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A role for chromatin topology in imprinted domain regulation

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Abbreviations: DMR, differentially methylated region; ESCs, embryonic stem cells; ICR, imprinting control region; LADs, Lamin-associated domains; lncRNA, long noncoding RNA; LOCKs, large organized chromatin K9 modifications; NADs, nucleolar-associated domains; NUPAC, nucleoporin-associated chromatin; RNAPII, RNA polymerase II; S/MARs, scaffold/matrix attachment regions; TADs, topologically-associating domains.

Draft

Abstract

Recently, many advancements in genome-wide chromatin topology and nuclear architecture have unveiled the complex and hidden world of the nucleus, where chromatin is organized into discrete neighbourhoods with coordinated gene expression. This includes the active and inactive X chromosomes. Using X chromosome inactivation as a working model, we utilized publicly available datasets together with a literature review to gain insight into topologically-associated domains, lamin-associated domains, nucleolar-associated domains, scaffold/matrix attachment regions, and nucleoporin-associated chromatin and their role in regulating monoallelic expression. Furthermore, we comprehensively review for the first time the role of chromatin topology and nuclear architecture in the regulation of genomic imprinting. We propose that chromatin topology and nuclear architecture are important regulatory mechanisms for directing gene expression within imprinted domains. Furthermore, we predict that dynamic changes in chromatin topology and nuclear architecture play roles in tissue-specific imprint domain regulation during early development and differentiation.

Key words: chromatin topology, genomic imprinting, long noncoding RNA, nuclear architecture, X chromosome inactivation.

Introduction

The mammalian genome is highly organized within the 3D space of the nucleus. On a macro scale, chromosomes are highly organized into distinct spatial chromosome territories (Cremer and Cremer 2001). Furthermore, contiguous regions along the chromosome are organized by chromatin topologies, which encompass local chromatin-chromatin interactions and their associations with the nucleus. These chromatin structures are facilitated by nuclear architecture, which comprises the scaffold on which chromosome territories and chromatin topologies are formed. Included are features such as the nuclear lamina, nuclear scaffold/matrix and the nucleolus. For example, disruption to nuclear lamina causes alterations to the organization of chromosome territories (Meaburn et al. 2007; Puckelwartz et al. 2011). This is a consequence of lost contacts between distinct regions of chromatin and the nuclear lamina. Thus, associations with nuclear architecture have a direct influence on chromatin topology. The inactive X chromosome and imprinted domains have been observed to localize specifically to the nuclear periphery or perinucleolar region (Zhang et al. 2007; Mohammad et al. 2008; Namekawa et al. 2010), suggesting that nuclear architecture is a component of monoallelic gene regulation.

X chromosome inactivation: a model for chromatin topology/nuclear architecture

X chromosome inactivation is a dosage compensation mechanism whereby one of the two X chromosomes in female mammals is silenced, thereby equalizing X-linked gene expression between females with two X chromosomes and males with a single copy of the X chromosome. In mice, X-inactivation is imprinted during a window in early

embryonic development. Here, X chromosome inactivation is directed by the paternally-expressed X inactive-specific transcript, known as the *Xist* long noncoding RNA (lncRNA) (Hong et al. 1999; Beletskii et al. 2001). At the 2-cell to 8-cell stage, X-inactivation is initiated with *Xist* lncRNA coating the future inactive X in *cis*, leading to the paternal X chromosome becoming inactivated (Huynh and Lee 2003; Patrat et al. 2009). Having said this, initiation of inactivation for some paternal X-linked genes has been reported to occur in a *Xist* lncRNA-independent manner within the early cleavage embryo (Kalantry et al. 2009). Furthermore, the timing of inactivation can occur in a stage-dependent manner on the paternal inactive X, with genes maintaining paternal expression until after the 8-cell, morula and blastocyst stages (Patrat et al. 2009). This suggests that other factors are required for initiating allelic silencing, such as chromatin modifiers (RNF12 ubiquitin ligase), chromatin structure (repeat-rich repressed chromatin), and nuclear substructure (nucleolus) (Shin et al. 2010; Patrat et al. 2009).

At the blastocyst stage, in epiblast cells, imprinted X inactivation is lost, allowing a switch to random X inactivation. Here, the *Xist* lncRNA is expressed at low levels from both the paternal and maternal alleles, as is the antisense *Tsix* lncRNA. The *Tsix* lncRNA regulates the silencing of *Xist* on the future active X chromosome, following which *Xist* becomes upregulated on the designated inactive X (Mak et al. 2004; Lee 2012). This leads to random X chromosome inactivation in the embryo. Extraembryonic cells retain imprinted X-inactivation for the remainder of development (Patrat et al. 2009; Williams et al. 2011).

In terms of initiation and propagation of X chromosome inactivation, the *Xist* lncRNA coats the X chromosome by spreading in *cis* from the X-inactivation center (Lee

2012). This spreading initiates the exclusion of RNAPII, the gradual loss of active histone and the gradual gain of repressive histone modifications, followed by the incorporation of the histone variant macroH2A (Chadwick et al. 2001; Chaumeil et al. 2006; Lee 2009).

The *Xist* lncRNA is proposed to act as a scaffold for the recruitment of epigenetic factors, including the Polycomb repressive complex 2 (PRC2), which catalyzes the deposition of histone 3 lysine 27 trimethylation (H3K27me3) (Plath et al. 2003; Silva et al. 2003). *Xist* spreading is facilitated by chromatin looping that seeds *Xist* at distant sites, enabling the spreading to continue in *cis* (Engreitz et al. 2013; Simon et al. 2013). Once the *Xist* lncRNA spreads along the designated inactive X chromosome, it induces a 3-dimensional (3D) conformational change, forming a silent nuclear compartment, also known as the Barr body (Chaumeil et al. 2006; Smeets et al. 2014). Genes that are repressed occupy the interior of this compartment, while genes that are active reside at the periphery. This *Xist*-mediated silencing of the designated inactive X chromosome points to the importance of chromatin structure to X chromosome inactivation (Nora et al. 2012; Engreitz et al. 2013).

Many aspects of the epigenetic regulation involved in X-inactivation are shared by imprinting domains (Lee 2003). Emerging evidence suggests that chromatin structure is also important for imprinted domain regulation. Here, we will use X-inactivation as a working model to explore linkages between monoallelic expression and features that regulate chromatin structure.

Topologically-associating domains (TADs)

Over the years, various terms have been used to describe the organization of chromatin into domains depending on the method used for demarcation. One emerging

methodology that utilizes chromatin capture revealed that the genome is organized into topologically-associating domains (TADs). TADs represent ~1 Mb chromatin domains, that are enriched for chromatin-chromatin interactions while interactions between neighbouring domains are low (Fig. 1) (Dixon et al. 2012; Nora et al. 2012; Pope et al. 2014). Remarkably, TADs are largely stable across cell types and conserved between species (Dixon et al. 2012; Nora et al. 2012). This suggests that generally TADs are not indicative of a particular transcriptional state, and that higher-order chromatin associations remain stable even with a transcriptional state change. Thus, TADs have been proposed to represent the linear segregation of the genome into regulatory neighbourhoods. By comparison, variations in subtopological regions within TADs and changes in DNA replication timing of a TAD, occur in distinct cell types (Phillips-Cremins et al. 2013; Pope et al. 2014). Generally, subtopological regions within TADs, known as subTADs, are achieved by specific looping of chromatin (Jin et al. 2013; Phillips-Cremins et al. 2013; Rao et al. 2014). There are two primary factors attributed to mediating chromatin looping in mammals; the CCCTC-binding factor protein, CTCF, and the cohesin complex. CTCF is a multifunctional protein known for its role as an insulator protein that defines chromatin boundaries and contributes to chromatin looping (Filippova et al. 1996; Bell et al. 1999; Hark et al. 2000; Phillips and Corces 2009). The cohesin complex forms a ring-like structure capturing and holding chromatin strands together, assisting the formation of chromatin loops (Gruber et al. 2003; Parelho et al. 2008; Wendt et al. 2008; Nasmyth and Haering 2009). While CTCF binding sites are found at TAD boundaries (Dixon et al. 2012), they are mostly enriched within subTADs (Phillips-Cremins et al. 2013). Interestingly, independent siRNA depletion of CTCF or

cohesins has distinct effects on chromatin interactions. CTCF depletion results in decreased interactions between subTADs as well as increased interactions between TADs, albeit not all TADs were disrupted (Zuin et al. 2014). Depletion of cohesin RAD21 also reduces subTAD chromatin interactions, although the TADs themselves are not disrupted. These results support previous findings that chromatin interactions can be independently regulated by CTCF or cohesins, and that 3D chromatin structure is supported by multiple factors (Phillips-Cremins et al. 2013).

The active and inactive X chromosomes are known to have distinct chromatin topologies (Splinter et al. 2011). Recently, the X-inactivation center in mouse embryonic stem cells (ESCs) was identified as being separated into multiple TADs, including one TAD containing the *Xist* transcriptional start site and another containing the *Tsix* transcriptional start site (Nora et al. 2012). A 58 kb-deletion spanning the boundary between these neighbouring TADs resulted in the ectopic associations between, but not merging of, the two TADs, which led to a loss of repression of genes within the *Xist*-containing TAD (Nora et al. 2012). These results demonstrate the importance of TADs and TAD boundaries in maintaining higher-order chromatin structure and, ultimately, transcriptional control.

Lamin-associated domains (LADs)

In general, higher order chromatin organization in the nucleus positions chromatin to the nuclear periphery where genes are generally silenced or in the nuclear interior where genes are typically active. At the nuclear periphery resides the nuclear lamina, an architectural framework that links chromatin to the inner nuclear membrane (Gruenbaum

et al. 2003). Lamin-associated domains (LADs) are regions of chromatin that are bound to Lamin B1, a major component of the nuclear lamina (Fig. 1), and frequently span ~1 Mb in size (Guelen et al. 2008). There is a close association of LADs with chromatin possessing low transcription levels. Mapping of LADs by a DamID-Lamin B1 fusion protein that marked lamin-associated DNA with adenine methylation (which does not naturally occur in eukaryotes), has shown as much as 30% to 40% of the mouse and human genomes constitutively reside within LADs, respectively (Guelen et al. 2008; Wu and Yao 2013). Constitutive LADs are stable across multiple undifferentiated and differentiated cell types. Unlike TADs that remain largely stable across cell and tissue types, there is a second group of LADs that are facultative. Facultative LADs have distinct patterns depending on cell type and represent ~29% of the genome (Meuleman et al. 2013). An example of the latter is the entire *IgH* domain. In fibroblast progenitors, the *IgH* domain resides within a LAD where its genes are repressed, while in B-lineage progenitors, it is localized to the nuclear interior where its genes are active (Zullo et al. 2012). Generally, inactive genes within a dynamic LAD in one cell type will move away from the nuclear periphery to become activated in another cell type (Peric-Hupkes et al. 2010). In total, over 60% of chromatin within the genome can be constituted by LADs (Meuleman et al. 2013).

LADs can also be mapped by an epigenetic signature known as large organized chromatin K9 modifications (LOCKS). LOCKs are ~40-90 kb in size depending on the cell type, and are characterized by the histone modification histone 3 lysine 9 dimethylation, H3K9me₂, which is catalyzed by the histone methyltransferase G9a (Wen et al. 2009). LOCKs overlap in size, distribution and localization of LADs. As such, one

role identified for G9a-dependent H3K9me₂ is to help tether chromatin to the lamina, supporting the nuclear periphery association of LADs (Kind et al. 2013). By comparison, H3K27me₃ is mostly excluded from the interior of LADs, instead demarcated LAD borders (Wen et al. 2009). Thus, H3K27me₃ and LOCKs further define structural components of LADs.

LADs typically have defined borders where lamin association markedly declines, and CTCF binding sites together with CpG islands are enriched (Guelen et al. 2008). Functionally, LADs are required to maintain chromosome territories. Loss of Lamin B1 in human colon cancer cells significantly altered chromosome territories due to chromatin decondensation, which was accompanied by altered chromosomal-wide distributions of histone H3K27me₃ (Camps et al. 2014). In addition to Lamin B1, other components of the nuclear lamina are important for maintaining chromatin association with the nuclear matrix and gene silencing, including Lamin A/C (Harr et al. 2015) and the Lamin B receptor (Polioudaki et al. 2001; Guarda et al. 2009). In a two step process, Lamin A/C followed by Lamin B receptor tethers heterochromatic to the nuclear matrix (Namekawa et al. 2010; Solovei et al. 2013). Loss of both proteins relocates heterochromatin from the nuclear periphery into the nuclear interior.

The Barr Body/inactive X chromosome has long been the quintessential example of 3D compacted chromatin structure, which primarily localizes at the nuclear periphery (Barr and Carr 1962). As stated above, the region spanning the X-inactivation center in ESCs is segmented into TADs (Nora et al. 2012). While these TADs are maintained in ES, neural progenitor and embryonic fibroblast cells, subTADs are altered between these cell types. These subtopological changes are accompanied by a gain in Lamin B1

association within TADs between undifferentiated and differentiated cell types, and with distinct Lamin B1 association within subTADs between different differentiated cell types. These data reinforce the idea that while chromatin topology can remain stable, associations with nuclear architecture can change resulting in transcriptional modulation.

Nucleolar-associated domains (NADs)

Besides the nuclear periphery, chromatin also associates with the nucleolus, in what are defined as nucleolar-associated domains (NADs) (Németh et al. 2010; van Koningsbruggen et al. 2010). The nucleolus is the largest subnuclear structure in the nucleus that is primarily responsible for the generation of rRNAs (Németh and Längst 2011). Recently, genome-wide mapping has revealed that all human chromosomes have NADs located at unique loci as well as at repetitive sequences (van Koningsbruggen et al. 2010; Németh and Längst 2011). NADs share common features of low gene density, low transcriptional activity, high AT-rich sequence elements density, and partially overlap with LADs (van Koningsbruggen et al. 2010). Interestingly, following cell division, chromatin associated with NADs either returns to the nucleolus or to the nuclear periphery. Thus, overlapping NADs and LADs suggest that defined regions shuttle between the nuclear periphery and nucleolus depending on cell type or cell cycle (van Koningsbruggen et al. 2010; Kind et al. 2013).

The inactivated X chromosome, as either the imprinted X or the randomly inactive X, can occupy two nuclear compartments, the nuclear periphery or perinucleolar region (Zhang et al. 2007; Namekawa et al. 2010). In the early preimplantation embryo, the X-inactivation center and repetitive elements along the paternal X contact the

nucleolus (Namekawa et al. 2010), indicating that these regions are NADs. While the *Xist* lncRNA partially spreads along the perinucleolar region, it is not required for the localization of the paternal X to the nucleolus. In differentiating mouse ESCs and mouse embryonic fibroblast cells, the inactive X contacts the nucleolus during mid-to-late S phase, residing within a SMARCA5 (also known as SNF2H) ring that encases the nucleolus (Collins et al. 2002; Zhang et al. 2007). The SWI/SNF-related matrix-associated actin-dependent regulator of chromatin A5, SMARCA5, enables replication through condensed chromatin and progression through replication forks. Deleting *Xist* not only leads to loss of nucleolar association but also maintenance of the heterochromatic inactive X, with reactivation of X-linked genes. These results point to the importance of NADs and the perinucleolar region in maintaining the inactive X nuclear compartment.

Scaffold/matrix attachment regions (S/MARs)

Chromosome territories are maintained in part through interactions with the nuclear scaffold/matrix. Scaffold/matrix attachment regions (S/MARs) are DNA loci that have a high affinity for the nuclear scaffold/matrix after the depletion of histones and other soluble factors (Fig. 1) (Mirkovitch et al. 1984; Cockerill and Garrard 1986). S/MARs are significant in that they provide a direct connection between chromatin and the nuclear scaffold/matrix that is independent from the histone state, indicating an important role in nuclear architecture. In human cell lines, disruption of the nuclear scaffold/matrix releases S/MAR associated proteins and leads to the disorganization of chromosomal territories (Ma et al. 1999). Almost 100 S/MAR-binding proteins have been

identified in model organisms, including lamins, topoisomerases, AT-rich binding proteins and histone deacetylases (Liebich et al. 2002a; Mika and Rost 2005). Not surprisingly, S/MARs have a significant overlap with LADs as Lamin B1 is a S/MAR associated protein. However, S/MARs are not as broadly localized, so it is common for S/MAR clusters to be found within LADs (Linnemann et al. 2009). The DNA sequences of S/MARs are highly polymorphic, without any common motif enrichment, suggesting a dynamic role for S/MAR association with the nuclear scaffold/matrix (Liebich et al. 2002b). That being said, S/MARs are enriched for AT-rich elements but not for CTCF binding sites (Goetze et al. 2005). This suggests that S/MARs are unlikely to primarily serve as anchors for CTCF/cohesin mediated chromatin loop formation but may serve specific roles for chromatin access and loop scaffolding. Recently, human S/MARs have been associated with the recruitment of RNA polymerase II and transcription factors (Arope et al. 2013). This supports a role for S/MARs facilitating an active transcriptional environment, independent of LADs. Interestingly, the S/MAR protein AT-rich sequence-binding protein 1 (SATB1) has been linked to chromatin looping and transcriptional activation at the mouse T-Helper 2 cytokine locus, where SATB1 acts as an anchor for the loop (Cai et al. 2006). CTCF is also required for proper transcriptional activation at this locus, likely supporting secondary chromatin structure or enhancer function, as the SATB1 and CTCF binding sites do not overlap (Ribeiro de Almeida et al. 2009). Human S/MARs are able to protect transgenes from heterochromatic silencing in *Drosophila*, suggesting that S/MARs are able to support insulator function (Namciu et al. 1998). Similar to facultative LADs, S/MARs also exhibit cell type specificity, indicating a dynamic association with cellular identity (Linnemann et al. 2009). The precise function

of S/MARs in relation to gene regulation remains an area of active research. Further investigation will be required to delineate S/MAR classes, their specificity and interacting partners, and their role in regulating repressed and active chromatin.

S/MARs associated proteins have been identified as critical components of X chromosome inactivation. SATB1 is recruited to the site of *Xist* transcription in both mice and humans (Agrelo et al. 2009). Loss of *SATB1* disrupts the initiation of *Xist*-mediated silencing on the inactive X in undifferentiated mouse ESCs (Agrelo et al. 2009). The nuclear scaffold/matrix protein, heterogeneous nuclear ribonucleoprotein U (hnRNPU; also known as scaffold attachment factor A) is also enriched on the inactive X chromosome and specifically binds to the *Xist* lncRNA as well as to DNA on the inactive X chromosome (Helbig and Fackelmayer 2003; Pullirsch et al. 2010). Both DNA and RNA binding domains are required for *Xist* lncRNA localization to the inactive X (Hasegawa et al. 2010). Mouse ESCs lacking hnRNPU fail to produce an inactive X chromosome (Helbig and Fackelmayer 2003; Pullirsch et al. 2010). These data suggest that the S/MARs along the X chromosome and the *Xist* lncRNA interact with nuclear scaffold/matrix proteins to form a silent nuclear compartment at the nuclear matrix.

Transmission electron and super-resolution microscopy have produced higher resolution images that provide further insight into the organization of the inactive X chromosome. The inactive X chromosome is architecturally structured into regions of highly compacted chromatin fibres separated by invaginations or intrachromosomal channels (Rego et al. 2008; Smeets et al. 2014). Interestingly, about 60-100 distinct *Xist* lncRNA foci were found to interdigitate with the condensed chromatin fibres rather than coat or paint the inactive X chromosome. Surprisingly, the *Xist* lncRNA foci mapped at

spatially distinct locations from H2K27me3 (Smeets et al. 2014) as well as PRC1 and PRC2 proteins (Cerase et al. 2014). These results indicate that the *Xist* lncRNA does not directly contact PRC2 when it is recruited for X chromosome inactivation, leaving open the question about how lncRNAs mediate gene repression. Instead, the *Xist* lncRNA foci closely localize with hnRNPU, forming chain-like structures in the intrachromosomal channels. Thus, it was proposed that the *Xist* lncRNA-hnRNPU chains provided structural organization to the Barr body.

Nucleoporin-associated chromatin (NUPAC)

Nuclear pore complexes are highly conserved multimer structures, that are composed of 30 different proteins known as nucleoporins, which permeate the nuclear membrane to facilitate nuclear transport (Fig. 1) (Raices and D'Angelo 2012). However, nuclear transport is not the only function ascribed to nuclear pore complexes. Several studies in yeast and *Drosophila* have identified a role for nuclear pore complexes in gene regulation (Mendjan et al. 2006; Capelson et al. 2010; Vaquerizas et al. 2010; Pascual-Garcia et al. 2014). For example, nucleoporin 98 associates with the methyl-binding domain related protein 2/NLS and with trithorax/MLL H3K4me2 methyltransferase histone modifying complexes at target genes, where they activate gene expression (Capelson et al. 2010; Pascual-Garcia et al. 2014). In rat cardiomyocytes, HDAC4 has been found to associate with nucleoporin 155, dissociating nucleoporin 155-chromatin interactions and abrogating gene expression (Kehat et al. 2011). Non-transport functions may be conserved in higher eukaryotes, as nucleoporins, including nucleoporin 98, associate with chromatin in mammals and have been linked to gene activation (Faria et al.

2006; D'Angelo et al. 2012; Light et al. 2013). In yeast, it has been proposed that interactions between chromatin at nuclear pore complexes and the adjacent nuclear lamina forms chromatin boundaries that protect euchromatic regions from heterochromatin spreading (Ishii et al. 2002). These nucleoporin-chromatin interactions are associated with SWR1 chromatin remodelling complexes (SWI/SNF related ATPase) at sites predicted to act as boundaries between active and repressed chromatin (Dilworth et al. 2005). Human nucleoporin 98 behaves in a similar manner to its yeast homologue nucleoporin 100, altering chromatin structure to an active state (Light et al. 2013). More specifically, induction with interferon gamma results in target gene activation that is accompanied by H3K4me2 and RNAPII enrichment at target gene promoters. Moreover, gene activation is dependent on nucleoporin 98 association, suggesting that nucleoporin 98 and the nuclear pore complex participate in chromatin organization into an active state. While the precise function of nucleoporins and nuclear pore complexes in the organization of chromatin domains remains to be fully realized, they appear to function as chromatin boundaries and facilitate chromatin looping that promotes gene transcription.

As stated above, at the high-super resolution level, the inactive X chromosome is structurally organized into regions of highly compacted chromatin fibres separated by intrachromosomal channels (Rego et al. 2008; Smeets et al. 2014). These intrachromosomal channels weave through the Barr body to the nuclear pores (Rego et al. 2008; Smeets et al. 2014). Located within and at the borders of these channels are *Xist* lncRNA/hnRNPU chains and chromatin marked by RNAPII and H3K4me3 (Smeets et al. 2014). The highest staining for H3K4me3 is at the nuclear pores. These data suggest that genes escaping inactivation are located in the intrachromosomal channels, with active

chromatin loops forming at the nuclear pores.

Chromatin associations with different parts of the nucleus such as the nuclear lamina, nuclear scaffold/matrix or nucleolus, are often considered in isolation. However, all contribute locally to chromatin topography and globally to chromosome territories. From the examples provided using X chromosome inactivation, it is clear that maintenance of chromatin topology is critical for chromosome-wide regulation. Similarly, imprinted domains provide an opportunity to examine chromatin topology and nuclear architecture associations as regulatory mechanisms for parent-specific gene activation and repression. Here, we provide a detailed examination of chromatin structure at imprinted domains using data available from public datasets for nuclear architecture associations and chromatin topology.

Genomic imprinting

Genomic imprinting is an epigenetic phenomenon where gene regulation depends on the sex of the transmitting parent, resulting in allelic silencing when transmitted by one parent and allelic activation when transmitted by the other. The hallmark of most imprinted domains is the presence of a germline differentially methylated region (gDMR). Imprinted gDMRs act as master switch elements, regulating imprinted expression of multiple imprinted genes within a domain. Currently, over 100 genes in mice and humans are known to be regulated by genomic imprinting (Bartolomei and Ferguson-Smith 2011). Most of these genes are located within imprinted domains that are controlled from a single gDMR. Imprinted domains can range in size from single retrotransposed gene sites to large regions spanning up to ~3 Mb. In the mouse, 21 maternal and 3 paternal gDMRs have been identified. Imprinted gDMRs are often referred to as or imprinting control

regions (ICRs) because gDMR deletion results in a domain-wide loss of imprinting (Spahn and Barlow 2003). Parental-specific DNA methylation of ICRs is established in the germline and maintained during preimplantation development (Bartolomei and Ferguson-Smith 2011; MacDonald and Mann 2014). Histone modification are also associated with ICRs, including the repressive marks, H3K9me3, H3K27me3 and H4K20me3, and active modifications, such as H3K4me2/3 (McEwen and Ferguson-Smith 2010). Thus, imprinted domains have parental-specific chromatin states, with the unmethylated gDMR possessing active euchromatic epigenetic modifications while the methylated gDMR bears repressive epigenetic modifications of heterochromatin.

Many imprinted domains also harbour long noncoding RNAs. Imprinted lncRNAs range in length from ~1.6 kb to ~1000 kb, and thus, many imprinted lncRNAs are also classified as macro lncRNAs due to their extraordinary length (Guenzl and Barlow 2012). To date, seven imprinted lncRNAs have been identified that are regulated by imprinted gDMRs and are conserved between mice and humans; *Airn*, *Gtl2*, *H19*, *Kcnq1ot1*, *Nespas*, *Peg13*, and *Ube3a-as* (Brannan et al. 1990; Gray et al. 1999; Smilinich et al. 1999; Lyle et al. 2000; Paulsen et al. 2001; Coombes et al. 2003; Court et al. 2014). Similar to *Xist*, these lncRNAs are thought to have a role in mediating long-range control of imprinted gene transcription.

Overall, an intriguing aspect of imprinted domains is the allelic duality of chromatin states, which results in allelic differences in replication timing and nuclear localization (Gribnau et al. 2003; Mohammad et al. 2008; Redrup et al. 2009)., Many questions remain including how boundaries of imprinted domains are determined, how some genes within imprinted domain escape imprinted expression altogether, what

controls or specifies parental-specific chromatin looping, and how tissue-specific imprinted expression is mediated. It is likely that a better understanding of chromatin structure at imprinting domains will provide insight into these outstanding questions.

TADs at imprinted domains

The *H19* and *Kcnq1ot1* imprinted domains are located adjacent to each other on mouse chromosome 7 (human chromosome 11) (Fig. 2). TAD data that we mined from Dixon et al. 2012 showed that the *Kcnq1ot1* imprinted domain is divided into 3 TADs in both mouse and human ESCs. The neighbouring *H19* domain is in a separate TAD. As an exception to the TAD stability rule, the entire *Kcnq1ot1* imprinted domain and neighbouring *H19* domain become one TAD in differentiated cells from the mouse brain or human IMR-90 fetal lung cells. This suggests a dynamic shift in chromatin topology occurs between these two imprinted domains upon differentiation.

The *Kcnq1ot1* imprinted domain spans a 1 Mb-region in mice and is characterized by a maternally methylated ICR and two paternally methylated secondary somatic DMRs (at and upstream of the *Cdkn1c* gene) (Yatsuki et al. 2002; Bhogal et al. 2004; Lewis et al. 2004; Umlauf et al. 2004; Golding et al. 2011; Mohammad et al. 2012) (Fig. 2). Within the *Kcnq1ot1* imprinted domain, there are 9 maternally expressed protein-coding genes, 5 genes that escape imprinted regulation, and the paternally expressed *Kcnq1ot1* lncRNA (Umlauf et al. 2004; Golding et al. 2011; Mohammad et al. 2012). These genes are classified by their expression during mid-gestation embryo development as inner/ubiquitously imprinted genes (imprinted expression in embryonic and extraembryonic tissues), *Phlda2*, *Slc22a18*, *Cdkn1c*, *Kcnq1* and *Kcnq1ot1*, and outer

extraembryonic-specific imprinted genes, *Osbp15*, *Nap114*, *Tssc4*, *Cd81*, *Ascl2* and *Th* (Lewis et al. 2004; Umlauf et al. 2004; Golding et al. 2011; Mohammad et al. 2012). In mouse ESCs, *Phlda2*, *Cdkn1c* and *Kcnq1* display maternal-specific expression and *Kcnq1ot1* harbours paternal-specific expression. *Slc22a18* display maternal-biased expression, while *Osbp15*, *Tssc4*, *Cd81*, *Ascl2* and *Th* have biallelic expression (Lewis et al. 2004; Umlauf et al. 2004; Lewis et al. 2006; Golding et al. 2011). What is interesting about the TAD arrangement at the *Kcnq1ot1* domain in these ESCs is that the ubiquitously imprinted genes are within one TAD while the extraembryonic-specific imprinted genes are separated into neighbouring TADs (Fig. 2). After differentiation, the *Kcnq1ot1* imprinted domain becomes one TAD, possibly allowing for the coordinate expression of imprinted genes within the *Kcnq1ot1* domain. This raises the possibility that chromatin interactions during early development establish an epigenetic framework at imprinted domains that does not need to be further supported by the same chromatin topology later in development.

The *H19* imprinted domain contains the paternally methylated ICR, three paternally methylated secondary somatic DMRs (DMR1 proximal to *Igf2*, DMR2 within *Igf2*, and a proximal DMR at the *H19* promoter), the maternally expressed *H19* lncRNA gene, the paternally expressed *Igf2as*, and the paternally expressed protein coding genes, *Igf2* and *Ins2* (Rivkin et al. 1993; Constancia et al. 2000; Srivastava et al. 2000; Murrell et al. 2001) (Fig. 2). Distinct chromatin looping is a characteristic feature of the *H19* imprinted domain. On the maternal allele, CTCF binds the unmethylated ICR, acting as an enhancer blocker, that directs maternal expression of *H19* (Lopes et al. 2003; Fedoriw et al. 2004), and maintains the maternal ICR in an unmethylated state (Schoenherr et al.

2003). Together with cohesins, CTCF mediates chromatin looping such that the maternal ICR interacts with DMR1 to isolate *Igf2* and *Ins2* away from an enhancer, and protects somatic DMRs from becoming methylated on the maternal allele (Lopes et al. 2003; Yoon et al. 2007; Li et al. 2008; Nativio et al. 2009). On the paternal allele, DNA methylation prevents CTCF binding the *H19* ICR, leading to paternal expression of *Igf2* and *Ins2* (Leighton et al. 1995; Kurukuti et al. 2006; Yoon et al. 2007).

The *H19* imprinted domain is smaller than the *Kcnq1ot1* imprinted domain, spanning over 100 kb. This may contribute to the *H19* imprinted domain always being represented within a single TAD. The *H19* ICR is critical for mediating parental-specific chromatin loops and enhancer insulator function. Maternal inheritance of a deleted *H19* ICR results in a loss of insulator function and reactivation of the normally silent maternal *Igf2* and *Ins2* alleles, while paternal inheritance of the deleted *H19* ICR results in reactivation of the normally silent paternal *H19* allele and reduction in *Igf2* expression (Leighton et al. 1995; Thorvaldsen et al. 1998). Maternal transmission of CTCF binding site deletions within the *H19* ICR leads to a loss in *H19* expression, as well as activation of the normally silent maternal *Igf2* gene (Engel et al. 2006). However, CTCF depletion did not yield changes to imprinted regulation of the *H19* lncRNA (Lin et al. 2011). Furthermore, depletion of cohesins in mouse embryonic fibroblasts results in increased *Igf2* expression, yet *Igf2* remained paternally expressed, suggesting that additional mechanisms beyond chromatin looping are required for the regulation of allelic expression (Lin et al. 2011). In humans, chromatin topology changes occur at the *H19* imprinted domain in Beckwith-Wiedemann syndrome and Silver-Russell syndrome patients with imprinting errors (Nativio et al. 2011). Beckwith-Wiedemann syndrome

patients with a gain of DNA methylation at the maternal *H19* ICR have altered chromatin looping where the downstream enhancer is brought into proximity with the maternal *Igf2* gene. For Silver-Russell syndrome patients, a loss of DNA methylation at the paternal *H19* ICR generates a chromatin loop that sequestered the downstream enhancer away from the paternal *Igf2* gene and into proximity with *H19* (Nativio et al. 2011). As the *H19* imprinted domain is within the same TAD in both mice and humans, such alterations to chromatin looping are likely the consequence of changes in subTAD interactions.

The *Snrpn* imprinted domain is ~3 Mb in size on mouse chromosome 7 and human chromosome 15, containing a maternally methylated ICR and three maternally methylated secondary somatic DMRs at the *Peg12*, *Mkrn3* and *Ndn* genes (Fig. 3) (Sutcliffe et al. 1994; Barr et al. 1995; Shemer et al. 1997; Johnstone et al. 2006; Xie et al. 2012). Within the *Snrpn* domain, there are 5 paternally expressed genes, *Peg12* (*Frat3*), *Mkrn3*, *Magel2*, *Ndn* and *Snrpn*, 4 distinct paternally expressed small nucleolar RNAs (including *Snord107*, *Snord46*, *Snord116* and *Snord115*), the paternally expressed long noncoding antisense transcript (LCNAT) of which a subset produce the *Ube3a-as* (*Ube3a-ATS*) lncRNA, and the maternally expressed genes *Ube3a* and *Atp10a* (conflicting reports regarding imprint status) (Kashiwagi et al. 2003; Kayashima et al. 2003; Landers et al. 2004; Powell et al. 2013; Peters 2014). In data that we mined from mouse ESCs (Dixon et al. 2012), the *Snrpn* imprinted domain is divided into three TADs with their boundaries falling between *Mkrn3* and *Magel2*, and within the *Snord116* genes (Fig. 3). In the cortex, there are 2 TADs, which places the downstream paternally expressed imprinted genes in a different TAD from the maternally expressed *Ube3a* and *Atp10a* genes, while the *Peg12-Ndn* gene cluster falls into a inter-TAD region. This may

account for *Snrpn* being a ubiquitously imprinted gene (Barr et al. 1995), while *Ube3a* displays tissue-specific imprinted expression; *Ube3a* is biallelically expressed in all tissues except in brain where there is maternal-specific *Ube3a* expression (Rougeulle et al. 1997; Vu and Hoffman 1997).

In both mice and human brain cells, neuronal-specific paternal *Ube3a-as* transcription has been linked to silencing of the downstream *Ube3a* gene (Chamberlain and Brannan 2001; Johnstone et al. 2006; Meng et al. 2012), although it has also been reported that *Ube3a* silencing can occur independently of *Ube3a-as* in a subset of neurons (Le Meur et al. 2005). Depletion of the *Ube3a-as* lncRNA via very large and small *Snrpn/Ube3a-as* promoter deletions or through truncation of the *Ube3a-as* transcript results in reactivation of the paternally-silenced *Ube3a* allele in neurons differentiated from ESCs and in the mouse brain in a dose-dependent manner (Bressler et al. 2001; Chamberlain and Brannan 2001; Dubose et al. 2011; Meng et al. 2012; Meng et al. 2013). Recent evidence suggests that *Ube3a-as* disrupts *Ube3a* transcription through a mechanism of transcriptional collision (Meng et al. 2013). In both mouse ESCs and cortex, *Ube3a* resides within a different TAD than the *Ube3a-as* transcriptional start site. The distinct TADs in these cell types suggest that *Ube3a-as* transcription does not initiate a chromatin loop between the *Snrpn* ICR and *Ube3a*, which is consistent with the proposed mechanism of transcriptional collision-based silencing. Furthermore, in human lymphoblastoid cells, *SNRPN* and the non-imprinted *GABRB3* gene are in closer proximity than with *UBE3A*, which is more distantly located (Kawamura et al. 2012). While it is important to note that *UBE3A* is biallelically expressed in this cell type, it does support a general model where *UBE3A* remains distant from the *SNRPN* ICR.

Interestingly, while other genes in the domain remain unchanged, *Ube3a-as* truncation produces increased *Ndn* expression in the mouse brain (Meng et al. 2013). This is consistent with the coordinated expression of *Ube3a-as* and *Ndn* in postmitotic neurons (Le Meur et al. 2005). The *Ndn* and *Ube3a-as* transcriptional start sites reside within the same TAD in ESCs, while *Ndn* lies at the TAD boundary in the cortex (Fig. 3), suggesting that the *Ube3a-as* lcrRNA or its transcription may facilitate chromatin looping between the *Snrpn* ICR and the proximal *Ndn* gene.

LADs at imprinted domains

Genome-wide data of LADs that we mined from Peric-Hupkes et al. 2010 and Meuleman et al. 2013 showed that the *Kncq1ot1* and *H19* imprinted domains are largely devoid of LADs in the cell types investigated. That being said, there are two small LADs, one of which is a constitutive LAD while the other is a facultative LAD. The ~20 kb-constitutive LAD is located in the *Kncq1ot1* imprinted domain, residing within intron 10 of the *Kncq1* gene (Fig. 2). This LAD is present in mouse ESCs, embryonic fibroblasts, and neuronal cells. A second larger LAD, ~170 kb in size, is situated between the *Kncq1ot1* and *H19* imprinted domains in embryonic fibroblasts and neuronal cells (Fig. 2). Additionally, LOCKs data that we mined from Wen et al. 2009 placed LOCKs within the dynamic LAD in differentiated mouse ESCs, as well as brain and liver tissues. No other LOCKs were present within the *Kncq1ot1* and *H19* imprinted domains. As stated above, G9a-dependent H3K9me2 helps tether chromatin to the lamina (Kind et al. 2013). Thus, it is tempting to postulate that in differentiated cells, the facultative LAD/LOCKs

function are to maintain the boundary between the *Kncq1ot1* and *H19* imprinted domains once they merge into one TAD.

In contrast to the embryonic liver, in the placenta, the *Kcnq1ot1 lncRNA* transcript interact with the polycomb complex PRC2 and histone methyltransferase G9a (Pandey et al. 2008). Furthermore, the *Kcnq1ot1 lncRNA* interacts with the *Osbpl5*, *Cdkn1c*, *Kcnq1*, *Cd81* and *Ascl2* promoters, providing a functional role in directing the repressive epigenetic marks H3K27me3 and H3K9me2 to paternally silent genes in the placenta (Pandey et al. 2008). As the presence of G9a and H3K9me2 are features of LOCKs and LADs, it is possible that LOCKs and LADs may be more widely distributed across the *Kcnq1ot1* imprinted domain in the placenta, supporting extraembryonic-specific imprinted expression of genes within the domain, although this remains to be investigated.

Falling within the dynamic LAD between the *Kncq1ot1* and *H19* imprinted domains is a targeted insertion of a transgenic GFP reporter. This reporter is situated 2.6 kb downstream of *Ins2*, leaving open the question of whether the transgene resides within the *H19* or *Kcnq1ot1* imprinted domain (Fig. 2) (Jones and Lefebvre 2009; Jones et al. 2011). While considerably closer to the *H19* imprinted domain, maternal-specific expression of the GFP reporter in postimplantation embryos is instead driven by maternal methylation at the *Kncq1ot1* ICR and *Kncq1ot1* lncRNA expression (Jones and Lefebvre 2009; Jones et al. 2011). Interestingly, the GFP reporter is expressed from both parental alleles in mouse ESCs, with maternal-specific silencing being acquired in differentiated ESCs (Jones et al. 2011). In undifferentiated ESCs, the GFP reporter lies within the *H19* imprinted domain TAD. The merging of *Kncq1ot1* and *H19* imprinted domains TADs into one large combined TAD in differentiated cell types, represented by the cortex, may

account for the acquisition of GFP reporter imprinted expression. Together, these findings suggest that merging of TADs may facilitate *Kncq1ot1* lncRNA chromatin-chromatin interactions, bringing the distant GFP reporter under *Kncq1ot1* domain regulation. Recent analysis of intergenic lncRNAs suggests that most interact with fewer than 30 chromatin sites, primarily within the same TAD, leading to the idea that lncRNAs may contribute to 3D organization through chromatin-chromatin interactions within TADs as well as insulation of TADs by maintaining chromatin structural associations (Jin et al. 2013; Ma et al. 2015). Further investigation will be required to determine whether imprinted lncRNAs play a similar role for chromatin interactions within imprinted domain TADs.

Unlike the *H19* and *Kncq1ot1* imprinted domains, the *Snrpn* imprinted domain has a wider distribution of constitutive LADs (Fig. 3), suggesting a strong domain association with the nuclear matrix. Interestingly, in mouse ESCs, there is a 20 kb-intervening region between constitutive LADs that contains the *Snrpn* ICR and most of the *Snrpn* gene. In differentiated cell types, embryonic fibroblasts, neural progenitors, and astrocytes, this region is a facultative LAD. This suggests that the *Snrpn* ICR may have dynamic associations with the nuclear matrix during development, which may influence epigenetic regulation within the *Snrpn* imprinted domain. There are two regions within the *Snrpn* imprinted domain that are outside of the constitutive and facultative LADs, residing near the ends of the domain. The first region spans ~90 kb and includes the paternally-expressed *Peg12*, *Mkrn3*, and *Magel2* genes and coincides with TAD-free *Peg12-Ndn* region. The second region extends ~120 kb and includes the maternally-expressed *Ube3a* gene. In the mouse cortex, this region closely aligns with the TAD

boundary that separates the neighbouring TADs within the *Snrpn* imprinted domain, perhaps demarcating regions with paternally versus maternally expressed genes. The close association between LAD and TAD boundaries in neuronal tissues raises the question of how long-range *Ube3a* imprinted regulation is mediated. Interestingly, LOCKs were primarily situated within the constitutive LADs between *Snrpn* and *Ube3a* in mouse ESCs, and within the *Snrpn-Ube3a* and *Ube3a-Atp10c* constitutive LADs in differentiated ESCs and the brain. Notably, a LOCK-free zone occurs over the *Ube3a* gene (Fig. 3). Furthermore, in mouse differentiated ESCs and liver, LOCKs extend across the domain within constitutive and facultative LADs. A similar pattern occurs in the human placenta, where *MKRN3*, *NDN* and *SNRPN* are located within LOCKs, while *UBE3A* is devoid of LOCKs (Wen et al. 2009). Further studies are required to determine specific chromatin features that demarcate LAD and LOCK free zones, and whether LOCKs and LADs mark chromatin topologies within TADs.

NADs at imprinted domains

Currently, limited information is available for the role and location of NADs at imprinted domains. Data mined from a human fibrosarcoma cell line provides maps of NADs at a 250 kb-resolution (van Koningsbruggen et al. 2010). While it is not possible to detect discrete NADs at this resolution, the *SNRPN* imprinted domain was observed to have a high ratio of NADs while the *H19* and *KNQ1OT1* imprinted domains had intermediate NAD levels compared to domain flanking regions. Data that we mined from Németh et al. 2010 showed that in HeLa cells, the *SNRPN* imprinted domain has two large NADs encompassing the entire domain, with the intervening region between the

NADs enclosing the *UBE3A* gene. Thus, this is reminiscent of the LAD- and LOCK-free zone that overlaps the *Ube3a* gene in mouse. By comparison, coordinates from HeLa cells showed an absence of NADs at the *H19* and *KNCQ1OT1* imprinted domains (Németh et al. 2010). This may be specific to this cell type or may be cell cycle-specific, since the *Kncqlot1* imprinted domain localizes to the nucleolar region in mouse placental and embryonic liver cells (Pandey et al. 2008), and in human trophoblast choriocarcinoma cells during S-phase (Mohammad et al. 2008). This is reminiscent of X-inactivation center and repetitive elements along the paternal X that contact the nucleolus (Namekawa et al. 2010), and the *Xist* lncRNA which associates with the nucleolar region during S-phase of the cell cycle (Collins et al. 2002; Zhang et al. 2007). Thus, further studies are required to map NADs within imprinted domains in other cell types, with an emphasis on those associations that occur during S-phase.

S/MARs at imprinted domains

Multiple S/MARs have been detected across the *Kcnqlot1*, *H19* and *Snrpn* imprinted domains with some conservation between mouse and humans (Greally et al. 1999; Kagotani et al. 2002; Weber et al. 2003; Purbowasito et al. 2004). A total of 52 S/MARs have been identified across the genomic region encompassing both the *Kcnqlot1* and *H19* imprinted domains in adult mouse liver (Purbowasito et al. 2004). Of these, 20 S/MARs map within a 58 kb-region within intron 9 of *Kcnql*, while a second cluster of 19 S/MARs are localized within a 169 kb-intergenic region between *Th* and *Ins2*. The first cluster colocalizes within constitutive LADs and the mouse ESC TAD containing the inner/ubiquitous imprinted genes, possibly pointing to a role in chromatin

looping within this TAD. The second cluster flanks the dynamic LAD and LOCKs sites at or near the TAD juncture between the *Kcnqlot1* and *H19* imprinted domains, perhaps supporting a role in boundary formation between these domains.

S/MARs have been proposed to assist in maintaining parental-specific expression at imprinted domains, since a subset of S/MARs have allele-specific associations with the nuclear scaffold/matrix (Kagotani et al. 2002; Weber et al. 2003; Purbowasito et al. 2004). In mice, the *H19* imprinted domain contains four S/MARs, two of which reside within DMRs at the *Igf2* gene, while two S/MARs are outside the DMRs, within the *Igf2* gene and 8 kb downstream of *Igf2* (Weber et al. 2003). The non-DMR S/MARs associates with both parental alleles while the DMR S/MARs associate with the active paternal allele. Furthermore, the paternal-specific S/MARs association is tissue-specific. Paternal-specific S/MAR association of one DMR corresponds with paternal-specific expression in placenta while the other DMR-S/MAR association is linked to paternal-specific expression in the liver (Weber et al. 2003). Functionally, a paternally inherited *H19* ICR deletion results in loss of the *Igf2* paternal-specific S/MARs association, suggesting that the ICR and/or CTCF binding contribute to the S/MAR (Weber et al. 2003). These results point to the complexity of domain-specific chromatin structure, and suggest that the presence of both allele-specific and biparental S/MARs contribute to the greater chromatin topology of *H19* imprinted domain.

With regard to the *SNRPN*, S/MARs extend across both the human *SNRPN* and mouse *Snrpn* genes. In human HeLa cells, the silent *SNRPN* maternal domain is associated with the nuclear scaffold/matrix (Greally et al. 1999), while in a second study involving mouse embryonic fibroblasts, the active paternal *SNRPN* domain associates

with the nuclear scaffold/matrix (Kagotani et al. 2002). Thus, depending on the species, cell type, stage or particular S/MAR being investigated, it is possible that both the active and silent alleles independently associate the nuclear scaffold/matrix to reinforce distinct chromosomal territories. Conservation of S/MARs within the *H19* and *SNRPN* imprinted domain between mice and humans points to their importance in chromatin structure. Studies are required to determine whether S/MARs contribute to chromatin loop formation at imprinted domains and to identify parental-allelic S/MARs as well as the specific scaffold/matrix proteins involved in these interactions.

Conclusion

Evidence presented here indicates that chromatin topology and nuclear architecture are likely important regulatory mechanisms for directing imprinted gene regulation within domains. Furthermore, dynamic changes in these chromatin structures may direct cell-type specific imprint domain regulation. However, many of the factors that contribute to chromatin topology and nuclear architecture at imprinted domains remain to be resolved. Increasing the resolution of TAD maps will provide greater insight into subTAD chromatin interactions within imprinted domains that are likely critical for maintaining imprinted expression across large domains. While nuclear lamin-chromatin associations have been documented in mammals (Guelen et al. 2008), high-resolution LAD, LOCK, NAD and S/MAR maps are still lacking. Furthermore, the role nucleoporins play in mammalian chromatin topology and more specifically in imprinted domain regulation remains largely unanswered, although evidence suggests that nucleoporins interact with chromatin, generate chromatin boundaries and possibly assist

the recruitment of epigenetic modifiers (Capelson et al. 2010; D'Angelo et al. 2012; Light et al. 2013; Pascual-Garcia et al. 2014). Finally, high-resolution studies need to be performed in multiple tissues to gain an understanding about chromatin structure and nuclear architecture changes that occur within imprinted domains during development and between different tissues.

As presented here, a body of literature has emerged to support the role of chromatin structure and nuclear architecture in imprinted domain regulation. However, the data provided to date relate to imprinted domains as a whole, while direct allelic information is lacking. Leveraging SNP databases to generate allele-specific chromatin structural maps of imprinted domains will greatly enhance our understanding of imprinted domain regulation. Advancement in super-resolution microscopy may also open further avenues for investigation of allele-specific interactions with nuclear architecture.

Generally, TADs remain largely stable across cell and tissue types and conserved between species (Dixon et al. 2012; Nora et al. 2012). Here, we document that TAD boundary shift and merger at the *Kcnq1ot1*, *H19* and *Snrpn* domains in different cell types, which also occur at other imprinted domains including *Airn*, *Grb10*, *AK008011/Nhlrc1*, *Trappc9*, and the imprinted retrotransposed gene *Zrs1*. Further investigations are required to determine whether TAD mergers and shifts are a general property of imprinted domains, and whether inter-TAD deletions will result in inappropriate inter-TAD interactions and aberrant imprinted expression, as was observed with the X inactivation center (Nora et al. 2012). Other interesting questions that remain to be answered are whether TADs arise from gDMR-directed interactions or whether

TADs support imprinted domains independent of the gDMR. The *Snrpn* ICR has been shown to be critical for maintenance of imprinted expression of genes within the domain in the preimplantation embryo, yet is dispensable for maintaining *Peg12*, *Mkrn3*, *Magel2* and *Ndn* imprinted expression in the neonatal brain (Dubose et al. 2012), supporting the latter. In this case, it is possible that at the *Snrpn* imprinted domain, TADs supported by LADs and LOCKs, are sufficient to maintain paternal-specific expression in the neonatal brain. Consistent with this, large deletions of ~4.8-35 kb encompassing the *Snrpn* ICR result in imprint maintenance disruption, including increased *Ndn* expression and a gain of maternal *Ndn* and *Mkrn3* DMR methylation (Bressler et al. 2001; Dubose et al. 2011). These deletion regions remove the majority of a facultative LAD that is present in embryonic fibroblasts, neural progenitors, and astrocytes, but is not present in ESCs (Fig. 3). Thus, LAD abrogation may disrupt TAD formation, leading to the disruption of imprint maintenance at the *Snrpn* imprinted domain.

In conclusion, deciphering the interplay between imprinting control regions, imprinted lncRNAs, chromatin modifications, chromatin topology, and nuclear architecture remains a difficult challenge. However, they are likely key to understanding epigenetic regulation at imprinted domains. Understanding how chromatin structure and nuclear architecture affect imprinted gene expression will have implications for novel models of imprinted domain regulation.

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Fig. 1. The mammalian genome is highly organized into chromatin topologies via interactions with the nuclear architecture. Topologically-associated domains (TADs) segregate the genome into regulatory neighbourhoods, which are enriched for chromatin-chromatin interactions that facilitate subTAD chromatin looping. CTCF and cohesins play a role in generating these chromatin interactions. Lamin-associated domains (LADs) are regions of chromatin that are bound to Lamin B1, a major component of the nuclear lamina, linking chromatin with low transcription levels to the inner nuclear membrane. Nucleolar-associated domains (NADs) describe chromatin with low transcriptional activity that localize to the perinucleolar region. NADs partially overlap with LADs, with a possible shift of chromatin between LADs and NADs during the cell cycle or in different tissues. Scaffold/matrix attachment regions (S/MARs) are DNA loci that have a high affinity for the nuclear scaffold/matrix and have been associated with repressed and active chromatin. Nucleoporin-associated chromatin (NUPAC) describes nucleoporin/nuclear pore complex interactions with active chromatin, demarcating chromatin boundaries and facilitating chromatin looping.

Fig. 2. Chromatin topology of the mouse *Kcnq1ot1* and *H19* imprinted domains. Yellow boxes with circles denote imprinted ICRs, representing the gDMR, with maternal-specific (red circle) or paternal-specific (blue circle) DNA methylation. Yellow boxes without circles represent secondary DMRs. Genes in red are maternally expressed, genes in pink are maternally expressed in extraembryonic tissues, genes in blue are paternally expressed, and genes in black escape imprinting. Arrowheads on genes indicate direction of transcription and arrows denoted lncRNAs, with red indicating

maternal expression and blue indicating paternal expression. The green asterisk indicates the location of GFP transgenic reporter (Jones and Lefebvre 2009; Jones et al. 2011). In ESCs, the *Kcnq1ot1* and *H19* imprinted domains are within independent TADs. The *Kcnq1ot1* imprinted domain is further divided into three TADs, with ubiquitously maternally expressed genes residing within the same TAD. In ESCs, the *Kcnq1ot1* TAD containing *Osbpl5-Cars* continues ~270 kb upstream with a boundary before the *Krtap5-1* gene (not depicted). The ESCs *H19* imprinted domain TAD extends ~70 kb downstream, with a boundary within intron 5 of the *Nadsyn1* gene (not depicted). In the mouse cortex, the entire region covering both the *Kcnq1ot1* and *H19* imprinted domains are within a single TAD, extending ~1 Mb from the *Chid1* gene to the *Nadsyn1* gene (not depicted). The constitutive LAD is present in ESCs and cortex while the facultative LAD is present in this differentiated cell type but not in ESCs, where there is a lack of LOCKs. S/MARs are represented by brown blocks, with the blue asterisks representing paternal-specific S/MAR association.

Fig. 3. Chromatin topology of the mouse *Snrpn* imprinted domain.

See Figure legend 2 for details of schematic. In mouse ESCs, paternally expressed genes in the *Snrpn* domain are contained within 2 distinct TADs that terminate within the *Snord116* cluster. In the mouse cortex, there is a shift in TAD location with maternally expressed *Ube3a* and *Atp10a* genes now residing in a TAD independent from the TAD containing the paternally-expressed genes. In ESCs, the TAD containing *Peg12* extends ~380 kb upstream in a gene desert, while the TAD containing *Ube3a* and *Atp10a* extends ~780 kb downstream ending prior to the *Gabrb3* gene (not depicted). In the cortex, the

Peg12-Ndn gene cluster is no longer enclosed with a TAD while the TAD containing *Ube3a* and *Atp10a* extends ~1130 kb downstream, containing the *Gabrb3* gene (not depicted). The dynamic LAD overlapping the *Snrpn* ICR is not present in ESCs, but is present in differentiated cell types. Constitutive and dynamic LADs cover a majority of the *Snrpn* imprinted domain, with two notable exceptions that are free of LADs, the region spanning *Peg12-Magel2*, and the region overlapping *Ube3a*. The *Ube3a* region is also notable for the lack of LOCKs. There are currently no S/MAR sites mapped for the mouse *Snrpn* imprinted domain.

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Fig. 1. The mammalian genome is highly organized into chromatin topologies via interactions with the nuclear architecture. Topological-associated domains (TADs) segregate the genome into regulatory neighbourhoods, which are enriched for chromatin-chromatin interactions that facilitate intraTAD chromatin looping. CTCF and cohesins play a role in generating these chromatin interactions. Lamin-associated domains (LADs) are regions of chromatin that are bound to Lamin B1, a major component of the nuclear lamina, linking chromatin with low transcription levels to the inner nuclear membrane. Nucleolar-associated domains (NADs) describe chromatin with low transcriptional activity that localize to the perinucleolar region. NADs partially overlap with LADs, with a possible shift of chromatin between LADs and NADs during the cell cycle or in different tissues. Scaffold/matrix attachment regions (S/MARs) are DNA loci that have a high affinity for the nuclear scaffold/matrix and have been associated with repressed and active chromatin. Nucleoporin-associated chromatin (NUPAC) describes nucleoporin/nuclear pore complex interactions with active chromatin, demarcating chromatin boundaries and facilitating chromatin looping.

595x446mm (72 x 72 DPI)

104x60mm (300 x 300 DPI)

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182x96mm (300 x 300 DPI)