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Pluripotency in 3D: genome organization in pluripotent cells

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Cells face the challenge of storing two meters of DNA in the three-dimensional (3D) space of the nucleus that spans only a few microns. The nuclear organization that is required to overcome this challenge must allow for the accessibility of the gene regulatory machinery to the DNA and, in the case of embryonic stem cells (ESCs), for the transcriptional and epigenetic changes that accompany differentiation. Recent technological advances have allowed for the mapping of genome organization at an unprecedented resolution and scale. These breakthroughs have led to a deluge of new data, and a sophisticated understanding of the relationship between gene regulation and 3D genome organization is beginning to form. In this review we summarize some of the recent findings illuminating the 3D structure of the eukaryotic genome, as well as the relationship between genome topology and function from the level of whole chromosomes to enhancer–promoter loops with a focus on features affecting genome organization in ESCs and changes in nuclear organization during differentiation.

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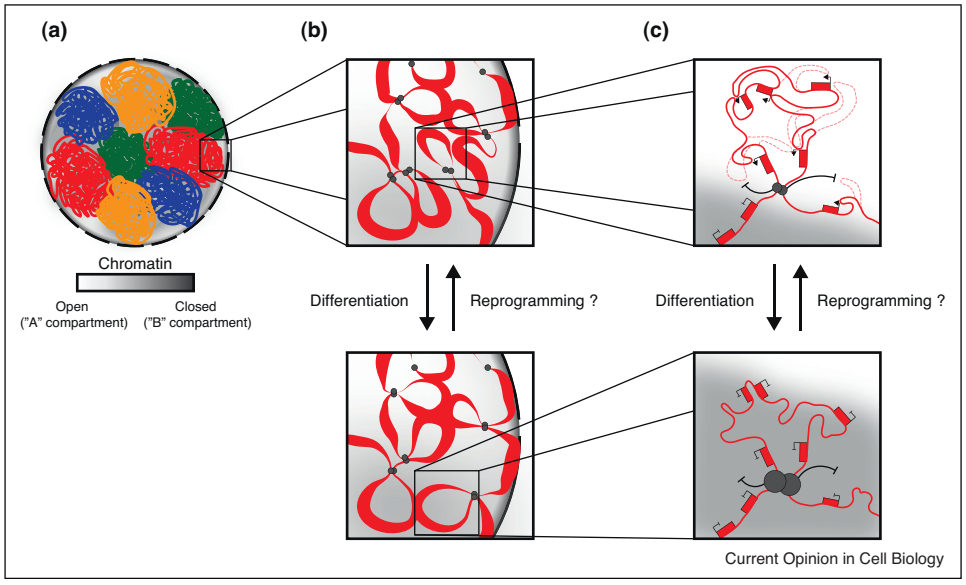
Introduction

Embryonic stem cells (ESCs), isolated from the inner cell mass of pre-implantation blastocysts, self-renew indefinitely under appropriate culture conditions and have the ability to produce cell types from all three germ layers upon induction of differentiation *in vivo* and *in vitro* [1,2]. Linear genomic features, such as the location of transcription factors, the basic transcriptional machinery, and chromatin modifications, as well as DNase hypersensitivity, expression state, and replication timing have been extensively mapped in ESCs. Therefore, gene regulatory processes which control the transcriptional program of ESCs are relatively well characterized (reviewed recently elsewhere [3]) and center on three core transcriptional networks: the pluripotency network, made up of highly expressed, ESC-specific genes bound by the transcription factors Oct4, Sox2, and Nanog which, together, control pluripotency through co-binding of many enhancers and promoters including their own [3]; the cMyc network, formed by transcription factors of the Myc family which drives gene expression by promoting the release of paused polymerase at its target genes [4]; and, the Polycomb group (PcG) protein network, which represses developmental and lineage-specific genes [5] through the tri-methylation of lysine 27 of histone 3 (H3K27me3) [6], H2AK119 ubiquitylation [7], and chromatin compaction [8]. These transcriptional networks work in concert with external signaling pathways to maintain the pluripotent state, most notably the LIF-Jak-Stat pathway in mouse ESCs [9] and bFGF-signaling in human ESCs [10]. Highlighting the importance of these transcriptional networks to pluripotent cell identity, ectopic expression of Oct4, Sox2, cMyc, and the pluripotency-associated transcription factor Klf4 is sufficient to reprogram somatic cells to induced pluripotent stem cells (iPSCs) [11]. iPSCs carry all the typical characteristics of ESCs including self-renewal, expression of the endogenous pluripotency program, and differentiation in both the teratoma and chimera formation assays [11]. More recently, there has been a push towards determining genome organization and correlating 3D topology with genomic functions such as transcriptional regulation. Because transcriptional networks and gene regulation are well studied in ESCs, these cells are an excellent model system with which to understand 3D genome organization and its changes upon cell fate change. In this review, we will first summarize general aspects of genome organization revealed from work with various cell types and then focus on new findings that begin to address genome organization in ESCs and its changes upon induction of differentiation.

Widely conserved features of genome organization: a top down view

Years of research from many groups utilizing a variety of cell types from numerous species have defined a number of general features of eukaryotic genome organization. Interphase chromosomes reside in discrete, minimally overlapping chromosome territories (CTs, reviewed exhaustively by the Cremer brothers [12], Figure 1a). CTs are organized such that small, gene rich chromosomes tend to pair and localize to the nuclear interior [13,14]. Cell type-specific radial positioning of CTs within the nucleus has also been reported [15], although

Figure 1



Hierarchical levels of genome organization and changes upon ESC differentiation. Model of the 3D organization of the genome in ESCs and its changes during the course of differentiation. We infer this model by combining findings from many different cell types. **(a)** Chromosomes exist as discrete, minimally overlapping territories. At the megabase level, compartments of open (white) and closed (gray) chromatin coarsely divide the genome into regions enriched for features of euchromatin and those depleted of euchromatic features, respectively. Locus positioning within the open or closed compartment defines probable interaction partners, with loci in the open compartment interacting more frequently with other open loci, and those in closed more frequently with other closed loci. **(b)** Below the megabase, level the genome is divided into topological domains, which, we speculate, exist as large chromatin loops created by the juxtaposition of CTCF binding sites at TAD boundaries (gray circles). These TADs function as modular units of genome organization whose member genes are often co-regulated, and we propose, localize as units within the nucleus and potentially switch between open and closed compartments during differentiation, somatic cell reprograming, or between different cell types as their euchromatic character changes. **(c)** Within TADs, enhancers and promoters loop together extensively and promiscuously to orchestrate cell type-specific gene expression profiles. Genes are more likely to be co-regulated if they lie within the same TAD, as opposed to between TADs. During differentiation and transcription factor induced reprogramming, all genes within a TAD may switch their transcriptional state.

the extent to which CT pairing and positioning are conserved through mitosis varies depending on the cell type analyzed [16,17]. Individual genes are largely confined to their respective chromosome's territory, however, in certain developmental contexts, such as Hox gene activation [18] and X chromosome inactivation [19••] (XCI, discussed in more detail below), gene loci have been shown to loop out or move to the outer edges of their CTs.

Localization of genomic regions to the nuclear periphery, specifically the nuclear lamina, is correlated with gene silencing across the eukaryotic kingdom [20–22], and ectopic targeting of genetic loci to the nuclear envelope (NE) can induce transcriptional silencing in some cases [20,23,24]. NE-mediated gene silencing is thought to function partly through the interaction of heterochromatin protein 1 (HP1) with repressive protein complexes localized to the NE through interactions with the B-type lamins, the major constituents of the NE (reviewed extensively elsewhere [25]), as well as through histone-Lamin A interactions [26]. Sequestration of the transcriptional machinery away from the nuclear periphery has been suggested as an additional mechanism of NE-mediated transcriptional silencing, although it is unclear if this phenomenon is a general feature of eukaryotic genome organization [27]. Recent work has added a new player in targeting specific genomic regions to the NE, the vertebrate homologue of the *Drosophila* GAGA factor, cKrox. cKrox binds GA repeat-enriched lamina associating DNA sequences (LASs) in a cell type-specific manner, targeting these regions to the NE, although it is currently unclear how cKrox is targeted to specific LASs [28].

Early studies of genome organization relied on cytological methods such as fluorescence *in situ* hybridization (FISH), and as such were limited in the number of gene loci that could be analyzed in a single experiment. The past decade has witnessed the introduction of molecular techniques and high-throughput mapping to the field of genome organization in the form of chromosome conformation capture (3C)-based techniques. 3C allows for a molecular view of genome organization via chemical fixation, restriction enzyme digestion, ligation of juxtaposed DNA fragments and detection of ligation events by PCR. The juxtaposition frequency of two DNA fragments in 3D space can be inferred based on the quantity

of the PCR product produced upon amplifying a given ligation event [29]. In recent years a number of groups have expanded 3C-based molecular techniques [30] to include 4C – which allows for the identification of all chromatin contacts made by a single locus with the rest of the genome [31,32], 5C – enabling the identification of all pair-wise chromatin interactions for a given genomic region [33], Hi–C [34] and its technical variants [35,36,37••] — which identify all pairwise chromatin interactions genome-wide, and ChIA-PET [38] — allowing for the identification of all pairwise chromatin interactions genome-wide, which share binding of a protein of interest.

These techniques have revealed a previously unappreciated hierarchical organization of eukaryotic genomes. As expected from the CT-based structure of the genome, intra-chromosomal (*cis*) chromatin interactions mapped by 3C-based techniques are much more frequent than inter-chromosomal (*trans*) ones [31,34]. Apart from verifying the existence of CTs and the preferential pairing of small, gene rich chromosomes, mapping of genome-wide chromatin interactions with Hi–C in human lymphoblasts [34], mouse pro-B cells [39], and *Drosophila* embryos [37••] demonstrated the existence of a further organizational subdivision of the genome into 'A' and 'B' compartments, where the A compartment is enriched for features of euchromatin and the B compartment is depleted of these features [34]. From an organizational standpoint, chromatin interactions within compartments are much more frequent than those between compartments (Figure 1a and b).

The comparatively smaller size of the *Drosophila* genome allowed for higher resolution DNA topology mapping than was previously accomplished in mammalian genomes and led to the identification of a further organizational subdivision of the genome into linear domains with shared epigenetic features, ranging in size from 10 kilobases (kb) to 500 kb [37••]. These domains appear to act modularly in governing global genome organization in *Drosophila*. Interactions of loci within a given domain are more frequent than interactions between loci in different domains. However, where inter-domain interactions occur, active domains preferentially interact with other active domains, inactive domains predominantly with inactive domains regulated domains with other domains of PcG enrichment [37••]. Recent work with a number of different cell lines has identified analogous domains in mammalian genomes [40•,41••,42••], termed topological domains or topologically associating domains (TADs). TADs delimit the range within which enhancers can affect their target genes, as co-regulated enhancer–promoter groups tend to form extended clusters of interacting chromatin that align with TADs [43••] (Figure 1c). Additionally, changes in gene expression upon differentiation are more likely to occur in the same direction for genes within a TAD than for genes in different TADs [41••]. It has long been appreciated that enhancer–promoter interactions are responsible for regulating the cell type-specific expression of genes. The importance of looping between promoter and enhancers for gene regulation is highlighted by data from the ENCODE consortium showing that genes whose transcriptional start sites are contacted by an enhancer are more highly transcribed than those that are not [44•].

The locations of TAD boundaries are strongly conserved between the mouse and the human genomes, particularly within syntenic regions; and TADs of both species' are largely conserved across different cell types [40•,41••]. CP190, a critical contributor to the function of various *Drosophila* insulator proteins through its mediation of DNA looping [45], is enriched at TAD boundaries in *Drosophila*, and the vertebrate insulator protein CTCF [46] is similarly enriched at the boundaries of a large subset of mammalian TADs [40•,42••], suggesting an evolutionarily conserved mechanism of TAD boundary formation by insulator proteins, similar to what has been proposed for mammalian insulators in general [47]. In ESCs, CTCF has been shown to mediate DNA looping events which partition the genome into physical domains each characterized by distinct epigenetic states [42••], supporting a model of DNA organization wherein many TADs function as large, independently regulated DNA loops (Figure 1b, c). Although the data arguing for the role of insulator proteins in delimiting TAD boundaries are strong, it is worth noting that only a portion of insulator binding sites function as TAD boundaries in mammalian and *Drosophila* cells [37••,40•], and that many enhancer–promoter interactions cross CTCF binding events in a variety of mammalian cells types [44•]. More work will therefore be required to determine the necessary and sufficient constituents of TAD boundary delimiters.

Albeit in flies interactions between TADs have been described [37••] (see above), the extent to which mammalian TADs interact with each other, and the mechanistic logic behind these interactions, remains unclear. Long-range, distal chromatin interactions between loci many millions of bases (Mb) apart, or in *trans*, have been demonstrated in a number of mammalian cell types by various 3C-based studies [31,32,34,42••,44•], but these interactions have not been examined in the context of TADs. It has been show that these long-range chromatin contacts can be cell type-specific and can occur between regions of the genome-enriched for the DNA binding motif of a given transcription factor or for genes regulated by the same *trans* acting factors [48,49], or by binding of gene regulatory factors as it has been demonstrated for PcG-regulated distal chromatin interactions in *Drosophila* [37••,50] (Figure 1). One may speculate that co-regulated TADs are brought together in physical space in mammalian genomes as a general rule. Comprehensive analysis of

long-range interactions in a well-annotated cell type such as ESCs should contribute to a better understanding of this question, as gene regulatory networks are well understood [3], and — in the case of mouse ESCs — are amenable to genetic manipulations, which can be used to test causal links between linear genomic features and genome organization both in pluripotency and during the course of differentiation.

The ESC genome in pluripotency and differentiation

The genomes of ESCs have a number of unique characteristics that distinguish them from somatic cell genomes. The contribution of these features to the different layers genome organization described above is currently unclear, however they may have an effect on the interpretation of organizational data in ESCs and thus are important to note. Among features unique to the genome of mouse ESCs are a hyper-dynamic association of chromatin proteins with the chromatin polymer [51], enhanced global transcriptional activity [52], a lack of condensed heterochromatin at the NE and peri-nucleolar regions [53], and two active X-chromosomes in female cells. Upon differentiation, chromatin protein association becomes more stable [51], wide-spread transcription of both protein coding and non-coding regions is restricted, repeat elements are silenced [51,52], and heterochromatic regions of the genome compact and localize to the nuclear periphery [53]. At the same time, a subset of pluripotency gene loci is silenced and moves to the nuclear periphery even before germ layer restriction occurs [53–56]. These processes occur contemporaneously with large-scale changes in DNA replication timing [54,55], silencing of a single X-chromosome in female cells [57], and the onset of Lamin A expression, which stabilizes histone H1 in heterochromatin and is required for the establishment of the large number of heterochromatin foci characteristic of differentiated cells [58]. Together, these data indicate that the dramatic changes in gene expression that occur upon pluripotent cell differentiation are accompanied by large-scale changes in genome topology.

Despite the correlation between NE localization and gene silencing in ESCs [56], LaminB1/B2 double knockout ESCs and trophectoderm cells show few changes in gene expression compared to their respective wild-type cells, and those genes that do change expression levels are not bound by B-type Lamins in wild-type cells [59]. This suggests that LaminB does not directly regulate expression of its interacting genes in ESCs or trophectoderm cells. Alternatively, unidentified redundant mechanisms may work to maintain gene silencing at the NE in the absence of B-type lamins in these cells. Additionally, LaminB-null ESCs show none of the NE morphology defects typical of somatic cells with mutations in nuclear lamina proteins [59,60]. During the course of differentiation of ESCs to neural precursor cells, many pluripotency-specific genes are re-localized to the nuclear lamina and many NPC-specific genes detach from the lamina [56]. In contrast to the phenotypically wild-type ESCs, upon embryonic development, LaminB1/B2-null mice display severe organogenesis and neural migration defects [59]. Implicated as a major player in somatic cell genome organization, it will be important to understand the role of the nuclear lamina in regulating genome organization of ESCs, or alternatively, to determine if chromatin-NE co-localization is only required upon differentiation.

In contrast to the transcriptionally repressive NE, in yeast, gene localization to the nuclear pore complex is associated with transcriptional activation in certain inducible systems [61]. In metazoans, however, some of the nucleoporins (Nups), the major constituents of the nuclear pore complex, have been implicated as regulators of gene expression through direct binding of chromatin in the nucleoplasm, mostly away from the nuclear pore [62–64]. Specifically, Nup133-null mice display defects in neural differentiation and Nup133-null ESCs differentiate inefficiently along neural lineages and do not contribute to the neural tube of chimeric embryos [65]. Similarly, the integral membrane protein Nup210 is expressed cell type specifically and is not essential for nuclear pore function, but is required for ESC differentiation into neural progenitors as well as for myogenesis. Nup210 depletion abrogates the upregulation of differentiation-associated genes and its overexpression facilitates the expression of essential differentiation genes. Notably, the authors argue against a role for Nup210 in tethering genes to the nuclear pore complex, as they do not see changes in NE localization of Nup210 regulated genes upon induction [66]. It will be important to understand the differing roles of Nups when they are chromatin bound in the nucleoplasm, versus when they are part of the nuclear pore complex, as well as their role in genome organization or re-organization upon differentiation in metazoans.

Re-organization of the Xi during ESC differentiation

The X-inactivation process is a striking example for topology changes associated with differentiation. The equalization of X-linked gene expression between sexes in mammals occurs via the silencing of one of two X-chromosomes upon induction of differentiation of ESCs. This process, induced by the upregulation and spreading of the non-coding RNA *Xist* on the future inactive X-chromosome (Xi), leads to the transcriptional silencing of the majority of X-linked genes on the Xi, and the establishment of a number of repressive chromatin modifications along the Xi, including PcG protein-mediated H3K27 methylation, DNA methylation, and deposition of the histone variant macroH2A [57].

At the onset of XCI homologous Xi's co-localize allowing for the pairing of the *Xist*-encoding X-inactivation centers (XIC), a process thought to be necessary for the initiation of XCI on one of the two X-chromosomes [67–69]. Following pairing, the future Xi preferentially localizes to the NE and peri-nucleolar regions of the nucleus [70], both of which are enriched for autosomal heterochromatin in differentiated cells [71]. This localization occurs predominantly during S phase of the cell cycle, and is dependent on *Xist* expression. Deletion of *Xist* in fibroblasts causes a re-localization of the Xi away from the nucleolus, with concomitant re-activation of a subset of genes in a small proportion of cells [70].

In addition to these large scale movements of the Xi upon induction of X-inactivation, *Xist* expression leads to the formation of an *Xist* RNA domain over the future Xi and the immediate exclusion of RNA polymerase II (RNAPII) and transcription machinery from the future Xi territory [19••]. Interestingly, the exclusion of transcription machinery precedes the completion of transcriptional silencing. At the time of transcription machinery exclusion from the territory of the future Xi, genes localize to the periphery of the future Xi territory, where they can contact the transcriptional machinery. As these genes are silenced during the course of differentiation and X-inactivation, they localize to the interior of the Xi territory. Silencing and sequestration of X-linked genes into the Xi territory requires the A-repeat [19••], a portion of *Xist* necessary for transcriptional silencing [72]. Genes that escape X-inactivation remain localized to the periphery of the Xi territory [19••]. A subsequent 4C study has shown that these escaping genes co-localize with other escaping genes as well as with gene loci on other chromosomes [73]. Conversely, silenced genes in the center of the Xi territory make few preferential interactions with other genomic regions, suggesting a random localization or restricted movement of these loci within the Xi [73]. Xi-specific 3D chromatin organization is partially dependent on *Xist* RNA coating, as *Xist* deletion results in an organizational state of the Xi resembling the active X-chromosome [19••,73].

The mechanisms regulating the dramatic re-organization of the Xi upon silencing are unclear, however, SatB1/B2 are implicated in this process [74]. In thymocytes, the SatB1 protein is organized in a cage-like structure throughout the nucleus [75] where it regulates gene expression through the anchoring of looped chromatin structures and the recruitment of chromatin modifying enzymes [76,77]. Upon induction of *Xist* expression in thymocytes and ESCs, *Xist* RNA accumulates in a region delimited by SatB1, and SatB1 depletion during ESC differentiation reduces the efficiency of XCI [74], although MEFs derived from SatB1/B2-null embryos display normal XCI [78,79], calling into question an essential role for SatB1 in the organization of chromatin and gene silencing during XCI.

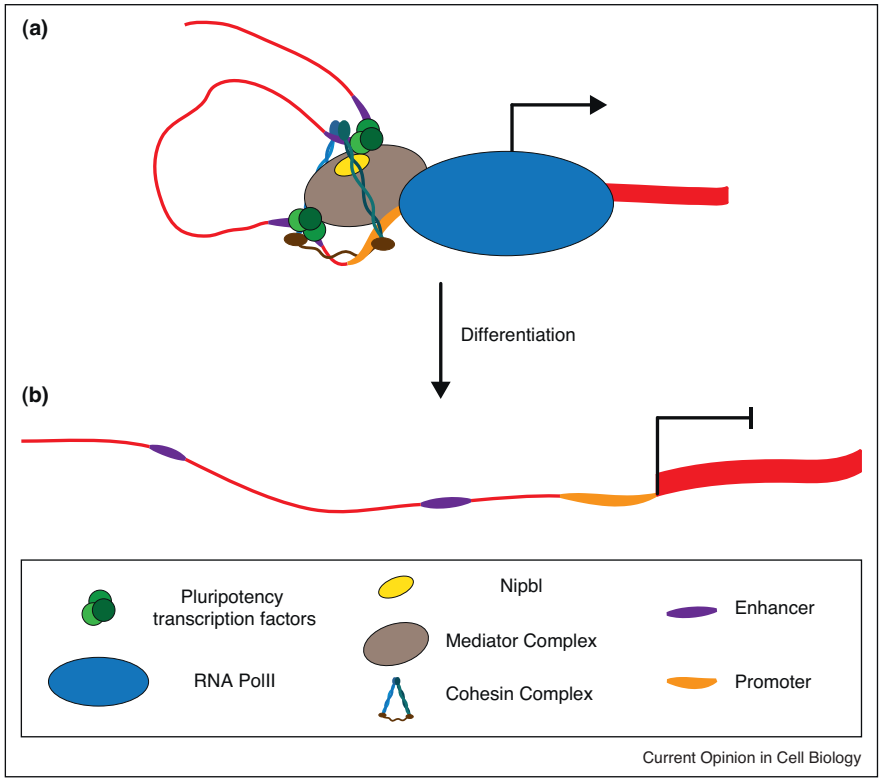
Despite the large-scale re-organization of the Xi during the course of inactivation, the existence of the two TADs encompassing the XIC does not change. However, specific intra-TAD interactions are lost upon X-inactivation, suggesting a random organization of the intra-TAD space within the Xi [41••], similar to that shown for long-range interactions within the Xi by 4C analysis [73]. Alternatively, molecular 'gluing' of these TADs to the nuclear lamina could lead to a very limited interactome. Genetic deletions of G9a, an H3K9 methyltransferase, or Eed, an essential component of the Polycomb repressive complex 2, have no effect on the chromatin conformation or TAD structure within the XIC, suggesting that epigenetic modifications function downstream of TAD formation. By contrast, deletion of the TAD boundary region in the XIC, specifically between *Xist* and *Tsix*, resulted in the partial merger of neighboring TADs in mouse ESCs [41••], although cells lacking this TAD boundary are still capable of undergoing random X-inactivation upon differentiation [80], leaving open the question of whether a specific organization of the XIC is required for X-inactivation.

Together, these data argue that X-inactivation is an essential developmental process that is associated with topology changes at various levels and may be a powerful model system to dissect the molecular mechanisms underlying genome organization and its dynamics during the course of differentiation. Notably, the 3D organization of the Xi during Xi-reactivation events *in vitro* or *in vivo*, either in the context of somatic cell reprogramming [81] or germ cell development [82], has not been investigated.

Mechanistic insights into genome organization

On the basis of studies of promoter and enhancer interactions by DNA looping, it is clear that gene expression is facilitated and regulated through distal chromatin contacts. The mode and mechanism of action of enhancer elements has been the subject of a large body of work over the years, and recent experiments have brought to light various molecular mechanisms underlying this phenomenon. In particular, the Cohesin complex — which, canonically, forms a ring around sister chromatids during mitosis [83] — has been shown to play a major role in organizing DNA topology and affecting gene regulatory processes at the level of enhancer–promoter interactions. It was initially characterized at the developmentally regulated *IFNG* locus in T-cells where it is required for enhancer–promoter looping and expression of *IFNG* [84], and at the *H19/IF2* loci in humanized mouse cells where it is required for insulator activity [85]. Cohesin binding sites overlap significantly with CTCF binding sites genome wide [85–88], many of which are conserved across cell types and species [47], leading to a model wherein CTCF-associated Cohesin localization is largely

Figure 2



The Mediator complex recruits Cohesin to chromatin and facilitates cell-type-specific enhancer–promoter looping and gene expression **(a)** Mediator-recruited Cohesin complexes orchestrate cell-type-specific enhancer–promoter loops, providing a mechanism for the cell-type-specific action of an enhancer on a given promoter and, by extension, cell-type-specific gene expression patters. In ESCs, many Oct4 (O), Sox2 (S), and Nanog (N) bound regions of the genome coincide with Mediator and Cohesin occupancy, indicating that these transcription factors recruit mediator to enhancers and promoters. **(b)** In fibroblasts, where OSN are not expressed, Mediator and Cohesin show differential DNA binding patterns and enhancer–promoter looping events at ESC-specific gene loci are absent.

cell type invariant [89••] (Figure 1b, c), potentially explaining the conservation of TAD boundaries across cell types and species, as hypothesized by Dixon *et al.* [40•,47].

In order to generate cell-type-specific DNA topologies for the facilitation of specific transcriptional programs, cells appear to utilize non-CTCF mediated recruitment of Cohesin to interphase chromatin. For instance, Cohesin is co-bound with the transcription factor CEBPA in Hep2G cells and with the estrogen receptor (ER) in MCF7 cells, where Cohesin binding persists in the absence of CTCF [90]. In the case of MCF7 cells, Cohesin binding is particularly enriched at regions involved in ER-mediated chromatin interactions [38]. Mounting evidence suggests that, similar to its role during mitosis, Cohesin functions by holding functional DNA elements together in the nucleus (Figure 2), and additionally, may stabilize TF binding to highly occupied *cis* regulatory elements [91].

A major advance in our understanding of the mechanistic underpinnings of promoter-enhancer interactions in ESCs was achieved recently through an shRNA screen for loss of *Oct4* gene expression [89••]. This screen identified numerous subunits of Mediator — a massive protein complex that regulates the activity of RNAPII [92] — and Cohesin subunits, as well as the Cohesin loading factor Nipbl, as regulators of *Oct4* gene expression. The authors found that Cohesin and Mediator co-immunoprecipitate with each other and Nipbl in ESCs, potentially allowing Cohesin to enable ESC-specific enhancer–promoter interactions upon recruitment of Mediator to chromatin by various transcription factors (Figure 2). Unlike CTCF and Cohesin co-bound sites, Mediator and Cohesin co-bound sites are cell type-specific and often overlap with locations of pluripotency transcription factors Oct4, Sox2, and Nanog in ESCs. In MEFs, among loci where Mediator binding is different compared to ESCs, enhancer–promoter looping interactions are likewise different, as shown using 3C at a number of candidate loci [89••]. These findings suggest a mechanistic explanation for previous work demonstrating a chromatin topology that brings together a variety of DNase HS sites and co-regulated genes within the extended 150 kb *Nanog* locus,

a topology that is lost upon Oct4 depletion [93]. Although it has not been explicitly demonstrated outside of ESCs, we speculate that recruitment of mediator to binding sites occupied by cell type-specific transcription factors facilitates the recruitment of Cohesin to interphase chromatin where it mediates enhancer–promoter interactions, and potentially even more long-range chromatin contacts.

Conclusions and outlook

The synthesis of recently published data leads us to propose the following speculative model of mammalian genome organization (Figure 1): within TADs [37••,40•,41••], enhancers and promoters dynamically co-localize with and co-regulate each other [44•,94] in a cell type-specific manner [44•], limited in range along the chromatin polymer by TAD boundaries [41••,43••]. These TADs, existing as topologically isolated loops [42••], can re-locate to various subnuclear compartments [18,34,41••,56] in response to specific developmental and gene regulatory cues, but apart from limited cases where specific genes (and probably entire TADs) loop out of their CTs, TAD localization is limited to its own CT. An important piece of information missing from this model is the mode and mechanism of preferential TAD–TAD interactions that we infer from 4C data. Owing to their well-defined transcriptional networks, chromatin states, and gene expression data sets, ESCs — in pluripotency and during the course of differentiation — will be an ideal cell type for studying this question with 3C-based methodologies. In combination with a transcriptionally permissive nuclear environment [52] and a lack of highly condensed heterochromatin [53], future studies may also help us to understand whether an ESC-specific 3D genomic organization contributes to the developmental plasticity of ESCs.

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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. Evans MJ, Kaufman MH: **Establishment in culture of pluripotential cells from mouse embryos**. *Nature* 1981, **292**:154-156.

2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: **Embryonic stem cell lines derived from human blastocysts**. *Science* 1998, **282**:1145-1147.

3. Young RA: **Control of the embryonic stem cell state**. *Cell* 2011, **144**:940-954.

4. Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA: **c-Myc regulates transcriptional pause release**. *Cell* 2010, **141**:432-445.

5. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK *et al.*: **Polycomb complexes repress developmental regulators in murine embryonic stem cells**. *Nature* 2006, **441**:349-353.

6. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y: **Role of histone H3 lysine 27 methylation in Polycomb-group silencing**. *Science* 2002, **298**:1039-1043.

7. Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, Zhang Y: **Role of histone H2A ubiquitination in Polycomb silencing**. *Nature* 2004, **431**:873-878.

8. Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, Gilbert N, Fan Y, Skoultchi AI, Wutz A *et al.*: **Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination**. *Mol Cell* 2010, **38**:452-464.

9. Niwa H, Ogawa K, Shimosato D, Adachi K: **A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells**. *Nature* 2009, **460**:118-122.

10. Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA: **Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells**. *Nat Methods* 2005, **2**:185-190.

11. Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors**. *Cell* 2006, **126**:663-676.

12. Cremer T, Cremer C: **Rise, fall and resurrection of chromosome territories: a historical perspective. Part II. Fall and resurrection of chromosome territories during the 1950s to 1980s. Part III. Chromosome territories and the functional nuclear architecture: experiments and models from the 1990s to the present**. *Eur J Histochem* 2006, **50**:223-272.

13. Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA: **The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells**. *Hum Mol Genet* 2001, **10**:211-219.

14. Warburton D, Naylor AF, Warburton FE: **Spatial relations of human chromosomes identified by quinacrine fluorescence at metaphase. I. Mean interchromosomal distances and distances from the cell center**. *Humangenetik* 1973, **18**:297-306.

15. Parada LA, McQueen PG, Misteli T: **Tissue-specific spatial organization of genomes**. *Genome Biol* 2004, **5**:R44.

16. Cremer T, Cremer C: **Chromosome territories, nuclear architecture and gene regulation in mammalian cells**. *Nat Rev Genet* 2001, **2**:292-301.

17. Cremer T, Cremer M: **Chromosome territories**. *Cold Spring Harb Perspect Biol* 2010, **2**:a003889.

18. Chambeyron S, Bickmore WA: **Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription**. *Genes Dev* 2004, **18**:1119-1130.

19. •• 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 Dev* 2006, **20**:2223-2237.
This work describes the localization of X-linked genes relative to the territory of the X chromosome during initiation of X-inactivation. It additionally characterizes the role of the *Xist* silencing domain in the relocalization of genes during X-inactivation

20. Andrulis ED, Neiman AM, Zappulla DC, Sternglanz R: **Perinuclear localization of chromatin facilitates transcriptional silencing**. *Nature* 1998, **394**:592-595.

21. Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, Singh H: **Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development**. *Science* 2002, **296**:158-162.

22. Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B: **Characterization of the *Drosophila melanogaster* genome at the nuclear lamina**. *Nat Genet* 2006, **38**:1005-1014.

23. Reddy KL, Zullo JM, Bertolino E, Singh H: **Transcriptional repression mediated by repositioning of genes to the nuclear lamina**. *Nature* 2008, **452**:243-247.

24. Kumaran RI, Spector DL: **A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence**. *J Cell Biol* 2008, **180**:51-65.

25. Schirmer EC, Foisner R: **Proteins that associate with lamins: many faces, many functions**. *Exp Cell Res* 2007, **313**:2167-2179.

26. Mattout A, Goldberg M, Tzur Y, Margalit A, Gruenbaum Y: **Specific and conserved sequences in *D. melanogaster* and *C. elegans* lamins and histone H2A mediate the attachment of lamins to chromosomes**. *J Cell Sci* 2007, **120**:77-85.

27. Yao J, Fetter RD, Hu P, Betzig E, Tjian R: **Subnuclear segregation of genes and core promoter factors in myogenesis**. *Genes Dev* 2011, **25**:569-580.

28. Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB, Spooner CJ, Luperchio TR, Bernstein BE, Pritchard JK, Reddy KL *et al.*: **DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina**. *Cell* 2012, **149**:1474-1487.

29. Dekker J, Rippe K, Dekker M, Kleckner N: **Capturing chromosome conformation**. *Science* 2002, **295**:1306-1311.

30. Hakim O, Misteli T, SnapShot:: **Chromosome confirmation capture**. *Cell* 2012, **148** 1068 e1–e2.

31. 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.

32. Zhao Z, Tavoosidana G, Sjolinder M, Gondor A, Mariano P, Wang S, Kanduri C, Lezcano M, Sandhu KS, Singh U *et al.*: **Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions**. *Nat Genet* 2006, **38**:1341-1347.

33. 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 Res* 2006, **16**:1299-1309.

34. 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.

35. 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.

36. Kalhor R, Tjong H, Jayathilaka N, Alber F, Chen L: **Genome architectures revealed by tethered chromosome conformation capture and population-based modeling**. *Nat Biotechnol* 2012, **30**:90-98.

37. •• 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.
This study was the first to identify topological domains as a unit of metazoan genome organization, and presented evidence for a heirarchical organization of the genome as illustrated in Figure 1, as well as for Polycomb-mediated long-range chromatin interactions genome wide

38. Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH *et al.*: **An oestrogen-receptor-alpha-bound human chromatin interactome**. *Nature* 2009, **462**:58-64.

39. Zhang Y, McCord RP, Ho YJ, Lajoie BR, Hildebrand DG, Simon AC, Becker MS, Alt FW, Dekker J: **Spatial organization of the mouse genome and its role in recurrent chromosomal translocations**. *Cell* 2012, **148**:908-921.

40. • 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.
This study demonstrated the existence of TADs in mammalian cells and showed them to be conserved across species and cell types.

41. •• 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.
This work showed that genes within TADs, specifically within the Xist/Tsix containing TADs of the X-inactivation centre, are coordinately regulated during the course of differentiation, that TADs remain static during the course of XCI, and that deletion of a TAD boundary region results in ectopic chromatin interactions.

42. •• Handoko L, Xu H, Li G, Ngan CY, Chew E, Schnapp M, Lee CW, Ye C, Ping JL, Mulawadi F *et al.*: **CTCF-mediated functional chromatin interactome in pluripotent cells**. *Nat Genet* 2011, **43**:630-638.
This study demonstrated the role of CTCF in ESCs as an organizer of large chromatin loops containing distinct epigenetic modifications

43. •• Shen Y, Yue F, McCleary DF, Ye Z, Edsall L, Kuan S, Wagner U, Dixon J, Lee L, Lobanenkov VV *et al.*: **A map of the *cis*-regulatory sequences in the mouse genome**. *Nature* 2012, **488**:116-120.
Using ChIP-seq and RNA-seq across a wide variety of cell lines for a large number of transcription factors and chromatin modifications, this study demonstrated that TADs function as domains of coordinately regulated enhancers and promoters in mammalain cells.

44. • Sanyal A, Lajoie BR, Jain G, Dekker J: **The long-range interaction landscape of gene promoters**. *Nature* 2012, **489**:109-113.
As part of the recently released ENCODE consortium data, this work demonstrated the promiscuity of enhancer elements in contacting numerous gene promoters, explicitly demonstrating that genes in contact with enhancers are more highly expressed than those without enhancer contacts across ENCODE analyzed regions of the genome, and explored the enrichment of numerous chromatin modifications taking part in looping interactions across a number of cell types

45. Wood AM, Van Bortle K, Ramos E, Takenaka N, Rohrbaugh M, Jones BC, Jones KC, Corces VG: **Regulation of chromatin organization and inducible gene expression by a Drosophila insulator**. *Mol Cell* 2011, **44**:29-38.

46. Bell AC, West AG, Felsenfeld G: **The protein CTCF is required for the enhancer blocking activity of vertebrate insulators**. *Cell* 1999, **98**:387-396.

47. Schmidt D, Schwalie PC, Wilson MD, Ballester B, Goncalves A, Kutter C, Brown GD, Marshall A, Flicek P, Odom DT: **Waves of retrotransposon expansion remodel genome organization and CTCF binding in multiple mammalian lineages**. *Cell* 2012, **148**:335-348.

48. Noordermeer D, de Wit E, Klous P, van de Werken H, Simonis M, Lopez-Jones M, Eussen B, de Klein A, Singer RH, de Laat W: **Variegated gene expression caused by cell-specific long-range DNA interactions**. *Nat Cell Biol* 2011, **13**:944-951.

49. Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, Andrews S, Kurukuti S, Mitchell JA, Umlauf D, Dimitrova DS *et al.*: **Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells**. *Nat Genet* 2010, **42**:53-61.

50. Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, Tixier V, Mas A, Cavalli G: **Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila**. *Cell* 2011, **144**:214-226.

51. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T: **Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells**. *Dev Cell* 2006, **10**:105-116.

52. Efroni S, Duttagupta R, Cheng J, Dehghani H, Hoeppner DJ, Dash C, Bazett-Jones DP, Le Grice S, McKay RD, Buetow KH *et al.*: **Global transcription in pluripotent embryonic stem cells**. *Cell Stem Cell* 2008, **2**:437-447.

53. Ahmed K, Dehghani H, Rugg-Gunn P, Fussner E, Rossant J, Bazett-Jones DP: **Global chromatin architecture reflects pluripotency and lineage commitment in the early mouse embryo**. *PLoS ONE* 2010, **5**:e10531.

54. Hiratani I, Ryba T, Itoh M, Rathjen J, Kulik M, Papp B, Fussner E, Bazett-Jones DP, Plath K, Dalton S *et al.*: **Genome-wide dynamics of replication timing revealed by *in vitro* models of mouse embryogenesis**. *Genome Res* 2010, **20**:155-169.

55. Hiratani I, Ryba T, Itoh M, Yokochi T, Schwaiger M, Chang CW, Lyou Y, Townes TM, Schubeler D, Gilbert DM: **Global reorganization of replication domains during embryonic stem cell differentiation**. *PLoS Biol* 2008, **6**:e245.

56. Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, Graf S, Flicek P, Kerkhoven RM, van Lohuizen M *et al.*: **Molecular maps of the reorganization of genome–nuclear lamina interactions during differentiation**. *Mol Cell* 2010, **38**:603-613.

57. Minkovsky A, Patel S, Plath K: **Concise review: pluripotency and the transcriptional inactivation of the female Mammalian X chromosome**. *Stem Cells* 2012, **30**:48-54.

58. Melcer S, Hezroni H, Rand E, Nissim-Rafinia M, Skoultchi A, Stewart CL, Bustin M, Meshorer E: **Histone modifications and lamin A regulate chromatin protein dynamics in early embryonic stem cell differentiation**. *Nat Commun* 2012, **3**:910.

59. Kim Y, Sharov AA, McDole K, Cheng M, Hao H, Fan CM, Gaiano N, Ko MS, Zheng Y: **Mouse B-type lamins are required for proper organogenesis but not by embryonic stem cells**. *Science* 2011, **334**:1706-1710.

60. Capell BC, Collins FS: **Human laminopathies: nuclei gone genetically awry**. *Nat Rev Genet* 2006, **7**:940-952.

61. Tan-Wong SM, Wijayatilake HD, Proudfoot NJ: **Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex**. *Genes Dev* 2009, **23**:2610-2624.

62. Capelson M, Liang Y, Schulte R, Mair W, Wagner U, Hetzer MW: **Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes**. *Cell* 2010, **140**:372-383.

63. Kalverda B, Pickersgill H, Shloma VV, Fornerod M: **Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm**. *Cell* 2010, **140**:360-371.

64. 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.

65. Lupu F, Alves A, Anderson K, Doye V, Lacy E: **Nuclear pore composition regulates neural stem/progenitor cell differentiation in the mouse embryo**. *Dev Cell* 2008, **14**:831-842.

66. D'Angelo MA, Gomez-Cavazos JS, Mei A, Lackner DH, Hetzer MW: **A change in nuclear pore complex composition regulates cell differentiation**. *Dev Cell* 2012, **22**:446-458.

67. Bacher CP, Guggiari M, Brors B, Augui S, Clerc P, Avner P, Eils R, Heard E: **Transient colocalization of X-inactivation centres accompanies the initiation of X inactivation**. *Nat Cell Biol* 2006, **8**:293-299.

68. Xu N, Tsai CL, Lee JT: **Transient homologous chromosome pairing marks the onset of X inactivation**. *Science* 2006, **311**:1149-1152.

69. Masui O, Bonnet I, Le Baccon P, Brito I, Pollex T, Murphy N, Hupe P, Barillot E, Belmont AS, Heard E: **Live-cell chromosome dynamics and outcome of X chromosome pairing events during ES cell differentiation**. *Cell* 2011, **145**:447-458.

70. Zhang LF, Huynh KD, Lee JT: **Perinucleolar targeting of the inactive X during S phase: evidence for a role in the maintenance of silencing**. *Cell* 2007, **129**:693-706.

71. Zhao R, Bodnar MS, Spector DL: **Nuclear neighborhoods and gene expression**. *Curr Opin Genet Dev* 2009, **19**:172-179.

72. Wutz A, Rasmussen TP, Jaenisch R: **Chromosomal silencing and localization are mediated by different domains of Xist RNA**. *Nat Genet* 2002, **30**:167-174.

73. 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.

74. Agrelo R, Souabni A, Novatchkova M, Haslinger C, Leeb M, Komnenovic V, Kishimoto H, Gresh L, Kohwi-Shigematsu T, Kenner L *et al.*: **SATB1 defines the developmental context for gene silencing by Xist in lymphoma and embryonic cells**. *Dev Cell* 2009, **16**:507-516.

75. Cai S, Han HJ, Kohwi-Shigematsu T: **Tissue-specific nuclear architecture and gene expression regulated by SATB1**. *Nat Genet* 2003, **34**:42-51.

76. Cai S, Lee CC, Kohwi-Shigematsu T: **SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes**. *Nat Genet* 2006, **38**:1278-1288.

77. Yasui D, Miyano M, Cai S, Varga-Weisz P, Kohwi-Shigematsu T: **SATB1 targets chromatin remodelling to regulate genes over long distances**. *Nature* 2002, **419**:641-645.

78. Nechanitzsky R, Dávila A, Savarese F, Fietze S, Grosschedl R: **Satb1 and Satb2 are dispensable for X chromosome inactivation in mice**. *Dev Cell* 2012, **23**:866-871.

79. Wutz A, Agrelo R, Response:: **The diversity of proteins linking xist to gene silencing**. *Dev Cell* 2012, **23**:680.

80. Monkhorst K, Jonkers I, Rentmeester E, Grosveld F, Gribnau J: **X inactivation counting and choice is a stochastic process: evidence for involvement of an X-linked activator**. *Cell* 2008, **132**:410-421.

81. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R *et al.*: **Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution**. *Cell Stem Cell* 2007, **1**:55-70.

82. Sasaki H, Matsui Y: **Epigenetic events in mammalian germ-cell development: reprogramming and beyond**. *Nat Rev Genet* 2008, **9**:129-140.

83. Haering CH, Farcas AM, Arumugam P, Metson J, Nasmyth K: **The cohesin ring concatenates sister DNA molecules**. *Nature* 2008, **454**:297-301.

84. Hadjur S, Williams LM, Ryan NK, Cobb BS, Sexton T, Fraser P, Fisher AG, Merkenschlager M: **Cohesins form chromosomal *cis*-interactions at the developmentally regulated IFNG locus**. *Nature* 2009, **460**:410-413.

85. Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, Schirghuber E, Tsutsumi S, Nagae G, Ishihara K, Mishiro T *et al.*: **Cohesin mediates transcriptional insulation by CCCTC-binding factor**. *Nature* 2008, **451**:796-801.

86. Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, Gregson HC, Jarmuz A, Canzonetta C, Webster Z, Nesterova T *et al.*: **Cohesins functionally associate with CTCF on mammalian chromosome arms**. *Cell* 2008, **132**:422-433.

87. Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, Baliga NS, Aebersold R, Ranish JA, Krumm A: **CTCF physically links cohesin to chromatin**. *Proc Natl Acad Sci U S A* 2008, **105**:8309-8314.

88. Stedman W, Kang H, Lin S, Kissil JL, Bartolomei MS, Lieberman PM: **Cohesins localize with CTCF at the KSHV latency control region and at cellular c-myc and H19/Igf2 insulators**. *EMBO J* 2008, **27**:654-666.

89. •• Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS *et al.*: **Mediator and cohesin connect gene expression and chromatin architecture**. *Nature* 2010, **467**:430-435.

Kagey *et al.* described the role of Mediator and Cohesin in mediating enhancer promoter looping in ESCs

90. Schmidt D, Schwalie PC, Ross-Innes CS, Hurtado A, Brown GD, Carroll JS, Flicek P, Odom DT: **A CTCF-independent role for cohesin in tissue-specific transcription**. *Genome Res* 2010, **20**:578-588.

91. Faure AJ, Schmidt D, Watt S, Schwalie PC, Wilson MD, Xu H, Ramsay RG, Odom DT, Flicek P: **Cohesin regulates tissue-specific expression by stabilizing highly occupied *cis*-regulatory modules**. *Genome Res* 2012, **22**:2163-2175.

92. Taatjes DJ: **The human Mediator complex: a versatile, genome-wide regulator of transcription**. *Trends Biochem Sci* 2010, **35**:315-322.

93. Levasseur DN, Wang J, Dorschner MO, Stamatoyannopoulos JA, Orkin SH: **Oct4 dependence of chromatin structure within the extended Nanog locus in ES cells**. *Genes Dev* 2008, **22**:575-580.

94. Li G, Ruan X, Auerbach RK, Sandhu KS, Zheng M, Wang P, Poh HM, Goh Y, Lim J, Zhang J *et al.*: **Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation**. *Cell* 2012, **148**:84-98.