BRAF. A mouse model revealed that induced overexpression of Braf-rs1 (the murine homologue of BRAFP1) leads to DLBCL (diffuse large B cell lymphomas)-like tumors. However, when the transgene was first induced to form tumors and then shut down again, the tumors regressed demonstrating the functional significance of the pseudo gene [158]. Braf-rs1 was shown to cause upregulation of Braf by acting as a ceRNA (competing endogenous RNA) and by “sponging” miRNAs that would otherwise target Braf transcripts.

One mechanism that cells can employ to prevent tumor formation is oncogene-induced senescence (OIS) [159]. The INK4B-ARF-INK4A locus, which plays a major role in OIS, is silenced in proliferating cells by Polycomb group proteins. This mechanism is dependent on the lncRNA
ANRIL, which is transcribed from within the locus and acts in cis [58, 59, 160]. Furthermore, the MIR31HG gene located 400 kb upstream of the INK4B-ARF-INK4A locus, also encodes a lncRNA involved in the regulation of INK4A [161]. A third lncRNA functioning in OIS, UCA1, stabilizes mRNAs of protein-coding genes involved in the senescence pathway, probably by sequestering hnRNP A1 [162]. Also, the tumor suppressor p53 and its associated signaling pathways include lncRNAs. p53 binds and regulates numerous lncRNAs, such as the linc-p21, which in turn regulates gene expression in concert with p53 and p21 [163–166].

Cardiovascular diseases

LncRNAs have also been linked to cardiovascular diseases (CVD), which are a major cause of death in western societies, and have been studied both in patient data, as well as in mouse models. It was observed that several genomic regions significantly associated with myocardial infarction encode lncRNAs, for example MIAT and ANRIL [167, 168]. Deletion of a 70 kb region including a large proportion of the ANRIL locus led to increased mortality in mice, particularly in combination with a high fat diet [169].

In other studies, massive parallel RNA sequencing revealed numerous lncRNAs displaying altered expression following surgically-induced myocardial infarction in mice [170, 171]. In cellular differentiation models, the depletion of selected lncRNAs impaired cardiomyocyte formation making them promising candidates for in vivo assessment of their functions [172]. Analysis of the noncoding transcripts originating from the myosin heavy chain 7 (Myh7) locus, an important structural protein required for heart contractions, revealed that one, the lncRNA Mhrt, was down-regulated after induced myocardial infarction. Ectopic expression of Mhrt in mice, which had been subjected to this operation reduced the severity of symptoms observed in control mice during recovery [68]. When lncRNAs in plasma from myocardial infarction patients were profiled, a mitochondrial lncRNA named long intergenic noncoding RNA predicting cardiac remodeling (LIPCAR) was found to significantly correlate with mortality caused by CVD and could thus be considered as a biomarker [173]. Whether this lncRNA also plays a causative role in infarction or heart failure remains to be investigated.

LncRNAs have been widely suggested as biomarkers and therapeutic targets in certain pathological settings. One reason for this is the extremely specific expression of many lncRNAs, opening the potential of targeting cellular subgroups rather than affecting patients systemically as in conventional treatments. Even if a lncRNA itself is not the disease driver, it can serve as drug target in cases where it acts as co-factor for a driver gene, which is more broadly expressed. Also, since lncRNAs often play quantitative roles rather than acting as switches in genetic programs, side effects of therapeutic treatments might be easier to control.

Concluding remarks

In recent years, the analysis of lncRNAs has highlighted the large diversity of cellular functions involving RNA molecules and has greatly expanded the catalog of functions previously attributed to noncoding RNAs. By now, we can conclude that noncoding RNAs play roles at all levels of gene control, including epigenetic mechanisms and nuclear organization, as well as in RNA processing, stability and translation. The recent discoveries of lncRNA functions provide good reasons for the notion that further investigation of lncRNAs bears high chances for the discovery of novel gene regulatory mechanisms in the future.

So far, only few lncRNAs playing an essential role in embryonic development, organogenesis or tissue homeostasis have been reported. Studies based on in vitro differentiation systems can provide important insights into the molecular modes of lncRNA function in local gene control or in regulatory networks. However, with regard to the roles of lncRNAs, for example in lineage decisions and cellular differentiation many findings are hampered by the lack of definitive proof in vivo. In particular, data derived from in vitro differentiated stem cells, whose genomic integrity and quality is hard to control, have to be interpreted cautiously. Also, the type of genetic change introduced into a lncRNA gene/locus for functional analysis (transcription stop, knockin, deletion etc.) needs to be considered carefully in order to draw the right conclusions [174–176]. Since lncRNAs do not only act as transcripts in cis or trans, but can also be side products of transcription affecting the expression of overlapping genes or accommodate regulatory DNA elements in their genomic loci, the analysis of their exact mode of action and functional roles in development and disease processes is more complex and difficult than that of protein-coding genes. It is now up to the responsibility of journal editors and reviewers to enforce the high standards of investigation this new and exciting field of research deserves.

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References

1. Okazaki Y, Furuno M, Kasukawa T et al (2002) Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. Nature 420:563–573. doi:10.1038/nature01266
2. Djebali S, Davis CA, Merkel A et al (2012) Landscape of transcription in human cells. Nature 489:101–108. doi:10.1038/nature11233
3. Derrien T, Johnson R, Bussotti G et al (2012) The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 22:1775–1789. doi:10.1101/gr.132159.111
4. Cabili MN, Trapnell C, Goff L et al (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 25:1915–1927. doi:10.1101/gad.174466.111
5. Guo X, Gao L, Wang Y et al (2015) Advances in long noncoding RNAs: identification, structure prediction and function annotation. Brief Funct Genomics. doi:10.1093/bfgp/elv022
6. Harrow J, Frankish A, Gonzalez JM et al (2012) GENCODE v7: the reference human genome annotation for The ENCODE Project. Genome Res 22:1760–1774. doi:10.1101/gr.135350.111
7. Ingolia NT, Lareau LF, Weissman JS (2011) Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. Cell 147:789–802. doi:10.1016/j.cell.2011.10.002
8. Ruiz-Orera J, Messeguer X, Subirana JA, Alba MM (2014) Long non-coding RNAs as a source of new peptides. elife 3:e03523. doi:10.7554/eLife.03523
9. Guttmann M, Russell P, Ingolia NT et al (2013) Ribosome profiling provides evidence that large noncoding RNAs do not encode proteins. Cell 154:240–251. doi:10.1016/j.cell.2013.06.009
10. Bănfai B, Jia H, Khatun J et al (2012) Long noncoding RNAs are rarely translated in two human cell lines. Genome Res 22:1646–1657. doi:10.1101/gr.134767.111
11. Bazzini AA, Johnstone TG, Christiano R et al (2014) Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation. EMBO J. doi:10.1002/embj.201488411
12. Frith MC, Forrest AR, Nourbakhsh E et al (2006) The abundance of short proteins in the mammalian proteome. PLoS Genet 2:e52. doi:10.1371/journal.pgen.0020052
13. Anderson DM, Anderson KM, Chang C-L et al (2015) A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. Cell 160:595–606. doi:10.1016/j.cell.2015.01.009
14. Pauli A, Norris ML, Valen E et al (2014) Toddler: an embryonic signal that promotes cell movement via Apelin receptors. Science 343:1248636. doi:10.1126/science.1248636
15. Yan Y, Cooper C, Hamedani MK et al (2015) The steroid receptor RNA activator protein (SRAP) controls cancer cell migration/motility. FEBS Lett 589:4010–4018. doi:10.1016/j.fedslet.2015.11.007
16. Rupaimoole R, Lee J, Haemmerle M et al (2015) Long noncoding RNA ceruloplasmin promotes cancer growth by altering glycolysis. Cell Rep 13:2395–2402. doi:10.1016/j.celrep.2015.11.047
17. Francis J, Hennelly SP, Tung C-S, Sanbonmatsu KY (2013) Rise of the RNA machines: exploring the structure of long noncoding RNAs. J Mol Biol 425:3731–3746. doi:10.1016/j.jmb.2013.02.030
18. Weeks KM (2015) Review toward all RNA structures, consisely. Biopolymers 103:438–448. doi:10.1002/bip.22601
19. Smola MJ, Rice GM, Busan S et al (2015) Selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) for direct, versatile and accurate RNA structure analysis. Nat Protoc 10:1643–1669. doi:10.1038/nprot.2015.103
20. Spitale RC. Flynn RA, Zhang QC et al (2015) Structural imprints in vivo decode RNA regulatory mechanisms. Nature. doi:10.1038/nature14263
21. Guttmann M, Amit I, Garber M et al (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458:223–227. doi:10.1038/nature07672
22. Sun M, Gadad SS, Kim D-S, Kraus WL (2015) Discovery, annotation, and functional analysis of long noncoding RNAs controlling Cell-cycle gene expression and proliferation in breast cancer cells. Mol Cell. doi:10.1016/j.molcel.2015.06.023
23. Marques AC, Hughes J, Graham B et al (2013) Chromatin signatures at transcriptional start sites separate two equally populated yet distinct classes of intergenic long noncoding RNAs. Genome Biol 14:R131. doi:10.1186/gb-2013-14-11-r131
24. ENCODE Project Consortium (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489:57–74. doi:10.1038/nature11247
25. Iyer MK, Niknafs YS, Malik R et al (2015) The landscape of long noncoding RNAs in the human transcriptome. Nat Genet 47:199–208. doi:10.1038/ng.3192
26. Hansen TB, Jensen TI, Clausen BH et al (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495:384–388. doi:10.1038/nature11993
27. Memczak S, Jens M, Eleftheriou A et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333–338. doi:10.1038/nature11928
28. Werner MS, Ratunen BJ (2015) Nuclear fractionation reveals thousands of chromatin-tethered noncoding RNAs adjacent to active genes. Cell Rep 12:1089–1098. doi:10.1016/j. celrep.2015.07.033
29. Herman RC, Williams JG, Pennm S (1976) Message and non-message sequences adjacent to poly(A) in steady state heterogeneous nuclear RNA of HeLa cells. Cell 7:429–437
30. Yue F, Cheng Y, Breschi A et al (2014) A comparative encyclopedia of DNA elements in the mouse genome. Nature 515:355–364. doi:10.1038/nature13992
31. Haerty W, Ponting CP (2015) Unexpected selection to retain high GC content and splicing enhancers within exons of multiexicnt IncRNA loci. RNA 21:333–346. doi:10.1261/rna.047324.114
32. Nitsche A, Rose D, Fasold M et al (2015) Comparison of splice sites reveals that long noncoding RNAs are evolutionarily well conserved. RNA 21:801–812. doi:10.1261/rna.046342.114
33. Ulitsky I, Shkumatava A, Jan CH et al (2011) Conserved. RNA 17:801–812. doi:10.1101/gad.174466.111
34. Necsulea A, Soumillon M, Warnefors M et al (2014) The evolution of IncRNA repertoire and expression patterns in tetrapods. Nature 505:635–640. doi:10.1038/nature12943
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35. Diederichs S (2014) The four dimensions of noncoding RNA conservation. Trends Genet 30:121–123. doi:10.1016/j.tig.2014.01.004
36. Mercer TR, Dinger ME, Sun SS et al (2008) Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci USA 105:716–721. doi:10.1073/pnas.0706729105
37. Goff LA, Groff AF, Sauvageau M et al (2015) Spatiotemporal expression and transcriptional perturbations by long noncoding RNAs in the mouse brain. Proc Natl Acad Sci USA 112:6855–6862. doi:10.1073/pnas.1411263112
38. Grote P, Herrmann BG (2013) The long non-coding RNA Gepac and embryonic stem cell pluripotency. elife 3:e02046. doi: 10.7554/eLife.02046
39. Li L, Liu B, Wapinski OL et al (2013) Role of histone H3 lysine 27 methylation in X inactivation. Science 300:131–135. doi:10.1126/science.1084274
40. da Rocha ST, Boeva V, Escamilla-Del-Arenal M et al (2014) Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 152:1298–1307. doi:10.1016/j.cell.2013.09.003
41. Zhao J, Sun BK, Erwin JA et al (2008) Polycomb proteins target a short repeat RNA to the mouse X chromosome. Science 322:750–756. doi:10.1126/science.1163045
42. Rinn JL, Kertesz M, Wang JK et al (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 129:1311–1323. doi:10.1016/j.cell.2007.05.022
43. Li L, Liu B, Wapinski OL et al (2013) Targeted disruption of Hotair leads to homeotic transformation and gene derepression. Cell Rep 5:3–12. doi:10.1016/j.celrep.2013.09.003
44. Schorderet P, Duboule D (2011) Structural and functional differences in the long non-coding RNA hotair in mouse and human. PLoS Genet 7:e1002071. doi:10.1371/journal.pgen.1002071
45. Klintenhoff CA, Scheuermann JC, Surface LE et al (2013) Jarid2 is implicated in the initial Xist-induced targeting of PRC2 to the inactive X chromosome. Mol Cell 53:301–316. doi:10.1016/j.molcel.2014.01.002
46. Grote P, Herrmann BG (2013) The long non-coding RNA Fendrr links epigenetic control mechanisms to gene regulatory lineage. Trends Genet 30:121–123. doi:10.1016/j.tig.2013.01.003
47. Grote P, Herrmann BG (2015) Long noncoding RNAs in organogenesis: making the difference. Trends Genet 31:329–335. doi:10.1016/j.tig.2015.02.002
48. Marı´n-Be´jar O, Marchese FP, Athie A et al (2013) Pint lincRNA action of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. Genes Dev 24:2264–2269. doi:10.1101/gad.159090.109
49. Brockdorff N (2013) Noncoding RNA and Polycomb recruitment of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. Cancer Res 67:3963–3969. doi:10.1158/0008-5472.CAN-06-2004
50. Prensner JR, Iyer MK, Sahu A et al (2013) The long noncoding RNA TARID directs demethylation and activation of the tumor suppressor TCF21 via GADD45A. Mol Cell. doi: 10.1016/j.molcel.2014.06.031
51. Guttman M, Donaghey J, Carey BW et al (2011) lincRNAs act in the circuity controlling pluripotency and differentiation. Nature 477:295–300. doi:10.1038/nature10398
52. Zhao J, Ohsumi TK, Kung JT et al (2010) Genome-wide identification of polycoding-associated RNAs by RIP-seq. Mol Cell 40:939–953. doi:10.1016/j.molcel.2010.12.011
53. Schmitz K-M, Mayer C, Postepska A, Grummt I (2010) Interaction of noncoding RNA with the rDNA promoter mediates
remodeling inhibition. Development 142:2641–2652. doi:10.1242/dev.126318
71. Minajigi A, Froberg JE, Wei C et al (2015) A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation. Science 349:aab2276. doi:10.1126/science.aab2276
72. Wang Y, He L, Du Y et al (2015) The long noncoding RNA IncTCF7 promotes self-renewal of human liver cancer stem cells through activation of Wnt signaling. Cell Stem Cell 16:413–425. doi:10.1016/j.stem.2015.03.003
73. Kaneko S, Bonasio R, Saldana-Meyer R et al (2013) Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin. Mol Cell. doi:10.1016/j.molcel.2013.11.012
74. Merry CR, Forrest ME, Sabers JN et al (2015) DNMT1-associated long non-coding RNAs regulate global gene expression and DNA methylation in colon cancer. Hum Mol Genet. doi:10.1093/hmg/ddv343
75. Davidovich C, Wang X, Cifuentes-Rojas C et al (2015) Toward a Consensus on the Binding Specificity and Promiscuity of PRC2 for RNA. Mol Cell. doi:10.1016/j.molcel.2014.12.017
76. Jiang W, Liu Y, Liu R et al (2015) The IncRNA DEANR1 facilitates human endoderm differentiation by activating FOXA2 expression. Cell Rep 11:137–148. doi:10.1016/j.celrep.2015.03.008
77. Kurian L, Aguirre A, Sancho-Martinez I et al (2015) Identification of novel long noncoding RNAs underlying vertebrate cardiovascular development. Circulation 131:1278–1290. doi:10.1161/CIRCULATIONAHA.114.013303
78. Ng S Y, Bogu GK, Soh BS, Stanton LW (2013) The long noncoding RNA RMST interacts with SOX2 to regulate neurogenesis. Mol Cell 51:349–359. doi:10.1016/j.molcel.2013.07.017
79. Rapicavoli NA, Qu K, Zhang J et al (2013) A mammalian pseudogene IncRNA at the interface of inflammation and anti-inflammatory therapeutics. elife 2:e00762. doi:10.7554/eLife.00762
80. Krawczyk M, Emerson BM (2014) p50-associated COX-2 extragenic RNA (PACER) activates COX-2 gene expression by occluding repressive NF-kB complexes. elife 3:e01776
81. Wang P, Xue Y, Han Y et al (2014) The STAT3-binding long noncoding RNA Inc-DC controls human dendritic cell differentiation. Science 344:310–313. doi:10.1126/science.1251456
82. Xing Z, Lin A, Li C et al (2014) IncRNA directs cooperative epigenetic regulation downstream of chemokine signals. Cell 159:1110–1125. doi:10.1016/j.cell.2014.10.013
83. Kung JT, Kesner B, An JY et al (2015) Locus-specific targeting to the X chromosome revealed by the RNA interactome of CTCF. Mol Cell. doi:10.1016/j.molcel.2014.12.006
84. Sun S, Del Rosario BC, Szanto A et al (2013) Jpx RNA activates Xist by evicting CTCF. Cell 153:1537–1551. doi:10.1016/j.cell.2013.05.028
85. Kim T-K, Hemberg M, Gray JM et al (2010) Widespread transcription at neuronal activity-regulated enhancers. Nature 465:182–187. doi:10.1038/nature09033
86. Koch F, Fenuil R, Gut M et al (2011) Transcription initiation platforms and GTF recruitment at tissue-specific enhancers and promoters. Nat Struct Mol Biol 18:956–963. doi:10.1038/nsmb.2085
87. Natoli G, Andrau J-C (2012) Noncoding transcription at enhancers: general principles and functional models. Annu Rev Genet 46:1–19. doi:10.1146/annurev-genet-110711-155459
88. Collis P, Antoniou M, Groversel F (1990) Definition of the minimal requirements within the human beta-globin gene and the dominant control region for high level expression. EMBO J 9:233–240
89. Ashe HL, Monks J, Wijgerde M et al (1997) Intergenic transcription and transinduction of the human beta-globin locus. Genes Dev 11:2494–2509
90. Orom UA, Shiekhattar R (2011) Long non-coding RNAs and enhancers. Curr Opin Genet Dev 21:194–198. doi:10.1016/j.gde.2011.01.020
91. Andersson R, Gebhard C, Miguel-Escalada I et al (2014) An atlas of active enhancers across human cell types and tissues. Nature 507:455–461. doi:10.1038/nature12787
92. Ounzain S, Pezuto I, Micheletti R et al (2014) Functional importance of cardiac enhancer-associated noncoding RNAs in heart development and disease. J Mol Cell Cardiol 76:55–70. doi:10.1016/j.yjmcc.2014.08.009
93. Orom UA, Derrien T, Beringer M et al (2015) Long noncoding RNAs with enhancer-like function in human cells. Cell 163:46–58. doi:10.1016/j.cell.2015.09.001
94. Li W, Notani D, Ma Q et al (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. Nature 498:516–520. doi:10.1038/nature12210
95. Lai F, Orom UA, Cesaroni M et al (2013) Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. Nature 494:497–501. doi:10.1038/nature11884
96. Schaukowitch K, Joo J-Y, Liu X et al (2014) Enhancer RNA facilitates NELF release from immediate early genes. Mol Cell 56:29–42. doi:10.1016/j.molcel.2014.08.023
97. Mousavi K, Zare H, Dell’orso S et al (2013) eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. Mol Cell 51:606–617. doi:10.1016/j.molcel.2013.07.022
98. Yin Y, Yan P, Lu J et al (2015) Opposing roles for the IncRNA haunt and its genomic locus in regulating HOXA Gene activation during embryonic stem cell differentiation. Cell Stem Cell 16:504–516. doi:10.1016/j.stem.2015.03.007
99. Maamar H, Cabili MN, Rinn J, Raj A (2013) linc-HOXA1 is a noncoding RNA that represses Hoxa1 transcription in cis. Genes Dev 27:1260–1271. doi:10.1101/gad.217018.113
100. Welsh IC, Kwak H, Chen FL et al (2015) Chromatin architecture of the Pitx2 locus requires CTCF- and Pitx2-dependent asymmetry that mirrors embryonic gut laterality. Cell Rep. doi:10.1016/j.celrep.2015.08.075
101. Yang L, Lin C, Jin C et al (2013) IncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. Nature 500:598–602. doi:10.1038/nature12451
102. Yang F, Deng X, Ma W et al (2015) The IncRNA Firre anchors the inactive X chromosome to the nucleolus by binding CTCF and maintains H3K27me3 methylation. Genome Biol 16:52. doi:10.1186/s13059-015-0618-0
103. Hacisuleyman E, Goff LA, Trapnell C et al (2014) Topological organization of multichromosomal regions by the long intergenic noncoding RNA Firre. Nat Struct Mol Biol 21:198–206. doi:10.1038/nsmb.2764
104. Bergmann JH, Li J, Eckersley-Maslin MA et al (2015) Regulation of the ESC transcriptome by nuclear long non-coding RNAs. Genome Res 25:1336–1346. doi:10.1101/gr.189027.114
105. Ramos AD, Andersen RE, Liu SJ et al (2015) The long non-coding RNA Pnky regulates neuronal differentiation of embryonic and postnatal neural stem cells. Cell Stem Cell 16:439–447. doi:10.1016/j.stem.2015.02.007
106. Lin N, Chang K-Y, Li Z et al (2014) An evolutionarily conserved long noncoding RNA TUNA controls pluripotency and neural lineage commitment. Mol Cell 53:1005–1019. doi:10.1016/j.molcel.2014.01.021
107. Aprea J, Prenninger S, Dori M et al (2013) Transcripome sequencing during mouse brain development identifies long non-coding RNAs functionally involved in neurogenic commitment. EMBO J 32:3145–3160. doi:10.1038/emboj.2013.245
Mechanisms of long noncoding RNA function in development and disease

108. Barry G, Briggs JA, Vanichkina DP et al (2014) The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing. Mol Psychiatry 19:486–494. doi:10.1038/mp.2013.45

109. Tripathi V, Ellis JD, Shen Z et al (2010) The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol Cell 39:925–938. doi:10.1016/j.molcel.2010.08.011

110. Eißmann M, Gutschner T, Hämmerle M et al (2012) Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development. RNA Biol 9:1076–1087. doi:10.4161/rna.21089

111. Lee S, Kopp F, Chang T-C et al (2015) Noncoding RNA NORAD regulates genomic stability by sequestering PUMIIL1 proteins. Cell. doi:10.1016/j.cell.2015.12.017

112. Chen CY, Sarnow P (1995) Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs. Science 268:415–417. doi:10.1126/science.7536344

113. Yan L, Yang M, Guo H et al (2013) Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol 20:1131–1139. doi:10.1038/nsmb.2660

114. Brannan CI, Dees EC, Ingram RS, Tilghman SM (1990) The product of the H19 gene may function as an RNA. Mol Cell Biol 10:28–36

115. Bartolomei MS, Zemel S, Tilghman SM (1991) Parental imprinting of the mouse H19 gene. Nature 351:153–155. doi:10.1038/351153a0

116. Leighton PA, Ingram RS, Egenschwiler J et al (1995) Disruption of imprinting caused by deletion of the H19 gene region in mice. Nature 375:34–39. doi:10.1038/375034a0

117. Dey BK, Pfeifer K, Dutta A (2014) The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev 28:491–501. doi:10.1101/gad.234419.113

118. Latos PA, Pauler FM, Koerner MV et al (2012) Airm transcriptional overlap, but not its lncRNA products, induces imprinted Igf2r silencing. Science 338:1469–1472. doi:10.1126/science.1228110

119. Kanduri C (2016) Long noncoding RNAs: lessons from genomic imprinting. Biochim Biophys Acta 1859:102–111. doi:10.1016/j.bbamcr.2015.08.006

120. Penny GD, Kay GP, Sheardown SA et al (1996) Requirement for Xist in X chromosome inactivation. Nature 379:131–137. doi:10.1038/379131a0

121. Marahrens Y, Panning B, Dausman J et al (1997) Xist-deficient mice are defective in dosage compensation but not spermato- genesis. Genes Dev 11:156–166

122. Zhou Y, Cheusunchon P, Nakayama Y et al (2010) Activation of paternally expressed genes and perinatal death caused by deletion of the Gtl2 gene. Development 137:2643–2652. doi:10.1242/dev.045724

123. Sauvageau M, Goff LA, Lodato S et al (2013) Multiple knockout mouse models reveal lncRNAs are required for life and brain development. elife 2:e01749. doi:10.7554/elife.01749

124. Nakagawa S, Nagamura T, Shioi G, Hirose T (2011) Paraspeckles are subjacent-specification nuclear bodies that are not essential in mice. J Cell Biol 193:31–39. doi:10.1083/jcb.201011110

125. Olivier PL, Chodroff RA, Gosal A et al (2015) Disruption of Viscl-2, a brain-expressed conserved long noncoding RNA, does not elicit an overt anatomical or behavioral phenotype. Cereb Cortex 25:3572–3585. doi:10.1093/cercor/bhu196

126. Li L, Chang HY (2014) Physiological roles of long noncoding RNAs: insight from knockout mice. Trends Cell Biol. doi:10.1016/j.tcb.2014.06.003

127. Nakagawa S, Shimada M, Yanaka K et al (2014) The IncRNA Neat1 is required for corpus luteum formation and the establishment of pregnancy in a subpopulation of mice. Development. doi:10.1242/dev.110544

128. Herriges MJ, Swarr DT, Morley MP et al (2014) Long noncoding RNAs are spatially correlated with transcription factors and regulate lung development. Genes Dev 28:1363–1379. doi:10.1101/gad.238782.114

129. Ng S-Y, Johnson R, Stanton LW (2012) Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. EMBO J 31:522–533. doi:10.1038/emboj.2011.450

130. Ramos AD, Diaz A, Nelllore A et al (2013) Integration of genome-wide approaches identifies IncRNAs of adult neural stem cells and their progeny in vivo. Cell Stem Cell 12:616–628. doi:10.1016/j.stem.2013.03.003

131. Chalei V, Sanson SN, Kong L et al (2014) The long non-coding RNA Dali is an epigenetic regulator of neural differentiation. elife 3:e04530. doi:10.7554/elife.04530

132. Wang W et al (2013) Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and H3K27me3. EMBO J 31:522–533. doi:10.1038/emboj.2011.459

133. Krol J, Krol I, Alvarez CPP et al (2015) A network comprising IncRNAs and TFs is required for development. EMBO Rep 16:1448–1457. doi:10.15252/embr.201540313

134. Ji P, Diederichs S, Wang W et al (2003) MALAT-1, a novel abundant nuclear non-coding RNA, is associated with chromatin modifiers and transcription factors. EMBO J 22:8031–8041. doi:10.1038/sj.onc.1206928

135. Kretz M, Webster DE, Fleckhart RJ et al (2012) Suppression of progenitor differentiation requires the long non-coding RNA ANCR. Genes Dev 26:338–343. doi:10.1101/gad.182121.111

136. Rapicavoli NA, Poth EM, Blackshaw S (2010) The long non-coding RNA RNR2 directs mouse retinal cell specification. BMC Dev Biol 10:49. doi:10.1186/1471-213X-10-49

137. Sone M, Hayashi T, Tarui H et al (2007) The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. J Cell Sci 120:249–250. doi:10.1242/jcs.009357

138. Kretz M, Webster DE, Fleckhart RJ et al (2012) Suppression of progenitor differentiation requires the long non-coding RNA ANCR. Genes Dev 26:338–343. doi:10.1101/gad.182121.111

139. Zhu L, Xu P-C (2013) Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. Biochem Biophys Res Commun 432:612–617. doi:10.1016/j.bbrc.2013.02.036

140. Lopez-Pajares V, Qu K, Zhang J et al (2015) A LncRNA-MAF:MAFB transcription factor network regulates epidermal differentiation. Dev Cell 32:693–706. doi:10.1016/j.devcel.2015.01.028

141. Kretz M, Siprashvili Z, Chu C et al (2013) Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature 493:231–235. doi:10.1038/nature11661

142. Ji P, Diederichs S, Wang W et al (2003) MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 22:8031–8041. doi:10.1038/sj.onc.1206928
Srikantan V, Zou Z, Petrovics G et al (2000) PCGEM1, a prostate-specific gene, is overexpressed in prostate cancer. Proc Natl Acad Sci USA 97:12216–12221. doi:10.1073/pnas.97.22.12216

Brunner AL, Beck AH, Edris B et al (2012) Transcriptional profiling of long non-coding RNAs and novel transcribed regions across a diverse panel of archived human cancers. Genome Biol 13:R75. doi:10.1186/gb-2012-13-8-475

Yan X, Hu Z, Feng Y et al (2015) Comprehensive genomic characterization of long non-coding RNAs across human cancers. Cancer Cell 28:529–540. doi:10.1016/j.ccell.2015.09.006

Du Z, Fei T, Verhaak RGW et al (2013) Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. Nat Struct Mol Biol 20:908–913. doi:10.1038/nsmb.2591

Pandey GK, Mitra S, Subhash S et al (2014) The risk-associated long noncoding RNA NBAT-1 controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation. Cancer Cell 26:722–737. doi:10.1016/j.ccell.2014.09.014

Gutschner T, Hämmerle M, Eißmann M et al (2013) The non-coding RNA HOTAIR reprograms chromatin state to promote cancer progression by regulating cell proliferation and neuronal differentiation. Cancer Cell 26:722–737. doi:10.1016/j.ccell.2014.09.014

Gupta RA, Shah N, Wang KC et al (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464:1071–1076. doi:10.1038/nature08975

Xue S, Li Q-W, Che J-P et al (2015) Decreased expression of long non-coding RNA NBAT-1 is associated with poor prognosis in patients with clear cell renal cell carcinoma. Int J Clin Exp Pathol 8:3765–3774

Trimarchi T, Bilul E, Ntziachristos P et al (2014) Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia. Cell 158:593–606. doi:10.1016/j.cell.2014.05.049

Gupta RA, Shah N, Wang KC et al (2010) Long non-coding RNA HOTAIIR reprograms chromatin state to promote cancer metastasis. Cancer Cell 27:370–381. doi:10.1016/j.ccell.2010.02.004

Dijkstra JM, Alexander DB (2015) The ‘‘NF-kB interacting long noncoding RNA blocks I kappa B inter-acting long noncoding RNA blocks I kappa B interaction’’ cell 20.14.05.049

Dimitrova N, Zamudio JR, Jong RM et al (2014) LincRNA-p21 regulates p21 in cis to promote Polycomb target gene expression and to enforce the G1/S checkpoint. Mol Cell 54:777–790. doi:10.1016/j.molcel.2014.04.025

Wu G, Cui J, Han Y et al (2014) LincRNA-p21 regulates neoinitima formation, vascular smooth muscle cell proliferation, apoptosis, and atherosclerosis by enhancing p53 activity. Circulation 130:1452–1465. doi:10.1161/CIRCULATIONA-HA.114.011675

Ishii N, Ozaki K, Sato H et al (2006) Identification of a novel non-coding RNA, MIAT, that confers risk of myocardial infarction. J Hum Genet 51:1087–1099. doi:10.1007/s10038-006-0070-9

Broadbent HM, Peden JP, Lorkowski S et al (2008) Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet 17:806–814. doi:10.1093/hmg/ddm352

Visel A, Zhu Y, May D et al (2010) Targeted deletion of the 9p21 non-coding coronary artery disease risk interval in mice. Nature 464:409–412. doi:10.1038/nature08801

Matkovich SJ, Edwards JR, Grossenbider TC et al (2014) Epigenetic coordination of embryonic heart transcription by dynamically regulated long noncoding RNAs. Proc Natl Acad Sci USA 111:12264–12269. doi:10.1073/pnas.1401022111

Ounziain S, Micheletti R, Beckmann T et al (2015) Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. Eur Heart J 36:353–368a. doi:10.1093/eurheartj/ehu180

Ounziain S, Micheletti R, Arnan C et al (2015) CARMEN, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. J Mol Cell Cardiol. doi:10.1016/j.yjmcc.2015.09.016

Kumarswamy R, Bauters C, Volkman I et al (2014) Circulating long non-coding RNA, LIPCAR, predicts survival in patients with heart failure. Circ Res 114:1569–1575. doi:10.1161/CIRCRESAHA.114.303915

Bassett AR, Akhtar A, Barlow DP et al (2014) Considerations when investigating lncRNA function in vivo. elife 3:e03058

Janowski BA, Huffman KE, Schwartz JC et al (2005) Inhibiting gene expression at transcription start sites in chromosomal DNA with antigenic RNAs. Nat Chem Biol. doi:10.1016/j.chembiol.2008-04-007

Goff LA, Rinn JL (2015) Linking RNA biology to lncRNAs. Genome Res 25:1456–1465. doi:10.1101/gr.191122.115

Stadtfeld M, Apostolou E, Ferrari F, et al. (2012) Ascorbic acid prevents loss of Dlk1-Dio3 imprinting and facilitates generation of all-IPS cell mice from terminally differentiated B cells. Nat Genet 44:398–405, S1–S2. doi:10.1038/ng.1110

Zhang X, Lian Z, Padden C et al (2009) A myelopoiesis-associated regulatory intergenic noncoding RNA transcript within the human HOXA cluster. Blood 113:2526–2534. doi:10.1182/blood-2008-06-162164

Delpratti S, Montavon T, Leleu M et al (2013) Multiple enhancers regulate Hoxd genes and the Hotdog LncRNA during cecum budding. Cell Rep. doi:10.1016/j.celrep.2013.09.002
180. Gong C, Li Z, Ramanujan K et al (2015) A long non-coding RNA, LncMyoD, regulates skeletal muscle differentiation by blocking IMP2-mediated mRNA translation. Dev Cell 34:181–191. doi:10.1016/j.devcel.2015.05.009

181. Prensner JR, Sahu A, Iyer MK et al (2014) The IncRNAs PCGEM1 and PRNCR1 are not implicated in castration resistant prostate cancer. Oncotarget 5:1434–1438

182. McHugh CA, Chen C-K, Chow A et al (2015) The Xist IncRNA interacts directly with SHARP to silence transcription through HDAC3. Nature 521:232–236. doi:10.1038/nature14443

183. Kay GF, Penny GD, Patel D et al (1993) Expression of Xist during mouse development suggests a role in the initiation of X chromosome inactivation. Cell 72:171–182