Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Spatial orchestration of the genome: topological reorganisation during X-chromosome inactivation

Alexandra Martitz[1,2] and Edda G Schulz[1,*]

Genomes are organised through hierarchical structures, ranging from local kilobase-scale *cis*-regulatory contacts to large chromosome territories. Most notably, (sub)-compartments partition chromosomes according to transcriptional activity, while topologically associating domains (TADs) define *cis*-regulatory landscapes. The inactive X chromosome in mammals has provided unique insights into the regulation and function of the three-dimensional (3D) genome. Concurrent with silencing of the majority of genes and major alterations of its chromatin state, the X chromosome undergoes profound spatial rearrangements at multiple scales. These include the emergence of megadomains, alterations of the compartment structure and loss of the majority of TADs. Moreover, the *Xist* locus, which orchestrates X-chromosome inactivation, has provided key insights into regulation and function of regulatory domains. This review provides an overview of recent insights into the control of these structural rearrangements and contextualises them within a broader understanding of 3D genome organisation.

Addresses
[1] Systems Epigenetics, Otto Warburg Laboratories, Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany
[2] Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, 14195 Berlin, Germany

Corresponding author: Schulz, Edda G (edda.schulz@molgen.mpg.de)
* Twitter account: @EddaGSchulz

Current Opinion in Genetics & Development 2024, **86**:102198

This review comes from a themed issue on **Genome Architecture and Expression**

Edited by **Maya Capelson** and **Eda Yildirim**

For complete overview of the section, please refer to the article collection, "Genome Architecture and Expression (2024)"

Available online 24 April 2024

https://doi.org/10.1016/j.gde.2024.102198

Introduction

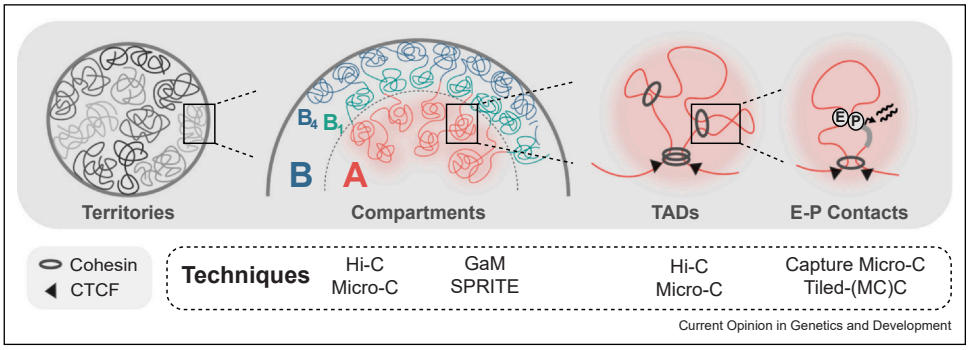
In higher eukaryotes, genes are embedded in *cis*-regulatory landscapes that govern their spatiotemporal expression. These landscapes contain *cis*-regulatory elements, such as enhancers, which are activated through the binding of sequence-specific transcription factors in defined cellular contexts. This triggers the assembly of the transcription machinery at their target gene promoters to drive gene activation. However, enhancer–promoter (E–P) distances in mammals can range up to several hundred kilobases. Therefore, dedicated mechanisms control their physical proximity in three-dimensional (3D) space [1]. In addition to these local mechanisms, gene activity is also modulated at the level of larger genomic structures, which provide transcriptionally permissive or repressive environments [2]. For instance, gene silencing can be established through compact domains of heterochromatin that can even extend to an entire chromosome, as exemplified by the inactive X chromosome (Xi) in mammals [3]. These various levels of gene regulation are closely linked to the spatial organisation in the genome across multiple scales (Figure 1).

Spatial genome organisation

E–P contacts are primarily controlled by so-called topologically associating domains (TADs), which are megabase-sized regions defined by insulating boundaries (Figure 1) [4,5]. They restrict enhancer activity to genes within the same TAD, thereby contributing to E–P specificity [6]. TAD boundaries are largely conserved across species and invariant across cell types [7]. Intra-TAD structure, by contrast, such as less insulating sub-TADs and *cis*-regulatory contacts, is dynamically formed or released during cell differentiation [8–12].

Another layer of the 3D organisation consists of so-called compartments, where regions with similar chromatin properties interact over large genomic distances (Figure 1), both in *cis* (intrachromosomal) and *trans* (interchromosomal) [13]. The A compartment exhibits high transcriptional activity, active histone marks (H3K9ac and H3K27ac), early replication timing and frequent (homotypic) contacts with A compartment domains in *cis* and in *trans* [14,15]. The B compartment represents heterochromatin, contains transcriptionally inactive genes and replicates late in S-phase [14,15]. Both A and B compartments can be further subdivided into regions with specific transcriptional and chromatin properties. For the B compartment in particular, several subcompartments (B_0, B_1, B_2, B_3, and B_4) have been described, of which B_1 and B_4 are the best understood (we follow the nomenclature used in Ref. [15]). The B_1 subcompartment contains regions enriched for the Polycomb mark H3K27me3, while the B_4 subcompartment is marked

Figure 1



3D organisation of the genome across scales. Chromosomes, which occupy distinct chromosome territories in the nucleus (left), are segmented in compartments (A/B), where chromatin regions with similar properties preferentially interact in *cis* and in *trans* (middle left). At the megabase scale, chromatin is folded into TADs (middle right), within which E–P contacts preferentially occur (right). These structures can be detected by a range of technologies developed in the last decade (bottom). The widely used Hi-C method [13], which is increasingly replaced by higher resolution techniques like MNase-based Micro-C [94], as well as ligation-free methods (e.g. Genome Architecture Mapping (GaM) and Split-Pool Recognition of Interactions by Tag Extension (SPRITE), Refs. [95,96]) are used to detect the (sub)compartment and/or TAD structure genome-wide. Recent technological advancements have further revealed the fine-scale structures between short- and long-range regulatory elements by capturing specific regions or viewpoints through Capture Micro-C, Micro-Capture-C (MCC) or Tiled-MCC [29,32,97–99].

by H3K9me3 and HP1, associates with the nuclear lamina, replicates very late in S-phase and exhibits very strong homotypic interactions in *cis* (Figure 2) [15,16]. Although A/B compartments were initially described to be significantly larger than TADs [14], more recent analyses at higher resolution have revealed A/B compartmentalisation also at smaller scales within TADs [17]. Unlike TADs, compartments vary between cell types, reflecting cell type–specific transcriptional programmes [15].

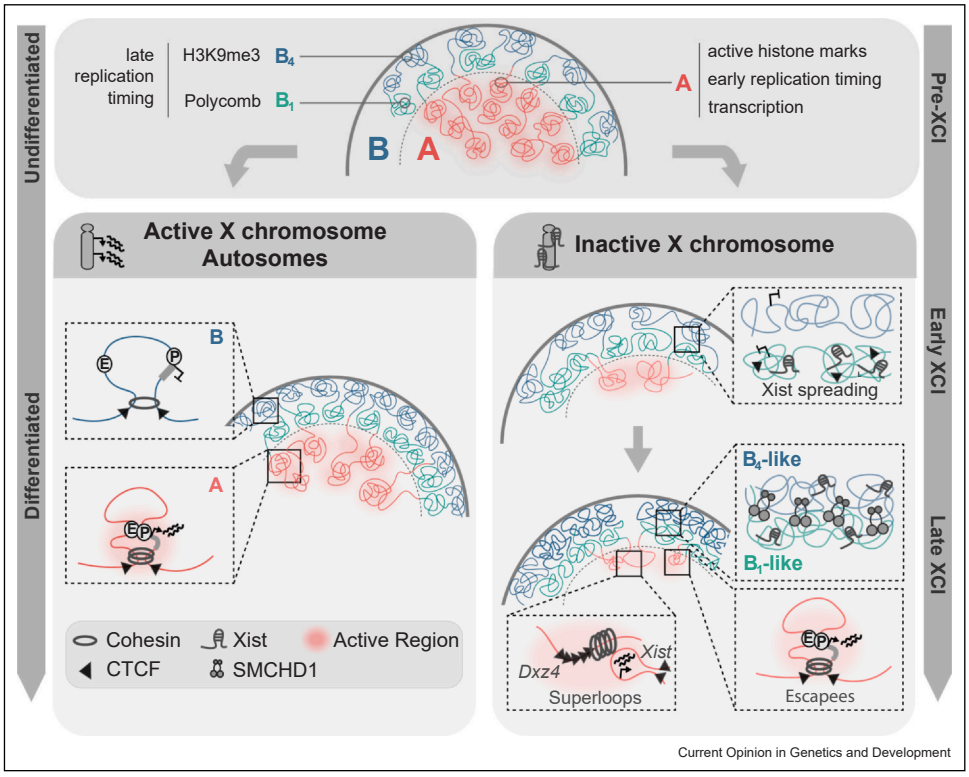
Various mechanisms establish the spatial organisation of the genome across different scales. The most extensively studied mechanism is loop extrusion, which not only underlies the formation of TADs but also modulates intra-TAD contacts. Here, a loop-extruding factor, like the ring-shaped Cohesin complex, moves along the DNA fibre in opposite directions, gradually generating larger loops [18]. The process continues until it encounters an extrusion barrier, such as the sequence-specific DNA-binding protein CCCTC-binding factor (CTCF) [19–22]. Although less well understood, RNA polymerase II, the enhancer-associated Mediator complex as well as R loops (RNA/DNA hybrids) have additionally been identified as extrusion barriers [23–26]. Surprisingly, acute ablation of loop extrusion has little effect on steady-state gene expression but does affect gene activation when it relies on *de novo* contact formation over large genomic distances [27,28]. Once established, E–P contacts might be partially maintained by Cohesin-independent mechanisms [28–31]. As this involves the interaction of active chromatin regions (active enhancers and promoters), the underlying mechanisms might resemble those driving the formation of the A compartment [32]. Compartments are formed independently of loop extrusion [19,22,33,34] and are thought to arise from homotypic interactions, where chromatin with a similar composition engages [35]. Homotypic interactions may play a role in maintaining or strengthening transcriptional states rather than initiating them, as they seem contingent upon the establishment of the activity pattern within a region.

An intriguing phenomenon, where an entire chromosome undergoes massive 3D reconfiguration at all organisational scales is X-chromosome inactivation (XCI). In the next sections, we will discuss recent progress in our understanding of the spatial organisation of the Xi and draw parallels with findings for other parts of the genome.

X-chromosome inactivation

XCI has evolved to ensure dosage compensation for X-linked genes between male and female mammals. In females, the genes on one randomly chosen X chromosome are silenced with the exception of so-called escape genes, which maintain some expression from the Xi [36]. XCI is established during early embryonic development, when cells are still pluripotent and can be recapitulated in differentiating mouse embryonic stem cells *in vitro*. Concurrently with XCI onset, a global genome reorganisation takes place with an increase of heterochromatin and A-to-B compartment changes [37–39]. While only a distinct set of regions on autosomes and the active X chromosome (Xa) are moved into the B compartment, almost the entire Xi undergoes heterochromatisation and becomes visible as a DNA-dense region in the nucleus, called the Barr body (Figure 2).

Figure 2



Restructuring of the Xi during the establishment of XCI. Autosomes and the active X chromosome (top and left) are partitioned in A (euchromatin, red) and B (heterochromatin, green and blue) compartments and some of their chromosomal domains migrate from the A into the B compartment upon differentiation. In contrast, the future inactive X (right) forms a largely repressed B-like compartment. Upon upregulation of Xist, active regions are preferentially covered by Xist RNA (early XCI) and recruited into the B_1-like subcompartment by Polycomb deposition. In the late phase of XCI, SMCHD1 is recruited to the Xi, promoting partial merging of the B subcompartments, allowing Xist to spread into the B_4-like subcompartment, resulting in near-complete silencing of the chromosome. TADs are lost from the Xi due to attenuated CTCF binding. The few regions that remain euchromatic (e.g. escape regions) remain in the A-like compartment, thereby forming superloops.

XCI is initiated by the master regulator Xist, a long non-coding RNA (lncRNA), which is transcribed specifically from the Xi. The Xist RNA acts as a modular binding platform for numerous proteins that allow it to coat the X chromosome, recruit chromatin modifiers to establish gene silencing and compact the chromatin to a transcriptionally inactive structure [40,41]. The A-repeat region in Xist binds the transcriptional repressor SPEN, which is required to initiate gene silencing, partly by promoting histone deacetylation [42,43]. Another region in Xist, the B/C-repeat, recruits Polycomb repressive complex 1 (PRC1), mediated by the RNA-binding protein heterogeneous nuclear Ribonucleoprotein K (hnRNPK) [44,45]. PRC1 deposits the repressive H2AK119ub mark, which in turn attracts PRC2, leading to the deposition of the repressive H3K27me3 mark. The Polycomb pathway contributes to initiating gene silencing to a much lesser extent than the SPEN pathway [46,47]. In addition, PRC1 mediates recruitment of the structural maintenance of chromosomes hinge domain containing 1 (SMCHD1) protein, which in recent years has been identified as a central player in Xi 3D organisation [48–51]. While the SPEN pathway is the main mechanism for initiating gene silencing on the Xi, Polycomb and SMCHD1 together with additional mechanisms, such as promoter DNA methylation, are primarily involved in XCI maintenance [3].

Three-dimensional organisation of the inactive X chromosome

The Xi not only acquires a heterochromatic landscape distinct from the Xa but also undergoes a major 3D reorganisation (Figure 2). The most prominent feature, when interrogating the Xi by Hi-C, is a bipartite structure, consisting of two so-called megadomains [14,52–54]. The megadomain boundary is formed by the *Dxz4* macrosatellite repeat region, which contains a high number of CTCF-binding sites that are occupied only on the Xi, potentially due to their Xi-specific DNA hypomethylation [52,53,55–57]. The *Dxz4* region is one of several sites that exhibit very long-range contacts on the Xi (also called superloops) and are generally euchromatic, which include *Xist* and *Firre* as well as other

escapees [54,58,59]. Megadomains arise from strong, loop extrusion-dependent insulation at the *Dxz4* repeat since they are lost upon Cohesin depletion [60]. In contrast, superloops are Cohesin-independent [60] and might rather represent the remainder of the A compartment, where active regions interact. Although they are conserved between mice and humans, megadomains are not essential for proper XCI or escape. It has been demonstrated that a *Dxz4* deletion or inversion disrupts the Xi bipartite structure, but is insufficient to cause major changes in gene expression, despite some Xi decondensation [52,56,61,62].

Because megadomains are the dominant large-scale structure, initial studies in mice did not observe compartments on the Xi [49,50,52]. Moreover, its largely heterochromatic state would suggest that the entire chromosome, with the exception of escape genes, would reside in the B compartment [37]. More in-depth analyses have revealed a compartment structure also on the Xi in both mice and humans [54,63,64]. These are distinct from the A/B compartments on the Xa since they are larger, less pronounced and more variable across cell types. We suggest that these might represent B_1- and B_4-like subcompartments since the Xi has been shown to be segmented into large, partially overlapping domains of H3K27me3 and H3K9me3 in both mice and humans, which align with the compartment structure [54,65,66]. The B_1-like subcompartment exhibits strong Xist coating, resulting in the deposition of H3K27me3, while the B_4-like subcompartment is covered by H3K9me3 and HP1, has reduced Xist coating and is associated with the nuclear lamina [49,50]. In the absence of SMCHD1, the B_1/B_4-like subcompartment structure (then also called S1/S2 compartments) becomes more pronounced, indicating a role for SMCHD1 in merging the two subcompartments [49,50]. Thus, the Xi adopts a distinct structure largely consisting of B_1/B_4-like subcompartments with a small number of regions that escape XCI and retain characteristics of the A compartment (Figure 2).

The establishment of XCI and the topological rearrangements of the Xi occur in multiple steps, with distinct groups of genes being silenced in each phase [3,46]. In the first phase, Xist coats gene-dense regions on the X chromosome and recruits SPEN to establish gene silencing of a substantial fraction of the X chromosome. This results in the emergence of B subcompartments (also called S1/S2), with Xist residing in the B_1-like domain [46,49]. Gene silencing is accompanied by a loss of binding of RNA Polymerase to chromatin, resulting in its depletion from the Xi territory [67]. Concurrently, Polycomb complexes are recruited to the Xi. Surprisingly, parts of the X chromosome that gain H3K27me3 now detach from the nuclear lamina [68]. In the second phase, which can only be initiated when cells differentiate, SMCHD1 is initially recruited to the B_1-like regions through Xist and PRC1 and then spreads to the B_4-like subcompartment. This is potentially mediated via its interaction partner Ligand-dependent nuclear Receptor Interacting Factor 1 (LRIF1), which binds HP1 and is therefore preferentially recruited to the B_4-like subcompartment [69,70]. Consequently, this leads to the SMCHD1/LRIF1-dependent partial merging of both subcompartments, which appears to help the Xist RNA diffusing from the B_1-like into the B_4-like domains [48]. Additionally, SMCHD1 might promote the spreading of H3K9me3 from sites that are already enriched for this chromatin mark on the X before its inactivation [48,65,71,72], thereby indirectly promoting LRIF1 recruitment [65]. These SMCHD1-dependent mechanisms are essential for complete silencing of a specific subset of genes on the Xi and for preserving the silenced state of a broader set of genes [46,49,65]. When SMCHD1 is absent at the onset of XCI, a small silencing defect is observed during early differentiation, which becomes more prominent in more differentiated cell types [46,49,50,65]. SMCHD1-dependent genes are normally silenced late in development, gain CpG island methylation with slow dynamics, are bound by the YY1 transcription factor and are located in the B_1-like subcompartment [46,65,73,74]. The silencing defect results in female-specific embryonic lethality of *SmcHD1* mutant mice [75]. Defective silencing is associated with focal loss of Polycomb marking at the gene body, failure to establish late replication timing and enhanced 3D contacts in *cis* and in *trans*, suggesting that the affected genes remain in the A compartment [49,50,65,73,76]. SMCHD1 is however not required for maintenance of silencing in differentiated cells, once promoter methylation has been established [48,71,72].

Apart from the compartment structure, reorganisation also occurs at the submegabase level, where the Xi exhibits a drastic attenuation of TADs, accompanied by reduced CTCF and Cohesin occupancy [49,50,52,77]. TADs are only maintained in regions that remain expressed on the Xi, such as the *Xist* locus and escape genes [52,78]. TAD loss is in part mediated by SMCHD1, since its deletion results in heightened levels of CTCF and Cohesin as well as partial TAD restoration [49,50]. Although CTCF binding can be modulated by DNA methylation, and SMCHD1 affects global DNA methylation levels at the Xi, the majority of affected CTCF binding sites are not methylated in wild-type cells. This points towards an additional DNA methylation-independent mechanism of CTCF depletion from the Xi [50]. In other contexts, several recent studies have reported CTCF loss from H3K9me3 domains, potentially mediated by nucleosome exclusion [15,79,80]. Since SMCHD1 loss also leads to a reduction of H3K9me3 on the Xi, similar mechanisms might be involved. Importantly, the apparent loss of Cohesin (peaks) from the Xi [49,50] does not necessarily indicate

an absence of loop extrusion, but rather a loss of extrusion barriers, as shown for the B_4 subcompartment on autosomes [15].

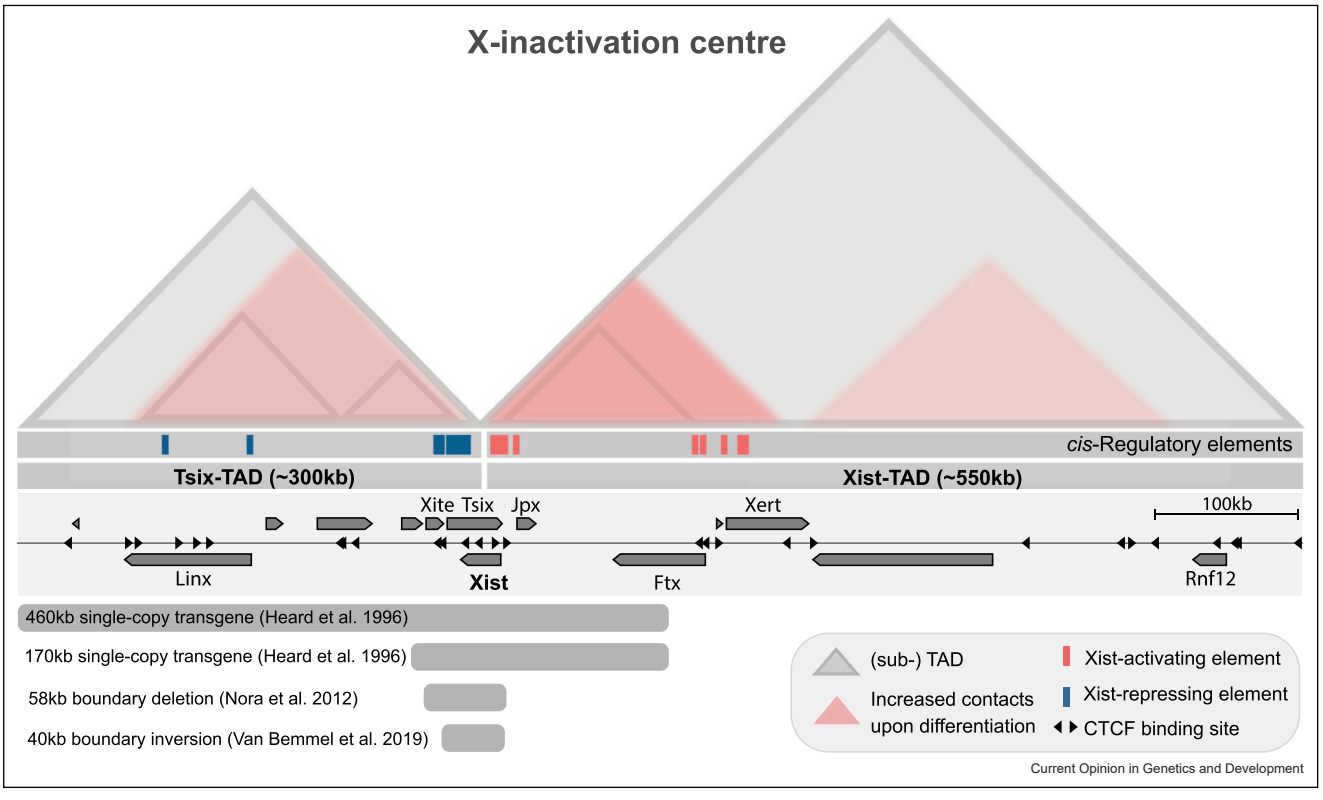
Taken together, the Xi assumes a largely heterochromatic structure with a spatial chromatin organisation resembling aspects of two B subcompartments on autosomes. However, SMCHD1, which affects only a small number of autosomal regions, including protocadherins and Hox genes [71,81], is specifically recruited to the Xi and helps to condense the subcompartments into the characteristic dense Barr body.

Chromatin architecture at the X-inactivation centre

A locus that has provided important insights into 3D genome organisation is the genomic region surrounding the *Xist* gene, also called the X-inactivation centre (Figure 3). Its bipartite TAD structure with opposing regulatory states makes it an excellent model for studying the relationship between genome architecture and gene regulation [82]. One TAD, often called the Xist-TAD, contains the promoter and the 5′ portion of the *Xist* gene, along with a series of enhancer elements and lncRNA loci (Jpx, Ftx, and Xert) implicated in Xist activation [40]. The neighbouring TAD contains the promoter of *Tsix*, the antisense transcript and *cis*-repressor of *Xist*, together with additional transcribed loci (Xite, Linx) that have a repressive function on *Xist* [40]. Both *Xist* and *Tsix* are transcribed across the TAD boundary, which contains a series of CTCF sites in their gene bodies.

The two TADs ensure spatial segregation of contrary developmental expression patterns, as *Tsix* is highly expressed at the pre-XCI state, where *Xist* is silent, and is gradually downregulated concomitantly with Xist upregulation on the Xi, when cells differentiate [83]. The first indication pointing towards a role of TADs in gene regulation was the observation that gene expression dynamics are highly correlated within each of the two TADs, as well as within neighbouring TADs [4]. Such intra-TAD coordination of gene expression was later shown to occur across the genome upon progesterone stimulation [84], and

Figure 3



The *cis*-regulatory landscape of the X-inactivation centre. The genomic region surrounding the mouse *Xist* gene is shown (chrX:103,134,000–104,034,000, mm10). At the top, the bipartite TAD structure with intra-TAD contacts is indicated, and regions with increased contacts upon differentiation are coloured red [87]. Red and blue vertical bars indicate activating and repressive *cis*-regulatory regions of *Xist*, respectively. In the middle, CTCF-binding sites (black triangles, after [100]) and genes are annotated with *Xist* and its regulators being labelled. All labelled genes except *Rnf12* are noncoding RNAs. On the bottom, some genomic regions that have previously been investigated through transgenesis, deletion or inversion are marked [4,86,90].

TADs were identified as the genome folding scale where gene co-regulation is most prominent [85]. This is likely due to the ability of enhancers to co-regulate multiple genes within the same TAD [6].

A prominent role for long-range enhancers in Xist regulation was initially suggested based on the analysis of large single-copy transgenes *in vivo* [86]. Even a 460 kb region containing all of the Tsix-TAD and ~120 kb of the Xist-TAD did not upregulate Xist in the embryonic lineage. This transgene is missing the Xert region and part of Ftx, and deletion of these regions from the endogenous locus impairs Xist upregulation [87,88], confirming their role as distal regulatory regions. As expected, the long transgene expresses Tsix *in vivo*, but a shorter ~170 kb version, lacking most of the Tsix-TAD except Tsix itself and its enhancer Xite, fails to express Tsix [4]. This underscores the crucial role of long-range Tsix regulatory elements, like the distal Linx locus [89].

Multiple structural perturbation experiments have helped to further dissect the importance of TAD-induced physical separation between the two *cis*-regulatory landscapes. Deletion of the TAD boundary containing *Xist* and *Tsix* resulted in increased inter-TAD contacts and premature upregulation of genes in the Xist-TAD before differentiation [4]. Similarly, inversion of the boundary, which placed the *Xist* promoter into the Tsix-TAD, led to ectopic Xist expression in undifferentiated cells [90]. Upon differentiation, Xist expression initially remained increased compared with the control, when Tsix is normally highly expressed, but was reduced at later time points, when Tsix is downregulated. This finding exemplifies how a gene promoter responds to the active enhancer landscape within the same TAD. Such gene missexpression due to enhancer adoption upon structural rearrangements at TAD boundaries has also been shown to underlie genetic disease phenotypes [91]. Although communication between the Xist- and Tsix-TADs is mainly mediated by Tsix transcription across the TAD boundary, some recent findings suggest additional Tsix-independent inter-TAD communication through mechanisms yet to be identified [89,92].

While the bipartite TAD structure is invariant across cellular states, the contact landscape within the two TADs is rewired when Xist is upregulated [4]. A sub-TAD appears, encompassing the *Xist* promoter and all its *cis*-regulatory elements (Jpx, Ftx, Xert, and enhancers), which might help to facilitate E–P interactions [87]. The sub-TAD appears concomitantly with activation of distal enhancers and upregulation of lncRNAs in the region, and is most pronounced, when both *Xist* promoter and its distal enhancers are active. Sub-TAD formation might thus be driven by Cohesin loading at active enhancers, by Cohesin stalling at transcribed loci or by the Mediator complex enabling E–P communication, as recently suggested in other contexts [24–26,93]. Whether TAD rewiring has a functional role in Xist regulation remains to be investigated. Taken together, the *Xist* locus has provided important insights into the role of 3D genome organisation in gene regulation.

Conclusions and perspectives

The intricate spatial organisation of the genome, exemplified by XCI in mice, highlights the interplay between chromatin architecture, E–P communication and gene expression during development. Several molecular pathways drive the formation of a robust heterochromatic structure on the Xi, which silences the inner core but permits transcription of specific genes located at the periphery. The interdependencies of 3D reorganisation, chromatin states and gene expression make it challenging to dissect whether the altered 3D structure has a functional role in establishing XCI, or whether it is rather a consequence of gene silencing. To address this challenge, we expect that the XCI field will benefit from future advances in our understanding of the molecular mechanisms that drive compartmentalisation on autosomes. This might eventually allow a more direct perturbation of the 3D structure of the Xi to study the functional consequences. Advancing our understanding of such Cohesin-independent mechanisms underlying 3D genome organisation will also be instrumental in dissecting the functional role of intra-TAD changes in contact frequency in gene regulation. We expect that the Xi will continue to contribute important insights into the structure and function of the 3D genome.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

This work is supported by the Max Planck Research Group Leader programme, the DFG (Deutsche Forschnungsgemeinschaft) priority programme SPP 2202 (508000619), and ERC (European Research Council) Starting Grant CisTune (948771).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Oudelaar AM, Higgs DR: **The relationship between genome structure and function**. *Nat Rev Genet* 2021, **22**:154-168.

2. McCarthy RL, Zhang J, Zaret KS: **Diverse heterochromatin states restricting cell identity and reprogramming**. *Trends Biochem Sci* 2023, **48**:513-526.

3. Żylicz JJ, Heard E: **Molecular mechanisms of facultative heterochromatin formation: an X-chromosome perspective**. *Annu Rev Biochem* 2020, **89**:255-282.

4. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, *et al*.: **Spatial partitioning of the regulatory landscape of the X-inactivation centre**. *Nature* 2012, **485**:381-385.

5. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, *et al*.: **Topological domains in mammalian genomes identified by analysis of chromatin interactions**. *Nature* 2012, **485**:376-380.

6. Pachano T, Haro E, Rada-Iglesias A: **Enhancer-gene specificity in development and disease**. *Development* 2022, **149**:1-14.

7. Dekker J, Heard E: **Structural and functional diversity of topologically associating domains**. *FEBS Lett* 2015, **589**:2877-2884.

8. Wang M, He B, Hao Y, Srinivasan D, Shrinet J, Fraser P: **Cellular reprogramming is driven by widespread rewiring of promoter-enhancer interactions**. *BMC Biol* 2023, **21**:264.

9. Winick-Ng W, Kukalev A, Harabula I, Zea-Redondo L, Szabó D, Meijer M, *et al*.: **Cell-type specialization is encoded by specific chromatin topologies**. *Nature* 2021, **599**:684-691.

10. Siersbæk R, Madsen JGS, Javierre BM, Nielsen R, Bagge EK, Cairns J, *et al*.: **Dynamic rewiring of promoter-anchored chromatin loops during adipocyte differentiation**. *Mol Cell* 2017, **66**:420-435 e5..

11. Chahar S, Zouari YB, Salari H, Kobi D, Maroquenne M, Erb C, *et al*.: **Transcription induces context-dependent remodeling of chromatin architecture during differentiation**. *PLoS Biol* 2023, **21**:e3002424.

12. Bonev B, Cohen NM, Szabo Q, Fritsch L, Papadopoulos GL, Lubling Y, *et al*.: **Multiscale 3D genome rewiring during mouse neural development**. *Cell* 2017, **171**:557-572 e24..

13. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, *et al*.: **Comprehensive mapping of long-range interactions reveals folding principles of the human genome**. *Science* 2009, **326**:289-293.

14. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, *et al*.: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping**. *Cell* 2014, **159**:1665-1680.

15. •• Spracklin G, Abdennur N, Imakaev M, Chowdhury N, Pradhan S, Mirny LA, *et al*.: **Diverse silent chromatin states modulate genome compartmentalization and loop extrusion barriers**. *Nat Struct Mol Biol* 2023, **30**:38-51.

Characterisation of A/B subcompartments in two different cell lines using a new segmentation algorithm provides a detailed definition of subcompartment properties. Moreover, the study describes an important role for DNA methylation and H3K9me3 in forming the B4 subcompartment, where CTCF binding is attenuated, but loop extrusion still occurs.

16. Siegenfeld AP, Roseman SA, Roh H, Lue NZ, Wagen CC, Zhou E, *et al*.: **Polycomb-lamina antagonism partitions heterochromatin at the nuclear periphery**. *Nat Commun* 2022, **13**:4199.

17. • Harris HL, Gu H, Olshansky M, Wang A, Farabella I, Eliaz Y, *et al*.: **Chromatin alternates between A and B compartments at kilobase scale for subgenic organization**. *Nat Commun* 2023, **14**:3303.

Using ultradeep sequencing and a newly developed algorithm, this study reports A/B compartmentalisation at the kilobase-scale, which has previously only been observed at a much larger scale.

18. Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA: **Formation of chromosomal domains by loop extrusion**. *Cell Rep* 2016, **15**:2038-2049.

19. Nora EP, Goloborodko A, Valton A-L, Gibcus JH, Uebersohn A, Abdennur N, *et al*.: **Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization**. *Cell* 2017, **169**:930-944 e22.

20. Li Y, Haarhuis JHI, Cacciatore ÁS, Oldenkamp R, van Ruiten MS, Willems L, *et al*.: **The structural basis for Cohesin-CTCF-anchored loops**. *Nature* 2020, **578**:472-476.

21. de Wit E, Vos ESM, Holwerda SJB, Valdes-Quezada C, Verstegen MJAM, Teunissen H, *et al*.: **CTCF binding polarity determines chromatin looping**. *Mol Cell* 2015, **60**:676-684.

22. Rao SSP, Huang S-C, St Hilaire BG, Engreitz JM, Perez EM, Kieffer-Kwon K-R, *et al*.: **Cohesin loss eliminates all loop domains**. *Cell* 2017, **171**:305-320 e24..

23. Zhang H, Shi Z, Banigan EJ, Kim Y, Yu H, Bai X-C, *et al*.: **CTCF and R-loops are boundaries of Cohesin-mediated DNA looping**. *Mol Cell* 2023, **83**:2856-2871 e8..

24. Banigan EJ, Tang W, van den Berg AA, Stocsits RR, Wutz G, Brandão HB, *et al*.: **Transcription shapes 3D chromatin organization by interacting with loop extrusion**. *Proc Natl Acad Sci USA* 2023, **120**:e2210480120.

25. Barshad G, Lewis JJ, Chivu AG, Abuhashem A, Krietenstein N, Rice EJ, *et al*.: **RNA polymerase II dynamics shape enhancer-promoter interactions**. *Nat Genet* 2023, **55**:1370-1380.

26. • Ramasamy S, Aljahani A, Karpinska MA, Cao TBN, Velychko T, Cruz JN, *et al*.: **The Mediator complex regulates enhancer-promoter interactions**. *Nat Struct Mol Biol* 2023, **30**:991-1000.

Using high-resolution targeted chromosome conformation capture approaches, this study identifies a functional role of the Mediator complex in promoting E–P contacts by modulating loop extrusion.

27. Karpinska MA, Oudelaar AM: **The role of loop extrusion in enhancer-mediated gene activation**. *Curr Opin Genet Dev* 2023, **79**:102022.

28. Hsieh T-HS, Cattoglio C, Slobodyanyuk E, Hansen AS, Darzacq X, Tjian R: **Enhancer-promoter interactions and transcription are largely maintained upon acute loss of CTCF, Cohesin, WAPL or YY1**. *Nat Genet* 2022, **54**:1919-1932.

29. Aljahani A, Hua P, Karpinska MA, Quililan K, Davies JOJ, Oudelaar AM: **Analysis of sub-kilobase chromatin topology reveals nano-scale regulatory interactions with variable dependence on Cohesin and CTCF**. *Nat Commun* 2022, **13**:2139.

30. Thiecke MJ, Wutz G, Muhar M, Tang W, Bevan S, Malysheva V, *et al*.: **Cohesin-dependent and -independent mechanisms mediate chromosomal contacts between promoters and enhancers**. *Cell Rep* 2020, **32**:107929.

31. Zhang S, Übelmesser N, Barbieri M, Papantonis A: **Enhancer-promoter contact formation requires RNAPII and antagonizes loop extrusion**. *Nat Genet* 2023, **55**:832-840.

32. Goel VY, Huseyin MK, Hansen AS: **Region Capture Micro-C reveals coalescence of enhancers and promoters into nested microcompartments**. *Nat Genet* 2023, **55**:1048-1056.

33. Schwarzer W, Abdennur N, Goloborodko A, Pekowska A, Fudenberg G, Loe-Mie Y, *et al*.: **Two independent modes of chromatin organization revealed by Cohesin removal**. *Nature* 2017, **551**:51-56.

34. Haarhuis JHI, van der Weide RH, Blomen VA, Yáñez-Cuna JO, Amendola M, van Ruiten MS, *et al*.: **The Cohesin release factor WAPL restricts chromatin loop extension**. *Cell* 2017, **169**:693-707 e14..

35. Solovei I, Thanisch K, Feodorova Y: **How to rule the nucleus: divide et impera**. *Curr Opin Cell Biol* 2016, **40**:47-59.

36. Galupa R, Heard E: **X-chromosome inactivation: a crossroads between chromosome architecture and gene regulation**. *Annu Rev Genet* 2018, **52**:535-566.

37. Poonperm R, Hiratani I: **Formation of a multi-layered 3-dimensional structure of the heterochromatin compartment during early mammalian development**. *Dev Growth Differ* 2021, **63**:5-17.

38. Miura H, Takahashi S, Poonperm R, Tanigawa A, Takebayashi S-I, Hiratani I: **Single-cell DNA replication profiling identifies spatiotemporal developmental dynamics of chromosome organization**. *Nat Genet* 2019, **51**:1356-1368.

39. Bonora G, Ramani V, Singh R, Fang H, Jackson DL, Srivatsan S, *et al.*: **Single-cell landscape of nuclear configuration and gene expression during stem cell differentiation and X inactivation**. *Genome Biol* 2021, **22**:279.

40. Loda A, Collombet S, Heard E: **Gene regulation in time and space during X-chromosome inactivation**. *Nat Rev Mol Cell Biol* 2022, **23**:231-249.

41. Keniry A, Blewitt ME: **Chromatin-mediated silencing on the inactive X chromosome**. *Development* 2023, **150**:1-13.

42. Dossin F, Pinheiro I, Żylicz JJ, Roensch J, Collombet S, Le Saux A, *et al.*: **SPEN integrates transcriptional and epigenetic control of X-inactivation**. *Nature* 2020, **578**:455-460.

43. McHugh CA, Chen C-K, Chow A, Surka CF, Tran C, McDonel P, *et al.*: **The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3**. *Nature* 2015, **521**:232-236.

44. Almeida M, Pintacuda G, Masui O, Koseki Y, Gdula M, Cerase A, *et al.*: **PCGF3/5-PRC1 initiates Polycomb recruitment in X chromosome inactivation**. *Science* 2017, **356**:1081-1084.

45. Pintacuda G, Wei G, Roustan C, Kirmizitas BA, Solcan N, Cerase A, *et al.*: **hnRNPK recruits PCGF3/5-PRC1 to the Xist RNA B-repeat to establish polycomb-mediated chromosomal silencing**. *Mol Cell* 2017, **68**:955-969 e10..

46. •• Bowness JS, Nesterova TB, Wei G, Rodermund L, Almeida M, Coker H, *et al.*: **Xist-mediated silencing requires additive functions of SPEN and Polycomb together with differentiation-dependent recruitment of SmcHD1**. *Cell Rep* 2022, **39**:110830.

Quantification of gene silencing dynamics in a series of mutant cell lines revealed that full silencing of the Xi requires differentiation-dependent recruitment of SMCHD1.

47. Bousard A, Raposo AC, Żylicz JJ, Picard C, Pires VB, Qi Y, *et al.*: **The role of Xist-mediated Polycomb recruitment in the initiation of X-chromosome inactivation**. *EMBO Rep* 2019, **20**:e48019.

48. Wang C-Y, Colognori D, Sunwoo H, Wang D, Lee JT: **PRC1 collaborates with SMCHD1 to fold the X-chromosome and spread Xist RNA between chromosome compartments**. *Nat Commun* 2019, **10**:2950.

49. Wang C-Y, Jégu T, Chu H-P, Oh HJ, Lee JT: **SMCHD1 merges chromosome compartments and assists formation of super-structures on the inactive X**. *Cell* 2018, **174**:406-421 e25..

50. Gdula MR, Nesterova TB, Pintacuda G, Godwin J, Zhan Y, Ozadam H, *et al.*: **The non-canonical SMC protein SmcHD1 antagonises TAD formation and compartmentalisation on the inactive X chromosome**. *Nat Commun* 2019, **10**:30.

51. Jansz N, Nesterova T, Keniry A, Iminitoff M, Hickey PF, Pintacuda G, *et al.*: **Smchd1 targeting to the inactive X is dependent on the Xist-HnrnpK-PRC1 pathway**. *Cell Rep* 2018, **25**:1912-1923 e9..

52. Giorgetti L, Lajoie BR, Carter AC, Attia M, Zhan Y, Xu J, *et al.*: **Structural organization of the inactive X chromosome in the mouse**. *Nature* 2016, **535**:575-579.

53. Deng X, Ma W, Ramani V, Hill A, Yang F, Ay F, *et al.*: **Bipartite structure of the inactive mouse X chromosome**. *Genome Biol* 2015, **16**:152.

54. Darrow EM, Huntley MH, Dudchenko O, Stamenova EK, Durand NC, Sun Z, *et al.*: **Deletion of DXZ4 on the human inactive X chromosome alters higher-order genome architecture**. *Proc Natl Acad Sci USA* 2016, **113**:E4504-E4512.

55. Minajigi A, Froberg J, Wei C, Sunwoo H, Kesner B, Colognori D, *et al.*: **A comprehensive Xist interactome reveals Cohesin repulsion and an RNA-directed chromosome conformation**. *Science* 2015, **349** aab2276-1-12.

56. Bonora G, Deng X, Fang H, Ramani V, Qiu R, Berletch JB, *et al.*: **Orientation-dependent Dxz4 contacts shape the 3D structure of the inactive X chromosome**. *Nat Commun* 2018, **9**:1445.

57. Chadwick BP: **DXZ4 chromatin adopts an opposing conformation to that of the surrounding chromosome and acquires a novel inactive X-specific role involving CTCF and antisense transcripts**. *Genome Res* 2008, **18**:1259-1269.

58. Hacisuleyman E, Goff LA, Trapnell C, Williams A, Henao-Mejia J, Sun L, *et al.*: **Topological organization of multichromosomal regions by the long intergenic noncoding RNA Firre**. *Nat Struct Mol Biol* 2014, **21**:198-206.

59. Splinter E, de Wit E, Nora EP, Klous P, van de Werken HJG, Zhu Y, *et al.*: **The inactive X chromosome adopts a unique three-dimensional conformation that is dependent on Xist RNA**. *Genes Dev* 2011, **25**:1371-1383.

60. Kriz AJ, Colognori D, Sunwoo H, Nabet B, Lee JT: **Balancing Cohesin eviction and retention prevents aberrant chromosomal interactions, Polycomb-mediated repression, and X-inactivation**. *Mol Cell* 2021, **81**:1970-1987 e9..

61. Andergassen D, Smith ZD, Lewandowski JP, Gerhardinger C, Meissner ZD, Rinn JL: **In vivo Firre and Dxz4 deletion elucidates roles for autosomal gene regulation**. *ELife* 2019, **8**:1-13.

62. Froberg JE, Pinter SF, Kriz AJ, Jégu T, Lee JT: **Megadomains and superloops form dynamically but are dispensable for X-chromosome inactivation and gene escape**. *Nat Commun* 2018, **9**:5004.

63. Miura H, Poonperm R, Takahashi S, Hiratani I: **Practical analysis of Hi-C Data: generating A/B compartment profiles**. *Methods Mol Biol* 2018, **1861**:221-245.

64. Bauer M, Vidal E, Zorita E, Üresin N, Pinter SF, Filion GJ, *et al.*: **Chromosome compartments on the inactive X guide TAD formation independently of transcription during X-reactivation**. *Nat Commun* 2021, **12**:3499.

65. •• Ichihara S, Nagao K, Sakaguchi T, Obuse C, Sado T: **SmcHD1 underlies the formation of H3K9me3 blocks on the inactive X chromosome in mice**. *Development* 2022, **149**:1-14.

Thorough characterisation of *Smchd1* mutant cell lines revealed that SMCHD1 is required for establishing H3K9me3 domains on the Xi, thereby providing an important step towards disentangling the intricate interplay of chromatin modifications, 3D genome structure and gene silencing during XCI.

66. Vallot C, Ouimette J-F, Makhlouf M, Féraud O, Pontis J, Côme J, *et al.*: **Erosion of X chromosome inactivation in human pluripotent cells initiates with XACT coating and depends on a specific heterochromatin landscape**. *Cell Stem Cell* 2015, **16**:533-546.

67. Collombet S, Rall I, Dugast-Darzacq C, Heckert A, Halavatyi A, Le Saux A, *et al.*: **RNA polymerase II depletion from the inactive X chromosome territory is not mediated by physical compartmentalization**. *Nat Struct Mol Biol* 2023, **30**:1216-1223.

68. • Kefalopoulou S, Rullens PMJ, de Luca KL, de Vries SS, Korthout T, van Oudenaarden A, *et al.*: **Time-resolved and multifactorial profiling in single cells resolves the order of heterochromatin formation events during X-chromosome inactivation**. *BioRxiv* 2023, 2003.12.15.571749.

Using a new method for multifactorial chromatin profiling within single cells, Dam&ChIC, this study reports the unexpected finding that large parts of the X chromosome detach from the nuclear lamina upon onset of XCI in particular at regions that subsequently gain Polycomb marking.

69. Nozawa R-S, Nagao K, Igami K-T, Shibata S, Shirai N, Nozaki N, *et al.*: **Human inactive X chromosome is compacted through a PRC2-independent SMCHD1-HBiX1 pathway**. *Nat Struct Mol Biol* 2013, **20**:566-573.

70. Brideau NJ, Coker H, Gendrel A-V, Siebert CA, Bezstarosti K, Demmers J, *et al.*: **Independent mechanisms target SMCHD1 to trimethylated histone H3 lysine 9-modified chromatin and the inactive X chromosome**. *Mol Cell Biol* 2015, **35**:4053-4068.

71. Jansz N, Keniry A, Trussart M, Bildsoe H, Beck T, Tonks ID, *et al.*: **Smchd1 regulates long-range chromatin interactions on the inactive X chromosome and at Hox clusters**. *Nat Struct Mol Biol* 2018, **25**:766-777.

72. Sakakibara Y, Nagao K, Blewitt M, Sasaki H, Obuse C, Sado T: **Role of SmcHD1 in establishment of epigenetic states**

required for the maintenance of the X-inactivated state in mice. *Development* 2018, **145**:1-13.

73. Gendrel A-V, Apedaile A, Coker H, Termanis A, Zvetkova I, Godwin J, *et al.*: **Smchd1-dependent and -independent pathways determine developmental dynamics of CpG island methylation on the inactive X chromosome**. *Dev Cell* 2012, **23**:265-279.

74. • Bowness JS, Almeida M, Nesterova TB, Brockdorff N: **YY1 binding is a gene-intrinsic barrier to Xist-mediated gene silencing**. *BioRxiv* 2023, 2003.11.22.568288.
This study reveals that slowly silenced genes are often associated with YY1 binding, which persists until late XCI, and suggests that YY1 could act as a barrier to Xist-mediated silencing on the Xi.

75. Blewitt ME, Gendrel A-V, Pang Z, Sparrow DB, Whitelaw N, Craig JM, *et al.*: **SmcHD1, containing a structural-maintenance-of-chromosomes hinge domain, has a critical role in X inactivation**. *Nat Genet* 2008, **40**:663-669.

76. Poonperm R, Ichihara S, Miura H, Tanigawa A, Nagao K, Obuse C, *et al.*: **Replication dynamics identifies the folding principles of the inactive X chromosome**. *Nat Struct Mol Biol* 2023, **30**:1224-1237.

77. Colognori D, Sunwoo H, Kriz AJ, Wang C-Y, Lee JT: **Xist deletional analysis reveals an interdependency between Xist RNA and Polycomb complexes for spreading along the inactive X**. *Mol Cell* 2019, **74**:101-117 e10..

78. Collombet S, Ranisavljevic N, Nagano T, Varnai C, Shisode T, Leung W, *et al.*: **Parental-to-embryo switch of chromosome organization in early embryogenesis**. *Nature* 2020, **580**:142-146.

79. Malachowski T, Chandradoss KR, Boya R, Zhou L, Cook AL, Su C, *et al.*: **Spatially coordinated heterochromatinization of long synaptic genes in fragile X syndrome**. *Cell* 2023, **186**:5840-5858 e36..

80. Jiang Y, Loh Y-HE, Rajarajan P, Hirayama T, Liao W, Kassim BS, *et al.*: **The methyltransferase SETDB1 regulates a large neuron-specific topological chromatin domain**. *Nat Genet* 2017, **49**:1239-1250.

81. Gendrel A-V, Tang YA, Suzuki M, Godwin J, Nesterova TB, Greally JM, *et al.*: **Epigenetic functions of smchd1 repress gene clusters on the inactive X chromosome and on autosomes**. *Mol Cell Biol* 2013, **33**:3150-3165.

82. Galupa R, Heard E: **X-chromosome inactivation: new insights into cis and trans regulation**. *Curr Opin Genet Dev* 2015, **31**:57-66.

83. Shiura H, Abe K: **Xist/Tsix expression dynamics during mouse peri-implantation development revealed by whole-mount 3D RNA-FISH**. *Sci Rep* 2019, **9**:3637.

84. Le Dily F, Baù D, Pohl A, Vicent GP, Serra F, Soronellas D, *et al.*: **Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation**. *Genes Dev* 2014, **28**:2151-2162.

85. Zhan Y, Mariani L, Barozzi I, Schulz EG, Blüthgen N, Stadler M, *et al.*: **Reciprocal insulation analysis of Hi-C data shows that TADs represent a functionally but not structurally privileged scale in the hierarchical folding of chromosomes**. *Genome Res* 2017, **27**:479-490.

86. Heard E, Kress C, Mongelard F, Courtier B, Rougeulle C, Ashworth A, *et al.*: **Transgenic mice carrying an Xist-containing YAC**. *Hum Mol Genet* 1996, **5**:441-450.

87. •• Gjaltema RAF, Schwämmle T, Kautz P, Robson M, Schöpflin R, Ravid Lustig L, *et al.*: **Distal and proximal cis-regulatory elements sense X chromosome dosage and developmental state at the Xist locus**. *Mol Cell* 2022, **82**:190-208.
Comprehensive enhancer identification and characterisation together with profiling of 3D contacts at the X-inactivation centre revealed the emergence of a sub-TAD encompassing the Xist promoter and all its enhancer elements, concomitantly with *Xist* upregulation.

88. Furlan G, Gutierrez Hernandez N, Huret C, Galupa R, van Bemmel JG, Romito A, *et al.*: **The ftx noncoding locus controls X chromosome inactivation independently of its RNA products**. *Mol Cell* 2018, **70**:462-472.e8.

89. Galupa R, Nora EP, Worsley-Hunt R, Picard C, Gard C, van Bemmel JG, *et al.*: **A conserved noncoding locus regulates random monoallelic Xist expression across a topological boundary**. *Mol Cell* 2020, **77**:352-367 e8..

90. van Bemmel JG, Galupa R, Gard C, Servant N, Picard C, Davies J, *et al.*: **The bipartite TAD organization of the X-inactivation center ensures opposing developmental regulation of Tsix and Xist**. *Nat Genet* 2019, **51**:1024-1034.

91. Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, *et al.*: **Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions**. *Cell* 2015, **161**:1012-1025.

92. Galupa R, Picard C, Servant N, Nora EP, Zhan Y, van Bemmel JG, *et al.*: **Inversion of a topological domain leads to restricted changes in its gene expression and affects interdomain communication**. *Development* 2022, **149**:1-14.

93. • Rinzema NJ, Sofiadis K, Tjalsma SJD, Verstegen MJAM, Oz Y, Valdes-Quezada C, *et al.*: **Building regulatory landscapes reveals that an enhancer can recruit Cohesin to create contact domains, engage CTCF sites and activate distant genes**. *Nat Struct Mol Biol* 2022, **29**:563-574.
Through an elegant approach based on constructing synthetic genomic loci, this study finds evidence that Cohesin is preferentially loaded onto chromatin at active enhancer elements.

94. Hsieh T-HS, Weiner A, Lajoie B, Dekker J, Friedman N, Rando OJ: **Mapping nucleosome resolution chromosome folding in yeast by Micro-C**. *Cell* 2015, **162**:108-119.

95. Beagrie RA, Thieme CJ, Annunziatella C, Baugher C, Zhang Y, Schueler M, *et al.*: **Multiplex-GAM: genome-wide identification of chromatin contacts yields insights overlooked by Hi-C**. *Nat Methods* 2023, **20**:1037-1047.

96. Quinodoz SA, Ollikainen N, Tabak B, Palla A, Schmidt JM, Detmar E, *et al.*: **Higher-order inter-chromosomal hubs shape 3D genome organization in the nucleus**. *Cell* 2018, **174**:744-757 e24..

97. Hua P, Badat M, Hanssen LLP, Hentges LD, Crump N, Downes DJ, *et al.*: **Defining genome architecture at base-pair resolution**. *Nature* 2021, **595**:125-129.

98. Gabriele M, Brandão HB, Grosse-Holz S, Jha A, Dailey GM, Cattoglio C, *et al.*: **Dynamics of CTCF- and Cohesin-mediated chromatin looping revealed by live-cell imaging**. *Science* 2022, **376**:496-501.

99. Pradhan B, Kanno T, Igarashi MU, Loke MS, Baaske MD, Wong JSK, *et al.*: **The Smc5/6 complex is a DNA loop-extruding motor**. *Nature* 2023, **616**:843-848.

100. Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, Schöler A, *et al.*: **DNA-binding factors shape the mouse methylome at distal regulatory regions**. *Nature* 2011, **480**:490-495.