Dosage Compensation and Nuclear Organization: Cluster to control chromosome-wide gene expressionRahul Sharma^{1,2} and Peter Meister^{1#}¹ Cell Fate and Nuclear Organization, Institute of Cell Biology, University of Bern, Switzerland² Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

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Abstract

In many species, male and female animals differ in the number of X chromosomes they possess. As a consequence, large scale differences in gene dosage exist between sexes; a phenomenon that is rarely tolerated by the organism for changes in autosome dosage. Several strategies have evolved independently to balance X-linked gene dosage between sexes, named dosage compensation (DC). The molecular basis of DC differs among the three best-studied examples: mammals, fruit fly and nematodes. In this short review, we summarize recent microscopic and chromosome conformation capture data that reveal key features of the compensated X chromosome and highlight the events leading to the establishment of a functional, specialized nuclear compartment, the X domain.

Introduction

Eukaryotic genomes are accommodated inside the small nuclear space while staying functional. A century of microscopic studies have demonstrated that this functionality is correlated to some degree to spatial organization of chromosomes. However these experiments had limited spatial resolution due to the use of light microscopy and the small number of loci whose location could be determined simultaneously. In the last 15 years, the advent of Chromosome Conformation Capture techniques (C-techniques, box 1) enabled the generation of genome-wide probabilistic physical contact maps, allowing investigation of the spatial organization of the entire genome [1]. One of the main discoveries using C-techniques is the folding of chromatin into large-scale (100 kb – 1 Mb) topologically associated domains (TADs, also named chromosomal interacting domains) in all studied genomes ([2,3] and [4] for review). Sequences inside TADs show higher contact frequencies among themselves than with sequences in neighboring TADs (also called insulation). TADs cluster together inside the nuclear space and preferential clustering is correlated with the chromatin state and the transcriptional status of genes located inside the TADs [2,5]. This is highly reminiscent of previously described nuclear “bodies” or “foci” of functional domains such as Polycomb or transcriptional factories [6,7].

Organization of genomes into TADs may contribute to long range transcriptional regulation. An example of such long range control is dosage compensation (DC), where simultaneous regulation of multiple genes on a single chromosome occurs. The X chromosome provides therefore a fascinating model to investigate the formation and function of a specialized nuclear body and how chromosome organization is linked to chromosome-wide gene regulation. In the three best studied model systems (mammals, fruit fly and nematodes), the common underlying feature of DC is the direct interaction of a Dosage Compensation Complex (DCC) with the X chromosome. The DCC binds the X at specific sites from which it spreads to control transcription. Recent microscopic and molecular data demonstrate that DCC binding modifies X chromosome conformation and nuclear positioning of the X chromosome, which in turn could modulate X-linked gene expression. Here we review the latest developments toward understanding the link between nuclear organization and dosage compensation in these model organisms.

Mammals: a long non-coding RNA-dependent silent compartment

In mammals, females randomly inactivate one of two X chromosomes during early development (X chromosome inactivation, XCI) and stably maintain this silenced state. Microscopically, the inactive X chromosome forms a compact heterochromatic structure, the Barr body while the active X is indistinguishable from an autosome [8]. XCI is caused by the monoallelic expression of the 17kb long non-coding *Xist* RNA from one of the two X chromosomes. The Barr body is coated with *Xist*, carries high levels of histone 3 lysine 27 methylation (deposited by the Polycomb complex 2), high levels of DNA methylation and is often localized at the nuclear periphery or close to the nucleolus - two places where autosomal heterochromatin is clustered (Fig 1A(c)) [9,10]. Super-resolution microscopy revealed that the Barr body is not uniformly compact, but contains several interchromatin spaces harboring active chromatin [9,11], suggesting a spatial separation of active segments (escapee genes) and a core repressive compartment within the inactive X territory (Fig 1A(a)) [12,13].

In mice, allele specific Hi-C experiments distinguished the active X from the inactive X, providing molecular evidence supporting microscopic differences between the active and the inactive X. The active X is structurally similar to autosomes and consists of several small TADs separated by boundary regions enriched for cohesin and CTCF. In contrast, the inactive X shows a bi-partite structure with two large megadomains devoid of cohesin and CTCF. These large domains include several long range contacts between active regions that escape silencing (Fig 1A(b)) [14-16]. This bi-partite structure is a consequence of *Xist* coating, as ablation of *Xist* from the inactive X resulted in both transcriptional reactivation and acquisition of several TADs separated by cohesion enriched boundaries, resembling the ones on the active X. Cohesin repulsion was therefore suggested as a way to establish the core repressive

sub compartment of the inactive X [16]. The boundary region between the two megadomains is the DXZ4 macrosatellite repeat locus [14-16]. Unlike most of the genes on the inactive X, this locus remains transcriptionally active and harbors long range contacts with other macrosatellite regions on the inactive X [15,17,18]. Moreover, the DXZ4 appears to anchor the inactive X close to the nucleolus, suggesting interplay between megadomain formation, nuclear localization and X chromosome silencing. Further experiments including Hi-C mapping in cells lacking DXZ4 locus will provide insights into the function of the megadomain boundary. The locus encoding *Xist*, named X inactivation center (Xic), encodes many XCI regulators. In mouse, the most important one is *Tsix*, an antisense non-coding RNA suppressing *Xist* transcription before XCI. If a *Tsix* homolog is present in humans, its function in human *XIST* regulation is still debated (for review [19]). The murine conformational landscape of Xic consists of two TADs spatially separating *Xist* and *Tsix* regulators [2,3]. This separation is essential for correct regulation of the locus, as deletion of the boundary between these TADs leads to transcriptional misregulation of Xic ([3,20], reviewed in [21]). Once *Xist* transcription initiates, the RNA spreads along the X chromosome, targeting gene-rich distant regions of the chromosome before spreading to gene-poor regions [22]. Correlation between Hi-C and genome-wide chromatin mapping of *Xist*-interacting sites showed that the primary regions bound by *Xist* are in close spatial proximity to the *Xist* locus [22-24]. This is likely because these regions are transcriptionally active, as is the *Xist* locus, and transcriptionally active regions tend to cluster inside the nucleus [2,5,22,23]. A notable exception is highly-expressed genes that seem to resist *Xist* interaction during the early stages of spreading (such as the *Ocr1* gene, [23]). They however integrate later into the *Xist* compartments once the ncRNA has coated most of the X chromosome. This selective binding of *Xist* is only observed during the establishment of inactivation. In the maintenance phase, once silencing is robust *Xist* binds homogeneously along the inactive X [22]. At this stage however, *Xist* appears dispensable for the maintenance of silencing [25].

Together, these observations suggest a two-step model for the establishment and maintenance of the inactive X compartment: *Xist* first targets moderately active regions located in close proximity to the *Xist* locus, recruiting downstream effectors mediating chromatin and DNA modifications, thus creating a silent core. *Xist* then progressively coats the entire chromosome, integrating highly expressed genes into the silent compartment while escapees stay at the periphery of the inactive X nuclear domain. Once the initial coating and silencing is achieved, chromatin and DNA modifications downstream of *Xist* can maintain silencing autonomously.

Three independent purification studies of *Xist*-associated proteins uncovered a number of interacting factors, some of which help explain structural and functional aspects of the inactive X domain (10-200 interactors, depending on the individual study) [16,26,27]. *Xist* recruits

Spn/SHARP, a transcriptional repressor that interacts with the histone deacetylase HDAC3. Deacetylated histones were proposed to recruit Polycomb complexes directly or *via* Polycomb/*Xist* interaction to mediate H3K27 methylation. This would ultimately lead to transcriptional silencing of X-linked genes [26,27]. Other factors repeatedly found associated with *Xist* are the nuclear matrix proteins hnRNP/SP120/SAF-A and SAF-B. hnRNP is essential for *Xist* spreading on the X and setup of the silent chromatin domain, requiring both the hnRNP nuclear matrix interaction domain and *Xist* RNA binding domain [28]. The exact function of the nuclear matrix interaction remains, however, elusive. Finally, another common *Xist* interactor was the lamin B Receptor (LBR), a nuclear envelope component, which could potentially mediate the perinuclear targeting of the inactive X (Fig 1A(d)) [16,26,29]. Knockdown of LBR led to desilencing of genes on the inactive X, even though the Polycomb complex is present [26]. Interestingly, such mechanisms in which sets of proteins mediate silencing and dictate nuclear localization are a recurrent theme linking nuclear domain formation and gene expression regulation [30].

***Drosophila*: a ribonucleoprotein-based active compartment**

In the fruit fly, males (XY) show two-fold transcriptional upregulation of X-linked genes to balance expression with females (XX). The male X chromosome is often located close to the nuclear periphery in S2 cultured cells [31] and harbors high level of histone 4 lysine 16 acetylation [32]. The *Drosophila* dosage compensation complex (DCC) consists of 5 subunits: MSL1, 2 and 3, MOF, an H4K16 histone acetyl-transferase, and MLE, an RNA/DNA helicase with ATPase activity. *msl2* is expressed exclusively in males and assembles the DCC. MLE associates with the rest of the MSL-MOF complex via two essential but redundant long non-coding X-encoded RNAs *roX1* and *roX2* (reviewed in [33]). The fly DCC is recruited to the X chromosome in males at High Affinity Sites (HAS) or Chromatin Entry Sites (CES) characterized by a 21 bp motif (MSL recognition element or MRE) including the *roX1* and *roX2* loci. 150 to 300 of these sites are present on the X chromosome [34,35]. MSL2 interacts directly with the MREs, however the number of MRE sites that effectively recruit the MSL complex *via* direct MSL-DNA interaction is not clear [36]. Most MRE sites seem to recruit DCC *via* CLAMP (chromatin-linked adaptor for MSL proteins), a protein expressed in both sexes and bound to the MREs [37]. Unlike *Xist*, when one of the *roX* RNAs is expressed from an autosome, it spreads in *trans* and this leads to MSL complex specifically coating the X chromosome [38,39]. An autosomal *roX* transgene containing HAS sites is also coated with MSL, yet the complex cannot spread and is restricted to active loci located nearby in three dimensions [40]. Together, this suggests a model in which MSL spreading results from a combination of targeting by HAS sites and local spreading from these sites (Fig 1B(g)) [41].

Unlike the mammalian inactive X, the overall TAD structure of the X chromosome in flies does not differ between male and female cell lines [39]. Interestingly, HAS are preferentially located at TAD boundaries and different HAS seem to interact independent of MSL binding. At least for one particular HAS, the chromosomal contact network is very similar in the presence or absence of the DCC (Fig 1B(e,g)) [39]. A similar tridimensional network of interacting HAS was independently observed by comparing *roX2*-bound loci and Hi-C data of mixed-sex embryos [42]. However, DCC loading led to increased long-range contacts (between different TADs along the chromosome), reminiscent of early FISH studies analyzing distances between HAS sites [43]. These data suggest that DCC takes advantage of spatially clustered HAS to target the male X chromosome and upon binding to HAS, further creates a specific 3D conformation of the entire X chromosome by increasing long range contacts. DCC-bound sites were scored as located closer to each other in males than in females, while DCC targets and unbound sequences (mostly inactive genes) were equally distant or even located further away in males than females. Together, these experiments suggest that similar to *Xist*, the MSL complex creates a nuclear domain with active genes clustered internally and spatially separated from inactive loci located at the edge or outside of the MSL territory (Fig 1B(f)). In this model, DCC bound regions form an active sub-compartment within the X territory for systematic transcriptional upregulation.

The nuclear organization of the X chromosome also appears affected by loading of the DCC. Two subunits of the nuclear pore basket, NUP153 and Megator/TPR, co-purify with the MSL complex [31,44]. These subunits bind autosomal regions, marking transcriptionally active regions of the genome named Nucleoporin Associated Domains (NARs) [44]. Knockdown of TPR or Nup153 led to downregulation of X-linked genes and impaired perinuclear localization of the X chromosome [31,44], but did not seem to change X chromosome conformation as measured by FISH [43]. Although the exact function of these proteins remains unclear and somewhat controversial, interaction with nuclear pores may provide a scaffold to build a stable active sub-compartment. Alternatively, as NUP153 and Megator are shuttling subunits between the pore and the nucleoplasm, they might simply facilitate pore-proximal positioning of the X chromosome, therefore indirectly activating transcription (Fig 1B (h)).

Nematodes: sex-specific nuclear compartments to fine-tune transcription

Another strategy for DC was selected in nematodes: in hermaphrodites (XX), expression from both X chromosomes is downregulated two-fold by DCC, thereby matching the expression of XO males [45]. DCC in worms comprises of a subset of proteins involved in sex-determination and dosage compensation (SDC-1, SDC-2 and SDC-3) and another subset exclusively involved in dosage compensation (MIX-1, DPY-21, -26, -27, -28 and DPY-30). Together, these subsets assemble to form a multiprotein complex that resembles the mitotic/meiotic condensin

complexes and is therefore also referred to as the Condensin I^{DC}. SDC-2 is essential for the loading of the complex onto X chromosomes and expressed exclusively in hermaphrodites [46]. The DCC is loaded at *rex* sites (*recruitment element on X*) characterized by a 12 bp MEX motif (Motif Enriched on X) [47]. About 38 of these sites have been formally characterized as able to recruit the DCC, although sequence analysis and chromatin immunoprecipitation experiments predict 200-300 *rex* sites on the X chromosome. Once loaded on *rex* sites, the DCC spreads in *cis*, leading to high levels of monomethylation of histone H4 lysine 20 and partial eviction of RNA polymerase II [48-51].

The precise molecular mechanism linking these DCC loading, chromatin modifications and transcriptional regulation remains unclear. Early on, the condensin-like structure of the DCC led to the suggestion that it could modify higher order chromatin structures to control gene expression [52]. Recent results show that indeed the DCC alters both the structural organization and the nuclear localization of the X chromosome.

Whole chromosome paints and distance measurements linked DCC binding to a slight increase in overall compaction of the X chromosome (Fig 1C) [53,54]. Hi-C maps revealed that the TADs on the X chromosome in hermaphrodites showed higher internal contact frequencies than on autosomes. X chromosome TADs spanned ~1Mb, separated by boundary regions that are enriched for *rex* sites. In addition, a subset of *rex* sites contacted each other more frequently compared to non-*rex* sites (Fig 1C(i,k)) [53,55]. Upon DCC knock-down, many but not all X TAD boundaries became weaker, in particular in the middle part of the chromosome [55]. Correlated with this partial disappearance of TAD boundaries, most *rex-rex* contacts disappeared, suggesting *rex* sites cluster in a DCC dependent manner. Although X chromosome large scale structure is clearly modified by DCC loading, the functional importance of these changes in terms of gene regulation remains, however, elusive.

DCC binding to the X chromosome changes its sub-nuclear positioning. In hermaphrodite embryos, the outer third of all autosomes show high levels of interaction with the nuclear lamina, while the X chromosome only loosely interacts with the lamina at sub-telomeric regions [56,57]. In contrast, in male embryos probes distributed along the X chromosome showed preferential perinuclear localization (Fig 1C(l)) [53]. Perinuclear enrichment of the X chromosome in males appeared to be at least partially mediated by *rex* sites. Indeed, a single *rex* site is able to autonomously target an autosomal locus towards the nuclear periphery in males specifically. However, there might be additional factors like non-coding RNA genes, highly enriched on the X chromosomes, some of which were shown previously to interact with nuclear pores [58]. The male X chromosome interacts broadly with a component of the nuclear pore (Fig 1C(l)), similar to *Drosophila* [31,44,53]. The loading of the DCC onto X chromosomes in hermaphrodites impaired both perinuclear chromosome localization as well as nucleoporin interaction [53].

Together, molecular and microscopy data suggest the presence of two distinct sex-specific compartments in *C. elegans*. In males, the X chromosome interacts with nucleoporins, creating an activating compartment able to increase X-linked genes expression. In hermaphrodites, DCC loading onto X chromatin on the one hand increases overall chromosome compaction and TAD structure while on the other hand it impairs activation at pores by masking the interaction sites and altering X chromosome position [53,59]. How these changes ultimately impact RNA polymerase levels on the X remains to be elucidated.

Nuclear organization and Dosage Compensation: where is the link?

Dosage compensation equates expression between X-linked and autosomal genes by either up- or down-regulating X-linked transcription. In the three systems where DC is mechanistically best understood, DC undoubtedly leads to changes in the X chromosome large scale organization, nuclear localization and chromatin composition. Interestingly, DC always makes use of chromosomal topology for DCC initial targeting and/or spreading along the X chromosome before modifying this organization to stabilize gene expression. Setting up a specialized compartment for the X chromosome provides an additional layer of gene regulation, segregating activating factors (male flies and nematodes) or silencing complexes (mammals and nematodes) from the rest of the genome in a self-reinforcing compartment. Understanding which factors setup the different X domains and stabilize these over the course of development will shed light on the importance of nuclear organization for X chromosome transcriptional regulation, providing useful paradigms for the plethora of other nuclear compartments.

Figure legend

Figure 1. X chromosome functional organization and nuclear positioning in mammals (humans, mice), *Drosophila melanogaster* and *Caenorhabditis elegans*.

A. In mammals, females inactivate one of the two X chromosomes. The inactive X (Xi) is coated with the long non-coding RNA *Xist*, compacted into a dense structure comprising two large contact domains (megadomains) often located at the nuclear periphery or close to the nucleolus. In contrast, the active X is euchromatic. **Inset:** (a) Inactive and active regions in the Xi territory are spatially separated, with silent regions clustered inside the Xi chromosome territory. (b) The active regions on the Xi engage in long-range contacts. (c) *Xist* coated regions tend to cluster together forming superdomains and interact with Polycomb to mediate the creation of the silent sub-compartment within the Xi. (d) *Xist* interacts with Lamin A/C and Lamin B receptor (LBR), which could position the Xi at the nuclear periphery.

B. In *Drosophila*, the single X chromosome in males is coated with the MSL complex which mediates two-fold upregulation of X-linked genes. **Inset:** (e) and (g) MSL binding sites (High Affinity Sites (HAS)/Chromatin Entry Sites (CES))) interact and cluster in both sexes, and the overall TAD organization of the X remains largely similar. (f) In males, transcriptionally unaffected or silent regions of the X chromosome are spatially excluded from the MSL territory. (g) Upon recruitment at HAS/CES, MSL spreading is restricted to spatially proximal regions. (h) MSL bound regions on the X chromosome are enriched for Nucleoporins interactions and the MSL complex co-purifies with two nuclear pore subunits.

C. In *C. elegans*, the two X chromosomes in hermaphrodites are bound by the condensin-like dosage compensation complex (DCC), which leads to overall chromatin compaction. Both X are randomly positioned and undergo a two-fold downregulation. The single X in males is less compact and transcriptionally upregulated. **Inset:** (i) and (k) In hermaphrodites, DCC spreads from its loading sites (recruitment element on X, *rex*). These *rex* sites cluster in a DCC dependent manner. (j) No spatial segregation of active/repressive regions has been reported in *C. elegans*, while the DCC binds preferentially active genes. (l) The male X chromosome is upregulated, located at the nuclear periphery and interacts with a nucleoporin.

Color codes for all systems: Black - the X territory; Color gradient represents transcriptional activity ranging from red (inactive/silent) to green (upregulated). Blue circles represent region of interest. -

Box 1. - Chromosome conformation capture techniques

Chromosome Conformation Capture (3C) techniques are based on the principle that in cross-linked chromatin, restriction fragments far apart on a chromosome or even on different chromosomes but close in the nuclear space can be ligated together. Different variations of 3C techniques exist (see below), but the initial steps are the same. Chromatin is cross-linked

with formaldehyde and cut with a restriction enzyme. Fragments are ligated together, leading to ligation products consisting of distant fragments of the linear genome.

One to one and one to many techniques

3C is based on (semi-)quantitative PCR with a pair of primers hybridizing near the ends of restriction fragments of interest [60]. When repeated for many pairs, this gives a matrix of relative ligation efficiency for all studied fragments.

The 4C methodology (Circularized 3C) is based on the creation of small DNA circles by another round of restriction digest and ligation [61]. The target locus (often called viewpoint) is circularized, capturing a ligated distant fragment. These circles are amplified using PCR with primers in the viewpoint pointing outwards. Amplicons are then either hybridized to microarrays or sequenced. This approach gives a genomic view of all possible contact partners from one site with the rest of the genome at high resolution.

Many to many technique

The 5C technology (Carbon Copy 3C) gives an overview of contacts between multiple sequences [62]. Instead of using a single oligonucleotide, numerous oligonucleotides corresponding to the different restriction sites in the genomic region of interest are hybridized. The 5' end of all these nucleotides carry the same T7/T3 sequence, which is used for PCR amplification. PCR products can be either hybridized to microarray or sequenced. The result is a matrix of contact frequencies for many sites.

All to all technique

For Hi-C, restriction ends are labelled using biotin-tagged nucleotides [63-65]. After ligation, purification and shearing, ligated fragments are pulled-down using biotin and sequenced. A matrix of contact frequencies between all restriction fragments in the genome can be constructed. Importantly, one restriction fragment can only ligate once in a given haploid cell. Therefore, contact maps constructed using C techniques are probabilistic and represent the likelihood of two given fragments contacting together.

The resolution of these techniques depends on the size in base pairs of the recognition sequence of the restriction enzyme used to create the fragments and the sequencing depth of libraries Hi-C. Whereas initial studies achieved only megabase resolution, the latest study with 15 billion contact reads reached kilobase resolution [15].

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Annotated references

Papers of Outstanding Interest: (two dots)

Minajigi et al Science 2015

Using a new technique iDRiP (identification of direct RNA interacting proteins), the authors identified ~200 unique interactors of *Xist* lncRNA. Apart from known interactors like hnRNP and ATRX, many structural proteins like Lamin B receptor, Cohesin, Condensin were found as novel interactors of *Xist* and knockdown of these influenced X-linked gene regulation. Allele-specific Hi-C experiments demonstrated a prominent loss of TAD structure on inactive X compared to active X, proposed to be a consequence of cohesion repulsion.

Chu et al Cell 2015

Authors performed Chromatin Isolation by RNA Purification coupled with Mass Spectrometry (ChiRP-MS) to uncover the *Xist*-associated proteome. They identified ~81 proteins including both known and novel *Xist* interactors. A key discovery was SHARP/SPEN, which binds to the A repeat region of *Xist* and recruits HDACs to silence gene expression. SPEN association with *Xist* also increases over differentiation. Using different systems like mouse ESC (No XCI) and differentiated cells (XCI established), trophoblast stem cells (imprinted X) and Epiblast stem cells (XCI initiated), the authors showed that the *Xist* interactome is unchanged between random and Imprinted XCI and evolves during XCI establishment.

Quinn et al Nat Biotech 2014

In this study, the authors isolated domain-specific chromatin using an RNA purification (dChiRP) technique to analyse RNA-RNA, RNA-DNA and RNA-protein interactions of specific domains of lncRNAs. Using dChiRP for U and D domains of *roX1* RNA in *Drosophila* clone 8 (male) cells, the authors first confirmed that only D domains interact with the MSL complex and then reported a novel *roX1* RNA conformation that clusters U domains together and exposes the D domains at the surface of the folded RNA to mediate interactions. Moreover, authors reported a positive correlation between *roX2* occupied regions (ChiRP) and *roX2* proximal regions (Hi-C). Conversely, the same analysis for *roX1* showed anticorrelation.

Ramirez et al Mol Cell 2015

The authors generated Hi-C profiles in a *Drosophila* male cell lines (clone 8 and S2) and compared these with previously published Hi-C profiles established in female Kc or embryonic mixed sex cells. They reported the striking similarity between TAD structures in both sexes. They also discovered that TAD boundaries are enriched for High affinity DCC binding sites (HAS). These HAS sites contact each other in a sex independent manner. Moreover, they showed that the *roX* RNA, part of the male DCC can coat the X chromosome with MSL even

when expressed from an autosomal locus. In contrast, the ectopic HAS site created by an autosomal insertion of the *roX* RNA locus is able to recruit MSL but the complex spreads only locally to spatially proximal regions.

Crane et al Nature 2015

This paper described the first Hi-C analysis in *C. elegans* hermaphrodite embryos. Compared to the autosomes, the two X chromosomes revealed prominent TADs separated by boundaries enriched for DCC recruitment sites (*rex* sites). Most *rex* sites appeared to interact and cluster together. Knockdown of the DCC (*sdC-2*) led to weakening of certain boundaries and partial disappearance of some TADs, as well as most *rex-rex* interactions. However, changes in gene expression upon DCC inhibition did not correlate with any particular change in TAD structures.

Sharma et al Gen Dev 2014

Using FISH and Live Imaging, the authors showed that the X chromosome is preferentially located at the nuclear periphery in males compared to a random localization in the hermaphrodite. DCC knockdown led to peripheral enrichment of the X in hermaphrodites. DCC recruitment sites (*rex* sites) could autonomously target to the nuclear periphery in males. DamID analysis revealed that the entire male X chromosome interacts broadly with a nuclear pore protein suggesting nuclear pore interaction as a mechanism for the reported transcriptional upregulation of the male X chromosome, while the DCC would impair this activation by masking pore-interaction sites.

Papers of special interest: (one dot)

McHugh et al Nature 2015

Using RNA antisense purification Mass spectrometry (RAP-MS) in mouse ES cells, authors identified 10 proteins that directly and specifically interact with the *Xist* lncRNA. The list includes previously known candidates such as hnRNP/SAF-A and new candidates like SHARP/SPEN, the Lamin B receptor, RBM15 and others. Using single molecule FISH (smFISH) to measure X-linked transcripts, they showed that most candidates lead to desilencing of X-linked genes upon knockdown. Moreover, knockdown of SHARP and HDAC3 revealed enhanced RNA polymerase II staining and exclusion of EZH2 (Polycomb complex) from the *Xist* territory, suggesting that histone deacetylation by HDAC3 might be important to recruit Polycomb and create a silent compartment. Interestingly, LBR knockdown led to desilencing in the absence of RNA PolIII and presence of EZH2, but the actual mechanism remains unexplained.

Rao et al Cell 2014

Authors performed High resolution Hi-C in different cell lines and revealed two large superdomains for the inactive X separated at the DXZ4 locus, in contrast the active X folds

into several TADs. Interestingly, the authors found new long range interactions on the inactive X and confirmed some previously reported ones.

Deng et al., Genome Biol.

This study uses F1 hybrid cell lines of a *Mus spretus* x *Mus musculus* cross which allows discrimination between Xi (from *musculus*) and Xa based on SNPs. DNase I Hi-C showed that the inactive X is split in two megadomains which boundary is located at the DXZ4 locus. Polymer modeling suggest the active loci on the inactive X are located at the periphery of the inactive X domain.

References

1. de Wit E, de Laat W: **A decade of 3C technologies: insights into nuclear organization.** *Genes & development* 2012, **26**:11-24.
2. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B: **Topological domains in mammalian genomes identified by analysis of chromatin interactions.** *Nature* 2012, **485**:376-380.
3. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, et al.: **Spatial partitioning of the regulatory landscape of the X-inactivation centre.** *Nature* 2012, **485**:381-385.
4. Dekker J, Heard E: **Structural and functional diversity of Topologically Associating Domains.** *FEBS letters* 2015, **589**:2877-2884.
5. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G: **Three-dimensional folding and functional organization principles of the Drosophila genome.** *Cell* 2012, **148**:458-472.
6. Cheutin T, Cavalli G: **Polycomb silencing: from linear chromatin domains to 3D chromosome folding.** *Current opinion in genetics & development* 2014, **25C**:30-37.
7. Ferrai C, de Castro IJ, Lavitas L, Chotalia M, Pombo A: **Gene positioning.** *Cold Spring Harbor perspectives in biology* 2010, **2**:a000588.
8. Barr ML, Bertram EG: **A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis.** *Nature* 1949, **163**:676.
9. Rego A, Sinclair PB, Tao W, Kireev I, Belmont AS: **The facultative heterochromatin of the inactive X chromosome has a distinctive condensed ultrastructure.** *Journal of cell science* 2008, **121**:1119-1127.
10. Yang F, Deng X, Ma W, Berletch JB, Rabaia N, Wei G, Moore JM, Filippova GN, Xu J, Liu Y, et al.: **The lncRNA Firre anchors the inactive X chromosome to the nucleolus by binding CTCF and maintains H3K27me3 methylation.** *Genome biology* 2015, **16**:52.
11. Smeets D, Markaki Y, Schmid VJ, Kraus F, Tattermusch A, Cerase A, Sterr M, Fiedler S, Demmerle J, Popken J, et al.: **Three-dimensional super-resolution microscopy of the inactive X chromosome territory reveals a collapse of its active nuclear compartment harboring distinct Xist RNA foci.** *Epigenetics & chromatin* 2014, **7**:8.
12. Chaumeil J, Le Baccon P, Wutz A, Heard E: **A novel role for Xist RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced.** *Genes & development* 2006, **20**:2223-2237.
13. Splinter E, de Wit E, Nora EP, Klous P, van de Werken HJ, Zhu Y, Kaaij LJ, van Ijcken W, Gribnau J, Heard E, et al.: **The inactive X chromosome adopts a unique three-dimensional conformation that is dependent on Xist RNA.** *Genes Dev* 2011, **25**:1371-1383.
14. Deng X, Ma W, Ramani V, Hill A, Yang F, Ay F, Berletch JB, Blau CA, Shendure J, Duan Z, et al.: **Bipartite structure of the inactive mouse X chromosome.** *Genome biology* 2015, **16**:152.
15. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al.: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping.** *Cell* 2014, **159**:1665-1680.
16. Minajigi A, Froberg JE, Wei C, Sunwoo H, Kesner B, Colognori D, Lessing D, Payer B, Boukhali M, Haas W, et al.: **A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation.** *Science* 2015, **349**.
17. 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 research* 2008, **18**:1259-1269.
18. Horakova AH, Moseley SC, McLaughlin CR, Tremblay DC, Chadwick BP: **The macrosatellite DXZ4 mediates CTCF-dependent long-range intrachromosomal interactions on the human inactive X chromosome.** *Human molecular genetics* 2012, **21**:4367-4377.

19. Romito A, Rougeulle C: **Origin and evolution of the long non-coding genes in the X-inactivation center.** *Biochimie* 2011, **93**:1935-1942.
20. Giorgetti L, Galupa R, Nora EP, Piolot T, Lam F, Dekker J, Tiana G, Heard E: **Predictive polymer modeling reveals coupled fluctuations in chromosome conformation and transcription.** *Cell* 2014, **157**:950-963.
21. Galupa R, Heard E: **X-chromosome inactivation: new insights into cis and trans regulation.** *Current opinion in genetics & development* 2015, **31**:57-66.
22. Simon MD, Pinter SF, Fang R, Sarma K, Rutenberg-Schoenberg M, Bowman SK, Kesner BA, Maier VK, Kingston RE, Lee JT: **High-resolution Xist binding maps reveal two-step spreading during X-chromosome inactivation.** *Nature* 2013, **504**:465-469.
23. Engreitz JM, Pandya-Jones A, McDonel P, Shishkin A, Sirokman K, Surka C, Kadri S, Xing J, Goren A, Lander ES, et al.: **The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome.** *Science* 2013, **341**:1237973.
24. Marks H, Kerstens HH, Barakat TS, Splinter E, Dirks RA, van Mierlo G, Joshi O, Wang SY, Babak T, Albers CA, et al.: **Dynamics of gene silencing during X inactivation using allele-specific RNA-seq.** *Genome biology* 2015, **16**:149.
25. Csankovszki G, Panning B, Bates B, Pehrson JR, Jaenisch R: **Conditional deletion of Xist disrupts histone macroH2A localization but not maintenance of X inactivation.** *Nature genetics* 1999, **22**:323-324.
26. McHugh CA, Chen CK, Chow A, Surka CF, Tran C, McDonel P, Pandya-Jones A, Blanco M, Burghard C, Moradian A, et al.: **The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3.** *Nature* 2015, **521**:232-236.
27. Chu C, Zhang QC, da Rocha ST, Flynn RA, Bharadwaj M, Calabrese JM, Magnuson T, Heard E, Chang HY: **Systematic discovery of Xist RNA binding proteins.** *Cell* 2015, **161**:404-416.
28. Hasegawa Y, Brockdorff N, Kawano S, Tsutui K, Nakagawa S: **The matrix protein hnRNP U is required for chromosomal localization of Xist RNA.** *Developmental cell* 2010, **19**:469-476.
29. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M, Cohen TV, Devys D, Foisner R, Peichl L, et al.: **LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation.** *Cell* 2013, **152**:584-598.
30. Meister P, Taddei A: **Building silent compartments at the nuclear periphery: a recurrent theme.** *Current opinion in genetics & development* 2013, **23**:96-103.
31. Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K, Mueller J, et al.: **Nuclear pore components are involved in the transcriptional regulation of dosage compensation in Drosophila.** *Mol Cell* 2006, **21**:811-823.
32. Smith ER, Pannuti A, Gu W, Steurnagel A, Cook RG, Allis CD, Lucchesi JC: **The drosophila MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation.** *Molecular and cellular biology* 2000, **20**:312-318.
33. Lucchesi JC, Kuroda MI: **Dosage compensation in Drosophila.** *Cold Spring Harbor perspectives in biology* 2015, **7**.
34. Alekseyenko AA, Peng S, Larschan E, Gorchakov AA, Lee OK, Kharchenko P, McGrath SD, Wang CI, Mardis ER, Park PJ, et al.: **A sequence motif within chromatin entry sites directs MSL establishment on the Drosophila X chromosome.** *Cell* 2008, **134**:599-609.
35. Straub T, Grimaud C, Gilfillan GD, Mitterweger A, Becker PB: **The chromosomal high-affinity binding sites for the Drosophila dosage compensation complex.** *PLoS genetics* 2008, **4**:e1000302.
36. Zheng S, Villa R, Wang J, Feng Y, Becker PB, Ye K: **Structural basis of X chromosome DNA recognition by the MSL2 CXC domain during Drosophila dosage compensation.** *Genes & development* 2014, **28**:2652-2662.
37. Soruco MM, Chery J, Bishop EP, Siggers T, Tolstorukov MY, Leydon AR, Sugden AU, Goebel K, Feng J, Xia P, et al.: **The CLAMP protein links the MSL complex to the**

- X chromosome during *Drosophila* dosage compensation.** *Genes & development* 2013, **27**:1551-1556.
38. Meller VH, Rattner BP: **The roX genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex.** *The EMBO journal* 2002, **21**:1084-1091.
 39. Ramirez F, Lingg T, Toscano S, Lam KC, Georgiev P, Chung HR, Lajoie BR, de Wit E, Zhan Y, de Laat W, et al.: **High-Affinity Sites Form an Interaction Network to Facilitate Spreading of the MSL Complex across the X Chromosome in *Drosophila*.** *Molecular cell* 2015, **60**:146-162.
 40. : !!! INVALID CITATION !!!
 41. Park Y, Kelley RL, Oh H, Kuroda MI, Meller VH: **Extent of chromatin spreading determined by roX RNA recruitment of MSL proteins.** *Science* 2002, **298**:1620-1623.
 42. Quinn JJ, Ilik IA, Qu K, Georgiev P, Chu C, Akhtar A, Chang HY: **Revealing long noncoding RNA architecture and functions using domain-specific chromatin isolation by RNA purification.** *Nature biotechnology* 2014, **32**:933-940.
 43. Grimaud C, Becker PB: **The dosage compensation complex shapes the conformation of the X chromosome in *Drosophila*.** *Genes Dev* 2009, **23**:2490-2495.
 44. Vaquerizas JM, Suyama R, Kind J, Miura K, Luscombe NM, Akhtar A: **Nuclear pore proteins nup153 and megator define transcriptionally active regions in the *Drosophila* genome.** *PLoS Genet* 2010, **6**:e1000846.
 45. Strome S, Kelly WG, Ercan S, Lieb JD: **Regulation of the X Chromosomes in *Caenorhabditis elegans*.** *Cold Spring Harbor perspectives in biology* 2014, **6**.
 46. Dawes HE, Berlin DS, Lapidus DM, Nusbaum C, Davis TL, Meyer BJ: **Dosage compensation proteins targeted to X chromosomes by a determinant of hermaphrodite fate.** *Science* 1999, **284**:1800-1804.
 47. McDonel P, Jans J, Peterson BK, Meyer BJ: **Clustered DNA motifs mark X chromosomes for repression by a dosage compensation complex.** *Nature* 2006, **444**:614-618.
 48. Ercan S, Dick LL, Lieb JD: **The *C. elegans* dosage compensation complex propagates dynamically and independently of X chromosome sequence.** *Current biology : CB* 2009, **19**:1777-1787.
 49. Vielle A, Lang J, Dong Y, Ercan S, Kotwaliwale C, Rechtsteiner A, Appert A, Chen QB, Dose A, Egelhofer T, et al.: **H4K20me1 contributes to downregulation of X-linked genes for *C. elegans* dosage compensation.** *PLoS genetics* 2012, **8**:e1002933.
 50. Kruesi WS, Core LJ, Waters CT, Lis JT, Meyer BJ: **Condensin controls recruitment of RNA polymerase II to achieve nematode X-chromosome dosage compensation.** *eLife* 2013, **2**:e00808.
 51. Wells MB, Snyder MJ, Custer LM, Csankovszki G: ***Caenorhabditis elegans* dosage compensation regulates histone H4 chromatin state on X chromosomes.** *Molecular and cellular biology* 2012, **32**:1710-1719.
 52. Chuang PT, Albertson DG, Meyer BJ: **DPY-27:a chromosome condensation protein homolog that regulates *C. elegans* dosage compensation through association with the X chromosome.** *Cell* 1994, **79**:459-474.
 53. Sharma R, Jost D, Kind J, Gomez-Saldivar G, van Steensel B, Askjaer P, Vaillant C, Meister P: **Differential spatial and structural organization of the X chromosome underlies dosage compensation in *C. elegans*.** *Genes & development* 2014, **28**:2591-2596.
 54. Lau AC, Nabeshima K, Csankovszki G: **The *C. elegans* dosage compensation complex mediates interphase X chromosome compaction.** *Epigenetics & chromatin* 2014, **7**:31.
 55. Crane E, Bian Q, McCord RP, Lajoie BR, Wheeler BS, Ralston EJ, Uzawa S, Dekker J, Meyer BJ: **Condensin-driven remodelling of X chromosome topology during dosage compensation.** *Nature* 2015, **523**:240-244.

56. Ikegami K, Egelhofer TA, Strome S, Lieb JD: **Caenorhabditis elegans chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2.** *Genome biology* 2010, **11**:R120.
57. Towbin BD, Gonzalez-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, Askjaer P, Gasser SM: **Step-Wise Methylation of Histone H3K9 Positions Heterochromatin at the Nuclear Periphery.** *Cell* 2012, **150**:934-947.
58. Ikegami K, Lieb JD: **Integral nuclear pore proteins bind to Pol III-transcribed genes and are required for Pol III transcript processing in C. elegans.** *Molecular cell* 2013, **51**:840-849.
59. Sharma R, Meister P: **Linking dosage compensation and X chromosome nuclear organization in C. elegans.** *Nucleus* 2015, **6**:266-272.
60. Dekker J, Rippe K, Dekker M, Kleckner N: **Capturing chromosome conformation.** *Science* 2002, **295**:1306-1311.
61. Simonis M, Klous P, Splinter E, Moshkin Y, Willemsen R, de Wit E, van Steensel B, de Laat W: **Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C).** *Nat Genet* 2006, **38**:1348-1354.
62. Dostie J, Richmond TA, Arnaout RA, Selzer RR, Lee WL, Honan TA, Rubio ED, Krumm A, Lamb J, Nusbaum C, et al.: **Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements.** *Genome research* 2006, **16**:1299-1309.
63. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al.: **Comprehensive mapping of long-range interactions reveals folding principles of the human genome.** *Science* 2009, **326**:289-293.
64. Rodley CD, Bertels F, Jones B, O'Sullivan JM: **Global identification of yeast chromosome interactions using Genome conformation capture.** *Fungal genetics and biology : FG & B* 2009, **46**:879-886.
65. Duan Z, Andronescu M, Schutz K, McIlwain S, Kim YJ, Lee C, Shendure J, Fields S, Blau CA, Noble WS: **A three-dimensional model of the yeast genome.** *Nature* 2010, **465**:363-367.

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