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► **To cite this version:**

David Llères, Yui Imaizumi, Robert Feil. Exploring chromatin structural roles of non-coding RNAs at imprinted domains. *Biochemical Society Transactions*, 2021, 49 (4), pp.1867-1879. 10.1042/BST20210758 . hal-03428250

HAL Id: hal-03428250

<https://hal.umontpellier.fr/hal-03428250>

Submitted on 15 Nov 2021

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Review Article

Exploring chromatin structural roles of non-coding RNAs at imprinted domains

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Different classes of non-coding RNA (ncRNA) influence the organization of chromatin. Imprinted gene domains constitute a paradigm for exploring functional long ncRNAs (lncRNAs). Almost all express an lncRNA in a parent-of-origin dependent manner. The mono-allelic expression of these lncRNAs represses close by and distant protein-coding genes, through diverse mechanisms. Some control genes on other chromosomes as well. Interestingly, several imprinted chromosomal domains show a developmentally regulated, chromatin-based mechanism of imprinting with apparent similarities to X-chromosome inactivation. At these domains, the mono-allelic lncRNAs show a relatively stable, focal accumulation *in cis*. This facilitates the recruitment of Polycomb repressive complexes, lysine methyltransferases and other nuclear proteins – in part through direct RNA–protein interactions. Recent chromosome conformation capture and microscopy studies indicate that the focal aggregation of lncRNA and interacting proteins could play an architectural role as well, and correlates with close positioning of target genes. Higher-order chromatin structure is strongly influenced by CTCF/cohesin complexes, whose allelic association patterns and actions may be influenced by lncRNAs as well. Here, we review the gene-repressive roles of imprinted non-coding RNAs, particularly of lncRNAs, and discuss emerging links with chromatin architecture.

Introduction

Diverse genetic and epigenetic systems of mono-allelic expression have evolved in mammals, together controlling thousands of genes [1]. These mono-allelic gene expression mechanisms provide unique identities to cells, such as in hematopoietic cells or olfactory neurons, or critically modulate the dosage of gene expression, such as in X-chromosome inactivation in females [1]. The epigenetic phenomenon of genomic imprinting is exceptional in that this kind of mono-allelic expression depends entirely on the parental origin of the gene [2]. Some imprinted genes are expressed from the maternally inherited copy only, others only from the paternal copy. About 150 genes are known to be imprinted in humans and mice [3,4] and their correct expression levels are important for fetal growth, development, homeostasis and behavior [5,6].

Imprinting is controlled by oocyte- and sperm-derived DNA methylation marks put onto specialized CpG islands called ‘imprinting control regions’ (ICRs). After fertilization, these epigenetic ‘imprints’ are maintained in the somatic lineages and bring about imprinted expression through diverse mechanisms [2,7,8].

Recent studies have shown that oocyte-acquired histone methylation, particularly histone H3 lysine-27 tri-methylation (H3K27me3), can give rise to parentally biased gene expression as well [9,10]. This non-canonical imprinting is limited to the pre-implantation embryo, and is maintained at only a handful of genes in the extra-embryonic lineages [11–16].

Virtually all the ‘classical’ imprinted genes that are controlled by DNA methylation imprints are clustered in large domains. Most of these imprinted chromosomal domains express one or more long non-coding RNAs (lncRNAs), defined as being more than 200 nucleotides in length [17,18].

Received: 19 May 2021
 Revised: 5 July 2021
 Accepted: 6 July 2021

Version of Record published:
 2 August 2021

Accumulating evidence indicates that these lncRNAs contribute to bringing about imprinted gene expression at close by and distant protein-coding genes. Here, we discuss how imprinted non-coding RNAs control gene expression *in cis*, with a particular emphasis on their putative roles in chromatin structure. We also discuss emerging insights into trans-regulatory functions.

Numerous non-coding RNAs are controlled by genomic imprinting

It is often not well appreciated that numerous non-coding RNAs are imprinted in mammals. For instance, about seven percent of all microRNAs (miRNAs) are imprinted in humans, more than hundred in total. These are mostly transcribed by large host transcription units, each expressing multiple miRNAs [19,20]. One example is the *DLK1-DIO3* imprinted domain on human chromosome 14 (mouse chromosome 12), which expresses 53 miRNAs from a 220 kb polycistronic transcription unit, on the maternal chromosome only. Several of these miRNAs control the levels and/or the translation of mRNAs transcribed by other imprinted genes [21–23]. This highlights the considerable interconnectivity between imprinted loci that has arisen during evolution [24,25]. Another large cluster of imprinted miRNAs maps to human chromosome 19. Interestingly, this ‘C19MC’ cluster is primate-specific and expressed in the placenta predominantly [19,26].

Members of one class of small nucleolar RNAs (snoRNAs) are imprinted as well. These so-called C/D-box snoRNAs are thought to guide 2'-O-methylation on specific RNAs, but their precise roles have remained unclear despite recent functional studies [27–29]. The snoRNA DNA sequences are embedded within large transcription units, similarly as the imprinted miRNAs, each expressing multiple C/D-box snoRNAs [19]. One such a host locus is the imprinted *DLK1-DIO3* domain, which besides many miRNAs, expresses 38 C/D-box snoRNAs from its maternally expressed ncRNA polycistron. The best-studied cluster of imprinted snoRNAs resides within the *SNRPN-UBE3A* imprinted domain, which expresses 81 C/D-box snoRNAs from a large polycistronic gene expressed on the paternal chromosome only [19,30].

With respect to chromatin regulation, the most relevant non-coding RNAs are the lncRNAs [2]. In fact, imprinted lncRNAs were amongst the first discovered long non-coding RNAs and have provided many broadly relevant insights [17]. Most imprinted chromosomal domains express at least one lncRNA and these are RNA Polymerase-II transcribed. The very first example was *H19* at the imprinted *Igf2-H19* domain. This maternally expressed lncRNA was originally described as one of the most highly expressed RNAs during embryonic development, exerting growth-regulating functions [31]. More recent, mechanistic studies revealed that it produces a miRNA (miR-675) that influences muscle development and exerts growth-repressive effects in the placenta [32–34].

Most imprinted lncRNAs originate from their domain's ICR, which acts as a promoter on the unmethylated parental copy. Some are spliced, others not, and several imprinted lncRNAs are retained in the nucleus. These nuclear lncRNAs show different degrees of *cis*-accumulation onto their locus and exert long-range repressive effects, at some loci across several megabases of chromatin [3,17,35].

Gene regulatory roles of imprinted lncRNAs

In general, lncRNA expression can influence the transcription of protein-coding genes in many different ways [36]. Despite tremendous efforts, however, it has remained complicated to conclude whether observed effects are due to an lncRNA itself or to its transcription [37].

Extensive research during the last years has evoked different models of how lncRNA transcription could interfere with the expression of close by other genes [36,38–40]. As concerns imprinted lncRNAs one transcription-linked mechanism is interference with an overlapping gene transcribed in the opposite orientation (Figure 1A). A well-studied example of this is the imprinted *Snrpn-Ube3a* domain, where a paternally expressed lncRNA crosses almost one megabase of chromatin, including a distally positioned protein-coding gene called *Ube3a*. Transcriptional stalling caused by the collision of RNA pol-II complexes coming from opposite directions may explain the lack of *Ube3a* expression on the paternal chromosome. lncRNA ablation, or expression of truncated forms of the lncRNA that do not overlap *Ube3A*, cause aberrant activation of this gene on the paternal chromosome [41,42]. Similarly, topoisomerase inhibitors that prevent unwinding of the DNA during transcription —and thereby prevent transcriptional elongation of the lncRNA — reactivate the paternal *Ube3A* gene [43]. Concordantly, antisense oligonucleotides against the long transcript crossing the domain result in

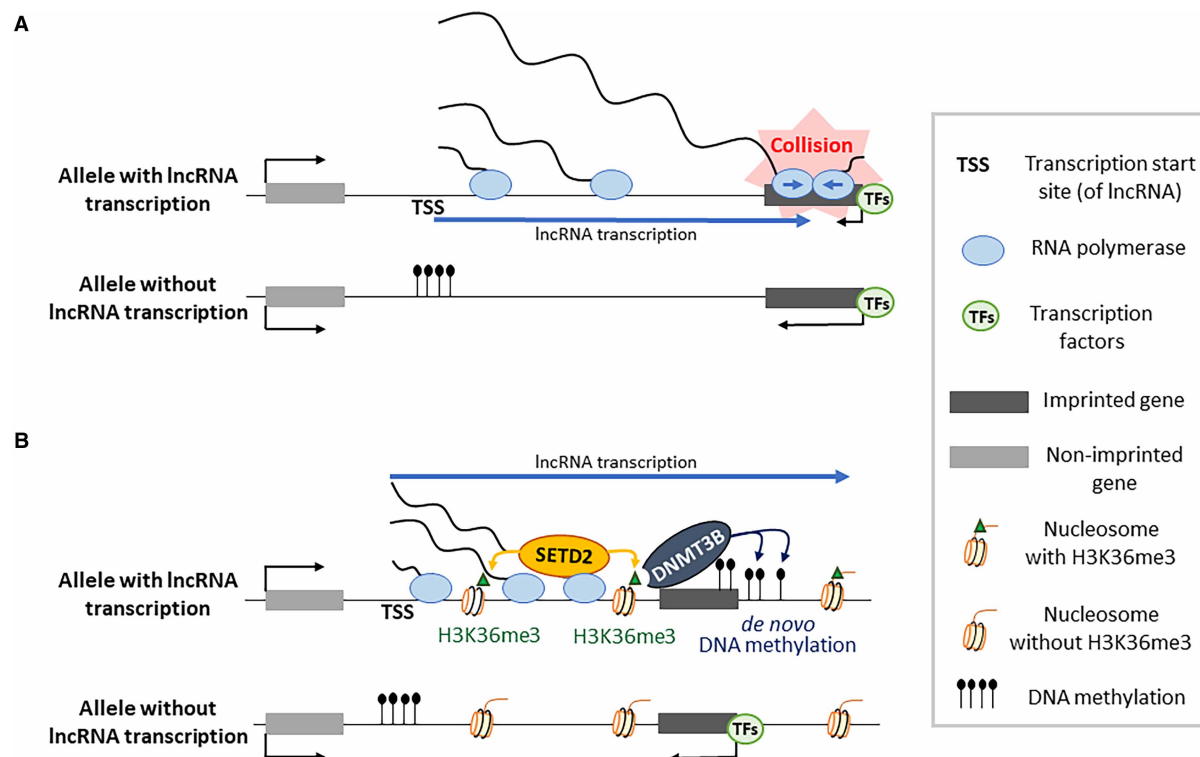


Figure 1. IncRNA-transcription-mediated interference mechanisms.

(A) One model of transcriptional interference involves collision of RNA Polymerase-II complexes (blue circles). High transcription of an imprinted lncRNA prevents elongation at an overlapping protein-coding gene (black rectangle) transcribed in the opposite direction. In this unidirectional repression model, the promoter of the target gene may show recruitment of transcription factors (TFs, green circles) on both the parental chromosomes. (B) Research on several imprinted genes has evoked another unidirectional model, involving promoter occlusion and repression. IncRNA transcription through a protein-coding gene mediates H3K36me3. This involves SETD2, a KMT brought to the chromatin through interaction with RNA-PolIII. This induces *de novo* DNA methylation by DNMT3B, a methyltransferase that recognizes H3K36me3 through its PWWP domain. Chromatin associated with the target promoter/CpG island may acquire other covalent histone modifications as well — particularly H3K9me3 — with the combined modifications preventing TF binding.

the activation of the paternal *UBE3A* gene, and such an approach is currently used in different clinical trials to treat Angelman Syndrome, a neuro-behavioural syndrome caused by loss of *UBE3A* expression [44,45].

A similar model has emerged from detailed studies on the imprinted IGF2-receptor (*Igf2r*) locus on mouse chromosome 17, which expresses a 117 kb non-spliced lncRNA called *Airn* that is transcribed oppositely to the *Igf2r* gene and overlaps its promoter [2]. The allelic lncRNA transcription across the paternal *Igf2r* promoter blocks RNA polymerase II recruitment, initially in the absence of repressive chromatin marks. Although in differentiating cells there is acquisition of DNA methylation and histone H3 lysine-9 trimethylation (H3K9me3), which provide an additional layer of repression, continued *Airn* expression is required to keep the paternal *Igf2r* promoter repressed [46–49].

At other imprinted domains, lncRNA transcription through promoters induces chromatin repression at promoters early in development [17]. At the *Gnas* locus on mouse chromosome 2, for instance, a lncRNA transcription unit called ‘Nesp-antisense’ (*Nespas*) overlaps an oppositely transcribed protein-coding gene called *Nesp* [50]. Diverse targeting studies in the mouse, including *Nespas* truncations, led to activation of the normally silent paternal *Nesp* allele. This highlights the importance of transcriptional overlap in the promoter repression (Figure 1B), which involves both histone and DNA methylation [51,52]. In a similar manner, at the imprinted *Zdbf2* locus on mouse chromosome 1, transient transcription during preimplantation development of a lncRNA (called *Liz*) brings about DNA methylation, close to the *Zdbf2* gene [53,54]. As part of the

mechanism, RNA polymerase-II could bring the KMT SETD2 to the chromatin, which induces histone H3 lysine-36 tri-methylation (H3K36me3) across the transcribed region. This histone modification is recognized by the DNA methyltransferase DNMT3B (through its PWWP domain) subsequently, which induces *de novo* DNA methylation [55–57].

LncRNA-controlled genes at several imprinted domains are located hundreds to thousands of kilobases away from the lncRNA gene [3,58]. These ‘long-distance’ effects have given rise to models in which the lncRNA itself brings about gene repression (Figure 2). Developmental studies have shown that such long-distance repression occurs in a tissue-specific manner at several of the domains [35]. At the *Igf2r* domain, in the extra-embryonic lineages, the paternally expressed *Airn* mediates the allelic repression of several non-overlapping genes positioned up to several megabases away [3,58]. Upon truncations of this lncRNA, this long-distance repressive effect no longer occurs [46,58,59].

At *Dlk1-Dio3*, similarly, the allelic expression of an lncRNA called *Meg3* is required to repress a distant protein-coding gene involved in Notch signaling, called *Dlk1*, in different somatic tissues [60–62]. Knock-out and overexpression studies have suggested that *Meg3* expression controls genes on other chromosomes as well, including TGF- β and p53 pathway genes in human cancer cells [63–67]. A similar *trans* effect has been reported for the lncRNA *IPW* generated from the *SNHG14* gene at the *SNRPN-UBE3A* domain (chromosome 15q11–13). This lncRNA dampens *in trans* the promoter of the *MEG3* non-coding polycistron at the *DLK1-DIO3* domain, a process that seems to involve repressive H3K9me3 [68]. This provides yet another example of the intricate regulatory links that exist between imprinted loci [25,69–71].

Another locus showing long-range repressive effects of an lncRNA is the *Kcnq1* domain on mouse chromosome 7. The integrity of a 91 kb lncRNA called *Kcnq1ot1*, particularly a 900 bp region at its 5' end, is important for the allelic repression of no fewer than eight genes at the proximal and distal parts of this multi-megabase domain [72–76]. Several of the target genes show placental-specific imprinting, indicating that lineage-specific factors likely contribute to the long-range repressive effects of this essential lncRNA [3,58,77]. Combined, the above examples illustrate that several imprinted lncRNAs repress protein-coding genes *in cis*, and that some control genes on other chromosomes as well.

Imprinted lncRNAs that mediate long-range chromatin repression

Genome-wide reporter-based studies have revealed that many non-imprinted lncRNA genes exert a positive effect on the expression of other genes in their neighborhood [78,79]. These ‘enhancer-like’ effects of lncRNA gene promoters contrast with the observed effects of imprinted lncRNAs, which mostly repress neighboring genes, through nucleation and spreading of repressive histone modifications across large regions [17].

For the imprinted lncRNAs *Kcnq1ot1*, *Airn* and *Meg3* evidence has been obtained for a direct role in chromatin repression. All three are retained in the nucleus and show a certain degree of *cis*-accumulation onto their imprinted domains. This focal accumulation is still detected hours after chemical inhibition of RNA polymerase-II, concordant with the reported intermediate stabilities of these nuclear lncRNAs [58,75,80,81].

Another similarity between *Kcnq1ot1*, *Airn* and *Meg3* is their reported interaction with components of chromatin regulatory complexes (Table 1). In preimplantation embryonic cells and in the placenta, the paternally expressed, 91 kb *Kcnq1ot1* (*Kcnq1* domain) co-localizes and interacts with components of the Polycomb repressive complexes 1 (PRC1) and -2 (PRC2) [74,75,82,83]. It also interacts with EHMT2 (also called G9A) [74], a lysine methyltransferase (KMT) that methylates lysine-9 on histone H3. Concordantly, there is allelic acquisition of EZH2 (PRC2)-mediated H3K27me3, RING1B (PRC1)-mediated H2AK119u1 and EHMT2-mediated H3K9me2 across the paternally repressed genes in trophoblast cells and in the placenta [58,75,77]. In trophoblast stem cells (TSCs) that expressed a truncated form of *Kcnq1ot1*, H3K27me3 levels were strongly reduced across the entire *Kcnq1* imprinted domain [58,74]. *Ehmt2* knock-out in mice gave biallelic expression of several of the placental-specific *Kcnq1ot1* targets in the placenta [84], and, similarly, the essential PRC2 component EED contributes to the process as well [85].

A recent study suggests that the *Kcnq1ot1* lncRNA interacts with the nuclear matrix protein hnRNPK. This RNA-interacting protein is essential for the PRC2-mediated H3K27me3 across the imprinted *Kcnq1* domain in TSCs [58]. One emerging model (Figure 2) is that hnRNPK enhances the recruitment and spreading of PRC1 complexes, a process that initiates at CpG islands that were already bound by PRC complexes beforehand [58,86].

Table 1 Chromatin repressive functions of imprinted lncRNAs

lncRNA	Imprinted gene domain	chromatin repressive effect(s) of the lncRNA	References
<i>Kcnq1ot1</i> (previously called <i>Lit1</i>)	<i>Kcnq1</i> domain	<ul style="list-style-type: none"> * Gene repression <i>in cis</i>. * Enhances allelic recruitment of PRC1/2, EHMT2 and hnRPNK. * Allelic enrichment of H3K27me3, H2AK119u1 and H3K9me2 across broad regions. * Interacts with CTCF and influences higher-order chromatin features. 	[58,74,75,83,84]
<i>Airn</i> (previously called <i>Air</i>)	<i>Igf2r</i> domain	<ul style="list-style-type: none"> * Mediates gene repression <i>in cis</i>. * Enhances allelic recruitment of PRC2, EHMT2 and hnRPNK. * Allelic enrichment of H3K27me2, H2AK119ub and H3K9me2 across broad regions. * Interacts with CTCF and influences higher-order chromatin features. 	[3,46–48,58,59,87]
<i>Meg3</i> (also called <i>Gtl2</i>)	<i>Dlk1-Dio3</i> domain	<ul style="list-style-type: none"> * Its expression mediates gene silencing <i>in cis</i>. * Likely represses genes <i>in trans</i> as well. * Interacts with PRC2 components (EZH2, JARID2) and possibly also with hnRPNK. * Maintains allelic H3K27me3 enrichment at target genes. 	[61,63,65,82,83,131]
<i>IPW</i> (?)	<i>SNRPN-UBE3A</i> domain	<ul style="list-style-type: none"> * Exerts a repressive effect <i>in trans</i>. * Influences H3K9me3 levels at its <i>trans</i> target (<i>Meg3</i> gene). 	[68]
<i>H19</i> (?)	<i>Igf2-H19</i> domain	<ul style="list-style-type: none"> * Gene repressive effects <i>in trans</i>, on other imprinted loci. * Modulates recruitment of the Methyl CpG Binding Domain-1 (MBD1) complex and its associated KMTs. * Modulates H3K9me3 levels at putative target loci. 	[130]

A similar picture has emerged for the paternally expressed, 117 kb lncRNA *Airn* (*Igf2r* domain), which in the extraembryonic lineages represses multiple genes across several megabases. In murine TSCs and in placenta, a truncated form of this lncRNA no longer gave gene repression *in cis* [3,58,59]. Recent gene targeting studies in mice show that the long-range repressive effects of *Airn* are not mediated by regulatory sequence elements within the *Airn* lncRNA gene, excluding transcriptional interference mechanisms at the distant non-overlapping genes controlled by *Airn*. Rather, these repressive effects correlate with the broad spreading of PRC2-mediated H3K27me3 and PRC1-mediated H2A-lysine-119 mono-ubiquitination (H2AK119u1) on the paternal chromosome predominantly [58,87]. *Airn* levels are crucial for the allelic recruitment of RING1B (PRC1) and EZH2 (PRC2). Enhancing lncRNA *Airn* copy numbers per cell, by CRISPR-VP16 mediated transcriptional activation, gave enhanced recruitment of PRC complexes onto the paternal chromosome [58]. *Airn* had been shown earlier to facilitate EHMT2 recruitment, which correlates with paternal allele-specific H3K9me2/3 enrichment [59]. Also *Airn* lncRNA seems to interact with hnRPNK and this could enhance recruitment of PRC complexes to the chromatin [88]. In agreement with this hypothesis, the allelic enrichment and spreading of H3K27me3 across the large *Igf2r* domain requires continued expression of the hnRPNK protein in TSCs [58].

Meg3 lncRNA seems to have a similar mode of action, in somatic tissues. Its expression represses *in cis* a developmental gene called *Dlk1*, located on the proximal side of the imprinted domain [61]. Different studies have reported *Meg3* association with PRC2 components (EZH2 and JARID2) and RNA precipitation assays on cross-linked chromatin suggest binding to hnRPNK as well [58,82,83]. In the absence of *Meg3* lncRNA, there is no longer acquisition of allelic *Dlk1* repression, and this is observed following depletion of EZH2 (PRC2 complex) as well [61]. Similarly as for *Airn* and *Kcnq1ot1* [58], the combined data suggest that *Meg3* lncRNA

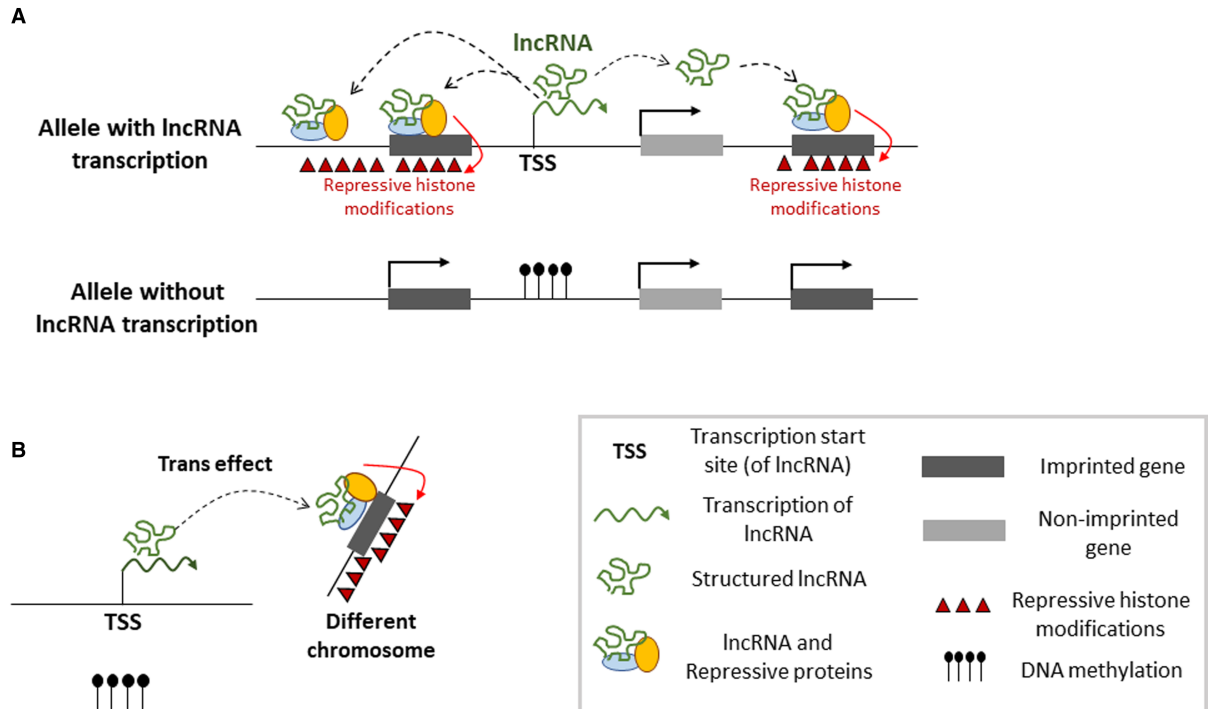


Figure 2. IncRNA-mediated chromatin repression across imprinted gene domains.

(A) Several imprinted lncRNAs mediate chromatin repression across megabases. This involves enhanced allelic recruitment of PRC1 and -2 complexes, EHMT2 and hnRNPk, in part through direct RNA–protein interactions, leading to allelic spreading of H3K27me3, H2AK119u1 and H3K9me2 across the domain. This bidirectional mechanism of chromatin repression, induced by the lncRNA itself is lineage-specific at several domains and shows certain similarities with X-chromosome inactivation [1].

(B) Some imprinted lncRNAs may repress genes on other chromosomes, in *trans*, possibly involving a chromatin-based mechanism that could be similar to *cis* repression [65–68,130].

enhances in an allelic manner the histone modifying activities and possibly also the spreading of PRC complexes, through still poorly understood mechanisms (Figure 2).

The above examples evoke similarities with X-chromosome inactivation in females, which is a *cis* repressive mechanism controlled by an lncRNA (called *Xist*) that involves PRC1, PRC2, EHMT2 and hnRNPk, and other proteins not yet been explored in genomic imprinting [1,88,89]. However, care needs to be taken before drawing firm conclusions. The methodologies used to explore *Xist*, for instance, have been more focused on the lncRNA itself, with functional identification of chromatin-binding RNA motifs. Complementary technologies have also confirmed a direct interaction between *Xist* and hnRNPk, which has not yet been shown for *Airn*, *Kcnq1ot1* or *Meg3* [90–93].

Emerging roles of imprinted lncRNAs in chromatin architecture

Because of the parental allele-specific DNA methylation imprints, at several imprinted domains there is allelic association of chromatin structural proteins. At several ICRs, and also at secondary DMRs at which the allelic methylation is acquired during development, there is binding of CCCTC-binding factor (CTCF) to the unmethylated allele only (the protein does not bind methylated DNA) [94–97]. This allelic CTCF binding and the CTCF-associated cohesin complexes contribute to imprinted gene expression [98]. Particularly, CTCF mediates long-range chromatin loops with distant other regions on the CTCF bound parental chromosome. Recent studies have explored these structural interactions by using allelic ‘chromosome conformation capture’ (3C) and 3D DNA FISH-based approaches [98].

At the *Igf2-H19* locus, CTCF binding to the unmethylated copy of the ICR brings this region in close proximity to distal regions on the maternal chromosome. Both in mice and humans, this insulates the *Igf2* gene from its distally located enhancers, thus leading to the imprinted *Igf2* expression from the paternal chromosome mostly [96,99–102].

At the *Dlk1-Dio3* domain, CTCF binds the promoter–CpG island of the *Meg3* gene, on its unmethylated maternal copy only [96,103]. Also here, allelic CTCF recruitment brings about specific long-distance structural interactions on the maternal chromosome predominantly. Particularly, the *Dlk1* gene shows close proximity to the lncRNA focus on the maternal chromosome, and this proximity effect contributes to its imprinted expression from the paternal chromosome predominantly [96]. Interestingly, 3D distance measurements between FISH probes show that the imprinted domain is more loosely compacted on the maternal chromosome (compared with the paternal chromosome), which may facilitate the observed CTCF-mediated looping patterns [96,104].

A similar picture has emerged for the *Kcnq1* imprinted domain. Here, CTCF binds the unmethylated paternal copy of the ICR, which also comprises the promoter that drives *Kcnq1ot1* expression on this parental chromosome [105]. The allelic CTCF binding mediates specific long-range interactions on the paternal chromosome, detected by 3C-based technology, that correlate with the allelic expression of several genes within the domain [75,102,106]. Another locus that shows both allelic CTCF binding and allelic lncRNA expression is the imprinted *Zdbf2* domain [107].

Could the allelic lncRNA expression and the allelic binding of CTCF be mechanistically linked? Possibly, transcription factor binding and lncRNA promoter activity keep CTCF binding sites unmethylated, thus ensuring the continued allelic association of this chromatin structural protein (which does not bind methylated DNA [97]). Continued promoter activity at *Meg3* protects indeed against the acquisition of *de novo* DNA methylation in early embryonic cells [108,109]. Point mutations within transcription-factor binding sites at the ICRs of the human *IGF2-H19* and *KCNQ1* domains have provided evidence for such a scenario as well [110–114]. Conversely, CTCF itself may protect the unmethylated allele against *de novo* DNA methylation [110,111,115,116], thus ensuring continued transcription of the lncRNA from the unmethylated parental allele only.

Since *Meg3*, *Kcnq1ot1* and *Airn* show a relatively stable focal accumulation onto their locus [58,61,75,80], this could locally influence CTCF-linked higher-order chromatin structure. CTCF comprises indeed a putative RNA binding domain (RBD) that is functionally important [117,118]. Recent studies suggest that binding of locally transcribed RNAs to the RBD is important for CTCF's association to many of its recognition sites in the genome. This impacts the 3D organization of the genome through the formation of specific chromatin loops [117,118]. It remains to be explored in mice on an F1 background between two phylogenetically distant strains whether there are direct allelic interactions between CTCF and imprinted lncRNAs and to what extent these may influence chromatin loop formation.

How and when lncRNA–protein compartments are formed at imprinted loci, and what controls their developmental regulation, remains unclear. Structural RNA features could be important. Several recent studies explored in detail the structure of *MEG3* *in vitro* and in cells [63,119], and interacting RNA loops within the lncRNA were shown to be essential for the *trans* effects of *MEG3* on the p53 pathway in cancer cells [63]. Whilst the RNA sequences of imprinted lncRNAs are generally not well conserved, specific secondary and tertiary structures may be comparable between different mammalian species, and may be important as docking sites for RNA–protein interactions.

Specific RNA sequence elements could be important as well; for instance in the association of lncRNAs to specific target genes in *trans*. In one interesting study on human cancer cells, expression of *MEG3* modulated the expression of TGF- β pathway genes, and this was linked to the formation of RNA–DNA triplex structures across several of these target genes [65]. Although further studies are required, such a process could provide specificity to the *trans* roles of lncRNAs.

The non-imprinted lncRNAs *MALAT1* and *NEAT1* are linked to the formation of membrane-less nuclear bodies called speckles and paraspeckles in specific cell types and under particular conditions [120–122]. Furthermore, emerging evidence on the heterochromatin-linked satellite RNAs and other non-imprinted RNAs suggest that RNA–protein aggregates can potentially form through liquid-liquid phase separation (LLPS) mechanisms (reviewed in [123]), an aspect that has not yet been explored in the context of imprinted domains.

Sub-nuclear localization could impact the process as well, given that at the *Dlk1-Dio3*, *Kcnq1* and other imprinted domains, the lncRNA-expressing parental chromosome displays a more central localization in the

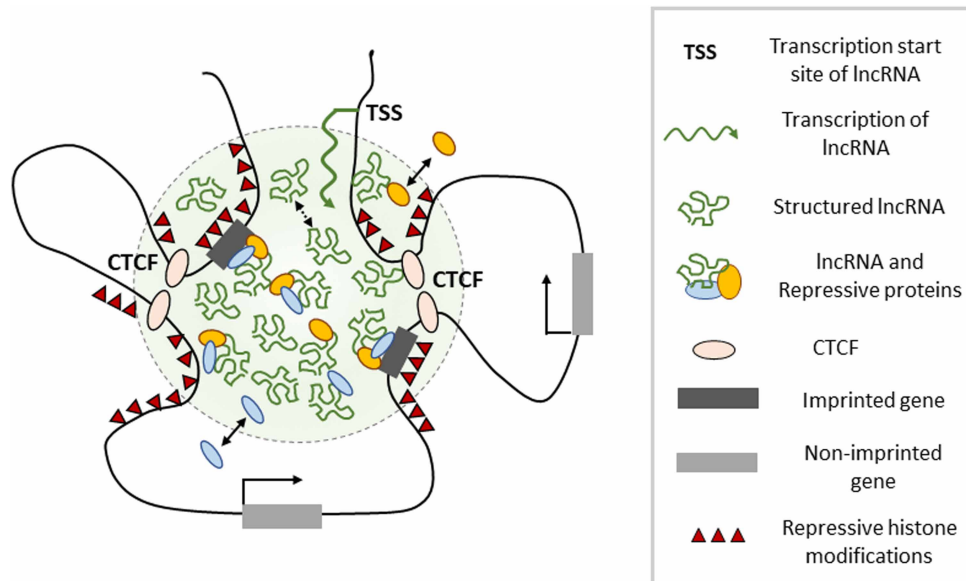


Figure 3. lncRNA–protein aggregates in chromatin architecture and gene expression.

The figure depicts a model in which the imprinted lncRNA accumulates in proximity to its transcription site and interacts with different chromatin repressive complexes (PRC1/2, EHMT2, others). This leads to the formation of relatively stable RNA–protein aggregates (green shading), possibly in part through LLPS. Chromosomal gene loci that are in close proximity to the aggregate acquire repressive histone modifications and become silenced. This process could be facilitated by positive effects of the lncRNA on CTCF binding, thereby ensuring appropriate long-range chromatin interactions that bring target gene(s) in close vicinity to the lncRNA–protein aggregate. The model could also explain lncRNA effects in *trans*, on genes located on other chromosomes, in case these are positioned close to the RNA–protein aggregate(s), at least part of the time.

nucleus than the opposite parental chromosome [80,124]. The available data so far evoke a model in which focal accumulation of lncRNA and associated chromatin-regulatory complexes creates an aggregate-like organization that brings specific loci in close proximity through protruding chromatin-loop formation and mediates gene repression (Figure 3). At some imprinted domains, interestingly, lncRNA/protein compartments seem to exclude RNA polymerase-II [75], which could be an important aspect of the imprinting process as well. lncRNA-mediated gene repression at imprinted domains is a rather complicated business, and we are only at the beginning of understanding its intricacies.

Perspectives

- Imprinted gene domains have provided strong paradigms for exploring the regulation and roles of lncRNAs in mammals. Ongoing research efforts unravel *cis*-regulatory chromatin mechanisms and explore how these compare to emerging *trans* roles of imprinted lncRNAs.
- Besides transcriptional interference mechanisms mediated by the expression of lncRNA genes, it is now well accepted that several imprinted lncRNAs themselves control gene repression. These *cis*-repressive actions of lncRNAs likely impact chromatin architecture, involve lncRNA–protein interactions, and specific RNA secondary and tertiary structures could be essential as well. In principle, reported *trans* effects involve the lncRNAs themselves as well [63,65,66,68]. One possibility is that *trans* targets would be transiently positioned in close proximity to lncRNA–protein aggregates, and several recent studies have started to explore this intriguing possibility [102,125].

- Novel CRISPR technologies may help to distinguish between the effects of lncRNA transcription and those of the imprinted lncRNA transcripts *per se* [126,127]. Future research should also unravel which sequence motifs and secondary structures within lncRNAs are important for chromatin repression and architecture, and how these control association of specific lncRNA-interacting proteins. Finally, it is timely to determine the importance of lncRNAs and chromatin architecture in human imprinting disorders (IDs) [4,69]. Initial studies have reported altered chromatin structural interactions within the *KCNQ1* and *IGF2-H19* domains in the growth disorders Beckwith-Wiedemann Syndrome (BWS) and Silver-Russell Syndrome (SRS) [102,128,129].

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

R.F., D.L. and Y.I. wrote the manuscript.

Acknowledgements

This review is dedicated to the memory of Denise Barlow, who pioneered research on functional long non-coding RNAs. We thank the reviewers for their excellent comments and suggestions and acknowledge grant funding from the Agence National de Recherche through project ANR-18-CE12-0022-02 ('IMP-REGULOME') and the Laboratory of Excellence EpiGenMed programme (ANR-10-LABX-12-01). Y.I. acknowledges Fellowship funding from the TOYOBO Biotechnology Foundation, Japan (2019-01) and the Japan Society for the Promotion of Science (JSPS-202160732).

Abbreviations

3C, chromosome conformation capture; CTCF, CCCTC-binding factor; DMR, differentially methylated domain; FISH, fluorescence *in situ* hybridization; ICR, imprinting control region; LLPS, liquid-liquid phase separation; MBD, Methyl CpG Binding Domain; PRC, Polycomb Repressive Complex; TAD, topologically associating domain.

References

- 1 Khamilichi, A.A. and Feil, R. (2018) Parallels between mammalian mechanisms of monoallelic gene expression. *Trends Genet.* **34**, 954–971 <https://doi.org/10.1016/j.tig.2018.08.005>
- 2 Barlow, D.P. and Bartolomei, M.S. (2014) Genomic imprinting in mammals. *Cold Spring Harb. Perspect. Biol.* **6**, a018382 <https://doi.org/10.1101/cshperspect.a018382>
- 3 Andergassen, D., Dotter, C.P., Wenzel, D., Sigl, V., Bammer, P.C., Muckenhuber, M. et al. (2017) Mapping the mouse allele reveals tissue-specific regulation of allelic expression. *elife* **6**, e25125 <https://doi.org/10.7554/eLife.25125>
- 4 Monk, D., Mackay, D.J.G., Eggermann, T., Maher, E.R. and Riccio, A. (2019) Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. *Nat. Rev. Genet.* **20**, 235–248 <https://doi.org/10.1038/s41576-018-0092-0>
- 5 Tucci, V., Isles, A.R., Kelsey, G., Ferguson-Smith, A.C. and Grp, E.I. (2019) Genomic imprinting and physiological processes in mammals. *Cell* **176**, 952–965 <https://doi.org/10.1016/j.cell.2019.01.043>
- 6 Peters, J. (2014) The role of genomic imprinting in biology and disease: an expanding view. *Nat. Rev. Genet.* **15**, 517–530 <https://doi.org/10.1038/nrg3766>
- 7 Kelsey, G. and Feil, R. (2013) New insights into establishment and maintenance of DNA methylation imprints in mammals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20110336 <https://doi.org/10.1098/rstb.2011.0336>
- 8 Pathak, R. and Feil, R. (2018) Environmental effects on chromatin repression at imprinted genes and endogenous retroviruses. *Curr. Opin. Chem. Biol.* **45**, 139–147 <https://doi.org/10.1016/j.cbpa.2018.04.015>
- 9 Chen, Z.Y. and Zhang, Y. (2020) Maternal H3K27me3-dependent autosomal and X chromosome imprinting. *Nat. Rev. Genet.* **21**, 555–571 <https://doi.org/10.1038/s41576-020-0245-9>
- 10 Pathak, R. and Feil, R. (2017) Oocyte-derived histone H3 lysine 27 methylation controls gene expression in the early embryo. *Nat. Struct. Mol. Biol.* **24**, 685–686 <https://doi.org/10.1038/nsmb.3456>
- 11 Hanna, C.W., Perez-Palacios, R., Gahurova, L., Schubert, M., Krueger, F., Biggins, L. et al. (2019) Endogenous retroviral insertions drive non-canonical imprinting in extra-embryonic tissues. *Genome Biol.* **20**, 225 <https://doi.org/10.1186/s13059-019-1833-x>
- 12 Chen, Z.Y., Yin, Q.Z., Inoue, A., Zhang, C.X. and Zhang, Y. (2019) Allelic H3K27me3 to allelic DNA methylation switch maintains noncanonical imprinting in extraembryonic cells. *Sci. Adv.* **5**, eaay7246 <https://doi.org/10.1126/sciadv.aay7246>

- 13 Inoue, A., Jiang, L., Lu, F., Suzuki, T. and Zhang, Y. (2017) Maternal H3K27me3 controls DNA methylation-independent imprinting. *Nature* **547**, 419–424 <https://doi.org/10.1038/nature23262>
- 14 Inoue, A., Chen, Z.Y., Yin, Q.Z. and Zhang, Y. (2018) Maternal Eed knockout causes loss of H3K27me3 imprinting and random X inactivation in the extraembryonic cells. *Genes Dev.* **32**, 1525–1536 <https://doi.org/10.1101/gad.318675.118>
- 15 Wanigasuriya, I., Gouil, Q., Kinkel, S.A., del Ferro, A.T., Beck, T., Roper, E.A. et al. (2020) Smchd1 is a maternal effect gene required for genomic imprinting. *eLife* **9**, e55529 <https://doi.org/10.7554/eLife.55529>
- 16 Santini, L., Halbritter, F., Titz-Teixeira, F., Suzuki, T., Asami, M., Ma, X. et al. (2021) Genomic imprinting in mouse blastocysts is predominantly associated with H3K27me3. *Nat. Commun.* **12**, 3804 <https://doi.org/10.1038/s41467-021-23510-4>
- 17 MacDonald, W.A. and Mann, M.R.W. (2020) Long noncoding RNA functionality in imprinted domain regulation. *PLoS Genet.* **16**, e1008930 <https://doi.org/10.1371/journal.pgen.1008930>
- 18 Barlow, D.P. (2011) Genomic imprinting: a mammalian epigenetic discovery model. *Annu. Rev. Genet.* **45**, 379–403 <https://doi.org/10.1146/annurev-genet-110410-132459>
- 19 Girardot, M., Cavaille, J. and Feil, R. (2012) Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease. *Epigenetics* **7**, 1341–1348 <https://doi.org/10.4161/epi.22884>
- 20 Malnou, E.C., Umlauf, D., Mouysset, M. and Cavaille, J. (2019) Imprinted microRNA gene clusters in the evolution, development, and functions of mammalian placenta. *Front. Genet.* **9**, 706 <https://doi.org/10.3389/fgene.2018.00706>
- 21 Whipple, A.J., Breton-Provencher, V., Jacobs, H.N., Chitta, U.K., Sur, M. and Sharp, P.A. (2020) Imprinted maternally expressed microRNAs antagonize paternally driven gene programs in neurons. *Mol. Cell* **78**, 85–95 <https://doi.org/10.1016/j.molcel.2020.01.020>
- 22 Gao, Y.Q., Chen, X., Wang, P., Lu, L., Zhao, W., Chen, C. et al. (2015) Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance. *Proc. Natl Acad. Sci. U.S.A.* **112**, 13627–13632 <https://doi.org/10.1073/pnas.1511448112>
- 23 Davis, E., Caiment, F., Tordoir, X., Cavaille, J., Ferguson-Smith, A., Cockett, N. et al. (2005) RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. *Curr. Biol.* **15**, 743–749 <https://doi.org/10.1016/j.cub.2005.02.060>
- 24 Ghousein, A. and Feil, R. (2020) Imprinted small RNAs unraveled: maternal MicroRNAs antagonize a paternal-genome-driven gene expression network. *Mol. Cell* **78**, 3–5 <https://doi.org/10.1016/j.molcel.2020.03.019>
- 25 Patten, M.M., Cowley, M., Oakey, R.J. and Feil, R. (2016) Regulatory links between imprinted genes: evolutionary predictions and consequences. *Proc. Biol. Sci.* **283**, 20152760 <https://doi.org/10.1098/rspb.2015.276>
- 26 Noguer-Dance, M., Abu-Amero, S., Al-Khtib, M., Lefevre, A., Coullin, P., Moore, G.E. et al. (2010) The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. *Hum. Mol. Genet.* **19**, 3566–3582 <https://doi.org/10.1093/hmg/ddq272>
- 27 Labialle, S., Marty, V., Bortolin-Cavaille, M.L., Hoareau-Osman, M., Pradere, J.P., Valet, P. et al. (2014) The miR-379/miR-410 cluster at the imprinted Dlk1-Dio3 domain controls neonatal metabolic adaptation. *EMBO J.* **33**, 2216–2230 <https://doi.org/10.15252/embj.201387038>
- 28 Marty, V., Labialle, S., Bortolin-Cavaille, M.L., De Medeiros, G.F., Moisan, M.P., Florian, C. et al. (2016) Deletion of the miR-379/miR-410 gene cluster at the imprinted Dlk1-Dio3 locus enhances anxiety-related behaviour. *Hum. Mol. Genet.* **25**, 728–739 <https://doi.org/10.1093/hmg/ddv510>
- 29 Hebras, J., Marty, V., Personnaz, J., Mercier, P., Krogh, N., Nielsen, H. et al. (2020) Reassessment of the involvement of Snord115 in the serotonin 2c receptor pathway in a genetically relevant mouse model. *eLife* **9**, e60862 <https://doi.org/10.7554/eLife.60862>
- 30 Bortolin-Cavaille, M.L. and Cavaille, J. (2012) The SNORD115 (H/MBII-52) and SNORD116 (H/MBII-85) gene clusters at the imprinted Prader-Willi locus generate canonical box C/D snoRNAs. *Nucleic Acids Res.* **40**, 6800–6807 <https://doi.org/10.1093/nar/gks321>
- 31 Gabory, A., Jammes, H. and Dandolo, L. (2010) The H19 locus: role of an imprinted non-coding RNA in growth and development. *Bioessays* **32**, 473–480 <https://doi.org/10.1002/bies.200900170>
- 32 Brannan, C.I., Dees, E.C., Ingram, R.S. and Tilghman, S.M. (1990) The product of the H19 gene may function as an RNA. *Mol. Cell Biol.* **10**, 28–36 <https://doi.org/10.1128/mcb.10.1.28-36.1990>
- 33 Keniry, A., Oxley, D., Monnier, P., Kyba, M., Dandolo, L., Smits, G. et al. (2012) The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat. Cell Biol.* **14**, 659–665 <https://doi.org/10.1038/ncb2521>
- 34 Dey, B.K., Pfeifer, K. and 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 <https://doi.org/10.1101/gad.234419.113>
- 35 Sanli, I. and Feil, R. (2015) Chromatin mechanisms in the developmental control of imprinted gene expression. *Int. J. Biochem. Cell Biol.* **67**, 139–147 <https://doi.org/10.1016/j.biocel.2015.04.004>
- 36 Statello, L., Guo, C.J., Chen, L.L. and Huarte, M. (2021) Gene regulation by long non-coding RNAs and its biological functions. *Nat. Rev. Mol. Cell Biol.* **22**, 96–118 <https://doi.org/10.1038/s41580-020-00315-9>
- 37 Bassett, A.R., Akhtar, A., Barlow, D.P., Bird, A.P., Brockdorff, N., Duboule, D. et al. (2014) Considerations when investigating lincRNA function in vivo. *eLife* **3**, e03058 <https://doi.org/10.7554/eLife.03058>
- 38 Kaikkonen, M.U. and Adelman, K. (2018) Emerging roles of Non-Coding RNA transcription. *Trends Biochem. Sci.* **43**, 654–667 <https://doi.org/10.1016/j.tibs.2018.06.002>
- 39 Pauler, F.M., Barlow, D.P. and Hudson, Q.J. (2012) Mechanisms of long range silencing by imprinted macro non-coding RNAs. *Curr. Opin. Genet. Dev.* **22**, 283–289 <https://doi.org/10.1016/j.gde.2012.02.005>
- 40 Kornienko, A.E., Guenzl, P.M., Barlow, D.P. and Pauler, F.M. (2013) Gene regulation by the act of long non-coding RNA transcription. *BMC Biol.* **11**, 59 <https://doi.org/10.1186/1741-7007-11-59>
- 41 Meng, L.Y., Person, R.E., Huang, W., Zhu, P.J., Costa-Mattioli, M. and Beaudet, A.L. (2013) Truncation of Ube3a-ATS unsilences paternal Ube3a and ameliorates behavioral defects in the Angelman syndrome mouse model. *PLoS Genet.* **9**, e1004039 <https://doi.org/10.1371/journal.pgen.1004039>
- 42 Hsiao, J.S., Germain, N.D., Wilderman, A., Stoddard, C., Wojenski, L.A., Villafano, G.J. et al. (2019) A bipartite boundary element restricts UBE3A imprinting to mature neurons. *Proc. Natl Acad. Sci. U.S.A.* **116**, 2181–2186 <https://doi.org/10.1073/pnas.1815279116>
- 43 Powell, W.T., Coulson, R.L., Gonzales, M.L., Cray, F.K., Wong, S.S., Adams, S. et al. (2013) R-loop formation at Snord116 mediates topotecan inhibition of Ube3a-antisense and allele-specific chromatin decondensation. *Proc. Natl Acad. Sci. U.S.A.* **110**, 13938–13943 <https://doi.org/10.1073/pnas.1305426110>

- 44 Meng, L.Y., Ward, A.J., Chun, S., Bennett, C.F., Beaudet, A.L. and Rigo, F. (2015) Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. *Nature* **518**, 409–412 <https://doi.org/10.1038/nature13975>
- 45 Lalevee, S. and Feil, R. (2015) Long noncoding RNAs in human disease: emerging mechanisms and therapeutic strategies. *Epigenomics* **7**, 877–879 <https://doi.org/10.2217/epi.15.55>
- 46 Sleutels, F., Zwart, R. and Barlow, D.P. (2002) The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* **415**, 810–813 <https://doi.org/10.1038/415810a>
- 47 Latos, P.A., Pauler, F.M., Koerner, M.V., Senergin, H.B., Hudson, Q.J., Stocsits, R.R. et al. (2012) Airn transcriptional overlap, but not its lncRNA products, induces imprinted Igf2r silencing. *Science* **338**, 1469–1472 <https://doi.org/10.1126/science.1228110>
- 48 Santoro, F., Mayer, D., Klement, R.M., Warczok, K.E., Stukalov, A., Barlow, D.P. et al. (2013) Imprinted Igf2r silencing depends on continuous Airn lncRNA expression and is not restricted to a developmental window. *Development* **140**, 1184–1195 <https://doi.org/10.1242/dev.088849>
- 49 Latos, P.A., Stricker, S.H., Steenpass, L., Pauler, F.M., Huang, R., Senergin, B.H. et al. (2009) An in vitro ES cell imprinting model shows that imprinted expression of the Igf2r gene arises from an allele-specific expression bias. *Development* **136**, 437–448 <https://doi.org/10.1242/dev.032060>
- 50 Peters, J. and Williamson, C.M. (2007) Control of imprinting at the Gnas cluster. *Epigenetics* **2**, 207–213 <https://doi.org/10.4161/epi.2.4.5380>
- 51 Williamson, C.M., Ball, S.T., Dawson, C., Mehta, S., Beechey, C.V., Fray, M. et al. (2011) Uncoupling antisense-mediated silencing and DNA methylation in the imprinted Gnas cluster. *PLoS Genet.* **7**, e1001347 <https://doi.org/10.1371/journal.pgen.1001347>
- 52 Mehta, S., Williamson, C.M., Ball, S., Tibbit, C., Beechey, C., Fray, M. et al. (2015) Transcription driven somatic DNA methylation within the imprinted Gnas cluster. *PLoS ONE* **10**, e0117378 <https://doi.org/10.1371/journal.pone.0117378>
- 53 Duffie, R., Aijan, S., Greenberg, M.V., Zamudio, N., del Arenal, M.E., Iranzo, J. et al. (2014) The Gpr1/Zdbf2 locus provides new paradigms for transient and dynamic genomic imprinting in mammals. *Genes Dev.* **28**, 463–478 <https://doi.org/10.1101/gad.232058.113>
- 54 Greenberg, M.V., Glaser, J., Borsos, M., Marjou, F.E., Walter, M., Teissandier, A. et al. (2017) Transient transcription in the early embryo sets an epigenetic state that programs postnatal growth. *Nat. Genet.* **49**, 110–118 <https://doi.org/10.1038/ng.3718>
- 55 Baubec, T., Colombo, D.F., Wirbelauer, C., Schmidt, J., Burger, L., Krebs, A.R. et al. (2015) Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **520**, 243–247 <https://doi.org/10.1038/nature14176>
- 56 Sendzikaitė, G., Hanna, C.W., Stewart-Morgan, K.R., Ivanova, E. and Kelsey, G. (2019) A DNMT3A PWWP mutation leads to methylation of bivalent chromatin and growth retardation in mice. *Nat. Commun.* **10**, 1884 <https://doi.org/10.1038/s41467-019-09713-w>
- 57 Li, Y.L., Chen, X. and Lu, C. (2021) The interplay between DNA and histone methylation: molecular mechanisms and disease implications. *EMBO Rep.* **22**, e51803 <https://doi.org/10.15252/embr.202051803>
- 58 Schertzer, M.D., Bracer, K.C.A., Starmer, J., Cherney, R.E., Lee, D.M., Salazar, G. et al. (2019) lncRNA-induced spread of polycomb controlled by genome architecture, RNA abundance, and CpG island DNA. *Mol. Cell* **75**, 523–537.e10 <https://doi.org/10.1016/j.molcel.2019.05.028>
- 59 Nagano, T., Mitchell, J.A., Sanz, L.A., Pauler, F.M., Ferguson-Smith, A.C., Feil, R. et al. (2008) The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* **322**, 1717–1720 <https://doi.org/10.1126/science.1163802>
- 60 Lin, S.P., Youngson, N., Takada, S., Seitz, H., Reik, W., Paulsen, M. et al. (2003) Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. *Nat. Genet.* **35**, 97–102 <https://doi.org/10.1038/ng1233>
- 61 Sanli, I., Lalevee, S., Cammisia, M., Perrin, A., Rage, F., Lleres, D. et al. (2018) Meg3 Non-coding RNA expression controls imprinting by preventing transcriptional upregulation in cis. *Cell Rep.* **23**, 337–348 <https://doi.org/10.1016/j.celrep.2018.03.044>
- 62 Zhou, Y., Cheunschou, P., Nakayama, Y., Lawlor, M.W., Zhong, Y., Rice, K.A. et al. (2010) Activation of paternally expressed genes and perinatal death caused by deletion of the Gtl2 gene. *Development* **137**, 2643–2652 <https://doi.org/10.1242/dev.045724>
- 63 Uroda, T., Anastasakou, E., Rossi, A., Teulon, J.M., Pellequer, J.L., Annibale, P. et al. (2019) Conserved pseudoknots in lncRNA MEG3 Are essential for stimulation of the p53 pathway. *Mol. Cell* **75**, 982–995.e9 <https://doi.org/10.1016/j.molcel.2019.07.025>
- 64 Zhou, Y., Zhong, Y., Wang, Y., Zhang, X., Batista, D.L., Gejman, R. et al. (2007) Activation of p53 by MEG3 non-coding RNA. *J. Biol. Chem.* **282**, 24731–24742 <https://doi.org/10.1074/jbc.M702029200>
- 65 Mondal, T., Subhash, S., Vaid, R., Enroth, S., Uday, S., Reinius, B. et al. (2015) MEG3 long noncoding RNA regulates the TGF-beta pathway genes through formation of RNA-DNA triplex structures. *Nat. Commun.* **6**, 7743 <https://doi.org/10.1038/ncomms8743>
- 66 Kuo, C.C., Hanzelmann, S., Cetin, N.S., Frank, S., Zajzon, B., Derks, J.P. et al. (2019) Detection of RNA-DNA binding sites in long noncoding RNAs. *Nucleic Acids Res.* **47**, e32 <https://doi.org/10.1093/nar/gkz037>
- 67 Habib, W.A., Brioude, F., Azzi, S., Rossignol, S., Lingart, A., Sobrier, M.L. et al. (2019) Transcriptional profiling at the DLK1/MEG3 domain explains clinical overlap between imprinting disorders. *Sci. Adv.* **5**, eaau9425 <https://doi.org/10.1126/sciadv.aau9425>
- 68 Stelzer, Y., Sagi, I., Yanuka, O., Eiges, R. and Benvenisty, N. (2014) The noncoding RNA IPW regulates the imprinted DLK1-DIO3 locus in an induced pluripotent stem cell model of prader-Willi syndrome. *Nat. Genet.* **46**, 551–557 <https://doi.org/10.1038/ng.2968>
- 69 Eggemann, T., Davies, J.H., Tauber, M., van den Akker, E., Hokken-Koelega, A., Johansson, G. et al. (2021) Growth restriction and genomic imprinting-overlapping phenotypes support the concept of an imprinting network. *Genes* **12**, 585 <https://doi.org/10.3390/genes12040585>
- 70 Varrault, A., Dantec, C., Le Digarcher, A., Chotard, L., Bilanges, B., Parrinello, H. et al. (2017) Identification of Plagl1/Zac1 binding sites and target genes establishes its role in the regulation of extracellular matrix genes and the imprinted gene network. *Nucleic Acids Res.* **45**, 10466–10480 <https://doi.org/10.1093/nar/gkx672>
- 71 Al Adhami, H., Evano, B., Le Digarcher, A., Gueydan, C., Dubois, E., Parrinello, H. et al. (2015) A systems-level approach to parental genomic imprinting: the imprinted gene network includes extracellular matrix genes and regulates cell cycle exit and differentiation. *Genome Res.* **25**, 353–367 <https://doi.org/10.1101/gr.175919.114>
- 72 Shin, J.Y., Fitzpatrick, G.V. and Higgins, M.J. (2008) Two distinct mechanisms of silencing by the KvDMR1 imprinting control region. *EMBO J.* **27**, 168–178 <https://doi.org/10.1038/sj.emboj.7601960>
- 73 Mohammad, F., Pandey, G.K., Mondal, T., Enroth, S., Redrup, L., Gyllensten, U. et al. (2012) Long noncoding RNA-mediated maintenance of DNA methylation and transcriptional gene silencing. *Development* **139**, 2792–2803 <https://doi.org/10.1242/dev.079566>
- 74 Pandey, R.R., Mondal, T., Mohammad, F., Enroth, S., Redrup, L., Komorowski, J. et al. (2008) Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Mol. Cell* **32**, 232–246 <https://doi.org/10.1016/j.molcel.2008.08.022>

- 75 Terranova, R., Yokobayashi, S., Stadler, M.B., Otte, A.P., van Lohuizen, M., Orkin, S.H. et al. (2008) Polycomb group proteins Ezh2 and Rnf2 direct genomic contraction and imprinted repression in early mouse embryos. *Dev. Cell* **15**, 668–679 <https://doi.org/10.1016/j.devcel.2008.08.015>
- 76 Mancini-DiNardo, D., Steele, S.J.S., Levorse, J.M., Ingram, R.S. and Tilghman, S.M. (2006) Elongation of the Kcnq1ot1 transcript is required for genomic imprinting of neighboring genes. *Genes Dev.* **20**, 1268–1282 <https://doi.org/10.1101/gad.1416906>
- 77 Umlauf, D., Goto, Y., Cao, R., Cerqueira, F., Wagschal, A., Zhang, Y. et al. (2004) Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of polycomb group complexes. *Nat. Genet.* **36**, 1296–1300 <https://doi.org/10.1038/ng1467>
- 78 Joung, J., Engreitz, J.M., Konermann, S., Abudayyeh, O.O., Verdine, V.K., Aguet, F. et al. (2017) Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. *Nature* **548**, 343–346 <https://doi.org/10.1038/nature23451>
- 79 Sanjana, N.E., Wright, J., Zheng, K.J., Shalem, O., Fontanillas, P., Joung, J. et al. (2016) High-resolution interrogation of functional elements in the noncoding genome. *Science* **353**, 1545–1549 <https://doi.org/10.1126/science.aaf7613>
- 80 Kota, S.K., Lleres, D., Bouschet, T., Hirasawa, R., Marchand, A., Begon-Pescia, C. et al. (2014) ICR noncoding RNA expression controls imprinting and DNA replication at the Dlk1-Dio3 domain. *Dev. Cell* **31**, 19–33 <https://doi.org/10.1016/j.devcel.2014.08.009>
- 81 Clark, M.B., Johnston, R.L., Inostroza-Ponta, M., Fox, A.H., Fortini, E., Moscato, P. et al. (2012) Genome-wide analysis of long noncoding RNA stability. *Genome Res.* **22**, 885–898 <https://doi.org/10.1101/gr.131037.111>
- 82 Kaneko, S., Bonasio, R., Saldana-Meyer, R., Yoshida, T., Son, J., Nishino, K. et al. (2014) Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin. *Mol. Cell* **53**, 290–300 <https://doi.org/10.1016/j.molcel.2013.11.012>
- 83 Zhao, J., Ohsumi, T.K., Kung, J.T., Ogawa, Y., Grau, D.J., Sarma, K. et al. (2010) Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol. Cell* **40**, 939–953 <https://doi.org/10.1016/j.molcel.2010.12.011>
- 84 Wagschal, A., Sutherland, H.G., Woodfine, K., Henckel, A., Chebli, K., Schulz, R. et al. (2008) G9a histone methyltransferase contributes to imprinting in the mouse placenta. *Mol. Cell. Biol.* **28**, 1104–1113 <https://doi.org/10.1128/MCB.01111-07>
- 85 Mager, J., Montgomery, N.D., de Villena, F.P. and Magnuson, T. (2003) Genome imprinting regulated by the mouse polycomb group protein Eed. *Nat. Genet.* **33**, 502–507 <https://doi.org/10.1038/ng1125>
- 86 Pintacuda, G., Wei, G., Roustan, C., Kirmizitas, B.A., Solcan, N., Cerase, A. et al. (2017) hnRNPK recruits PCGF3/5-PRC1 to the Xist RNA B-repeat to establish polycomb-mediated chromosomal silencing. *Mol. Cell* **68**, 955–69 e10 <https://doi.org/10.1016/j.molcel.2017.11.013>
- 87 Andergassen, D., Muckenhuber, M., Bammer, P.C., Kulinski, T.M., Theussl, H.C., Shimizu, T. et al. (2019) The Airn lncRNA does not require any DNA elements within its locus to silence distant imprinted genes. *PLoS Genet.* **15**, e1008268 <https://doi.org/10.1371/journal.pgen.1008268>
- 88 Almeida, M., Bowness, J.S. and Brockdorff, N. (2020) The many faces of polycomb regulation by RNA. *Curr. Opin. Genet. Dev.* **61**, 53–61 <https://doi.org/10.1016/j.gde.2020.02.023>
- 89 Loda, A. and Heard, E. (2019) Xist RNA in action: past, present, and future. *PLoS Genet.* **15**, e1008333 <https://doi.org/10.1371/journal.pgen.1008333>
- 90 da Rocha, S.T. and Heard, E. (2017) Novel players in X inactivation: insights into xist-mediated gene silencing and chromosome conformation. *Nat. Struct. Mol. Biol.* **24**, 197–204 <https://doi.org/10.1038/nsmb.3370>
- 91 Nesterova, T.B., Wei, G.F., Coker, H., Pintacuda, G., Bowness, J.S., Zhang, T.Y. et al. (2019) Systematic allelic analysis defines the interplay of key pathways in X chromosome inactivation. *Nat. Commun.* **10**, 3129 <https://doi.org/10.1038/s41467-019-11171-3>
- 92 Bousard, A., Raposo, A.C., Zyllicz, J.J., Picard, C., Pires, V.B., Qi, Y.Y. et al. (2019) The role of xist-mediated polycomb recruitment in the initiation of X-chromosome inactivation. *EMBO Rep.* **20**, e48019 <https://doi.org/10.15252/embr.201948019>
- 93 Colognori, D., Sunwoo, H., Kriz, A.J., Wang, C.Y. and Lee, J.T. (2019) Xist deletion analysis reveals an interdependency between xist RNA and polycomb complexes for spreading along the inactive X. *Mol. Cell* **74**, 101–117 <https://doi.org/10.1016/j.molcel.2019.01.015>
- 94 Kagey, M.H., Newman, J.J., Bilodeau, S., Zhan, Y., Orlando, D.A., van Berkum, N.L. et al. (2010) Mediator and cohesin connect gene expression and chromatin architecture. *Nature* **467**, 430–435 <https://doi.org/10.1038/nature09380>
- 95 Stadler, M.B., Murr, R., Burger, L., Ivanek, R., Lienert, F., Scholer, A. et al. (2011) DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* **480**, 490–495 <https://doi.org/10.1038/nature10716>
- 96 Lleres, D., Moindrot, B., Pathak, R., Piras, V., Matelot, M., Pignard, B. et al. (2019) CTCF modulates allele-specific sub-TAD organization and imprinted gene activity at the mouse Dlk1-Dio3 and Igf2-H19 domains. *Genome Biol.* **20**, 272 <https://doi.org/10.1186/s13059-019-1896-8>
- 97 Wang, H., Maurano, M.T., Qu, H.Z., Varley, K.E., Gertz, J., Pauli, F. et al. (2012) Widespread plasticity in CTCF occupancy linked to DNA methylation. *Genome Res.* **22**, 1680–1688 <https://doi.org/10.1101/gr.136101.111>
- 98 Noordermeer, D. and Feil, R. (2020) Differential 3D chromatin organization and gene activity in genomic imprinting. *Curr. Opin. Genet. Dev.* **61**, 17–24 <https://doi.org/10.1016/j.gde.2020.03.004>
- 99 Eun, B., Sampley, M.L., Good, A.L., Gebert, C.M. and Pfeifer, K. (2013) Promoter cross-talk via a shared enhancer explains paternally biased expression of Nctc1 at the Igf2/H19/Nctc1 imprinted locus. *Nucleic Acids Res.* **41**, 817–826 <https://doi.org/10.1093/nar/gks1182>
- 100 Ideraabdullah, F.Y., Thorvaldsen, J.L., Myers, J.A. and Bartolomei, M.S. (2014) Tissue-specific insulator function at H19/Igf2 revealed by deletions at the imprinting control region. *Hum. Mol. Genet.* **23**, 6246–6259 <https://doi.org/10.1093/hmg/ddu344>
- 101 Kernohan, K.D., Vernimmen, D., Gloor, G.B. and Berube, N.G. (2014) Analysis of neonatal brain lacking ATRX or MeCP2 reveals changes in nucleosome density, CTCF binding and chromatin looping. *Nucleic Acids Res.* **42**, 8356–8368 <https://doi.org/10.1093/nar/gku564>
- 102 Rovina, D., La Vecchia, M., Cortesi, A., Fontana, L., Pesant, M., Maitz, S. et al. (2020) Profound alterations of the chromatin architecture at chromosome 11p15.5 in cells from Beckwith-Wiedemann and Silver-Russell syndromes patients. *Sci. Rep.* **10**, 8275 <https://doi.org/10.1038/s41598-020-65082-1>
- 103 Lin, S., Ferguson-Smith, A.C., Schultz, R.M. and Bartolomei, M.S. (2011) Nonallelic transcriptional roles of CTCF and cohesins at imprinted loci. *Mol. Cell. Biol.* **31**, 3094–3104 <https://doi.org/10.1128/MCB.01449-10>
- 104 Vitali, P., Royo, H., Marty, V., Bortolin-Cavaille, M.L. and Cavaille, J. (2010) Long nuclear-retained non-coding RNAs and allele-specific higher-order chromatin organization at imprinted snoRNA gene arrays. *J. Cell Sci.* **123**, 70–83 <https://doi.org/10.1242/jcs.054957>
- 105 Fitzpatrick, G.V., Pugacheva, E.M., Shin, J.Y., Abdullaev, Z., Yang, Y.W., Khatod, K. et al. (2007) Allele-specific binding of CTCF to the multipartite imprinting control region KvDMR1. *Mol. Cell. Biol.* **27**, 2636–2647 <https://doi.org/10.1128/MCB.02036-06>
- 106 Korostowski, L., Raval, A., Breuer, G. and Engel, N. (2011) Enhancer-driven chromatin interactions during development promote escape from silencing by a long non-coding RNA. *Epigenetics Chromatin* **4**, 21 <https://doi.org/10.1186/1756-8935-4-21>

- 107 Greenberg, M., Teissandier, A., Walter, M., Noordermeer, D. and Bourc'his, D. (2019) Dynamic enhancer partitioning instructs activation of a growth-related gene during exit from naive pluripotency. *eLife* **8**, e44057 <https://doi.org/10.7554/eLife.44057>
- 108 Das, P.P., Hendrix, D.A., Apostolou, E., Buchner, A.H., Carver, M.C., Beyaz, S. et al. (2015) PRC2 is required to maintain expression of the maternal *Gtl2-Rian-Mirg* locus by preventing de novo DNA methylation in mouse embryonic stem cells. *Cell Rep.* **12**, 1456–1470 <https://doi.org/10.1016/j.celrep.2015.07.053>
- 109 Luo, Z., Lin, C., Woodfin, A.R., Bartom, E.T., Gao, X., Smith, E.R. et al. (2016) Regulation of the imprinted *Dlk1-Dio3* locus by allele-specific enhancer activity. *Genes Dev.* **30**, 92–101 <https://doi.org/10.1101/gad.270413.115>
- 110 Habib W, A., Azzi, S., Brioude, F., Steunou, V., Thibaud, N., Das Neves, C. et al. (2014) Extensive investigation of the *IGF2/H19* imprinting control region reveals novel *OCT4/SOX2* binding site defects associated with specific methylation patterns in Beckwith-Wiedemann syndrome. *Hum. Mol. Genet.* **23**, 5763–5773 <https://doi.org/10.1093/hmg/ddu290>
- 111 Demars, J., Shmela, M.E., Khan, A.W., Lee, K.S., Azzi, S., Dehais, P. et al. (2014) Genetic variants within the second intron of the *KCNQ1* gene affect CTCF binding and confer a risk of Beckwith-Wiedemann syndrome upon maternal transmission. *J. Med. Genet.* **51**, 502–511 <https://doi.org/10.1136/jmedgenet-2014-102368>
- 112 Hori, N., Kubo, S., Sakasegawa, T., Sakurai, C. and Hatsuzawa, K. (2021) *OCT3/4*-binding sequence-dependent maintenance of the unmethylated state of CTCF-binding sequences with DNA demethylation and suppression of de novo DNA methylation in the *H19* imprinting control region. *Gene* **769**, 144923 <https://doi.org/10.1016/j.gene.2020.144923>
- 113 Kubo, S., Murata, C., Okamura, H., Sakasegawa, T., Sakurai, C., Hatsuzawa, K. et al. (2020) Oct motif variants in Beckwith-Wiedemann syndrome patients disrupt maintenance of the hypomethylated state of the *H19/IGF2* imprinting control region. *FEBS Lett.* **594**, 1517–1531 <https://doi.org/10.1002/1873-3468.13750>
- 114 Kim, J.D., Kim, H., Ekram, M.B., Yu, S., Faulk, C. and Kim, J. (2011) *Rex1/Zfp42* as an epigenetic regulator for genomic imprinting. *Hum. Mol. Genet.* **20**, 1353–1362 <https://doi.org/10.1093/hmg/ddr017>
- 115 Hori, N., Kubo, S., Sakasegawa, T., Sakurai, C. and Hatsuzawa, K. (2020) *OCT3/4*-binding sequence-dependent maintenance of the unmethylated state of CTCF-binding sequences with DNA demethylation and suppression of de novo DNA methylation in the *H19* imprinting control region. *Gene* **743**, 144 <https://doi.org/10.1016/j.gene.2020.144606>
- 116 Schoenherr, C.J., Levorse, J.M. and Tilghman, S.M. (2003) CTCF maintains differential methylation at the *Igf2/H19* locus. *Nat. Genet.* **33**, 66–69 <https://doi.org/10.1038/ng1057>
- 117 Saldana-Meyer, R., Rodriguez-Hernaez, J., Escobar, T., Nishana, M., Jacome-Lopez, K., Nora, E.P. et al. (2019) RNA interactions are essential for CTCF-mediated genome organization. *Mol. Cell* **76**, 412–422 <https://doi.org/10.1016/j.molcel.2019.08.015>
- 118 Hansen, A.S., Hsieh, T.H.S., Cattoglio, C., Pustova, I., Saldana-Meyer, R., Reinberg, D. et al. (2019) Distinct classes of chromatin loops revealed by deletion of an RNA-binding region in CTCF. *Mol. Cell* **76**, 395–411.e13 <https://doi.org/10.1016/j.molcel.2019.07.039>
- 119 Sherpa, C., Rausch, J.W. and Le Grice, S.F.J. (2018) Structural characterization of maternally expressed gene 3 RNA reveals conserved motifs and potential sites of interaction with polycomb repressive complex 2. *Nucleic Acids Res.* **46**, 10432–10447 <https://doi.org/10.1093/nar/gky722>
- 120 Bond, C.S. and Fox, A.H. (2009) Paraspeckles: nuclear bodies built on long noncoding RNA. *J. Cell Biol.* **186**, 637–644 <https://doi.org/10.1083/jcb.200906113>
- 121 West, J.A., Davis, C.P., Sunwoo, H., Simon, M.D., Sadreyev, R.I., Wang, P.I. et al. (2014) The long noncoding RNAs *NEAT1* and *MALAT1* bind active chromatin sites. *Mol. Cell* **55**, 791–802 <https://doi.org/10.1016/j.molcel.2014.07.012>
- 122 Nakagawa, S., Ip, J.Y., Shioi, G., Tripathi, V., Zong, X.Y., Hirose, T. et al. (2014) *Malat1* is not an essential component of nuclear speckles in mice. *RNA* **18**, 1487–1499 <https://doi.org/10.1261/rna.033217.112>
- 123 Thakur, J. and Henikoff, S. (2020) Architectural RNA in chromatin organization. *Biochem. Soc. Trans.* **48**, 1967–1978 <https://doi.org/10.1042/BST20191226>
- 124 Gribnau, J., Hochedlinger, K., Hata, K., Li, E. and Jaenisch, R. (2003) Asynchronous replication timing of imprinted loci is independent of DNA methylation, but consistent with differential subnuclear localization. *Genes Dev.* **17**, 759–773 <https://doi.org/10.1101/gad.1059603>
- 125 Lahbib-Mansais, Y., Barasc, H., Marti-Marimon, M., Mompert, F., Iannucci, E., Robelin, D. et al. (2016) Expressed alleles of imprinted *IGF2*, *DLK1* and *MEG3* colocalize in 3D-preserved nuclei of porcine fetal cells. *BMC Cell Biol.* **17**, 35 <https://doi.org/10.1186/s12860-016-0113-9>
- 126 Perez-Pinera, P., Kocak, D.D., Vockley, C.M., Adler, A.F., Kabadi, A.M., Polstein, L.R. et al. (2013) RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nat. Methods* **10**, 973–976 <https://doi.org/10.1038/nmeth.2600>
- 127 Wang, H.W., Xu, X.S., Nguyen, C.M., Liu, Y.X., Gao, Y.C., Lin, X.Q. et al. (2018) CRISPR-mediated programmable 3D genome positioning and nuclear organization. *Cell* **175**, 1405–1417 <https://doi.org/10.1016/j.cell.2018.09.013>
- 128 Nativio, R., Sparago, A., Ito, Y., Weksberg, R., Riccio, A. and Murrell, A. (2011) Disruption of genomic neighbourhood at the imprinted *IGF2-H19* locus in Beckwith-Wiedemann syndrome and Silver-Russell syndrome. *Hum. Mol. Genet.* **20**, 1363–1374 <https://doi.org/10.1093/hmg/ddr018>
- 129 Naveh, N.S.S., Deegan, D.F., Huhn, J., Traxler, E., Lan, Y., Weksberg, R. et al. (2021) The role of CTCF in the organization of the centromeric 11p15 imprinted domain interactome. *Nucleic Acids Res.* **49**, 6315–6330 <https://doi.org/10.1093/nar/gkab475>
- 130 Monnier, P., Martinet, C., Pontis, J., Stancheva, I., Ait-Si-Ali, S. and Dandolo, L. (2013) *H19* lncRNA controls gene expression of the imprinted gene network by recruiting MBD1. *Proc. Natl Acad. Sci. U.S.A.* **110**, 20693–8 <https://doi.org/10.1073/pnas.1310201110>
- 131 Terashima, M., Tange, S., Ishimura, A. and Suzuki, T. (2017) *MEG3* long noncoding RNA contributes to the epigenetic regulation of epithelial-mesenchymal transition in lung cancer cell lines. *J. Biol. Chem.* **292**, 82–99 <https://doi.org/10.1074/jbc.M116.750950>