REVIEW

Long noncoding RNA functionality in imprinted domain regulation

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Abstract

Genomic imprinting is a parent-of-origin dependent phenomenon that restricts transcription to predominantly one parental allele. Since the discovery of the first long noncoding RNA (lncRNA), which notably was an imprinted lncRNA, a body of knowledge has demonstrated pivotal roles for imprinted lncRNAs in regulating parental-specific expression of neighboring imprinted genes. In this Review, we will discuss the multiple functionalities attributed to lncRNAs and how they regulate imprinted gene expression. We also raise unresolved questions about imprinted lncRNA function, which may lead to new avenues of investigation.

This Review is dedicated to the memory of Denise Barlow, a giant in the field of genomic imprinting and functional lncRNAs. With her passion for understanding the inner workings of science, her indomitable spirit and her consummate curiosity, Denise blazed a path of scientific investigation that made many seminal contributions to genomic imprinting and the wider field of epigenetic regulation, in addition to inspiring future generations of scientists.

Introduction

The H19 RNA was the first long noncoding RNA (lncRNA) within the mammalian genome to be discovered, followed shortly by the X-inactive-specific transcript (Xist) lncRNA [1,2]. Following pioneering work by Denise Barlow’s as well as other laboratories, imprinted lncRNAs were found to play pivotal roles in regulating parental-specific expression of neighboring imprinted genes (see references marked in bold for Denise Barlow’s contributions to the field). Importantly, identification of imprinted lncRNAs genes and their role in epigenetic regulation has ignited the field of research into lncRNAs. Saliently, thousands of lncRNAs have been found in mammals, with robust research efforts aimed at understanding their functionality and mechanism of action [3–8], including in X-inactivation (see recent review on Xist lncRNA function [9]). In this Review, we will describe the state of knowledge about imprinted lncRNAs, their function, and, in the spirit of Denise Barlow, address as well as pose the many fascinating and outstanding questions remaining to be answered.
Genomic imprinting

Genomic imprinting is an epigenetic mechanism whereby gene regulation depends on the sex of the transmitting parent [10]. Imprinted genes, which are governed by this mechanism, exhibit transcriptional silencing when inherited by one parent and active transcription when transmitted by the other parent. Often, imprinted genes reside together in clusters or imprinted domains (2 to 20 genes). Since expressed and repressed alleles of imprinted genes reside in the same nucleus, they must be governed by cis-regulatory mechanisms. One of the seminal discoveries in genomic imprinting was the identification of genetic elements called gametic differentially methylated regions (gDMRs) that harbor the “imprinting mark” acquired in oocytes and sperm [10]. These marks are subsequently inherited by embryos and offspring, directing parental-specific allelic expression. Deletions of various gDMRs, experimentally or naturally, have validated the gDMR as a master switch, which coordinately regulates the repressed or active state of multiple imprinted genes within an imprinted domain [11–16], thus, earning the name of imprinting center, imprinting control element, or imprinting control region (ICR). Saliently, within an imprinted domain, ICRs possess the “imprinting mark”, rather than genes per se [10]. As a result, Denise Barlow refined terminology in the field, classifying allelic expression as “maternally or paternally expressed or repressed” and not as “maternally or paternally imprinted.” Importantly, while less than 1% of genes in the mammalian genome are regulated by genomic imprinting [17,18], deletion or loss of imprinting at these genes can lead to lethality or cause a broad spectrum of effects, including growth, developmental, metabolic, and neurological phenotypes [See reviews 19, 20]. As such, genomic imprinting has become a central paradigm for understanding how epigenetic mechanisms control expression and repression of genes, gene clusters, and gene pathways involved in development, health, and disease.

Imprinted lncRNAs

Another seminal discovery in genomic imprinting was the identification of lncRNAs within most imprinted domains. As their name implies, lncRNAs are not known to code for proteins. In comparison to small noncoding RNAs, lncRNAs are distinguished by their long length (greater than 200 bp). Imprinted lncRNAs range in length from approximately 1.9 kb to approximately 1,000 kb, leading Barlow and colleagues to classify the very long imprinted lncRNAs as macro noncoding RNAs (ncRNAs) due to their extraordinary length [21]. Imprinted lncRNAs also differ from mRNAs in their capacity to be spliced. Long ncRNAs are spliced transcripts with a low intron to exon ratio that are present in the cytoplasm and/or are present predominantly as unspliced transcripts that are retained in the nucleus [4]. As a result, imprinted lncRNAs have short half-lives compared to mRNAs [22–28]. Another important aspect of imprinted lncRNAs is their intimate relationship to ICRs, with promoters either embedded within or closely positioned to the ICR. Generally, ICR-embedded promoters are maternally methylated, reflecting oocyte-specific acquisition of DNA methylation following transcription elongation and histone H3 lysine 36 trimethylation (H3K36me3) disposition [29–31], while lncRNA promoters near ICRs are paternally methylated, acquiring DNA methylation at intergenic sequences during spermatogenesis (Fig 1). Importantly, imprinted lncRNA expression is dependent on the ICR being in an unmethylated state. Another particular fascinating feature of nuclear lncRNAs is their accumulation as a large volume or “cloud” at the parental domain from which they are expressed. Here, imprinted lncRNAs generally function through cis-acting, long-range effects, silencing alleles of multiple neighboring genes, often bidirectionally, at megabase distances [32]. However, it is still uncertain whether transcription per se is a key feature of lncRNA function, with accumulation reflecting the lncRNAs...
Fig 1. Imprinted domains and their long noncoding RNAs. (A) Airn imprinted domain. (B) Kcnq1ot1 imprinted domain. (C) Nespas imprinted domain. (D) Snhgl14 imprinted domain. (E) H19 imprinted domain. (F) Gtl2 imprinted domain. Note: The domain sizes indicated likely represent minimum lengths of the domains [10]. Imprinted protein-coding genes, which are expressed from one parental allele while the other copy is silent, reside in clusters or imprinted domains. Maternally expressed genes are represented by a red arrow and text, while paternally expressed genes are represented by a blue arrow and text. Nonimprinted genes, where both alleles
are expressed, also localize to imprinted domains (black text). At least one lncRNA (wavy line) is present with a domain (dashed wavy line represents potential extension of a lncRNA transcript). Regulation of imprinting across an imprinted domain is through a cis-acting mechanism that is controlled by an ICR (large circles) and its associated lncRNA gene. Note: Some imprinted genes in these domain exhibit tissue-specific imprinted expression. The DNA methylated state of ICRs is depicted by large black circles, while an unmethylated ICR is denoted by white circles. Smaller circles represent methylation at secondary, somatic DMRs. Small ncRNAs housed within lncRNAs are depicted as diamonds for endogenous small interfering RNAs, arrowheads for microRNAs, and rhomboids for snoRNAs.

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unusual length and processing, or whether lncRNA accumulation is indicative of a physical role for lncRNAs in directing the silencing of neighboring imprinted genes [4,33]. Whichever the case, the mechanisms of repressive function are not yet completely understood. Below, we examine proposed mechanism for lncRNA function, focusing primarily on mouse models of the better-studied imprinted domains. For consistency, we have designated these domains according to the associated lncRNA gene: Airn (antisense of Igfr2; Fig 1A), Kcnq1ot1 (Kcnq1 opposite transcript 1; Fig 1B), Nespas (Nesp antisense; Fig 1C), Snhg14 (Small nucleolar RNA host gene 14, also known as Snrptn, Lnccat, IC-Snurf-Snrpn, Snrpn-Ube3a, U-Ube3aast, Ube3aast and Ube3a-as; Fig 1D), H19 (Fig 1E), and Gtl2 (also known as Meg3; Fig 1F).

Functional output linked to IncRNA transcription

Imprinted IncRNA gene transcription runs interference

How lncRNAs regulate parent-specific expression of multiple neighboring imprinted genes within imprinted domains is of major research interest. The simple act of transcription of a lncRNA is itself proposed to be a mechanism of action called transcriptional interference [34], where transcription of one gene directly suppresses transcription of a second gene in cis [35]. Transcriptional interference has been proposed for the Airn lncRNA gene in silencing Igf2r [36]. The Airn lncRNA gene is 108 to 118 kb in length with variable 3’ length [37], which originates from the Airn ICR within intron 2 of the Igf2r gene [38] (Fig 1A). This sets up a situation of convergent transcription of the paternal Airn lncRNA in an antisense direction across the paternal Igf2r promoter. Premature terminations of the Airn lncRNA before but not after the Igf2r promoter result in paternal Igf2r reactivation, suggesting that transcription through the promoter is the primary mechanism of paternal Igf2r silencing [36,39]. Furthermore, in embryonic stem cells (ESCs), inducible Airn transcription is both necessary and sufficient to silence paternal Igf2r expression [40]. Collectively, these findings established a clear role for transcriptional interference as a mechanism for silencing overlapping genes.

Several models have been proposed for this suppressive mechanism [35]. The first model involves promoter competition, where the stronger promoter, such as Airn, enables better recruitment of the transcription initiation complex than the weaker promoter, such as Igf2r (Fig 2A). Second is the promoter occlusion model, where one transcript, such as the Airn lncRNA, is more robustly and continuously expressed, causing the elongation complex to block the overlapping Igf2r promoter from recruiting the transcription initiation machinery (Fig 2B). The third model proposes dislodgement, where one transcript is faster at engaging the elongation complex, such as the Airn lncRNA, leading to dislocation of the transcription complex at the slower Igf2r promoter (Fig 2C). In addition to transcriptional interference between the paternal Airn and Igf2r alleles, a transcription interference mechanism has been proposed where transcription of the paternal Airn lncRNA would interfere with activators or enhancers within the Airn gene body, thereby preventing initiation and/or up-regulation of paternal, nonoverlapping upstream Slc22a2 and Slc22a3 alleles [32,41]. However, upon deletion of the entire Airn gene, no regulatory elements have been identified that lead to up-regulation of paternal Slc22a2 and Slc22a3 alleles [41]. This indicates that a transcription
Fig 2. Function of long noncoding RNA genes. (A–D) Expression of lncRNAs run transcription interference by directly suppressing transcription of a second gene in cis through multiple modes of action. (A) Promoter competition involves a strong promoter suppressing recruitment of the transcription initiation complex at a weaker promoter. (B) Promoter occlusion arises when transcription of a lncRNA blocks recruitment of the transcription initiation machinery at an overlapping promoter. (C) Dislodgement results when a lncRNA engages the elongation complex faster, leading to dislocation of the transcription complex at another transcript. (D) Collision
involves the crash of two convergent elongation complexes between the lncRNA transcript and a second transcript, leading to premature termination of the latter. (E-H) Long RNAs themselves also have direct functions in gene regulation. (E) Long ncRNAs serve as a host for small ncRNAs which are excised from the lncRNA transcript. (F) Scaffold function refers to lncRNAs acting as the framework for RNA-binding proteins and modifier complexes interactions with DNA and chromatin to carry out their function. (G) lncRNAs can function as guides by aligning with specific DNA sequences though lncRNA and DNA pairing, attracting chromatin modifiers and directing chromatin modification to target sequences. (H) Decoy function involves lncRNAs acting as a sink or sponge to sequester proteins away from a target site.

Another form of transcriptional interference is transcriptional collision, which by definition is limited to convergently transcribed genes. The collision model proposes the crash of two converging elongation complexes, leading to premature termination of one complex (Fig 2D) [35]. This mechanism has been proposed as a silencing mechanism within the Snhg14 imprinted domain [42] (Fig 1D). The Snhg14 lncRNA likely originates from exons upstream of the Snurf-Snrpn gene and extends approximately 1,000 kb through to the Ube3a gene [43,44]. In mouse neurons, the Snhg14 lncRNA gene is paternally expressed, while the Ube3a gene is maternally expressed. Deletions of the Snhg14 promoter or truncation of the paternal Snhg14 lncRNA between Snord115 and Ube3a result in reactivation of the paternally silenced Ube3a allele [42,45,46]. Since both parental Ube3a promoters engage the transcription initiation complex, transcriptional interference via promoter competition, promoter occlusion, and dislodgement are eliminated as mechanisms. Furthermore, the paternally expressed Snhg14 lncRNA extends as far as the intronic region of Ube3a between exons 4 and 5, coinciding with the position of paternal Ube3a termination [42]. Thus, transcriptional collision of RNA polymerases on the paternal allele could account for paternal Ube3a transcription stalling, incomplete elongation, and subsequent degradation of the paternal Ube3a transcript [42]. In support of a collision model, treatment with topoisomerase inhibitors, which block unwinding of DNA during transcription, leads to reactivation of paternal Ube3a, where it is proposed that stalling of elongation complexes prevents Snhg14 lncRNA transcription from extending through to Ube3a [47].

Another imprinted domain, Kcnq1ot1, may also function though a transcription interference mechanism (Fig 1B). Deletion or conditional deletion of the Kcnq1ot1 ICR (also known as KvDMR1); deletion of the Kcnq1ot1 promoter; and premature truncations of the paternal Kcnq1ot1 lncRNA to less than 3 kb all result in loss of paternal Kcnq1ot1 lncRNA expression and reactivation of paternally silent, protein-coding genes across the domain [48,49]. To differentiate between Kcnq1ot1 ncRNA transcription interference and Kcnq1ot1 lncRNA silencing function, posttranscriptional depletion of the Kcnq1ot1 lncRNA via RNA interference was carried out in embryonic and extraembryonic stem cells. No effect was observed on paternal allelic silencing of protein-coding genes in the domain [50]. As RNA interference acts posttranscriptionally, this strengthens a function through transcriptional interference. However, data are conflicting regarding the length of the Kcnq1ot1 lncRNA gene, extending from 83 [37], 91 [51], 121 [32,52], and 471 kb in length [50], suggesting that the Kcnq1ot1 lncRNA is at least 83 kb with a variable 3’ end (Fig 1B) and that transcriptional length of the Kcnq1ot1 lncRNA could reflect differences between cell types and developmental stages. As the Kcnq1ot1 lncRNA originates from the ICR within intron 11 of the Kcnq1 gene [53], a Kcnq1ot1 lncRNA transcript of 90 to 121 kb would not overlap the convergent Kcnq1 promoter, limiting transcriptional interference to a collision model that prematurely terminates the paternal Kcnq1 transcript but not other downstream paternally silenced genes (Fig 2D). On the other hand, longer transcription of the Kcnq1ot1 lncRNA gene up to 471 kb would extend through five
paternally repressed alleles, Kcnq1, Tssc4, Cd81, Ascl2, and Th [50]. All transcriptional interference mechanisms by the Kcnq1ot1 lncRNA may apply to the four convergently transcribed genes, while transcription dislodgement or occlusion would be possible mechanisms in paternal Th silencing (Fig 2A–2D). Finally, a posttranscriptional mechanism involving the RNA interference pathway has been ruled out for Kcnq1ot1 domain regulation following Dicer deletion [51].

Nespas is another imprinted domain that may operate through transcriptional interference (Fig 1C). The Nespas lncRNA gene is approximately 30 kb in length [28] (Fig 1C). The Nespas promoter is embedded within the Nespas ICR (also known as Nespas-Gnasxl gDMR), and when unmethylated on the paternal allele, Nespas is expressed [12]. Nesp and Nespas are convergently transcribed and overlapping sense-antisense genes. Paternal truncation of Nespas to 12.6 kb, after the Nesp gene and the Nesp somatic DMR, elicits no change in allelic expression or methylation of the paternal Nesp allele [28]. However, two additional paternal truncations, prior to the Nesp promoter at less than 100 bp, and just short of Nesp exon 2 at 10 kb, produce a loss of secondary methylation at the paternal Nesp DMR and a gain in H3K4me3 [28,54,55]. This indicates that Nespas lncRNA transcription across the Nesp promoter and DMR is required for a gain of de novo paternal methylation at the Nesp somatic DMR, likely via H3K9me3 deposition [54]. However, only the very premature Nespas truncation at 100 bp reactivated the silent paternal Nesp allele, supporting a mechanism of Nespas lncRNA collision of Nesp transcription between the promoter and exon 2 of the Nesp gene (Fig 2D). Both Nespas truncations also reduced expression of the paternal Gnasxl allele, likely through loss of Nesp DMR methylation [28,54,55]. Finally, only the very premature truncation leads to paternal silencing of Exon1A and paternal reactivation of the Gnas promoters, connecting paternal Nesp silencing with paternal-specific regulation of these genes.

Going forward, comprehensive studies are required to determine the exact mechanisms of specific transcriptional interference models at imprinted domains. How is transcriptional interference specifically mediated at the DNA sequence level? R-loops regulating the elongation complex have been proposed as one mechanism [47]. Do other structures such as G-quadruplexes play a role? Adding a twist (pun intended), how do these models function in the context of chromatin structure, since current models are mostly based on chromatin as a linear array?

**Functional output of posttranscriptional lncRNAs**

**Imprinted lncRNAs as hosts**

One function imprinted lncRNAs serve is as precursor transcripts for small regulatory RNAs (Fig 2E). Intriguingly, imprinted domains present a higher frequency of small ncRNAs compared to rest of the genome [56]. For example, within an intron of the mouse- and rat-specific, paternally expressed Sfmbt2 gene alone, there is a large cluster of approximately 65 to 72 microRNAs (interspersed with B1 retrotransposons and microsatellite repeats) [57,58]. Imprinted lncRNAs themselves also host multiple small ncRNAs, and in some cases, they hold all the small ncRNAs within an imprinted domain [56]. Within the Airn lncRNA resides the retro-Rangap1 (Au76) pseudogene, which harbors hairpin-loop structures that are processed into endogenous small interfering RNAs (485 sequences [59]; Fig 1A). Within the Snhg14 lncRNA lie two large C/D type small nucleolar RNA (snoRNA) clusters, Snord115 (approximately 71 copies) and Snord116 (approximately 136 copies) plus 3 additional snoRNAs (Fig 1D) [60]. Situated within the Nespas and H19 lncRNAs are 2 and 1 microRNAs, respectively [61,62] (Fig 1C and 1E). The approximately 220 kb-continuous, polycistronic Gtl2lt lncRNA (Meg3-Rian-Mirg), which includes the lncRNA-encoding sequences Rtl1as (microRNAs),
Rian (C/D snoRNAs), and Mirg (microRNAs), contains one of the largest collections of small ncRNA clusters (approximately 40–50 microRNAs) within the genome (Fig 1F), followed by the Snhg14 lncRNA [63–66]. Strikingly, an imprinted gene on human chromosome 19 is composed entirely of 46 microRNAs interspersed with Alu sequences, which are encoded by a single (or few) paternally expressed transcript [67]. While these small RNAs may have roles in regulating expression of multiple transcript targets [68], their action is through trans-acting function [69]. For example, the maternally expressed, Rtl1as ncRNA gene within the Glt2 domain, which harbors two maternally expressed microRNAs (miR-127 and miR-136), regulates the dosage of the paternally expressed Rtl1 gene [63,69–71], while miR-329 within the maternally expressed miR-379/miR-544 cluster (plus 6 other predicted miRs) regulates the paternally expressed Dlk1 gene [65,72]. To date, no role has been identified for cis-regulated function in silencing alleles of neighboring imprinted genes [56]. That being said, it has been proposed that the repetitive nature of imprinted small ncRNA clusters may play a role in gene silencing [56]. Future studies will need to address whether the repetitive and 3D structural nature of small ncRNAs embedded within imprinted lncRNAs contribute to allelic silencing of adjacent imprinted genes.

Imprinted lncRNAs as scaffolds

Transcriptional interference can account for suppressive function by lncRNA transcription for overlapping downstream genes. However, how lncRNAs silence upstream genes is still an outstanding question. A common feature of many imprinted lncRNAs is that transcripts localize as foci or “clouds” [4,73]. Given the short half-lives of imprinted lncRNAs [25–27], this would require a mechanism that is spatially driven. Thus, an alternative mechanism proposed for lncRNA function is as a scaffold for recruitment of chromatin modifiers to target promoters for allelic silencing [32,74]. In this capacity, lncRNAs act as a backbone onto which proteins are loaded to carry out their function [75,76] (Fig 2F). Within the paternal Airn domain, the Airn lncRNA accumulates and forms a RNA cloud on the paternal allele, spreading from the site of transcription to cover the upstream region containing the paternal silent Slc22a2 and Slc22a3 genes [77]. In trophoblast stem cells (TSCs), the paternal Airn lncRNA cloud associates with Polycomb repressive complex 1 (PRC1) and repressive modifications [H3K27me3, H4K20me1, H2AK119 monoubiquitination (u1)] [27]. In ESCs, PRC2 interacts with the Airn lncRNA [78]. Thus, it has been suggested that the Airn lncRNA recruits PRC2 and PRC1 to paternal alleles of imprinted genes within the domain, forming a repressive compartment [27,41]. In TSCs and visceral yolk sac endoderm, broad segments of parental-specific H3K27me3 and H2AK119u1 (20 kb sliding windows) stretch across a region that harbors the paternal Slc22a3 and Slc22a2 alleles [22,41]. Intriguingly, H3K27me3 levels at these broad segments appear to be dependent on Airn lncRNA levels, with overexpression increasing and reduced expression decreasing H3K27me3 levels in cis [22]. This points to a direct correlation between Airn lncRNA levels, H3K27me3 enrichment, and, potentially, PRC2 occupancy. In the placenta, the paternal Airn lncRNA may also acts as a scaffold with histone H3K9 methyltransferase, EHMT2 (also known as G9a) at the upstream paternal Slc22a3 promoter region, thereby contributing to paternal Slc22a3 silencing [77]. Premature truncation of the Airn lncRNA results in a loss of Airn lncRNA association with the Slc22a3 promoter, EHMT2 is not recruited, and the paternal Slc22a3 allele is reactivated [77]. Interestingly, the Airn lncRNA is not observed to recruit EHMT2 to the Igf2r promoter, reinforcing distinct mechanisms of action for the Airn lncRNA in paternal allelic silencing of imprinted genes within the domain [36,77]. Finally, HNRNPK (heterogeneous nuclear ribonucleoprotein K), an RNA-binding protein, may facilitate Airn lncRNA scaffold function. HNRNPK interacts with the Airn
lncRNA, with its deletion reducing H3K27me3 across the paternal Slc22a3 and Slc22a2 alleles in TSCs [22]. As a model, HNRNPK may bind the Airn lncRNA and then interact with PRC2 through protein to protein interactions. DNA and/or histone binding domains in PRC2 proteins would mediate H3K27me3 modification across the paternal Slc22a3 and Slc22a2 alleles.

The paternal Kcnq1ot1 lncRNA is also accredited with long-range repressive function of nine imprinted genes within the domain via scaffold functions (Fig 1B) [15,48,49,79]. As part of a lncRNA repressive function, the Kcnq1ot1 lncRNA accumulates on or “coats” the paternal domain [27,50,51,80,81]. During early development, the paternal Kcnq1ot1 imprinted domain harbors a contracted volume marked by PRC2 and PRC1 enrichment and repressive histone modifications (H2AK119u1, H3K9me3, and/or H4H20me1) [27]. In early embryos, stem cells and placentas, this is accompanied by repressive modifications (H3K27me3, H3K9me2/3, and/or H2AK119u1) at paternal alleles of upstream and downstream imprinted gene promoters [23,27,82–85] as well as broad segments of paternal-specific H3K27me3 and H2AK119u1 [22,41]. The Kcnq1ot1 lncRNA also interacts with PRC2 and EHMT2 in placentas [52,86]. These findings have led to the claim that the paternal Kcnq1ot1 lncRNA acts as a scaffold to recruit chromatin modifiers to target upstream and downstream gene promoters in cis, inducing their silencing by PRC2 and PRC1. An additional scaffold function for the Kcnq1ot1 lncRNA has been postulated in postimplantation embryos, where the Kcnq1ot1 lncRNA interacts with DNMT1 at the paternal Cdkn1c and Slc22a18 loci, catalyzing DNA methylation at the paternal secondary Cdkn1c/Slc22a18 DMRs [85]. Finally, the Kcnq1ot1 lncRNA is bound by HNRNPK in TSCs and its deletion reduces H3K27me3 across the paternal Kcnq1ot1 imprinted domain [22]. Thus, Kcnq1ot1 lncRNA scaffold function may facilitate HNRNPK: PRC2:DNA/chromatin interactions, eliciting H3K27me3 bidirectionally across the paternal Kcnq1ot1 imprinted domain.

At the Nespas imprinted domain, it is still unclear whether the Nespas lncRNA could play a role in domain regulation outside of transcription interference mechanisms (Fig 1C). However, two observation support Nespas scaffold function. Firstly, PRC2 has been found to interact with the Nespas lncRNA [78]. Secondly, premature termination of the Nespas lncRNA to less than 100 bp fails to repress the paternal Nesp allele, while truncation of the Nespas lncRNA at 10 kb retains paternal Nesp silencing [28,54,55]. These findings suggest that any scaffold function enabling repressive function of the Nespas lncRNA is contained within the first 10 kb of the Nespas lncRNA. Indeed, a major binding site for the PRC2 complex lies within 10 kb of the Nespas transcription start site [78]. Together, these data suggest a silencing role for the Nespas lncRNA through a scaffold function with PRC2.

Another imprinted domain for which a lncRNA may function as a scaffold is at the Gtl2 imprinted domain (Fig 1F). The Gtl2 lncRNA accumulates at the site of transcription, the maternal Gtl2 ICR (called IG-DMR) and the maternally silent Dlk1 allele [24,87]. In ESCs, Dlk1 is very lowly expressed from both the maternal and paternal alleles [24,87], with both showing EZH2 and H2K27me3 enrichment. The Gtl2 lncRNA directly interacts with PRC2 and JARID2 [78,88]. Upon differentiation into embryoid bodies, cortical neurons or neuronal progenitor cells, Dlk1 attains paternal-specific expression while the maternal allele remains repressed [24,87]. Deletions of the Gtl2 promoter or gene body, as well as Gtl2 depletion fail to repress the maternal Dlk1 allele with reduced EZH2 and H3K27me3 enrichment [24,87]. Finally, Ezh2 deletion also leads to lack of maternal Dlk1 repression, albeit without a change in Gtl2 lncRNA expression [87], suggesting that only scaffold functions of the Gtl2 lncRNA is perturbed by Ezh2 deletion.

Many fascinating questions emerge from the above data. With respect to lncRNA coating of an imprinted domain, is the entire domain coated or only specific regions? If varying lncRNA levels lead to different levels of histone modifications, what are the mechanisms.
controlling IncRNA levels? Does IncRNAs scaffold function lead to IncRNA tethering to chromatin? Alternatively, is tethering an integral component of scaffold function and does it enable spreading of repression across the imprinted domain? Does the IncRNA form multiple scaffolds for numerous independent chromatin modifiers or does the scaffold exist as one large multimeric, macromolecular complex, executing multiple silencing mechanisms simultaneously?

**Imprinted IncRNAs as guides**

As a guide, IncRNAs target specific DNA sequences though IncRNA and DNA pairing, attracting chromatin modifiers that directly modify target sequences (Fig 2G) [75,76,89]. As stated above, imprinted IncRNAs interact with neighboring imprinted genes in cis; the Airn IncRNA with the upstream paternal Slc22a3 promoter; the Kcnq1ot1 IncRNA with paternal promoters of at least six imprinted genes in the domain [52,85]; and the Gtl2 IncRNA with the maternal Dlk1 allele (Fig 1A, 1B and 1F) [24,87]. One mechanism for molecular guide function is through the IncRNA making contact with specific target genes in triplex IncRNA-DNA–DNA formation. In silico predictions in the human have revealed numerous triplex-forming DNA-binding sites within imprinted IncRNAs and target sites at promoters of imprinted genes, including the Airn IncRNA to the IGF2R, Slc22a2, and Slc22a3 promoters; the H19 IncRNA to multiple IGF2 promoters; and the Kcnq1ot1 IncRNA to eight imprinted gene promoters in the domain [90]. In the mouse, multiple DNA-binding sites within the Nespas IncRNA have predicted triplex formation at matched target sites at the Nesp, Gnasxl, and Gnas promoters [90]. For the human Gtl2 imprinted domain, the secondary structure of in vitro- and ex vivo-produced Gtl2 IncRNA (approximately 1,600 nt) has been mapped for guide function [91]. The highly structured, Gtl2 IncRNA possesses at least five RNA structural elements including triplex-forming DNA-binding sites in structural motif I and two PRC2 binding sites for EZH2 and SUZ12 in structural motif III [91]. 3D modeling of the DNA–RNA triplex in motif I and PRC2 and IncRNA in motif III place them in close proximity, suggesting that they act in concert to execute H3K27me3 modification of chromatin. However, as best we can tell, imprinted genes within the Gtl2 imprinted domain have not been assessed for triplex target sites.

The function of IncRNAs serving as guides leaves open many outstanding questions. Are there IncRNA specific sequences or 2D and/or 3D structural requirements for generating triplex formation and for modifier complex interactions? Four PRC2 proteins (EZH2, EED, SUZ12, and AEBP2) have known RNA-binding capacities [92–95] with high specificity to G-quadruplexes [96]. To anchor the RNA to chromatin and then target PRC2 to specific target regions, is RNA–DNA triplex formation required in conjunction with PRC2 binding to RNA? Do other chromatin modifying complexes also bind IncRNAs in a similar fashion? As multiple binding sites within a IncRNA align with different target sites within an imprinted domain, does this necessitate multiple copies of the imprinted IncRNA to target imprinted gene promoters or does the 3D structure of the IncRNA and of chromatin enable a single IncRNA to align with and silence multiple promoters simultaneously? In either case, does loss or interference with one target sequence simultaneously impact the alignment and silencing of other target sites?

**Imprinted IncRNAs as decoys**

Another function attributed to IncRNAs is that of a molecular decoy. Decoy function is described by IncRNAs acting as a sink or sponge to sequester proteins away from a target site (Fig 2H). This could include transcription factors, signaling proteins, splicing factors, and
chromatin modifying proteins or complexes [75,76]. At the Gtl2 imprinted domain, the Gtl2 lncRNA may function as a decoy (Fig 1F). In ES cells, blastocysts and neuronal cells, the maternal Gtl2 ICR harbors an enhancer signature and produces bidirectional enhancer transcripts as well as binds AFF3 (AF4/FMR2 family member 3). AFF3 is a core component of the superelongation complex that facilitates an active chromatin state and promotes transcription of the polycistronic Gtl2l transcript [24,97]. The Gtl2 lncRNA, in turn, may act as a decoy for the Gtl2 ICR, maintaining its active chromatin state [97,98]. This is supported by PRC2 and JARID2 interactions with the Gtl2 lncRNA (which contrasts with weak binding by EZH2 and JARID2 and the lack of SUZ12 and H3K27me3 enrichment at the maternal Gtl2 ICR) [88,99]. Upon maternal Gtl2 promoter deletion, Gtl2 lncRNA expression is abolished, leading to increased EZH2 binding at the Gtl2 ICR [87]. Furthermore, following Ezh2, Eed, and Jarid2 deletion, the maternal Gtl2 ICR is occupied by DNMT3A and DNMT3L and becomes de novo methylated [97,98]. Together, the data suggest that the Gtl2 lncRNA interacts with JARID2 and PRC2 to mitigate EZH2 methyltransferase activity and DNMT3A/3L recruitment at the maternal Gtl2 ICR.

There are many outstanding questions about lncRNA decoy function. As a molecular decoy, lncRNAs may maintain the ICR in an active state and at the same time, the active ICR directs lncRNA expression. What controls this apparent feedback loop? How susceptible is the active ICR to fluctuations in lncRNA levels? Is decoy function mediated via RNA-binding proteins? What is the relationship between weak and strong chromatin modifier interactions with chromatin and lncRNAs? If lncRNAs act as decoys to titrate away positive and negative regulatory proteins, what mechanisms are in place to balance decoy versus other lncRNA function such as scaffold function?

**Imprinted lncRNAs as transregulators**

In addition to imprinted lncRNAs operating in cis to regulate expression of imprinted genes within their respective domains, imprinted lncRNAs also regulate other genes or domains in trans [100]. Loss and gain of H19 lncRNA function alter expression of imprinted genes in the Kcnq1ot1, Airn, Nespas, and Gtl2 domains [101], while loss and overexpression of the human IPW lncRNA (from the SNHG14 lncRNA) lead to changes in GTL2 lncRNA, microRNA, and snoRNAs in the GTL2 domain [102]. One factor that may contribute to lncRNAs trans function is that imprinted domains can colocalize to the same 3D nuclear space [103,104], generating imprinted gene networks [105], where expression of an imprinted gene in one domain influences expression of imprinted genes in other domains. While upstream or downstream regulatory pathways could indirectly account for these linked expression patterns [105], imprinted lncRNAs are likely to be directly associated with interactions between imprinted domains. With respect to nonimprinted genes, both Gtl2 and H19 lncRNAs are involved in the regulation of the signaling transforming growth factor β (TGF-β) pathway [106,107], the cell fate Wnt/β-catenin pathway [108,109], and the tumor suppressor p53 response pathway [110,111]. Overall, the trans-actions of these lncRNAs point to either lncRNA scaffold or guide function. As a scaffold function, the H19 lncRNA interacts with the epigenetic modifier methyl-CpG-binding domain protein 1 (MBD1) and targets MBD1 to imprinted genes in trans, modulating repression through H3K9me3 [112]. In a similar fashion, IPW interacts with EHMT2, conferring H3K9me3 to the Gtl2 ICR [102]. The Gtl2 lncRNA interacts with JARID2 and PRC2, directing H3K27me3 to gene targets outside of the Gtl2 domain [88]. As lncRNA guide function, bioinformatic predictions of H19 lncRNA–DNA triplex binding sites in humans and mice identified promoter target sites at multiple imprinted genes, including Igf2r, Gnas, Dlk1, Peg1, and Cdkn1c [113], while the human GTL2 lncRNA has DNA triplex
binding sites that map to multiple genome-wide target sites in both human and mouse cell lines \[107,114\]. While the mechanisms of H19 lncRNA regulation of gene targets in \textit{trans} remain to be fully elucidated, the Gtl2 lncRNA–DNA triplex structures may act together with its PRC2-binding motifs to modulate gene targets in \textit{trans} \[107\].

As stated above, many outstanding questions regarding lncRNA scaffold and guide function remain to be resolved. More specifically for lncRNA \textit{trans} function, what mechanisms operate for imprinted lncRNAs to find their targets? Is lncRNA \textit{trans} function at imprinting gene networks and other potential chromosomal interactions left to random associations or are there active mechanisms to target imprinted lncRNAs to single gene promoters or to chromosomal networks?

**Emerging functions of posttranscriptional IncRNAs**

**Imprinted IncRNAs as higher-order structures**

IncRNAs are able to fold into secondary and tertiary higher-order 3D structures, confining them to the nucleus and influencing their regulatory function \[115\]. While the dynamics of RNA folding is an important component of human GTL2 lncRNA function \[111\], the recently identified tertiary structure of the GTL2 lncRNA has shed new light on the importance of higher-order tertiary structures \[116\]. Here, highly conserved motifs within the GTL2 lncRNA interact to generate pseudoknot structures, with specific loops imparting a stable 3D structure ("kissing loops") \[116\]. Surprisingly, point mutations to the conserved motifs disrupt formation of the pseudoknot and compromise GTL2 lncRNA function in \textit{trans}, which is independent of the ability of the GTL2 lncRNA to recruit proteins, such as PRC2. To the best of our knowledge, imprinted genes within the Gtl2 imprinted domain have not been assessed for the effects of altered Gtl2 secondary and tertiary structure on their expression. Specific conserved motifs within Kcnq1ot1 lncRNA also contribute to folding and secondary stem-loop structure of the lncRNA \[80\]. Mutations in this motif region perturbs the structure of Kcnq1ot1 lncRNA and leads to a relaxation of paternal allelic silencing of imprinted genes within the domain \[80\]. Whether the Kcnq1ot1 lncRNA forms tertiary structures to carry out its function remains to be determined.

The mechanism of how lncRNA higher-order structure contributes to its ability to regulate expression remains unknown. It has been postulated that spatial organization of lncRNA pilots their function by directing protein positioning and localizing lncRNAs to target sites \[80,116\]. Thus, is the tertiary-folded lncRNA involved in contributing to scaffold or guide function? If structural motifs with lncRNAs must be in close proximity to function, what would be the consequence of inserting a wedge between these structural motifs? Do all imprinted lncRNAs attain a higher-order, 3D structure?

**Imprinted IncRNAs as architectural components**

Even with the above lncRNA functions, the question still remains as to how lncRNAs navigate upstream of the lncRNA gene to ultimately carry out long-range silencing. The answers to this question usually involve explanations of chromatin interaction generating active and repressed chromatin loops. Emerging evidence suggests that lncRNAs may play a role by facilitating chromatin interactions. The classic model of chromatin looping at imprinted domains is the H19 imprinted domain. CCCTC-binding factor (CTCF) and the cohesin complex generate insulator activity and chromatin looping between the maternal H19 ICR and downstream enhancer, which leads to upstream maternal Igf2 silencing (Fig 1E) \[117–119\]. A recent analysis of topological-associated domains (TADs) at the H19 and Gtl2 domains revealed that while both parental domains possess the same CTCF-associated TAD organization, at the subTAD
level, a maternal-specific CTCF site within the unmethylated H19 ICR and Gtl2 promoter bisected the TAD into two smaller subTADs [120]. This physically isolates the active maternal H19 and Gtl2 alleles from the silent maternal Igf2, and Rtl1 and Dio3 alleles, respectively. However, the maternal subTAD that encloses the Gtl2 ICR and promoter, also includes the upstream, silent Dlk1 allele. Thus, the simple scenario of active alleles in one chromosomal loop/subTAD and repressed alleles in another loop/subTAD is unsubstantiated at this domain, requiring additional mechanisms for allelic silencing for the upstream Dlk1 allele. Since the Gtl2 lncRNA prevents activation of the maternal Dlk1 allele, it was proposed that the Gtl2 lncRNA recruits CTCF to the maternal Gtl2 promoter DMR though CTCF’s ability to bind RNA [121,122], focusing Gtl2 lncRNA repressive function within the subTAD to the upstream Dlk1 allele [120].

At the paternal Kcnq1ot1 domain, the Kcnq1ot1 lncRNA may function as an architectural component in chromatin looping. On the paternal allele, the Kcnq1ot1 lncRNA interacts with and coordinates chromatin looping between the paternal Kcnq1ot1 ICR and the Kcnq1 promoter. Loss of Kcnq1ot1 lncRNA through artificial methylation of the paternal Kcnq1ot1 ICR, Kcnq1ot1 promoter deletion, premature termination of the Kcnq1ot1 lncRNA, or post-transcriptional depletion of Kcnq1ot1 lncRNA disrupts chromatin interactions at the paternal Kcnq1ot1 ICR and Kcnq1 promoter in mouse embryonic fibroblasts (MEFs) and neonatal heart [123,124]. In MEFs, this results in paternal allelic reactivation of the downstream Kcnq1 and upstream Cdkn1c genes [123]. These data point to a role for the Kcnq1ot1 lncRNA in directing chromatin interactions that may generate an active loop for the Kcnq1ot1 lncRNA gene while exclusion of paternal upstream and downstream alleles may lead to their repression. This role may be developmentally dependent, as posttranscriptional depletion of Kcnq1ot1 lncRNA did not impact paternal allelic silencing of imprinted genes in ESCs and TSCs [50], and CTCF had little to no interaction with the Kcnq1ot1 lncRNAs in TSCs [22].

At the Airn domain, chromatin-chromatin interactions are observed at the maternal Airn domain between the Airn gene body and the Slc22a3 promoter in visceral yolk sac endoderm [41]. At the paternal domain, the Airn lncRNA disrupts these chromatin interactions, as demonstrated by premature truncation of the Airn lncRNA. Since cohesin complex and CTCF binding had little enrichment at the paternal domain, and CTCF had little to no interaction with the Airn lncRNA in TSCs [22], it is suggested that the paternal domain lacks paternal-specific chromatin loops, and that the Airn lncRNA lacks a role as an architectural component.

Many unresolved questions remain for lncRNAs as architectural components. Does CTCF bind (a subset) of imprinted lncRNAs? If so, do lncRNAs play a role in recruiting or biasing CTCF binding to lncRNA promoters and ICRs? Is there also a role for lncRNAs in inducing chromatin looping? What other lncRNA-protein or lncRNA-chromatin interactions could be involved in chromatin looping? Do imprinted lncRNA have chromatin-templating roles on which chromatin-chromatin interactions are mediated?

Discussion

Since the discovery of the first imprinted lncRNA, significant progress has been made in understanding the functional roles of imprinted lncRNAs in imprinted domain regulation. Compelling evidence described above supports multiple functions for how lncRNAs regulate long-range repressive function. Perhaps the most remarkable feature is that these regulatory mechanisms are not mutually exclusive, with a single lncRNA and its transcription regulating different genes within an imprinted domain. For example, the paternal Airn lncRNA gene and its transcript act through transcriptional interference to silence the paternal Igf2r allele, as a
Fig 3. Multiplicity of function for lncRNA genes and their transcripts at (A) the paternal Airn imprinted domain and (B) the maternal Gtl2 imprinted domain. Note that some lncRNA functions may be part of the same mechanism, for example, scaffold and guide function, incorporating higher-order lncRNA structure as well.

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host for small interfering RNAs, in long-range silencing of upstream paternal alleles through a lncRNA scaffold, and possibly as a guide to direct triplex formation (Fig 3A). By comparison, the maternal \textit{Gtl2} lncRNA functions as a scaffold, a decoy, and a host for microRNAs and snoRNAs. It may also act through higher-order structure and as an architectural component upstream in \textit{cis}, and as a guide in \textit{trans} (Fig 3B). However, it is also possible that there is less multiplicity of lncRNA functions than the above findings indicate. For example, multiple mechanisms, such as lncRNA scaffold and guide function, may be different sides of the same coin, just investigated from different experimental paradigms. Alternatively, more recent, sophisticated, and detailed analyses may be a better reflection of lncRNA functionalities than earlier studies, requiring future experiments to retest past interpretations.

Moving forward, more in-depth, comprehensive studies are required to understand the properties of imprinted lncRNAs as well as to delve deeper into the actual mechanisms pertaining to imprinted lncRNA function and their mode of action. Case in point, to establish the plausibility of posttranscriptional lncRNA functions, it is essential to determine how imprinted lncRNAs are specifically localized to their \textit{cis} and \textit{trans} targets as well as the stoichiometric relationships between lncRNAs, protein partners, and their target sites [125,126]. With regard to emerging imprinted lncRNAs functions, it remains to be seen how broadly applicable they are across imprinted domains. At the very least, these emerging functions emphasize the complexity of imprinted lncRNA regulation and stress the need for continued research into potentially novel lncRNA functions. Furthermore, important questions remain regarding lncRNA regulation of imprinted domains in the context of developmental stage-specific and cell-type specific regulation. Saliently, imprinted lncRNAs and their long-range regulation of imprinted domains will continue to be a rich model for bettering our understanding of the mechanisms of lncRNA functionality and increasing our knowledge of lncRNA-associated pathophysiology in human disease, which ultimately, may lead to medical advances in RNA therapeutics that target long noncoding RNAs.

References

1. Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. Mol Cell Biol. 1990; 10: 28–36. https://doi.org/10.1128/mcb.10.1.28 PMID: 1688465

2. Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, et al. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature. 1991; 349: 38–44. https://doi.org/10.1038/349038a0 PMID: 1985261

3. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. Cold Spring Harbor Lab; 2011; 25: 1915–1927. https://doi.org/10.1101/gad.17446611 PMID: 21890647

4. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 2012; 22: 1775–1789. https://doi.org/10.1101/gr.132159.111 PMID: 2295988

5. ENCODE Project Consortium, Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007; 447: 799–816. https://doi.org/10.1038/nature05874 PMID: 17571346

6. Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, et al. Antisense transcription in the mammalian transcriptome. Science. 2005; 309: 1564–1566. https://doi.org/10.1126/science.1120009 PMID: 16141073

7. Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, et al. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. Nature. 2002; 420: 563–573. https://doi.org/10.1038/nature01266 PMID: 12466851

8. Guttmann M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009; 458: 223–227. https://doi.org/10.1038/nature07672 PMID: 19182780
9. Loda A, Heard E. Xist RNA in action: Past, present, and future. PLoS Genet. 2019; 15: e1008333. https://doi.org/10.1371/journal.pgen.1008333 PMID: 31537017
10. Barlow DP, Bartolomei MS. Genomic imprinting in mammals. Cold Spring Harbor Perspect Biol. Cold Spring Harbor Lab; 2014; 6: a018382. https://doi.org/10.1101/cshperspect.a018382 PMID: 24492710
11. Wutz A, Smrzka OW, Schweifer N, Schellander K, Wagner EF, Barlow DP. Imprinted expression of the Igf2 gene depends on an intronic CpG island. Nature. 1997; 389: 745–749. https://doi.org/10.1038/39631 PMID: 9338788
12. Williamson CM, Turner MD, Ball ST, Nottingham WT, Glenister P, Fray M, et al. Identification of an imprinting control region affecting the expression of all transcripts in the Gnas cluster. Nat Genet. 2006; 38: 350–355. https://doi.org/10.1038/ng1731 PMID: 16462745
13. Thorvaldsen JL, Duran KL, Bartolomei MS. Deletion of the H19 differentially methylated domain results in loss of imprinted expression of H19 and Igf2. Genes Dev. 1998; 12: 3693–3702. https://doi.org/10.1101/gad.12.23.3693 PMID: 9851976
14. Lin S-P, Youngson N, Takada S, Seitz H, Reik W, Paulsen M, et al. Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. Nat Genet. 2003; 35: 97–102. https://doi.org/10.1038/ng1233 PMID: 12937418
15. Fitzpatrick GV, Soloway PD, Higgins MJ. Regional loss of imprinting and growth deficiency in mice with a targeted deletion of KvDMR1. Nat Genet. 2002; 32: 426–431. https://doi.org/10.1038/ng888 PMID: 12410230
16. Bielinska B, Blaydes SM, Bulting K, Yang T, Krajew ska-Walasek M, Horsthemme B, et al. De novo deletions of SNRPN exon 1 in early human and mouse embryos result in a paternal to maternal imprint switch. Nat Genet. 2000; 25: 74–78. https://doi.org/10.1038/75629 PMID: 10802660
17. Morison IM, Ramsay JP, Spencer HG. A census of mammalian imprinting. Trends in genetics: TIG. 2005; 21: 457–465. https://doi.org/10.1016/j.tig.2005.06.008 PMID: 15990197
18. Williamson CM, Blake A, Thomas S, Beechey CV, Hancock J, Cattanach BM, et al. MRC Harwell, Oxfordshire. World Wide Web Site—Mouse Imprinting Data and References - https://www.mousebook.org/mousebook-catalogs/imprinting-resource (2013).
19. Monk D, Mackay DJG, Eggermann T, Maher ER, Riccio A. Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. Nat Rev Genet. Nature Publishing Group; 2019; 20: 235–248. https://doi.org/10.1038/s41576-018-0092-0 PMID: 30647469
20. Tucci V, Isles AR, Kelsey G, Ferguson-Smith AC, Eric Imprinting Group. Genomic imprinting and Physiological Processes in Mammals. Cell. 2019; 176: 952–965. https://doi.org/10.1016/j.cell.2019.01.043 PMID: 30794780
21. Guenzl PM, Barlow DP. Macro IncRNAs: a new layer of cis-regulatory information in the mammalian genome. RNA biology. 2012; 9: 731–741. https://doi.org/10.4161/ma.19985 PMID: 22617879
22. Schertzzer MD, Braceros KCA, Starmer J, Cherney RE, Lee DM, Salazar G, et al. IncRNA-Induced Spread of Polycomb Controlled by Genome Architecture, RNA Abundance, and CpG Island DNA. Mol Cell. 2019; 75: 523–537.e10. https://doi.org/10.1016/j.molcel.2019.05.028 PMID: 31256989
23. Sachani SS, Landschoot LS, Zhang L, White CR, MacDonald WA, Golding MC, et al. Neuronal protein 107, 62 and 153 mediate Kcnq1ot1 imprinted domain regulation in extraembryonic endoderm stem cells. Nat Commun. 2018; 9: 2795. https://doi.org/10.1038/s41467-018-0092-0 PMID: 30622050
24. Kota SK, Lières D, Bouschet T, Hirasawa R, Marchand A, Begon-Pescia C, et al. ICR Noncoding RNA Expression Controls Imprinting and DNA Replication at the Dlk1-Dio3 Domain. Dev Cell. Elsevier; 2014; 31: 19–33. https://doi.org/10.1016/j.devcel.2014.08.009 PMID: 25263792
25. Seidl CIM, Stricker SH, Barlow DP. The imprinted Air ncRNA is an atypical RNApolII transcript that evades splicing and escapes nuclear export. EMBO J. 2006; 25: 3565–3575. https://doi.org/10.1038/sj.emboj.7601245 PMID: 16874305
26. Clark MB, Johnston RL, Inostroza-Ponta M, Fox AH, Fortini E, Moscato P, et al. Genome-wide analysis of long noncoding RNA stability. Genome Res. 2012; 22: 885–898. https://doi.org/10.1101/gr.131037.111 PMID: 22406755
27. Terranova R, Yokobayashi S, Stadler MB, Otte AP, van Lohuizen M, Orkin SH, et al. Polycomb group proteins Ezh2 and Rnf2 direct genomic contraction and imprinted repression in early mouse embryos. Dev Cell. 2008; 15: 668–679. https://doi.org/10.1016/j.devcel.2008.08.015 PMID: 18848501
28. Tibbit CJ, Williamson CM, Mehta S, Ball ST, Chotalia M, Nottingham WT, et al. Antisense Activity across the Nesf Promoter is Required for Nesf-Mediated Silencing in the Imprinted Gnas Cluster. Noncoding RNA. 2015; 1: 246–265. https://doi.org/10.3390/ncrna1030024 PMID: 29861426
29. White CR, MacDonald WA, Mann MRW. Conservation of DNA Methylation Programming Between Mouse and Syrian Gametes and Preimplantation Embryos. Biol Reprod. Society for the Study of
30. MacDonald WA, Mann MRW. Epigenetic regulation of genomic imprinting from germ line to preimplantation. 2014; 81: 126–140. https://doi.org/10.1002/mrd.22220 PMID: 23893518

31. Stewart KR, Veselovska L, Kelsey G. Establishment and functions of DNA methylation in the germline. Epigenomics. 2016; 8: 1399–1413. https://doi.org/10.2217/epi-2016-0056 PMID: 27659720

32. Pauler FM, Barlow DP, Hudson QJ. Mechanisms of long range silencing by imprinted macro non-coding RNAs. Curr Opin Genet Dev. 2012; 22: 283–289. https://doi.org/10.1016/j.gde.2012.02.005 PMID: 22386265

33. Furuno M, Pang KC, Ninomiya N, Fukuda S, Frith MC, Bull C, et al. Clusters of internally primed transcripts reveal novel long noncoding RNAs. PLoS Genet. 2006; 2: e37. https://doi.org/10.1371/journal.pgen.0020037 PMID: 16683026

34. Pauler FM, Koerner MV, Barlow DP. Silencing by imprinted noncoding RNAs: is transcription the answer? Trends in genetics: TIG. 2007; 23: 284–292. https://doi.org/10.1016/j.tig.2007.03.018 PMID: 17445943

35. Hao N, Palmer AC, Dodd IB, Shearwin KE. Directing traffic on DNA-How transcription factors relieve or induce transcriptional interference. Transcription. 2017; 8: 100–106. https://doi.org/10.14161/ma.6.2.7854 PMID: 19229135

36. Latos PA, Pauler FM, Koerner MV, Şenergin HB, Hudson QJ, Stocsits RR, et al. Airn transcriptional overlap, but not its lncRNA products, induces imprinted Igf2r silencing. Science. 2012; 338: 1469–1472. https://doi.org/10.1126/science.1228110 PMID: 23239737

37. Huang R, Jaritz M, Guenzl P, Vlatkovic I, Sommer A, Tamir IM, et al. RNA-Seq strategy to detect the complete coding and non-coding transcriptome including full-length imprinted macro ncRNAs. Huang R, Jaritz M, Guenzl P, Vlatkovic I, Sommer A, Tamir IM, et al., editors. PLoS ONE. 2011; 6: e27288. https://doi.org/10.1371/journal.pone.0027288 PMID: 22102886

38. Latos PA, Barlow DP. Regulation of imprinted expression by macro non-coding RNAs. RNA biology. 2009; 6: 100–106. https://doi.org/10.4161/rna.6.2.7854 PMID: 19229135

39. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature. 2002; 415: 810–813. https://doi.org/10.1038/415810a PMID: 11845212

40. Santoro F, Mayer D, Klement RM, Warczok KE, Stukalov A, Barlow DP, et al. Imprinted Igf2r silencing depends on continuous Airn IncRNA expression and is not restricted to a developmental window. Development. The Company of Biologists Limited; 2013; 140: 1184–1195. https://doi.org/10.1242/dev.088849 PMID: 2344351

41. Andergassen D, Muckenhuber M, Bammer PC, Kulinski TM, Theussl H-C, Shimizu T, et al. The Airn IncRNA does not require any DNA elements within its locus to silence distant imprinted genes. Bartolomei MS, editor. PLoS Genet. 2019; 15: e1008268. https://doi.org/10.1371/journal.pgen.1008268 PMID: 31329595

42. Meng L, Person RE, Huang W, Zhu PJ, Costa-Mattioli M, Beaudet AL. Truncation of Ube3a-ATS unsilences paternal Ube3a and ameliorates behavioral defects in the Angelman syndrome mouse model. Bartolomei MS, editor. PLoS Genet. 2013; 9: e1004039. https://doi.org/10.1371/journal.pgen.1004039 PMID: 24385930

43. Landers M, Bancescu DL, Le Meur E, Rougeulle C, Glaat-Deeley H, Brannan C, et al. Regulation of the large (approximately 1000 kb) imprinted murine Ube3a antisense transcript by alternative exons upstream of Snrpn. Nucleic Acids Res. Oxford University Press; 2004; 32: 3480–3492. https://doi.org/10.1093/nar/gkh670 PMID: 15226413

44. Lewis MW, Vargas-Franco D, Morse DA, Resnick JL. A mouse model of Angelman syndrome imprinting defects. Hum Mol Genet. 2019; 28: 220–229. https://doi.org/10.1093/hmg/ddy345 PMID: 30260400

45. Bressler J, Tsai TF, Wu MY, Tsai SF, Ramirez MA, Armstrong D, et al. The SNRPN promoter is not required for genomic imprinting of the Prader-Willi/Angelman domain in mice. Nat Genet. 2001; 28: 232–240. https://doi.org/10.1038/90067 PMID: 11431693

46. Meng L, Person RE, Beaudet AL. Ube3a-ATS is an atypical RNA polymerase II transcript that represses the paternal expression of Ube3a. Hum Mol Genet. Oxford University Press; 2012; 21: 3001–3012. https://doi.org/10.1093/hmg/ddz130 PMID: 22493002

47. Powell WT, Coulson RL, Gonzales ML, Crary FK, Wong SS, Adams S, et al. R-loop formation at Snord116 mediates topotecan inhibition of Ube3a-antisense and allele-specific chromatin decondensation. Proceedings of the National Academy of Sciences. National Acad Sciences; 2013; 110: 13938–13943. https://doi.org/10.1073/pnas.1309426110 PMID: 23918391
48. Mancini-Dinardo D, Steele SJ, Levorre JM, Ingram RS, Tilghman SM. Elongation of the Kcnq1ot1 transcript is required for genomic imprinting of neighboring genes. Genes Dev. Cold Spring Harbor Lab; 2006; 20: 1268–1282. https://doi.org/10.1101/gad.1416906 PMID: 16702402

49. Shin J-Y, Fitzpatrick GV, Higgins MJ. Two distinct mechanisms of silencing by the KvDMR1 imprinting control region. EMBO J. 2008; 27: 168–178. https://doi.org/10.1038/sj.emboj.7601960 PMID: 18079696

50. Golding MC, Magri LS, Zhang L, Lalone SA, Higgins MJ, Mann MRW. Depletion of Kcnq1ot1 non-coding RNA does not affect imprinting maintenance in stem cells. Development. 2011; 138: 3667–3678. https://doi.org/10.1242/dev.057778 PMID: 21775415

51. Redrup L, Branco MR, Perdeaux ER, Krueger C, Lewis A, Santos F, et al. The long noncoding RNA Kcnq1ot1 organises a lineage-specific nuclear domain for epigenetic gene silencing. Development. 2009; 136: 525–530. https://doi.org/10.1242/dev.031328 PMID: 19147178

52. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, et al. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol Cell. 2008; 32: 232–246. https://doi.org/10.1016/j.molcel.2008.08.022 PMID: 18951091

53. Yatsuki H, Jou K, Higashimoto K, Soejima H, Arai Y, Wang Y, et al. Domain regulation of imprinting in Kip2/Lit1 subdomain on mouse chromosome 7F4/F5: large-scale DNA methylation analysis reveals that DMR-Lit1 is a putative imprinting control region. Genome Research. 2002; 12: 1870–1870. https://doi.org/10.1101/gr.110702 PMID: 12466290

54. Williamson CM, Ball ST, Dawson C, Mehta S, Beechey CV, Fray M, et al. Uncoupling antisense-mediated silencing and DNA methylation in the imprinted Gnas cluster. PLoS Genet. 2011; 7: e1001347. https://doi.org/10.1371/journal.pgen.1001347 PMID: 21455290

55. Mehta S, Williamson CM, Ball S, Tibbit C, Beechey C, Fray M, et al. Transcription driven somatic DNA methylation within the imprinted Gnas cluster. El-Maarri O, editor. PLoS ONE. Public Library of Science; 2015; 10: e0117378. https://doi.org/10.1371/journal.pone.0117378 PMID: 25659103

56. Cai X, Cullen BR. The imprinted H19 noncoding RNA is a primary microRNA precursor. RNA. Cold Spring Harbor Lab; 2007; 13: 313–316. https://doi.org/10.1261/ma.351707 PMID: 17237358

57. Inoue K, Hirose M, Inoue H, Hatanaka Y, Honda A, Hasegawa A, et al. The Rodent-Specific MicroRNA Gene Is Imprinted and Essential for Placental Development. Cell Reports. 2017; 19: 949–956. https://doi.org/10.1016/j.celrep.2017.04.018 PMID: 28467908

58. Watanabe T, Totoki Y, Toyoda A, Kanemori-Miyagawa S, Obata Y, et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. Nature. 2008; 453: 539–543. https://doi.org/10.1038/nature06908 PMID: 18404146

59. Cavaille J, Butting K, Kiefmann M, Lalande M, Brannan CI, Horsthemke B, et al. Identification of brain-specific and imprinted small nuclear RNA genes exhibiting an unusual genomic organization. Proc Natl Acad Sci USA. National Academy of Sciences; 2000; 97: 14311–14316. https://doi.org/10.1073/pnas.250426397 PMID: 11106375

60. Cavaille J, Cailleau J, Kiefmann M, Lalande M, Brannan CI, Horsthemke B, et al. Identification of brain-specific and imprinted small nuclear RNA genes exhibiting an unusual genomic organization. Proc Natl Acad Sci USA. National Academy of Sciences; 2000; 97: 14311–14316. https://doi.org/10.1073/pnas.250426397 PMID: 11106375

61. Cai X, Cullen BR. The imprinted H19 noncoding RNA is a primary microRNA precursor. RNA. Cold Spring Harbor Lab; 2007; 13: 313–316. https://doi.org/10.1261/ma.351707 PMID: 17237358

62. Robson JE, Eaton SA, Underhill P, Williams D, Peters J. MicroRNAs 296 and 298 are imprinted and part of the Gnas/Gnas cluster and miR-296 targets IKBKE and Tmed9. RNA. 2012; 18: 135–144. https://doi.org/10.1261/rna.029561.111 PMID: 22114321

63. Seitz H, Royo H, Borotol M-L, Lin S-P, Ferguson-Smith AC, Cavaille J. A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. Genome Research. 2004; 14: 1741–1748. https://doi.org/10.1101/gr.2743304 PMID: 15310658

64. Labaille S, Marty V, Borotol-Cavaille M-L, Hoareau-Osman M, Pradère J-P, Valet P, et al. The miR-379/miR-410 cluster at the imprinted Dlk1-Dio3 domain controls neonatal metabolic adaptation. EMBO J. 2014; 33: 2216–2230. https://doi.org/10.15252/embj.201387038 PMID: 25124681

65. Gao Y-Q, Chen X, Wang P, Lu L, Zhao W, Chen C, et al. Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance. Proceedings of the National Academy of Sciences. 2015; 112: 13627–13632. https://doi.org/10.1073/pnas.1511448112 PMID: 26487685

66. Kircher M, Bock C, Paulsen M. Structural conservation versus functional divergence of maternally expressed microRNAs in the Dlk1/Gtl2 imprinting region. BMC Genomics. 2008; 9: 346. https://doi.org/10.1186/1471-2164-9-346 PMID: 18651963
67. Bortolin-Cavaillé M-L, Dance M, Weber M, Cavaillé J. C19MC microRNAs are processed from introns of large Pol-II, non-protein-coding transcripts. Nucleic Acids Res. Oxford University Press; 2009; 37: 3464–3473. https://doi.org/10.1093/nar/gkp205 PMID: 19393516

68. Girardot M, Cavaillé J, Feil R. Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease. Epigenetics. 2012; 7: 1341–1348. https://doi.org/10.4161/epi.22884 PMID: 23154539

69. Kumamoto S, Takahashi N, Nomura K, Fujiwara M, Kijoka M, Uno Y, et al. Overexpression of microRNAs from the Gtl2-Rian locus contributes to postnatal death in mice. Hum Mol Genet. 2017; 26: 3653–3662. https://doi.org/10.1093/hmg/ddx223 PMID: 28934383

70. Ito M, Sferruzzi-Perri AN, Edwards CA, Adalsteinsson BT, Allen SE, Loo T-H, et al. A trans-homologue interaction between reciprocally imprinted miR-127 and Rtl1 regulates placenta development. Development. Oxford University Press for The Company of Biologists Limited; 2015; 142: 2425–2430. https://doi.org/10.1242/dev.121996 PMID: 26138477

71. Davis E, Caiment F, Tordoir X, Cavaillé J, Ferguson-Smith A, Cockett N, et al. RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. Curr Biol. 2005; 15: 743–749. https://doi.org/10.1016/j.cub.2005.02.060 PMID: 15854907

72. Hagan JP, O’Neill BL, Stewart CL, Kozlov SV, Croce CM. At least ten genes define the imprinted Dlk1-Dio3 cluster on mouse chromosome 12qF1. Aramayo R, editor. PLoS ONE. Public Library of Science; 2009; 4: e4352. https://doi.org/10.1371/journal.pone.0004352 PMID: 19194500

73. Tsai M-C, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science. 2010; 329: 689–693. https://doi.org/10.1126/science.1192002 PMID: 20616235

74. Koerner MV, Pauler FM, Huang R, Barlow DP. The function of non-coding RNAs in genomic imprinting. Development. The Company of Biologists Limited; 2009; 136: 1771–1783. https://doi.org/10.1242/dev.030403 PMID: 19429783

75. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell. 2011; 43: 904–914. https://doi.org/10.1016/j.molcel.2011.08.018 PMID: 21925379

76. Yang Y, Wen L, Zhu H. Unveiling the hidden function of long non-coding RNA by identifying its major partner-protein. Cell Biosci. BioMed Central; 2015; 5: 59–10. https://doi.org/10.1186/s13578-015-0050-x PMID: 26500759

77. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science. 2008; 322: 1717–1720. https://doi.org/10.1126/science.1163802 PMID: 18988810

78. Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, et al. Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol Cell. 2010; 40: 939–953. https://doi.org/10.1016/j.molcel.2010.12.011 PMID: 21172659

79. Mohammad F, Pandey GK, Mondal T, Enroth S, Redrup L, Gyllensten U, et al. Long noncoding RNA-mediated maintenance of DNA methylation and transcriptional gene silencing. Development. 2012; 139: 2792–2803. https://doi.org/10.1242/dev.079566 PMID: 22721776

80. Mohammad F, Pandey RR, Nagano T, Chakalova L, Mondal T, Fraser P, et al. Kcnq1ot1/Litr1 noncoding RNA mediates transcriptional silencing by targeting to the perinucleolar region. Mol Cell Biol. American Society for Microbiology; 2008; 28: 3713–3728. https://doi.org/10.1128/MCB.02263-07 PMID: 18299392

81. Fedorov AM, Calabrese JM, Mu W, Yee D, Magnuson T. Differentiation-driven nucleolar association of the mouse imprinted Kcnq1 locus. Genetics Society of America; 2012; 2: 1521–1528. https://doi.org/10.1534/g3.112.004226 PMID: 23279875

82. Lewis A, Green K, Dawson C, Redrup L, Huynh KD, Lee JT, et al. Epigenetic dynamics of the Kcnq1 imprinted domain in the early embryo. Development. 2006; 133: 4203–4210. https://doi.org/10.1242/dev.022104 PMID: 17021040

83. Lewis A, Mitsuya K, Umlauf D, Smith P, Dean W, Walter J, et al. Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. Nat Genet. 2004; 36: 1291–1295. https://doi.org/10.1038/ng1468 PMID: 15516931

84. Umlauf D, Goto Y, Cao R, Cerqueira F, Wagshals A, Zhang Y, et al. Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone modification and recruitment of Pol-comb group complexes. Nat Genet. 2004; 36: 1296–1300. https://doi.org/10.1038/ng1467 PMID: 15516932

85. Mohammad F, Mondal T, Guseva N, Pandey GK, Kanduri C. Kcnq1ot1 noncoding RNA mediates transcriptional gene silencing by interacting with Dnmt1. Development. 2010; 137: 2493–2499. https://doi.org/10.1242/dev.048181 PMID: 20573698
86. Wagschal A, Sutherland HG, Woodfine K, Henckel A, Chebli K, Schulz R, et al. G9a histone methyltransferase contributes to imprinting in the mouse placenta. Mol Cell Biol. 2008; 28: 1104–1113. https://doi.org/10.1128/MCB.01111-07 PMID: 18039842

87. Sanli I, Lalevée S, Cammisso M, Perrin A, Rage F, Lières D, et al. Meg3 Non-coding RNA Expression Controls Imprinting by Preventing Transcriptional Upregulation in cis. Cell Reports. 2018; 23: 337–348. https://doi.org/10.1016/j.celrep.2018.03.044 PMID: 29641995

88. Kaneko S, Bonasio R, Saldaña-Meyer R, Yoshida T, Son J, Nishino K, et al. Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin. Mol Cell. 2014; 53: 290–300. https://doi.org/10.1016/j.molcel.2013.11.012 PMID: 24374312

89. Davidovich C, Cech TR. The recruitment of chromatin modifiers by long noncoding RNAs: lessons from PRC2. RNA. 2015; 21: 2007–2022. https://doi.org/10.1261/rna.053918.115 PMID: 26574518

90. He S, Zhang H, Liu H, Zhu H. LongTarget: a tool to predict lncRNA DNA-binding motifs and binding sites via Hoogsteen base-pairing analysis. Bioinformatics. 2015; 31: 178–186. https://doi.org/10.1093/bioinformatics/btu643 PMID: 25262155

91. Sherpa C, Rausch JW, Le Grice SF. Structural characterization of maternaly expressed gene 3 RNA reveals conserved motifs and potential sites of interaction with polycomb repressive complex 2. Nucleic Acids Res. 2018; 46: 10432–10447. https://doi.org/10.1093/nar/gky722 PMID: 30102382

92. Kanhere A, Jenner RG. Noncoding RNA localisation mechanisms in chromatin regulation. Silence. 2012; 3: 2. https://doi.org/10.1186/1758-907X-3-2 PMID: 22292981

93. Cifuentes-Rojas C, Hernandez AJ, Sarma K, Lee JT. Regulatory interactions between RNA and polycomb repressive complex 2. Mol Cell. 2014; 55: 171–185. https://doi.org/10.1016/j.molcel.2014.05.009 PMID: 24882207

94. Kaneko S, Li G, Son J, Xu C-F, Margueron R, Neubert TA, et al. Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. Genes Dev. Cold Spring Harbor Lab; 2010; 24: 2615–2620. https://doi.org/10.1101/gad.1983810 PMID: 21123648

95. Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science. American Association for the Advancement of Science; 2008; 322: 750–756. https://doi.org/10.1126/science.1163045 PMID: 18974356

96. Wang X, Goodrich KJ, Gooding AR, Naeem H, Archer S, Paucek RD, et al. Targeting of Polycomb Repressive Complex 2 to RNA by Short Repeats of Consecutive Guanines. Mol Cell. 2017; 65: 1056–1067.e5. https://doi.org/10.1016/j.molcel.2017.02.003 PMID: 28306504

97. Das PP, Hendrix DA, Apostolou E, Buchner AH, Canver MC, Beyaz S, et al. 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 Reports. 2015; 12: 1456–1470. https://doi.org/10.1016/j.celrep.2015.07.053 PMID: 26299972

98. Luo Z, Lin C, Woodfin AR, Bartom ET, Gao X, Smith ER, et al. Regulation of the imprinted Dlk1-Dio3 locus by allele-specific enhancer activity. Genes Dev. Cold Spring Harbor Lab; 2016; 30: 92–101. https://doi.org/10.1101/gad.270413.115 PMID: 26728555

99. Yen Y-P, Hsieh W-F, Tsai Y-Y, Lu Y-L, Liau ES, Hsu H-C, et al. Dlk1-Dio3 locus-derived lncRNAs perpetuate postmitotic motor neuron cell fate and subtype identity. Elife. 2018; 7: 178. https://doi.org/10.7554/eLife.38080 PMID: 30311912

100. Kornienko AE, Guenzl PM, Barlow DP, Pauer FM. Gene regulation by the act of long non-coding RNA transcription. BMC biology. BioMed Central; 2013; 11: 59. https://doi.org/10.1186/1741-7007-11-59 PMID: 23721193

101. Gabory A, Ripoche M-A, Le Digarcher A, Watrin F, Ziyat A, Forné T, et al. H19 acts as a trans regulator of the imprinted gene network controlling growth in mice. Development. 2009; 136: 3413–3421. https://doi.org/10.1242/dev.036061 PMID: 19762426

102. Stelzer Y, Sagi I, Yanuka O, Eiges R, Benvenisty N. The noncoding RNA IPW regulates the imprinted DLK1-DIO3 locus in an induced pluripotent stem cell model of Prader-Willi syndrome. Nat Genet. 2014; 46: 551–557. https://doi.org/10.1038/ng.2968 PMID: 24816254

103. Lahbib-Mansais Y, Barasch H, Marti-Marimon M, Mompart F, Iannuccelli E, Robelin D, et al. Expressed alleles of imprinted IGF2, DLK1 and MEG3 colocalize in 3D-preserved nuclei of porcine fetal cells. BMC Cell Biol. 2016; 17: 35. https://doi.org/10.1186/s12860-016-0113-9 PMID: 27716032

104. Marti-Marimon M, Vialaeneix N, Voillet V, Yerle-Bouissou M, Lahbib-Mansais Y, Liaubet L. A new approach of gene co-expression network inference reveals significant biological processes involved in porcine muscle development in late gestation. Sci Rep. Nature Publishing Group; 2018; 8: 10150–13. https://doi.org/10.1038/s41598-018-28173-8 PMID: 29977047
105. Varrauti A, Gueydan C, Delalbre A, Bellmann A, Houssami S, Aknin C, et al. Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell. 2006; 11: 711–722. https://doi.org/10.1016/j.devcel.2006.09.003 PMID: 17084352

106. Wang X, Cheng Z, Dai L, Jiang T, Jia L, Jing X, et al. Knockdown of Long Noncoding RNA H19 Represses the Progress of Pulmonary Fibrosis through the Transforming Growth Factor β/Mad3 Pathway by Regulating MicroRNA 140. Mol Cell Biol. American Society for Microbiology Journals; 2019; 39: 431. https://doi.org/10.1128/MCB.00143-19 PMID: 30988156

107. Mondal T, Subhash S, Vaid R, Enroth S, Uday S, Reinius B, et al. MEG3 long noncoding RNA regulates the TGF-β pathway genes through formation of RNA-DNA triplex structures. Nat Commun. Nature Publishing Group; 2015; 6: 7743. https://doi.org/10.1038/ncomms8743 PMID: 26205790

108. Liang W-C, Fu W-M, Wang Y-B, Sun Y-X, Xu L-L, Wong C-W, et al. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep. Nature Publishing Group; 2016; 6: 20121–11. https://doi.org/10.1038/srep20121 PMID: 26853553

109. Gao Y, Lu X. Decreased expression of MEG3 contributes to retinoblastoma progression and affects retinoblastoma cell growth by regulating the activity of Wnt/β-catenin pathway. Tumour Biol. 2016; 37: 1461–1469. https://doi.org/10.1007/s13277-015-4564-y PMID: 26662307

110. Yang F, Bi J, Xue X, Zheng L, Zhi K, Hua J, et al. Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. FEBS J. 2012; 279: 3159–3165. https://doi.org/10.1111/j.1742-4788.2012.08694.x PMID: 22776265

111. Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, et al. Activation of p53 by MEG3 non-coding RNA. J Biol Chem. American Society for Biochemistry and Molecular Biology; 2007; 282: 24731–24742. https://doi.org/10.1074/jbc.M702029200 PMID: 17569660

112. Monnier P, Martinet C, Pontis J, Stancheva I, Ait-Si-Ali S, Dandolo L. H19 IncRNA controls gene expression of the Imprinted Gene Network by recruiting MBD1. Proceedings of the National Academy of Sciences. 2013; 110: 20693–20698. https://doi.org/10.1073/pnas.1310201110 PMID: 24297921

113. Liu H, Shang X, Zhu H. LncRNA/DNA binding analysis reveals losses and gains and lineage specificity of genomic imprinting in mammals. Bioinformatics. 2017; 33: 1431–1436. https://doi.org/10.1093/bioinformatics/btx818 PMID: 28052924

114. Iyer S, Modali SD, Agarwal SK. Long Noncoding RNA MEG3 Is an Epigenetic Determinant of Oncogenic Signaling in Functional Pancreatic Neuroendocrine Tumor Cells. Mol Cell Biol. 2017; 37. https://doi.org/10.1128/MCB.00278-17 PMID: 28847847

115. Fabbri M, Girnita L, Varani G, Calin GA. Decrypting noncoding RNA interactions, structures, and functional networks. Genome Res. 2019; 29: 1377–1388. https://doi.org/10.1101/gr.247239.118 PMID: 31434680

116. Li Y, Qiu W, Zhao Y, Liu H, Guo X, Wang Y, et al. The CTCF-binding site regulates the expression of Igf2r in testis by recruiting MBD1. Biochem Biophys Res Commun. 2018; 501: 1455–1460. https://doi.org/10.1016/j.bbrc.2018.03.178 PMID: 29756569

117. Wang X, Cheng Z, Dai L, Jiang T, Jia L, Jing X, et al. Knockdown of Long Noncoding RNA H19 Represses the Progress of Pulmonary Fibrosis through the Transforming Growth Factor β/Mad3 Pathway by Regulating MicroRNA 140. Mol Cell Biol. American Society for Microbiology Journals; 2019; 39: 431. https://doi.org/10.1128/MCB.00143-19 PMID: 30988156

118. Wang X, Cheng Z, Dai L, Jiang T, Jia L, Jing X, et al. Knockdown of Long Noncoding RNA H19 Represses the Progress of Pulmonary Fibrosis through the Transforming Growth Factor β/Mad3 Pathway by Regulating MicroRNA 140. Mol Cell Biol. American Society for Microbiology Journals; 2019; 39: 431. https://doi.org/10.1128/MCB.00143-19 PMID: 30988156

119. Moscatello D, Moindrot B, Pathak R, Piras V, Matelot M, Pignard B, et al. CTCF modulates allele-specific sub-TAD organization and imprinted gene activity at the mouse Dlk1-Dio3 and Igf2-H19 domains. Mol Cell. 2019; 37. https://doi.org/10.1016/j.molcel.2019.07.025 PMID: 31444106

120. Kurukuti S, Tiwari VK, Tavoosidana G, Pugacheva E, Murrell A, Zhao Z, et al. CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. Proc Natl Acad Sci USA. 2006; 103: 10684–10689. https://doi.org/10.1073/pnas.0600326103 PMID: 16815976

121. Murrell A, Heeson S, Reik W. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. Nat Genet. 2004; 36: 889–893. https://doi.org/10.1038/ng1402 PMID: 15273689

122. Szabo P, Tang SH, Rentendorj A, Pfeifer GP, Mann JR. Maternal-specific footprints at putative CTCF sites in the H19 imprinting control region give evidence for insulator function. Current Biology. 2000; 10: 607–610. https://doi.org/10.1016/s0960-9822(00)00489-9 PMID: 10837224

123. Lièrè D, Moindrot B, Pathak R, Piras V, Matelot M, Pignard B, et al. CTCF modulates allele-specific sub-TAD organization and imprinted gene activity at the mouse Dlk1-Dio3 and Igf2-H19 domains. Mol Cell. 2019; 37. 202. https://doi.org/10.1016/j.molcel.2019.07.025 PMID: 31444106

124. Hansen AS, Hsieh T-HS, Cattoglio C, Pustova I, Saldaña-Meyer R, Reinberg D, et al. Distinct Classes of Chromatin Loops Revealed by Deletion of an RNA-Binding Region in CTCF. Mol Cell Biol. 2019; 39: 431–422.e5. https://doi.org/10.1128/MCB.00143-19 PMID: 31522988

125. Saldaña-Meyer R, Rodríguez-Hernández J, Escobar T, Nishana M, Jácome-López K, Nora EP, et al. RNA Interactions Are Essential for CTCF-Mediated Genome Organization. Mol Cell. 2019; 76: 412–422.e5. https://doi.org/10.1083/jcb.201304152 PMID: 24396636
124. Korostowski L, Sedlak N, Engel N. The Kcnq1ot1 long non-coding RNA affects chromatin conformation and expression of Kcnq1, but does not regulate its imprinting in the developing heart. PLoS Genet. 2012; 8: e1002956. https://doi.org/10.1371/journal.pgen.1002956 PMID: 23028363

125. Long Y, Wang X, Youmans DT, Cech TR. How do lncRNAs regulate transcription? Sci Adv. 2017; 3: eaao2110. https://doi.org/10.1126/sciadv.aao2110 PMID: 28959731

126. Kopp F, Mendell JT. Functional Classification and Experimental Dissection of Long Noncoding RNAs. Cell. 2018; 172: 393–407. https://doi.org/10.1016/j.cell.2018.01.011 PMID: 29373828