Chromatin-Driven Behavior of Topologically Associating Domains

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Abstract

Metazoan genomes are highly organized inside the cell nucleus. Topologically associating domains (TADs) represent the building blocks of genome organization, but their linear modularity does not explain alone their spatial organization. Indeed, the chromatin type adorning a TAD can shape its structure and drives its nuclear positioning and its function. Genome-wide association studies revealed mainly four chromatin types: active chromatin, Polycomb-repressed chromatin, null chromatin and constitutive heterochromatin. In this review, we will describe the main three-dimensional features of each chromatin type and finally their relationships with TAD organization and epigenetic memory

Introduction

Chromatin fiber is not randomly distributed inside the cell nucleus. Firstly, chromosomes are confined in discrete "territories" [1,2], and within them, further levels of spatial organization are imposed to chromatin. Metazoan genomes share a modular organization of their chromatin in structures called "topologically associating domains" (TADs). TADs can be defined as linear units of chromatin that fold as discrete three-dimensional (3D) structures tending to favor internal, rather than external, chromatin interactions. They are delimited by sharp boundaries, which contain housekeeping genes and insulator sites. TADs are detected by methods such as Hi-C [1,2], which allows genome-wide identification of chromatin contacts [3–5], and they correspond to chromosomal domains, previously identified by microscopy [6]. Remarkably, TADs are conserved among different cell types in animals, whereas they have not been detected in plants and yeasts [7–11], demonstrating that alternative modes of genome folding are possible. Besides their 3D organization, metazoan genomes are organized in linear clusters of co-expressed genes [12], although there is a certain degree of liberty in expression within gene clusters [13,14]. These co-expression clusters span on average about 100 kb in *Drosophila melanogaster* [15] and 1 Mb in humans [13]. Strikingly, these genomic ranges correspond to the average TAD sizes in both species.

The investigation of chromatin landscapes in *metazoa* through genome-wide association studies proved to be a fruitful approach [3,16–23]. In these studies, the presence of several chromatin factors and histone modifications were analyzed and finally combined, in order to obtain a global overview of the chromatin landscape. Theoretically, a huge number of chromatin type combinations were possible but, in fact, a very limited number of these were shown to cover the vast majority of the genome. The precise number of chromatin types varied among the studies, according to the algorithm used, the resolution and other parameters. Despite these technical differences, every report basically recapitulated the presence of an active chromatin environment, sometimes further subdivided, and three major types of repressive chromatin: a Polycomb-repressed environment, a null environment and a heterochromatic environment.

Strikingly, TADs were found to overlap with linear chromatin domains [3–5,24,25]; therefore, a defined chromatin type can be assigned to every single TAD. Consequently, the chromatin flavor of a given TAD can define its functional identity and drive its 3D organization. Moreover, the chromatin identity of a given TAD can guide its nuclear positioning and

therefore shape the genome architecture. Although TAD borders are generally not variable among different cell types, chromatin states can change dramatically their identity. The chromatin coating the genome of a given species can be reshaped in different cell types and conditions, hence reflecting its activity [26]. The conserved modularity of TAD organization can be combined with the variable nature of chromatin identity. In this review, we will recapitulate the current understanding about the 3D organization of the abovementioned chromatin types. Recent and older studies concerning the chromatin compaction, local interactions, long-range interactions and the nuclear positioning of each chromatin type will be reviewed. These studies have been conducted using a plethora of different methodologies, spanning from microscopy-based to cell-population approaches. We invite readers to refer to recent reviews on the technical aspects of these approaches [27–29]. Based on this multi-tiered evidence, a nuclear architecture model based on TADs and their chromatin types will be proposed.

Active Chromatin

Active chromatin is here referred as the fraction of chromatin that is in a competent state for gene expression and initiating DNA replication. Different cell types are characterized by specific sets of active and silenced genes. The specific portion of active chromatin residing in the nucleus of a given cell type reflects its identity [26]. Active chromatin is the most heterogeneous chromatin type, since it displays binding sites for many chromatin factors and it is adorned by a plethora of histone modifications as H3 methylations on lysine 4, lysine 36 and lysine 79 and acetylation of multiple lysines on H3 and H4 N-terminal tails [17,23]. The deposition of such marks reflects the identity of the underlying functional element (e.g., promoter, gene body, enhancer, etc.) and its degree of activity (e.g., active, poised, etc.). Several chromatin factors often work cooperatively and the surrounding chromatin environment can alter their binding [23]. Finally, the specific characteristics of a gene (e.g., length, presence of introns, etc.) or a promoter (e.g., housekeeping, developmentally regulated, etc.) can influence the local chromatin features as nucleosome positioning, binding of specific factors and deposition of histone modifications [17,30].

Active chromatin represents a highly accessible environment, displaying an increased density of DNase I hypersensitive sites when compared to other types of chromatin [31]. In *D. melanogaster*, active chromatin domains show a more rapid decay in contacts frequency as a function of genomic distance than other domain types, revealing a decondensed organization [3]. Genome-wide association studies confirmed the extensive heterogeneity and the higher accessibility of active chromatin. Depending on the algorithm used to categorize the different chromatin types, some studies grouped active chromatin as a single chromatin variety [3,20,21], whereas other studies identified subpatterns, characterizing elements such as promoters, gene bodies, active introns, enhancers and so on [16,18,19,22]. Finer classifications can uncover chromatin patterns that are specific for housekeeping genes and tissue-specific genes [23] or X-linked genes [17].

Active chromatin is organized in linear domains, on average, shorter than other chromatin types. Those domains cluster groups of active genes, which regulation relies on distal regulatory elements placed inside megabase-sized domains [24,32,33]. Active domains can be organized in TADs [3,4,34,35], suggesting that these clusters of active genes are spatially independent form nearby domains.

3D organization of active TADs

The chromatin fiber forming active TADs does not fold randomly but is highly organized in the 3D space. Although chromatin contacts are not random, they are quite variable between two cells [36]. The internal interaction profile is even more different between cells of a different type [5,37]. At the single gene level, promoters display preferential interactions with their downstream gene bodies rather than with upstream sequences [37–39]. This asymmetric distribution is independent of ongoing transcription [37]. Furthermore, some genes can form a promoter–terminator loop, which formation does not seem to be strictly dependent on the gene activity and its functional relevance has still to be elucidated [40,41]. In addition, promoters of coregulated genes often contact one another [38,39].

A typical metazoan gene has a complex regulatory network, which includes the presence of distal regulatory elements called enhancers. Although several models to explain the functional relation between enhancers and promoters were originally proposed, it is now accepted that enhancers work through a physical interaction with the promoter, therefore forming a chromatin loop [42–44]. Enhancer function is usually independent on orientation and distance to the target promoter [45] and can be located upstream, downstream or in intronic regions [30]. These interactions are mediated by both specific and general transcription factors such as the mediator complex, cohesins and CTCF [46–48]. Promoter–enhancer interaction networks are highly dependent on the nature of the gene. For instance, promoters of many housekeeping genes seem to have no enhancer [37] although this result might be affected by resolution issues, whereas promoters regulated by a single enhancer are usually controlling tissue-specific genes [49]. This kind of enhancers tends to be in close proximity to the promoter [50]. Alternatively, several enhancers can regulate a single promoter [51], usually

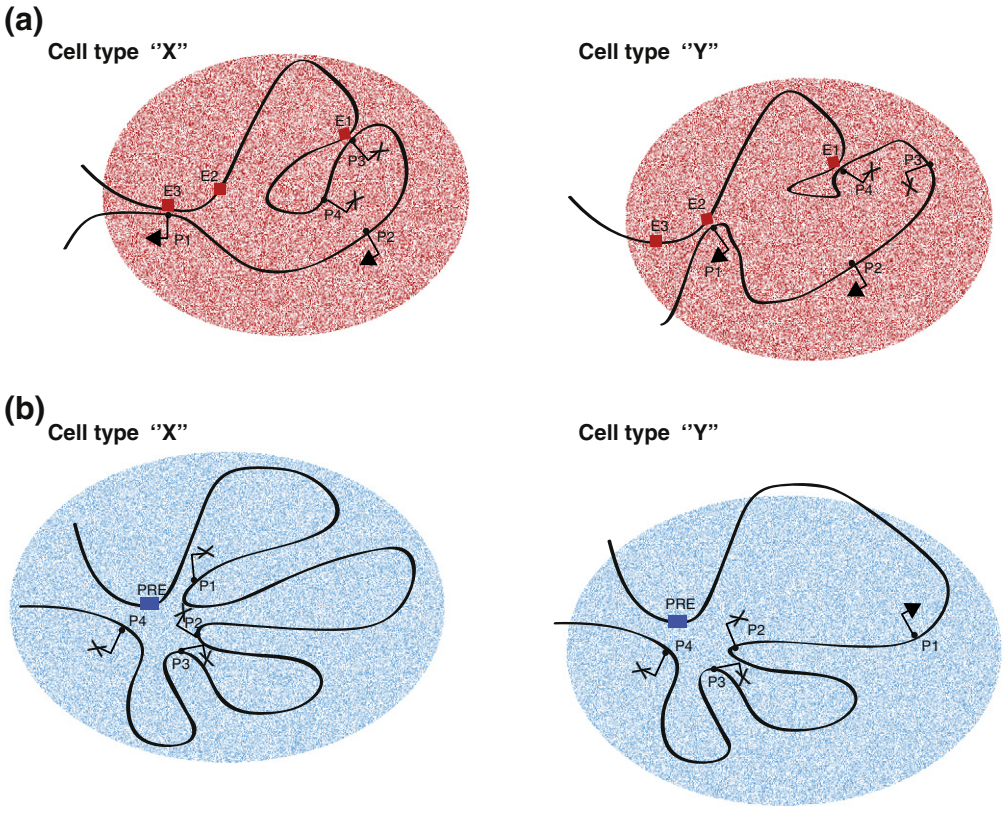

Fig. 1. Specific but variable interactions inside TADs. (a) Enhancer–promoter interactions occurring in an active TAD are represented. In the cell type "X", the enhancer 3 binds the promoter 1 despite that it is not the closest promoter linearly. The promoter 2 is active without being in contact with any enhancer. Despite the fact that enhancer 1 binds the promoter 3, it is not transcriptionally active yet. In the cell type "Y", the promoter 1 is bound by the enhancer 2 and not by the enhancer 3 anymore. The enhancer 1 switches its contacts from the promoter 3 and the promoter 4. (b) PRE–promoter interactions occurring in Polycomb-repressed TADs are represented. In the cell type "X", a PRE contacts four promoters, causing the silencing of their downstream genes. In the cell type "Y", the contacts between the promoter 1 and the PRE are lost, whereas the other promoters are still bound to the PRE. This release causes a looping-out of the chromatin associated with the gene 1 and concomitantly its transcriptional derepression.

controlling broadly expressed genes. In this case, different subsets of enhancers are active on their target gene in different cell types, revealing high cell-type specificity in promoter–enhancer interactions [24,38,39,52]. On the other hand, single enhancers can control several genes [37,53], which are usually coregulated. Finally, the association between an enhancer and its target promoter is not guided just by linear proximity, since most of the enhancers control promoters located beyond the nearest gene [39,52] (Fig. 1a). These association rules are in part driven by the presence of insulator sequences, which can form DNA loops through insulator binding proteins. In mammals, CTCF dimers mediate these DNA looping, whereas other organisms as *D. melanogaster* exhibit a more diverse set of insulator binding proteins [54]. Interestingly in a human fibroblast cell line, it was observed that enhancer–promoter looping is already preset before gene activation, since the stimulation of a set of inducible genes did not result in any consistent spatial reorganization [37]. A similar mechanism has been recently observed in *D. melanogaster* embryos. Here, promoter–enhancer interactions are very conserved between different developmental stages and they are often associated with paused RNA Pol II, arising before gene activation [55].

Long-range interactions and nuclear positioning of active TADs

Even though internal interactions are highly preferred, interactions between active loci located on

different TADs can occur [3,36]. Furthermore, specific interactions can involve loci on different chromosomes, and in some cases, they may have functional significance [56]. The existence of inter-chromosomal interactions between active domains was further confirmed by genome-wide studies [39,52]. The role of the insulator protein CTCF for these interactions appears to be crucial. In *D. melanogaster*, it was demonstrated that CTCF could promote long-range interactions of distant transgenes, whereas enhancer presence could only increase their colocalization [57].

Besides specific cases of regulation *in trans*, long-range interactions between active regions can have a strong impact on genome organization. Indeed, clustering of linearly distant RNA Pol II genes was observed in specific cases [43,58]. For example, coregulated genes sharing the same transcription factors or involved in the same pathways can colocalize when they are transcribed in the same moment [59–62]. Their colocalization frequencies are higher than with other transcribed genes, suggesting the presence of specialized factories dedicated to their transcription [58]. Transcription factories are described as proteinaceous structures transcribing several RNA Pol II genes and characterized by an average diameter of 130 nm in erythroid cells [63]. RNA Pol II activity is higher at the external surface of these structures [64], which form upon induction of transcription [65] by specific transcription factors [62]. In this context, long-range interactions could favor coregulation of distant genes by increasing the local amount of factors required for their transcription. These findings are consistent with a conserved tendency of active chromatin domains to interact specifically with other active domains, even when placed on other chromosomes. In contrast, other chromatin types are less prone to interact with other chromosomes [3,36,66,67].

A special case of transcription factory is the nucleolus, the largest compartment in the nucleus. Nucleoli are the places were rRNA is synthesized, accounting for up to 80% of the total RNA amount in a cell. In these compartments, rRNA is also processed and finally assembled with ribosomal proteins. Arrays of rRNA genes are arranged in several nucleolus organizer regions, which can be located in the same or in different chromosomes, depending on the species. These regions are not all active in every cell, but the active ones cluster together to form one to several nucleoli [68]. Their clustering is likely to be dependent on the essential transcription factor UBF, which does not bind the inactive nucleolus organizer regions [69]. Furthermore, active rRNA genes can form loops between promoter and terminator, enabling coordination of their expression [70].

Compartmentalization of the active chromatin was already observed in Hi-C studies as a spatial segregation from inactive chromatin [2]. Active chromatin is positioned preferentially in the nuclear interior [71,72]. This tendency is reflected at the chromosome scale, as gene-dense and more active chromosomes reside in a more internal position than gene-poor and silent ones [73]. An intriguing hypothesis is that, when several TADs are in an active state on a given chromosome, contacts between transcriptional complexes at active chromatin may cooperatively bring the chromosome toward a more internal position. In contrast, a chromosome depleted in active TADs would be relegated to a more peripheral location. The driving forces bringing the active TADs toward the center of the nucleus could be the transcriptional factories, which in a cell-specific way might bring together different active TADs. Alternatively, the internal positioning of active chromatin could be due to a passive force, resulting in preferential positioning of the null TADs to the nuclear periphery. These two hypotheses do not necessarily exclude each other and further investigation will be needed to better elucidate this point.

In conclusion, active chromatin can be described as a heterogeneous environment, highly accessible and decondensed. 3D interactions inside active TADs are specific and crucial for gene expression although variable at single cell level. The vast majority of those interactions occur within each active TAD, but inter-TAD interactions, especially among coregulated genes, are possible. Active TADs are placed preferentially in the inner part of the nucleus and have preferential contact with other active TADs, rather than inactive ones.

Polycomb-Repressed Chromatin

Polycomb group genes (PcG) were firstly identified in *D. melanogaster* as repressors of Hox genes [74]. Later, PcG proteins were found to be organized in mainly two multiprotein complexes, that is, Polycomb repressive complexes 1 and 2 (PRC1 and PRC2), which can respectively ubiquitylate H2AK119 and monomethylate, dimethylate and trimethylate H3K27 [75,76]. Finally, genome-wide studies revealed that PcG proteins are widespread transcriptional repressors, which are responsible for the silencing of a portion of metazoan genomes [77–79]. PRC1 and PRC2 lack DNA binding motifs; therefore, they have to be recruited by specific DNA-binding factors [80]. In *D. melanogaster*, these recruiters drive PRC1 and PRC2 to specific Polycomb responsive elements (PREs) [81]. Genomic regions containing arrays of PREs form large domains covered by the H3K27me3 mark. The most studied examples are the two homeotic complexes, that is, Antennapedia complex and Bithorax complex (ANT-C and BX-C), located on chromosome arm 3R. In mammals, Polycomb-bound regions are found mainly on Hox clusters, X-inactivation sites, imprinted regions and thousands of genes bearing CpG islands [82]. As expected, Polycomb-repressed

chromatin emerged as a distinct chromatin type in all the genome-wide studies aimed to identify chromatin domains in *metazoa* [3,16–23]. PcG proteins and H3K27me3 demarcate a significant portion of the *D. melanogaster* genome, which shows very low transcriptional activity and late replication timing [3,17,23]. Similar scenarios were observed in mammals [16,18,19,21] and *Caenorhabditis elegans* [22], demonstrating that this silencing strategy is evolutionary conserved.

In *D. melanogaster*, Polycomb-repressed domains correspond to a subset of TADs [3], linking their functional specificity with an independent spatial organization. The situation appears to be more complex in mammalian genomes. Here, the correspondence between Polycomb-repressed chromatin and specific TADs is not as straightforward. This is due to only a partial overlap in lengths' ranges between TADs (100 kb to 10 Mb) and H3K27me3 domains (1 kb to 100 kb) [83]. One explanation for this discrepancy might be the different resolution between fly and mammalian Hi-C studies. Since mammalian genomes are 10- to 20-fold larger than in flies, a sequencing depth of 100- to 400-fold would be required in order to reach the same resolution in contact mapping. Lacking this sequencing depth, it is possible that part of the TAD boundaries may have been missed in currently available maps. However, cases of H3K27me3-specific TADs have been reported on the inactive X chromosome [4] and on Hox clusters [34,35], suggesting that, at least in some cases, this correspondence can be similar to what was observed in *D. melanogaster*. Moreover, clusters of single Polycomb-repressed genes that do not form large and uniform H3K27me3 domains could in principle reside inside the same TAD and may form higher-order 3D structures that could be seen as “Polycomb-repressed TADs”.

Polycomb-repressed TAD compaction

While initial studies assessing accessibility to nucleases failed to detected Polycomb chromatin compaction [84], later reports showed that chromatin bound by PcG proteins has a reduced accessibility when compared to euchromatin [85–87], suggesting the presence of higher-order structures comparable with classic heterochromatin [85]. Fitzgerald *et al*. obtained similar results while testing the accessibility of the BX-C. They observed that GAL-4-dependent activation of RNA Pol II transcription and FLP-mediated recombination are specifically suppressed in the Polycomb-repressed segments, while transcription by the less bulky T7 RNAP is only partially blocked in a subset of PcG-repressed cells. This behavior was dependent on the presence of PcG proteins [88].

Several lines of evidences indicate that the major responsible for chromatin compaction is PRC1. PRC1 can compact nucleosomal arrays *in vitro*, independently of the presence of histone tails. This compaction is dependent on nucleosomes, rather than on linker DNA, with templates organized in a ratio of one PRC1 every three nucleosomes [89]. In *D. melanogaster*, the C-terminal region of the PRC1 subunit Psc plays a central role in this process [89,90]. Moreover, the presence of Psc mutant alleles alters PRC1 activity *in vitro* [90]. In mouse, however, a Polycomb ortholog called CBX2 is the main responsible for *in vitro* chromatin compaction [91]. The common feature shared by the *D. melanogaster* Psc and the mouse CBX2 is an unstructured highly basic domain, which firstly interacts with a single nucleosome, then tethers more nucleosomes together and finally promotes oligomerization through the formation of higher-order structures [91]. Ph, another PRC1 member, promotes oligomerization via its SAM domain, facilitating PRC1 and PRC2 binding and fostering chromatin compaction [92]. The lack of RING1B, the catalytic subunit of PRC1, is responsible for chromatin decompaction on HoxB and HoxD loci in mouse ESCs. This effect is not just a consequence of transcriptional induction and it is independent of its ubiquityltransferase activity [93]. RING1B is responsible for chromatin compaction also at Kcnq1 imprinted locus in mouse. This locus shows a 3D contracted state that differs from the maternal allele when paternally imprinted [94]. The authors conclude that RING1B is responsible for genomic contraction *in vivo*. Interestingly, they found that EZH2, the catalytic subunit of PRC2, also plays a role in this process independently on PRC1. Furthermore, a PRC2 complex containing EZH1 can compact chromatin independently on its enzymatic activity [95]. Finally, indications for a cooperative function between PRC1 and PRC2 suggest that the PRC1-mediated compaction could be facilitated by the PRC2 activity and *vice versa*. Indeed, reconstituted *D. melanogaster* PRC2 exhibits improved activity on dense rather than dispersed oligonucleosomes, indicating that chromatin, when compacted by PRC1, is a more suitable substrate to exert PRC2 function [96].

3D organization of Polycomb-repressed TADs

A complex issue is to determine how chromatin is organized 3D inside these compact domains. Pioneering studies shed light on the spatial organization of the Bithorax complex in *D. melanogaster*. The *Fab-7* PRE and the *Abd-B* promoter were found to interact specifically, although they are distant 35 kb. These interactions occur in nuclei where the gene is silenced by PcG proteins and they are dependent by the presence of a boundary element of the *Fab-7* region [97]. In 2007, Lanzuolo *et al*. suggested that the BX-C forms a topologically independent structure. When repressed by PcG proteins, the BX-C is organized in a multi-loop structure where specific interactions occur among PREs, promoters and 3′ end of the genes,

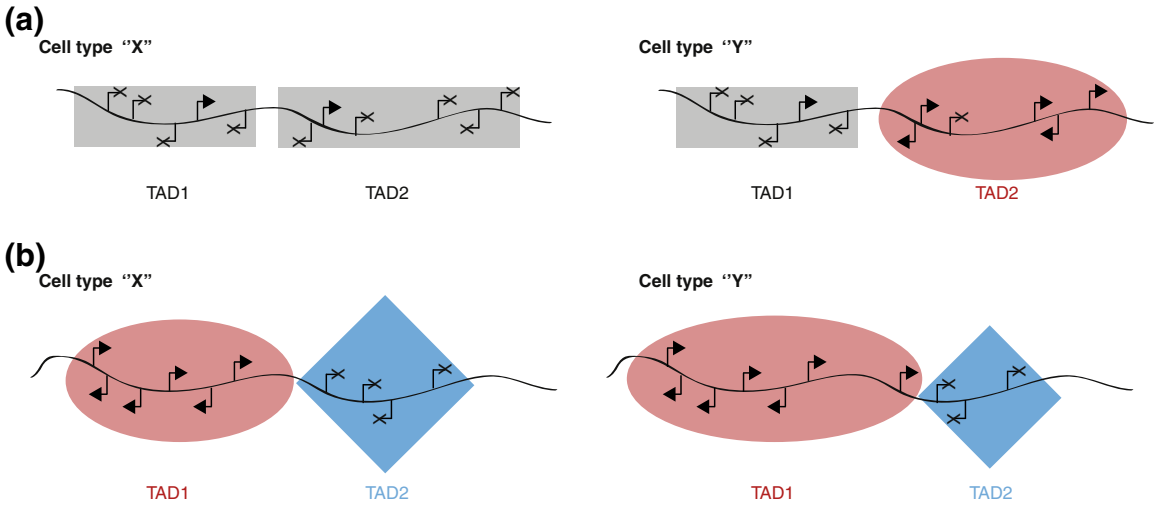

Fig. 2. Linear representation of TADs and their variations between cell types. (a) The cell type "X" displays two null TADs depicted in black (TAD1 and TAD2), harboring mainly inactive genes. In the cell type "Y", the TAD 2 is completely switched into an active TAD, depicted in red, whose genes are now mostly active. The TAD borders are fixed between the two cell types. (b) An active TAD (TAD1) and a Polycomb-repressed TAD (TAD2) are depicted in red and blue, respectively. In some instances, TAD borders can shift between different cell types. Indeed, in cell type "Y", the active TAD1 incorporates one gene that was part of the TAD2 in the cell type "X".

forming three subdomains corresponding to the three transcriptional units. When one of those transcriptional units is expressed, the PRE dedicated to its regulation loses most of its interactions within rest of the domain, particularly with its proximal promoter, suggesting a looping-out of the active gene [98]. These specific interactions inside the BX-C can also occur *in trans*, connecting regulatory sequences of homologous chromosomes [99]. Moreover, the presence of an insulator can block PRE–promoter contacts and interfere with gene silencing in a transgenic construct [100] (Fig. 1b).

3D organization of Hox genes was also studied in mammals. Mammalian genomes bear four Hox clusters that have evolved by duplication events from a common ancestor [101]. They are organized in multi-loop structures, preferentially interacting within the domain, even though external interactions can be observed at lower frequencies [34,35,102,103], as in their *D. melanogaster* counterpart [66,67]. Repression of groups of genes inside these clusters correlates with chromatin condensation and H3K27me3 enrichment [103], whereas transcriptional activation results in decreased interaction frequencies and loss of H3K27me3 [104]. Interestingly, transcriptional induction leads to the disruption of these looping contacts, not only at the induced genes but also in the whole HoxA cluster [102]. It was proposed that EZH2 might not be responsible for the DNA looping, whereas CTCF represents a good candidate for the looping function [102,104]. When a Hox cluster is completely repressed, it is covered with the H3K27me3 mark and it is organized in a single 3D compartment. On the other hand, when a portion of the cluster is transcriptionally active, a bimodal 3D organization appears, mirroring the underlying chromatin profiles. In other words, the linear epigenetic boundary reflects the spatial organization of two separated 3D compartments, a repressed one and an active one. Strikingly, when the expression profile changes, the TAD boundary shifts concomitantly [34,35]. These findings demonstrate that, at least in some cases, the boundary between two TADs can move linearly, according to the underlying chromatin environment (Fig. 2b). Outside Hox clusters, multi-looped conformations can be found in other PcG-repressed loci in human and mouse, such as the GATA-4 locus [105], the INK4-ARF locus [106] and the Meis2 locus [107]. In each case, transcription has a disruptive role on these interactions and Polycomb components are involved in their maintenance.

Polycomb-repressed TAD long-range interactions and nuclear positioning

In addition to intra-TAD contacts [3,34,66,67,98], Polycomb-repressed TADs show long-range interactions. Two studies used a 4C strategy in *D. melanogaster* with baits within the BX-C. Long-range interactions involved other Polycomb-bound domains placed from the same chromosome [66,67]. Larger Polycomb-repressed TADs having more PREs interact more often [67]. Moreover, long-range interactions are evolutionary conserved despite the fact that genomic rearrangements make the linear arrangement of the underlying chromosomes substantially different [66]. Earlier reports stated that long-range interactions, even across different chromosomes,

could occur at high frequency between transgenic PREs [108] or between the transgenic PRE and the endogenous copy [109]. This suggests that Polycomb TAD interactions are an intrinsic feature and not a mere reflection of the linear disposition along the chromosome. Furthermore, the destabilization of these interactions has a direct effect on gene expression, demonstrating their functional relevance [66,109]. In terms of molecular components involved in this process, long-range interactions were shown to be partially dependent on PcG proteins [109] and on insulator components such as CTCF that binds to regulatory regions flanking the interacting PREs [57,110]. Depletion of CTCF affects long-range interactions between the *D. melanogaster* homeotic complexes, without affecting their local clustering [57], while EZH2 was found to have a similar role in mammals [111]. Surprisingly, mutations in several components of the RNAi pathway also reduce contact frequency [57,112], although the molecular role of these factors at PREs is not known. Finally, long-range Polycomb-dependent contacts occur in regions of high Polycomb concentration, called Polycomb foci [57,66,113–115], which can be seen as the physical manifestation of Polycomb TAD interactions. Noteworthy, PcG proteins and H3K27me3 mark do not affect TAD presence [4] and do not alter higher-order nuclear compartmentalization [94,116], but rather they define the chromatin state and the 3D organization of Polycomb-repressed TADs.

In summary, Polycomb-repressed TADs represent a compact chromatin environment with a complex internal multi-looped interaction network. As for the other TADs, internal contacts are highly privileged but inter-TAD contacts within the same chromosome arm form a higher-order network, driving Polycomb-repressed TADs into specific nuclear compartments [116], placing them apart from active chromatin and from lamina-associated chromatin.

Null Chromatin

Genome-wide mapping studies in *D. melanogaster* revealed that nearly half of the genome is covered by a highly repressive chromatin state that appears independent from the other well-known silencing systems (i.e., PcG and HP1) [17,23]. This "obscure" type of chromatin, originally baptized "black chromatin" or "null chromatin", shows a lack of specific enrichments for the histone modifications tested [3,17,23]. The only proteins associated with this type of chromatin are shared with one or more different repressive chromatin types [23,117]. Among them, a strong enrichment is observed for lamin, which plays a central role for the silencing and the compartmentalization at nuclear periphery of null chromatin. This lamin-enriched chromatin type emerged also in similar studies in mammals, thus revealing a general strategy to keep chromatin peripherally compartmentalized and transcriptionally silenced [16,18,20,21]. Something reminding null chromatin was found also in *C. elegans*, where a large portion of silenced genes was enriched in lamin and H3K9me1, H3K9me2 and H3K9me3 marks [22]. These findings suggest that, at least in some species, null chromatin displays some histone modifications. Moreover, it could be speculated that, in some organisms, null chromatin and classic heterochromatin might have been merged. High histone H1 presence in null chromatin [3,17,23] corroborates with gene expression data, as H1 has a negative effect on transcription and can induce chromatin compaction [118]. In *D. melanogaster*, null chromatin is organized in chromatin domains that are on average larger than the other types [17,23,119], showing a good overlap with TADs [3]. Therefore, "null TADs" might be most likely located at the nuclear periphery because of their high enrichment in lamins.

The nuclear periphery has been described to interact with transcriptionally inactive chromatin domains. However, some active genes in *metazoa* were also found to be associated to the nuclear pore proteins (Nups). These findings led to the idea that the nuclear pore could be a favorable environment for gene expression in metazoan nuclei, as it is in yeast. Remarkably, genes associated with Nups were found to be active in the nucleoplasm but silenced when tethered to the nuclear pore complexes in both *D. melanogaster* and human cells, suggesting that nuclear periphery is a quite homogeneously repressive environment in *metazoa* (reviewed in Ref. [120]). Besides nuclear pores, the internal surface of the nucleus is paved by the nuclear lamina, an intricate meshwork of proteins that physically separates the inner nuclear membrane from the peripheral chromatin. Its main components are lamins, which are intermediate filament proteins, and lamin-associated proteins [121]. A-type lamins are mostly expressed in differentiating cells whereas B-type lamins are expressed ubiquitously. Mutations in lamin genes lead to severe developmental defects in *D. melanogaster* [122] and in humans [123], including notably the Hutchinson-Gilford progeria syndrome [124].

LADs

The fragments of the genome interacting with the nuclear lamina are called lamin-associated domains or LADs. In *D. melanogaster*, LADs usually span from 7 to 700 kb, with a median size of 90 kb, bearing on average 7 genes per LAD [125]. Similarly, mammalian LADs are large and gene-poor domains spanning from 0.1 to 10 Mb, covering about 40% of the genome in a cell population [126,127]. About 500 genes were found to interact with lamin in flies. Interestingly, those genes had a very low transcriptional activity, lacking any active histone mark or heterochromatin mark. Moreover, they showed late

replication timing and had intergenic regions 7 times larger than average genes, none of these features alone being sufficient to predict lamin binding [128]. FISH experiments in *D. melanogaster* showed that LADs are located preferentially but not exclusively at the nuclear periphery, indicating a dynamic nature of lamin tethering [128]. Single cell studies conducted in mammals confirmed this trend, showing that only roughly one-third of the LADs are located at less than 1 μm from the nuclear lamina at any point in time. Interestingly, the fraction of the LADs that is associated with the periphery does not move toward the center of the nucleus during a given cell cycle [129]. The evolutionary conserved repressive nature of LADs is supported by genome-wide studies, showing that the average gene expression levels inside the LADs are consistently lower than outside [125–127]. Furthermore, tethering experiments of genes to the nuclear lamina corroborated the idea of a transcriptionally silenced compartment [130].

Genes clustered in LADs usually show developmental coregulation, as they can change simultaneously their expression together with their nuclear repositioning (Fig. 2a). For example, testis-specific genes in *D. melanogaster* tend to be organized in clusters and they are embedded in repressive nuclear peripheral compartments in somatic cells. These clusters are highly enriched for lamin and H1 but depleted for any active, heterochromatin or Polycomb-related marks. Upon depletion of lamin, somatic derepression of those genes is observed, together with detachment from the nuclear envelope [131]. Depletion of lamin can also cause misexpression of key developmental genes. For instance, the Hunchback gene moves to the nuclear periphery when the competence time window to specify early-born neurons is over. Depletion of lamin leads to detachment of hunchback gene from the nuclear periphery with concomitant reduction of its silencing and extension of the time window for neuroblast competence [132]. LADs display a dynamic behavior during development also in *C. elegans* [133] and in mammals [127], even though some LADs are more conserved than others among different cell types [134]. Their developmental reorganization can happen, as in *D. melanogaster*, at single gene level or at the level of entire LADs [127]. Null chromatin domains found in genome-wide association studies in mammals show strong overlap with LADs: they are organized in large domains and span a large portion of genome; they have the lowest transcriptional levels, have the lowest DNase I accessibility, have the highest lamina enrichments, lack any active mark [16,18,20], replicate late and have high levels of DNA methylation [21].

In *D. melanogaster*, chromatin regulation plays a key role in LAD dynamics. Depletion of lamin results in higher chromatin accessibility, as well as increased H3 and H4 acetylation. HDAC1 is required for silencing, whereas HDAC3 has an auxiliary role in silencing but plays a key role in peripheral retention [135]. In a previous report, HDAC3 was found to interact at the nuclear periphery with LAP2β, an integral nuclear-envelope protein with repressive function [136]. The role of HDAC3 in LAD peripheral maintenance is strikingly conserved from *D. melanogaster* to mammals [137–139]. Indeed, the lamin-associated protein Emerin binds HDAC3 at the nuclear periphery and activates its function [138]. Emerin depletion releases HDAC3 from the nuclear periphery [138] and correlates with loss of peripheral chromatin association [138,139]. Mammalian LADs also exhibit specific features, such as enrichment in G9a-dependent H3K9me2 [126,129] that is required for tethering to the lamina [129]. H3K9me2 was previously found to cover large portions of the genome, initially called “Locks” [140] that mostly reflect LADs [130]. This reinforces the idea that the so-called null chromatin is not devoid of all histone marks in every species. LADs are decorated with histone modifications also in other *metazoa*. Indeed, in *C. elegans* LADs, H3K9 is initially monomethylated and dimethylated by MET-25 and successively trimethylated by SET-25. H3K9me1/me2 modifications are required for the anchoring at the nuclear periphery and the H3K9me3 mark is responsible for transcriptional repression [141]. These results show that the anchoring and silencing mechanisms are coordinated but can be uncoupled. Moreover, the attachment to the nuclear periphery is not just a passive process driven by external forces but is at least partially driven by biochemical mechanisms. Mammalian LADs show very sharp borders, wherein the occupancy of lamin drops drastically within few kilobases. These borders are genetically defined, since they show the presence of bidirectional transcription units, usually pointing outward from LADs and bearing CpG islands and CTCF binding sites [126]. These features seem to be conserved in *D. melanogaster*, where very strong transcriptional units pointing outward and insulator proteins mark the sharp boundaries of null TADs [3,119] and LADs [125]. This active chromatin environment demarcating LAD borders may block the spreading of chromatin marks outside LADs. Strikingly, the same genetic determinants, that is, CTCF binding sites and highly expressed housekeeping genes, were found also at human and mouse TAD borders [5].

NADs

Nucleoli, besides rDNA arrays, also associate with actively transcribed RNA Pol III genes, with constitutive centromeric and telomeric heterochromatin and with nucleolar-associated domains or NADs [142]. NADs cover about 4% of the human genome, comprising loci with tissue-specific repression, transposable elements and repetitive sequences. There are several evidences

that NADs and LADs actually overlap. NADs can relocate at the nuclear periphery after cell division [143] and their linear size range highly corresponds with LADs. Moreover, a subset of internalized LADs was shown to become associated with the nucleolus [129]. Finally, tissue-specific repressed olfactory receptor genes, which were already shown to cluster to the nuclear periphery [144], were shown to also associate at the periphery of the nucleolus [142]. Thus, nuclear lamina and nucleolar periphery provide physical platforms to host null TADs.

In addition to null chromatin, the nuclear and the nucleolar peripheries can host constitutive heterochromatin [145]. Moreover, Polycomb-repressed chromatin shows a certain degree of lamin enrichment in genome-wide association studies [3,16,18,20,23]. Nevertheless, null chromatin is likely to represent the major flavor of chromatin to be localized both at the nuclear periphery and around nucleoli.

Constitutive Heterochromatin

Heterochromatin was originally defined as the chromatin portion that maintains high condensation during interphase [146] and was subdivided in facultative and constitutive heterochromatins [147]. Facultative heterochromatin corresponds to euchromatic regions that can become silenced and condensed under specific circumstances. In contrast, constitutive heterochromatin preserves its compaction regardless of the cellular context. In *metazoa*, constitutive heterochromatin usually comes in large blocks that are contiguous to centromeres and it is also called pericentric heterochromatin. Depending on the species, constitutive heterochromatin can also be found in other genomic regions. For example, in *D. melanogaster*, constitutive heterochromatin is located at pericentric regions, telomeres, most of the Y chromosome and on the small fourth chromosome [148]. The amount of constitutive heterochromatin is highly variable among different organisms: it covers about 30% of *D. melanogaster* and human genomes [149] and up to 90% in certain nematodes bearing holocentric chromosomes [150]. Constitutive heterochromatin is highly enriched in tandem repeats, satellite DNA and silenced transposable elements. It has very low rates of meiotic recombination and transcription besides replicating during late S phase. Gene density in constitutive heterochromatin is low compared to the rest of the genome, approximately 10-fold less in *D. melanogaster* [151]. These genes embedded in constitutive heterochromatin are actively transcribed and often essential for viability. Moreover, they require a heterochromatic environment to be expressed [149,152,153]. Besides RNA Pol II genes, both small and long noncoding RNAs are transcribed from heterochromatin [154–156], indicative of an environment not completely averse to transcription.

Universal hallmarks of constitutive heterochromatin are the presence of H4K20me3, H3K9me2/me3 and HP1a (*h*eterochromatin *p*rotein *1a*), which binds to H3K9me2/me3 via its chromodomain [157]. H3K9me2/me3 marks are deposited in *D. melanogaster* pericentric heterochromatin by SU(VAR)3-9 and by its homologs in other species, but different heterochromatin environments can use different methyltransferases [148]. The borders between heterochromatin and euchromatin were initially described cytologically [158] and then by molecular approaches [159,160]. These borders are defined by sharp H3K9me2 transitions and they show a substantial overlap among different tissues [153]. Euchromatic genes placed near those borders display a mosaic expression, namely, a variable transcriptional state in different cells. This phenomenon, initially discovered in *D. melanogaster*, (reviewed in Ref. [161]) and recently reported also in humans [162], was called PEV (*p*osition *e*ffect *v*ariegation) and was explained through a spreading model, predicting that the linear distance of a euchromatic gene from the heterochromatin border influences its transcriptional state. Interestingly, this model does not explain cases where a marker gene closer to heterochromatin is active and a more distant one is repressed. Thus, an alternative model has been proposed, confirming a linear polarity but introducing the possibility that chromatin loops could bring linear distant genes 3D closer to the nucleation sites. This 3D explanation can solve the observed skipping in the silencing of nearer genes [163].

Genome-wide studies recapitulated many of the already-known features of constitutive heterochromatin. In *D. melanogaster*, heterochromatin domains show enrichment for H3K9me2/me3 and for already-known heterochromatin proteins, together with other proteins shared with different chromatin types [3,17,23]. Average transcriptional activity is lower than in active chromatin but higher than Polycomb-repressed chromatin and null chromatin [23]. Moreover, high H1 enrichment [23] and the lowest density of DNase I hypersensitive sites [17] predict a very compact chromatin environment. Similar studies in humans confirmed the presence of H3K9me3 domains enriched in repetitive sequences and devoid of active marks and H3K27me3 [16,18–21].

Constitutive heterochromatin compaction

As mentioned above, constitutive heterochromatin represents by definition a highly condensed environment. Surprisingly, the first studies aimed to determine the relative levels of heterochromatin accessibility to DNA methylating enzymes [164] could not identify strong differences in accessibility compared to control regions, but similar approaches performed with transgenes with a well-characterized chromatin structure showed substantial differences in chromatin accessibility between euchromatic and

heterochromatic insertions. Transgenes inserted in several heterochromatic loci showed a reduction in accessibility to restriction enzyme digestion, together with transcriptional silencing. HP1a presence influenced both compaction and silencing of transgenes inserted into pericentric heterochromatic loci and in the fourth chromosome [165]. Reduced subtelomeric heterochromatin accessibility was further confirmed [166]. Moreover, micrococcal nuclease assays revealed a more regular nucleosomal ladder in heterochromatin compared to euchromatin, indicating a higher-order compaction with a constant repeat length [167].

The higher compaction observed in constitutive heterochromatin may not just depend on the lack of transcription since non-transcribed euchromatic genes maintain the same DNA accessibility when they become transcribed [85]. Interestingly, when the relative DNA accessibilities of euchromatin, constitutive heterochromatin and Polycomb-repressed chromatin were compared in the same study, the latter two showed similar low levels, regardless of their different molecular nature and genomic positions. These findings indicate that the two different compaction mechanisms may involve similar higher-order chromatin organization [85]. Finally, it is important to mention that transcription factors can, in some cases, challenge the heterochromatic environment and even be crucial for its establishment [168]. This means that DNA accessibility, although lower than in active regions, does not represent an insurmountable barrier for DNA-binding factors.

Long-range interactions and nuclear positioning of constitutive heterochromatin

There is no formal proof that constitutive heterochromatin is organized in TADs and technical difficulties in mappability given by the huge amount of repetitive sequences make it hard to answer this question. Nonetheless, either a TAD arrangement or an alternative 3D organization could be possible. In general, constitutive heterochromatin regions interact poorly with any other chromatin type, whereas they readily interact with other heterochromatin regions *in cis* and *in trans* [3].

In *D. melanogaster*, centromeres and pericentric heterochromatin coalesce into nuclear structures called chromocenters. In polytene salivary gland nuclei, there is a single chromocenter that clusters centromeres, the Y and fourth chromosomes in a single heterochromatic structure, located at the periphery of the nucleus. This cytological organization has been recapitulated by Hi-C studies performed in late *D. melanogaster* embryos [3] and cultured cells of embryonic origin [119]. Recently, clustering of *D. melanogaster* centromeres was shown to be dependent on CTCF and nucleoplasmin-like proteins. These proteins, together with the nucleolin homolog Modulo, favor the interaction of centromeres with the periphery of the nucleolus [169]. In *D. melanogaster* embryos, heterochromatic telomeric sequences also cluster, but they cluster away from the chromocenter [3]. Mammalian nuclei display chromocenters in numbers and sizes that vary across species and cell types. For example, mouse chromocenters are more clustered than human counterparts [170]. Mammalian chromocenters reside at the nuclear periphery in late G1, then they disassemble and centromeres internalize in late S phase and G2. After mitosis, centromeres keep an internal position until early G1 [171,172]. Differentiation can either increase chromocenter clustering [170,172] or induce a de-clustering [173–175], according to the cell type.

Long-range interactions between heterochromatic foci and euchromatic genes can drive their silencing by a proximity effect. This phenomenon was called *trans*-inactivation in *D. melanogaster* [176]. The most famous example is the brown dominant allele (*bwD*), carrying a 1.6-Mb block of heterochromatic satellite sequence in its locus [177]. Whereas null alleles of *brown* are recessive, *bwD* can silence *in trans* the wild-type copy, dragging both alleles to pericentric heterochromatin [178]. The result is that the two alleles are repressed [176], even though the wild-type allele does not show the typical heterochromatic condensation or HP1a binding in polytene chromosomes [179]. Nevertheless, physical proximity to heterochromatin is not sufficient for silencing, since genes nearby *brown* do not get silenced and an active form of *brown* was found to be compatible with heterochromatin association [180]. Other examples of *trans*-inactivation were described in *D. melanogaster*, affecting protein coding genes [181,182] and transposable elements [183,184]. Similar long-range interactions between heterochromatin and silenced genes can also occur in mammals. For instance, in mouse thymocytes, specific loci are bound by the zinc-finger protein Ikaros and targeted to pericentric heterochromatin [185]. Ikaros firstly represses their transcription and then brings them to heterochromatin foci, where it multimerizes and physically bridges the repressed genes with the heterochromatin satellite repeats [186]. During the repositioning, deacetylation on H3K9ac occurs. After heterochromatic localization, firstly, there is demethylation of H3K4me3 and then methylation of H3K9, finally spreading bidirectionally and leading to an irreversible silencing [187]. Interestingly, genome-wide association studies in *D. melanogaster* have highlighted a special form of H3K9me2/me3 heterochromatin, not located at the classical genomic loci but rather embedded in euchromatin [17]. These ectopic heterochromatic domains vary among cell types and contain mostly silenced genes [17,153]. An intriguing hypothesis is that those tissue-specific silenced genes might be 3D tethered to pericentromeric heterochromatin, reflecting a looping mechanism.

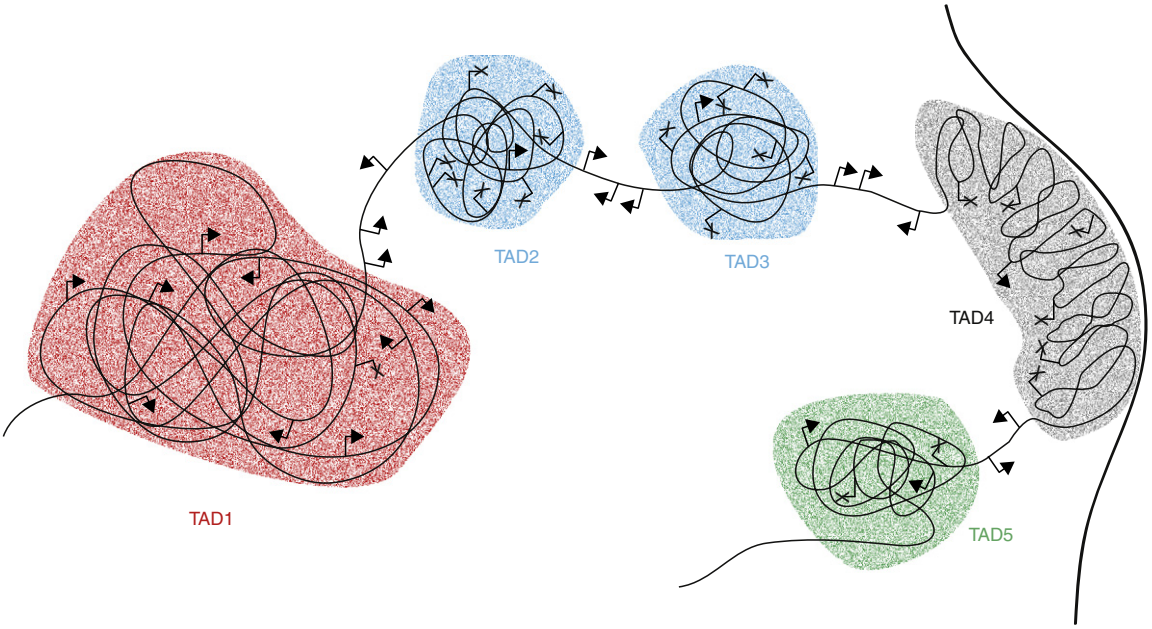

Fig. 3. 3D representation of TADs. The active TAD1 in red, the Polycomb-repressed TAD2 and TAD3 in blue, the null TAD4 in black and the heterochromatic TAD 5 in green are depicted inside the cell nucleus. TAD1 harbors mostly active genes and is more decondensed and its nuclear positioning is more internal. TAD2 and TAD3 are more condensed than TAD1 and they are mainly transcriptionally silent. TAD4 is associated to the nuclear periphery and its genes are mostly silent. TAD5 is coated with constitutive heterochromatin. TAD5 not only has repressed transposable elements but also has few genes, which are expressed, regardless of the surrounding chromatin environment. The domains' borders harbor actively transcribed housekeeping genes.

In summary, constitutive heterochromatin represents a special chromatin environment, usually covering the centromeric and telomeric regions. Its condensation is fundamental for its functions and its compartmentalization radically shapes the genome architecture, also providing a potential platform for tissue specific gene silencing.

Conclusion

There is still much to understand about chromatin organization in *metazoa*, but some principles are beginning to emerge. TADs are the basic units of metazoan chromosomes and they represent a physical compartment for chromatin interactions. TADs are robust entities, since their borders are generally conserved among different cell types [4,5], even though they can shift in some instances [34,35]. Genetically defined TADs are associated with a particular chromatin type that dramatically alters TADs identity. The main chromatin types are basically four: active chromatin, Polycomb-repressed chromatin, null chromatin and constitutive heterochromatin, but further sub-classifications are possible and their description will help understand the complexity of the system [3,20,21]. The chromatin type characterizing a TAD is switchable, giving a certain degree of freedom to these genomic entities (Fig. 2). This chromatin type confers to the TAD's some specific characteristics, which impact on TAD's functionality, even though a certain degree of internal heterogeneity is tolerated. The 3D organization of a TAD, adorned with a specific chromatin type, reflects its typical features as relative accessibility, chromatin compaction and looping contacts, which are characteristic of that particular chromatin environment. Despite these type-specific differences, the common feature of every TAD is its partial 3D separation from the rest of the genome (Fig. 3).

Moreover, the chromatin type of a TAD is a main factor for its nuclear positioning, favoring or disfavoring specific long-range contacts and driving it toward a preferential localization in the nucleus. The strong influence that chromatin types can give to TAD localization could be a major force in chromosome positioning and finally in genome architecture. For example, more gene-rich and transcriptionally active chromosomes occupy preferentially a more internal territory than gene-poor and silent ones do. Such a behavior at whole chromosome level may just reflect the tendencies of its own TADs, which finally could

drag the chromosome to different nuclear neighborhoods. Consequently, chromosomes with several null TADs could be more peripheral, as they will bind preferentially the nuclear lamina. This abundance of lamin binding TADs might lead to effective competition of inactive chromosomes against more active ones. Inactive chromosomes would thus locate at the periphery, at the expense of chromosomes carrying higher density of active TADs, which would place themselves in a more central position and would interact more easily with active TADs present on other chromosomes.

The chromatin type of TADs is switchable, and this change will influence its function together with its 3D internal organization. This feature should also affect nuclear architecture, as different cell types sharing their TADs but coated with different combination of chromatin types can have a completely different genome organization (Fig. 4). In this regard, it is important to mention that even two cells of the same type show a high variability in contacts between TADs [36], demonstrating that two cells displaying exactly the same nuclear organization do not exist. In summary, a given cell type, with a genome defined by the linear succession of different chromatin types, can have several possible 3D organizations. This is due to a certain degree of stochasticity and to the dynamics of the process. Nonetheless, two different cell types, displaying different chromatin landscapes, will have an even less similar nuclear organization.

The chromatin coating the genome has also been defined as the “epigenome”. This is due to the fact that chromatin states, and particularly histone modifications, have been considered as potential carriers of epigenetic inheritance. In favor of this idea, many chromatin modifiers have been found on the replication forks, suggesting that the epigenome could be replicated along with the genome [188]. Remarkably, nucleosomes relocate after DNA replication. While duplicating chromatin, the parental histones are repositioned in a place that can be about 400 bp (corresponding to 2–3 nucleosomes) away from the original position [189]. In light of this fact, large domains of repressive marks (e.g., H3K27me3, H3K9me2, H3K9me3), rather than sharp peaks of active marks (e.g., H3K4me3, lysine acetylations), would represent better candidate for this epigenetic role. Since TAD's organization is completely lost during metaphase [190], a faithful epigenome replication would allow two daughter cells to have very similar chromatin landscapes, which may recapitulate a related genome organization. This principle, although partially true, cannot be universally applied. Indeed in mammals, LADs tethered to the nuclear periphery in the mother cell do not associate preferentially to the periphery in the daughter cell, meaning that there is no epigenetic mechanism to restore nuclear positioning. LAD association to the nuclear periphery is linked to a certain degree of stochasticity in the acquirement of dedicated histone marks, that is, H3K9me2, which finally drives the peripheral compartmentalization [129]. Older reports already showed that the same loci in daughter cells do not share the same nuclear positioning and therefore

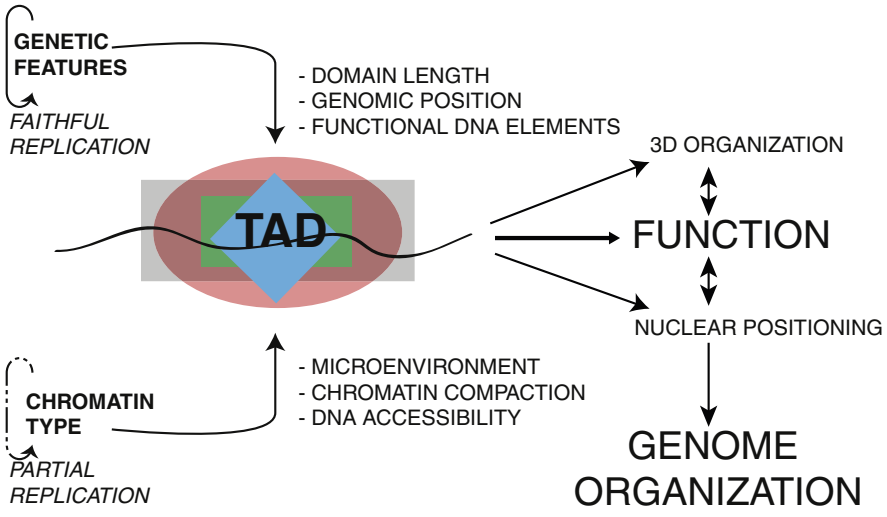

Fig. 4. Impact of genetic features and chromatin type on the TAD. Fixed genetic elements define TAD borders, determining its length. The presence of genes and of functional elements inside a TAD are also genetically determined, as well as its genomic site. These features do not vary among different cell types. The chromatin type surrounding the TAD defines features as the DNA accessibility and the chromatin compaction. The chromatin environment can vary among different cell types and its partial replication will determine differences between different cells of the same type. Together, these genetic and epigenetic parameters will affect TAD 3D organization and nuclear positioning that will finally determine TAD function. The TAD tendency to occupy preferential nuclear positions will finally drive the global genome organization.

cannot be inherited from the mother cell [191]. The same principle can be applied to the conservation of chromosome territories, as their relative positions observed in daughter cells differ significantly from that of the mother cell. Apparently, chromosome territories are established in early G1 and stably maintained throughout the cell cycle, but during early prophase, a reshuffling occurs [182].

In conclusion, genome architecture relies on TAD organization. TADs are primarily defined by genetic determinants and further shaped by their chromatin environment. Epigenome replication processes will ensure the maintenance of their chromatin landscape and, consequently, of part of their 3D properties. Although the genome is faithfully replicated, the epigenome could be in part variable between two daughter cells. This variability, along with a high stochasticity in genome conformations, will lead to a partial conservation of genome architecture between two cells of the same type (Fig. 4).

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Abbreviations used:
TAD, topologically associating domain; 3D, three-dimensional; PRE, Polycomb responsive element.

References

[1] Cremer T, Cremer M. Chromosome territories. Cold Spring Harb Perspect Biol 2010;2:a003889.
[2] Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 2009;326:289–93.
[3] Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, et al. Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 2012;148:458–72.
[4] Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 2012;485:381–5.
[5] Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012;485:376–80.
[6] Cmarko D, Verschure PJ, Martin TE, Dahmus ME, Krause S, Fu XD, et al. Ultrastructural analysis of transcription and splicing in the cell nucleus after bromo-UTP microinjection. Mol Biol Cell 1999;10:211–23.
[7] Feng S, Cokus SJ, Schubert V, Zhai J, Pellegrini M, Jacobsen SE. Genome-wide Hi-C Analyses in Wild-Type and Mutants Reveal High-Resolution Chromatin Interactions in Arabidopsis. Mol Cell 2014;55:694–707.
[8] Grob S, Schmid MW, Grossniklaus U. Hi-C Analysis in Arabidopsis Identifies the KNOT, a Structure with Similarities to the flamenco Locus of Drosophila. Mol Cell 2014;55:678–93.
[9] Moissiard G, Cokus SJ, Cary J, Feng S, Billi AC, Stroud H, et al. MORC family ATPases required for heterochromatin condensation and gene silencing. Science 2012;336:1448–51.
[10] Tanizawa H, Iwasaki O, Tanaka A, Capizzi JR, Wickramasinghe P, Lee M, et al. Mapping of long-range associations throughout the fission yeast genome reveals global genome organization linked to transcriptional regulation. Nucleic Acids Res 2010;38:8164–77.
[11] Tjong H, Gong K, Chen L, Alber F. Physical tethering and volume exclusion determine higher-order genome organization in budding yeast. Genome Res 2012;22:1295–305.
[12] Hurst LD, Pal C, Lercher MJ. The evolutionary dynamics of eukaryotic gene order. Nat Rev Genet 2004;5:299–310.
[13] Caron H, van Schaik B, van der Mee M, Baas F, Riggins G, van Sluis P, et al. The human transcriptome map: clustering of highly expressed genes in chromosomal domains. Science 2001;291:1289–92.
[14] Gierman HJ, Indemans MH, Koster J, Goetze S, Seppen J, Geerts D, et al. Domain-wide regulation of gene expression in the human genome. Genome Res 2007;17:1286–95.
[15] Spellman PT, Rubin GM. Evidence for large domains of similarly expressed genes in the Drosophila genome. J Biol 2002;1:5.
[16] Ernst J, Kellis M. Discovery and characterization of chromatin states for systematic annotation of the human genome. Nat Biotechnol 2010;28:817–25.
[17] Kharchenko PV, Alekseyenko AA, Schwartz YB, Minoda A, Riddle NC, Ernst J, et al. Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. Nature 2011;471:480–5.
[18] Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, et al. Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 2011;473:43–9.
[19] Ram O, Goren A, Amit I, Shoresh N, Yosef N, Ernst J, et al. Combinatorial patterning of chromatin regulators uncovered

by genome-wide location analysis in human cells. Cell 2011;147:1628–39.
[20] Zhu J, Adli M, Zou JY, Verstappen G, Coyne M, Zhang X, et al. Genome-wide chromatin state transitions associated with developmental and environmental cues. Cell 2013;152: 642–54.
[21] Julienne H, Zoufir A, Audit B, Arneodo A. Human genome replication proceeds through four chromatin states. PLoS Comput Biol 2013;9:e1003233.
[22] Liu T, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre I, Cheung MS, et al. Broad chromosomal domains of histone modification patterns in C. elegans. Genome Res 2011;21: 227–36.
[23] Filion GJ, van Bemmel JG, Braunschweig U, Talhout W, Kind J, Ward LD, et al. Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. Cell 2010;143:212–24.
[24] Shen Y, Yue F, McCleary DF, Ye Z, Edsall L, Kuan S, et al. A map of the cis-regulatory sequences in the mouse genome. Nature 2012;488:116–20.
[25] Lan X, Witt H, Katsumura K, Ye Z, Wang Q, Bresnick EH, et al. Integration of Hi-C and ChIP-seq data reveals distinct types of chromatin linkages. Nucleic Acids Res 2012;40: 7690–704.
[26] Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan KK, Cheng C, et al. Architecture of the human regulatory network derived from ENCODE data. Nature 2012;489:91–100.
[27] de Wit E, de Laat W. A decade of 3C technologies: insights into nuclear organization. Genes Dev 2012;26:11–24.
[28] Dekker J, Marti-Renom MA, Mirny LA. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. Nat Rev Genet 2013;14:390–403.
[29] Rouquette J, Cremer C, Cremer T, Fakan S. Functional nuclear architecture studied by microscopy: present and future. Int Rev Cell Mol Biol 2010;282:1–90.
[30] Harmston N, Lenhard B. Chromatin and epigenetic features of long-range gene regulation. Nucleic Acids Res 2013;41: 7185–99.
[31] Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, et al. The accessible chromatin landscape of the human genome. Nature 2012;489:75–82.
[32] Nobrega MA, Ovcharenko I, Afzal V, Rubin EM. Scanning human gene deserts for long-range enhancers. Science 2003;302:413.
[33] Kikuta H, Laplante M, Navratilova P, Komisarczuk AZ, Engstrom PG, Fredman D, et al. Genomic regulatory blocks encompass multiple neighboring genes and maintain conserved synteny in vertebrates. Genome Res 2007;17: 545–55.
[34] Noordermeer D, Leleu M, Splinter E, Rougemont J, De Laat W, Duboule D. The dynamic architecture of Hox gene clusters. Science 2011;334:222–5.
[35] Andrey G, Montavon T, Mascrez B, Gonzalez F, Noordermeer D, Leleu M, et al. A switch between topological domains underlies HoxD genes collinearity in mouse limbs. Science 2013;340:1234167.
[36] Nagano T, Lubling Y, Stevens TJ, Schoenfelder S, Yaffe E, Dean W, et al. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature 2013;502:59–64.
[37] Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, et al. A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature 2013;503:290–4.
[38] Kieffer-Kwon KR, Tang Z, Mathe E, Qian J, Sung MH, Li G, et al. Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. Cell 2013;155:1507–20.
[39] Li G, Ruan X, Auerbach RK, Sandhu KS, Zheng M, Wang P, et al. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell 2012;148:84–98.
[40] Tan-Wong SM, French JD, Proudfoot NJ, Brown MA. Dynamic interactions between the promoter and terminator regions of the mammalian BRCA1 gene. Proc Natl Acad Sci U S A 2008;105:5160–5.
[41] O'Reilly D, Greaves DR. Cell-type-specific expression of the human CD68 gene is associated with changes in Pol II phosphorylation and short-range intrachromosomal gene looping. Genomics 2007;90:407–15.
[42] Cullen KE, Kladde MP, Seyfred MA. Interaction between transcription regulatory regions of prolactin chromatin. Science 1993;261:203–6.
[43] Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, et al. Active genes dynamically colocalize to shared sites of ongoing transcription. Nat Genet 2004;36: 1065–71.
[44] Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W. Looping and interaction between hypersensitive sites in the active beta-globin locus. Mol Cell 2002;10:1453–65.
[45] Maston GA, Evans SK, Green MR. Transcriptional regulatory elements in the human genome. Annu Rev Genomics Hum Genet 2006;7:29–59.
[46] Drissen R, Palstra RJ, Gillemans N, Splinter E, Grosveld F, Philipsen S, et al. The active spatial organization of the beta-globin locus requires the transcription factor EKLF. Genes Dev 2004;18:2485–90.
[47] Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, et al. Mediator and cohesin connect gene expression and chromatin architecture. Nature 2010;467: 430–5.
[48] Degner SC, Verma-Gaur J, Wong TP, Bossen C, Iverson GM, Torkamani A, et al. CCCTC-binding factor (CTCF) and cohesin influence the genomic architecture of the Igh locus and antisense transcription in pro-B cells. Proc Natl Acad Sci U S A 2011;108:9566–71.
[49] Chepelev I, Wei G, Wangsa D, Tang Q, Zhao K. Characterization of genome-wide enhancer-promoter interactions reveals co-expression of interacting genes and modes of higher order chromatin organization. Cell Res 2012;22:490–503.
[50] Roider HG, Lenhard B, Kanhere A, Haas SA, Vingron M. CpG-depleted promoters harbor tissue-specific transcription factor binding signals–implications for motif overrepresentation analyses. Nucleic Acids Res 2009;37: 6305–15.
[51] Montavon T, Soshnikova N, Mascrez B, Joye E, Thevenet L, Splinter E, et al. A regulatory archipelago controls Hox genes transcription in digits. Cell 2011;147:1132–45.
[52] Zhang Y, Wong CH, Birnbaum RY, Li G, Favaro R, Ngan CY, et al. Chromatin connectivity maps reveal dynamic promoter-enhancer long-range associations. Nature 2013; 504:306–10.
[53] Lower KM, Hughes JR, De Gobbi M, Henderson S, Viprakasit V, Fisher C, et al. Adventitious changes in long-range gene expression caused by polymorphic structural variation and promoter competition. Proc Natl Acad Sci U S A 2009;106:21771–6.
[54] Yang J, Corces VG. Insulators, long-range interactions, and genome function. Curr Opin Genet Dev 2012;22:86–92.

[55] Ghavi-Helm Y, Klein FA, Pakozdl T, Ciglar L, Noordermeer D, Huber W, et al. Enhancer loops appear stable during development and are associated with paused polymerase. Nature; 2014.
[56] Noordermeer D, de Wit E, Klous P, van de Werken H, Simonis M, Lopez-Jones M, et al. Variegated gene expression caused by cell-specific long-range DNA interactions. Nat Cell Biol 2011;13:944–51.
[57] Li HB, Ohno K, Gui H, Pirrotta V. Insulators target active genes to transcription factories and polycomb-repressed genes to polycomb bodies. PLoS Genet 2013;9: e1003436.
[58] Jackson DA, Hassan AB, Errington RJ, Cook PR. Visualization of focal sites of transcription within human nuclei. EMBO J 1993;12:1059–65.
[59] Apostolou E, Thanos D. Virus Infection Induces NF-kappaB-dependent interchromosomal associations mediating monoallelic IFN-beta gene expression. Cell 2008;134: 85–96.
[60] de Wit E, Bouwman BA, Zhu Y, Klous P, Splinter E, Verstegen MJ, et al. The pluripotent genome in three dimensions is shaped around pluripotency factors. Nature 2013;501:227–31.
[61] Hu Q, Kwon YS, Nunez E, Cardamone MD, Hutt KR, Ohgi KA, et al. Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules. Proc Natl Acad Sci U S A 2008;105:19199–204.
[62] Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, Andrews S, et al. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. Nat Genet 2010;42:53–61.
[63] Eskiw CH, Fraser P. Ultrastructural study of transcription factories in mouse erythroblasts. J Cell Sci 2011;124: 3676–83.
[64] Eskiw CH, Rapp A, Carter DR, Cook PR. RNA polymerase II activity is located on the surface of protein-rich transcription factories. J Cell Sci 2008;121:1999–2007.
[65] Mitchell JA, Fraser P. Transcription factories are nuclear subcompartments that remain in the absence of transcription. Genes Dev 2008;22:20–5.
[66] Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, et al. Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. Cell 2011;144:214–26.
[67] Tolhuis B, Blom M, Kerkhoven RM, Pagie L, Teunissen H, Nieuwland M, et al. Interactions among Polycomb domains are guided by chromosome architecture. PLoS Genet 2011; 7:e1001343.
[68] Nemeth A, Langst G. Genome organization in and around the nucleolus. Trends Genet 2011;27:149–56.
[69] Kalmarova M, Smirnov E, Masata M, Koberna K, Ligasova A, Popov A, et al. Positioning of NORs and NOR-bearing chromosomes in relation to nucleoli. J Struct Biol 2007;160: 49–56.
[70] Shiue CN, Berkson RG, Wright AP. c-Myc induces changes in higher order rDNA structure on stimulation of quiescent cells. Oncogene 2009;28:1833–42.
[71] Foster HA, Bridger JM. The genome and the nucleus: a marriage made by evolution Genome organisation and nuclear architecture. Chromosoma 2005;114:212–29.
[72] Kosak ST, Scalzo D, Alworth SV, Li F, Palmer S, Enver T, et al. Coordinate gene regulation during hematopoiesis is related to genomic organization. PLoS Biol 2007;5:e309.
[73] Croft JA, Bridger JM, Boyle S, Perry P, Teague P, Bickmore WA. Differences in the localization and morphology of chromosomes in the human nucleus. J Cell Biol 1999;145: 1119–31.
[74] Lewis EB. A gene complex controlling segmentation in Drosophila. Nature 1978;276:565–70.
[75] Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, et al. Stabilization of chromatin structure by PRC1, a Polycomb complex. Cell 1999;98:37–46.
[76] Muller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell 2002;111: 197–208.
[77] Tolhuis B, de Wit E, Muijrers I, Teunissen H, Talhout W, van Steensel B, et al. Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in Drosophila melanogaster. Nat Genet 2006;38:694–9.
[78] Negre N, Hennetin J, Sun LV, Lavrov S, Bellis M, White KP, et al. Chromosomal distribution of PcG proteins during Drosophila development. PLoS Biol 2006;4:e170.
[79] Schuettengruber B, Ganapathi M, Leblanc B, Portoso M, Jaschek R, Tolhuis B, et al. Functional anatomy of polycomb and trithorax chromatin landscapes in Drosophila embryos. PLoS Biol 2009;7:e13.
[80] Schuettengruber B, Cavalli G. Recruitment of polycomb group complexes and their role in the dynamic regulation of cell fate choice. Development 2009;136:3531–42.
[81] Kassis JA, Brown JL. Polycomb group response elements in Drosophila and vertebrates. Adv Genet 2013;81:83–118.
[82] Schwartz YB, Pirrotta V. A new world of Polycombs: unexpected partnerships and emerging functions. Nat Rev Genet 2013;14:853–64.
[83] Bickmore WA, van Steensel B. Genome architecture: domain organization of interphase chromosomes. Cell 2013;152:1270–84.
[84] Schlossherr J, Eggert H, Paro R, Cremer S, Jack RS. Gene inactivation in Drosophila mediated by the Polycomb gene product or by position-effect variegation does not involve major changes in the accessibility of the chromatin fibre. Mol Gen Genet 1994;243:453–62.
[85] Boivin A, Dura JM. In vivo chromatin accessibility correlates with gene silencing in Drosophila. Genetics 1998;150: 1539–49.
[86] Marchetti M, Fanti L, Berloco M, Pimpinelli S. Differential expression of the Drosophila BX-C in polytene chromosomes in cells of larval fat bodies: a cytological approach to identifying in vivo targets of the homeotic Ubx, Abd-A and Abd-B proteins. Development 2003;130:3683–9.
[87] Zink D, Paro R. Drosophila Polycomb-group regulated chromatin inhibits the accessibility of a trans-activator to its target DNA. EMBO J 1995;14:5660–71.
[88] Fitzgerald DP, Bender W. Polycomb group repression reduces DNA accessibility. Mol Cell Biol 2001;21:6585–97.
[89] Francis NJ, Kingston RE, Woodcock CL. Chromatin compaction by a polycomb group protein complex. Science 2004;306:1574–7.
[90] King IF, Emmons RB, Francis NJ, Wild B, Muller J, Kingston RE, et al. Analysis of a polycomb group protein defines regions that link repressive activity on nucleosomal templates to in vivo function. Mol Cell Biol 2005;25:6578–91.
[91] Grau DJ, Chapman BA, Garlick JD, Borowsky M, Francis NJ, Kingston RE. Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. Genes Dev 2011;25:2210–21.

[92] Isono K, Endo TA, Ku M, Yamada D, Suzuki R, Sharif J, et al. SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. Dev Cell 2013;26:565–77.
[93] Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, et al. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. Mol Cell 2010;38:452–64.
[94] Terranova R, Yokobayashi S, Stadler MB, Otte AP, van Lohuizen M, Orkin SH, et al. Polycomb group proteins Ezh2 and Rnf2 direct genomic contraction and imprinted repression in early mouse embryos. Dev Cell 2008;15:668–79.
[95] Margueron R, Li G, Sarma K, Blais A, Zavadil J, Woodcock CL, et al. Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. Mol Cell 2008;32:503–18.
[96] Yuan W, Wu T, Fu H, Dai C, Wu H, Liu N, et al. Dense chromatin activates Polycomb repressive complex 2 to regulate H3 lysine 27 methylation. Science 2012;337:971–5.
[97] Cleard F, Moshkin Y, Karch F, Maeda RK. Probing long-distance regulatory interactions in the Drosophila melanogaster bithorax complex using Dam identification. Nat Genet 2006;38:931–5.
[98] Lanzuolo C, Roure V, Dekker J, Bantignies F, Orlando V. Polycomb response elements mediate the formation of chromosome higher-order structures in the bithorax complex. Nat Cell Biol 2007;9:1167–74.
[99] Ronshaugen M, Levine M. Visualization of trans-homolog enhancer-promoter interactions at the Abd-B Hox locus in the Drosophila embryo. Dev Cell 2004;7:925–32.
[100] Comet I, Schuettengruber B, Sexton T, Cavalli G. A chromatin insulator driving three-dimensional Polycomb response element (PRE) contacts and Polycomb association with the chromatin fiber. Proc Natl Acad Sci U S A 2011;108:2294–9.
[101] Holland PW. Evolution of homeobox genes. Wiley Interdiscip Rev Dev Biol 2013;2:31–45.
[102] Ferraiuolo MA, Rousseau M, Miyamoto C, Shenker S, Wang XQ, Nadler MB, et al. The three-dimensional architecture of Hox cluster silencing. Nucleic Acids Res 2010;38:7472–84.
[103] Fraser J, Rousseau M, Shenker S, Ferraiuolo MA, Hayashizaki Y, Blanchette M, et al. Chromatin conformation signatures of cellular differentiation. Genome Biol 2009;10:R37.
[104] Rousseau M, Crutchley JL, Miura H, Suderman M, Blanchette M, Dostie J. Hox in motion: tracking HoxA cluster conformation during differentiation. Nucleic Acids Res 2014;42:1524–40.
[105] Tiwari VK, McGarvey KM, Licchesi JD, Ohm JE, Herman JG, Schubeler D, et al. PcG proteins, DNA methylation, and gene repression by chromatin looping. PLoS Biol 2008;6:2911–27.
[106] Kheradmand Kia S, Solaimani Kartalaei P, Farahbakhshian E, Pourfarzad F, et al. EZH2-dependent chromatin looping controls INK4a and INK4b, but not ARF, during human progenitor cell differentiation and cellular senescence. Epigenetics Chromatin 2009;2:16.
[107] Kondo T, Isono K, Kondo K, Endo TA, Itohara S, Vidal M, et al. Polycomb Potentiates Meis2 Activation in Midbrain by Mediating Interaction of the Promoter with a Tissue-Specific Enhancer. Dev Cell 2013.
[108] Vazquez J, Muller M, Pirrotta V, Sedat JW. The Mcp element mediates stable long-range chromosome-chromosome interactions in Drosophila. Mol Biol Cell 2006;17:2158–65.
[109] Bantignies F, Grimaud C, Lavrov S, Gabut M, Cavalli G. Inheritance of Polycomb-dependent chromosomal interactions in Drosophila. Genes Dev 2003;17:2406–20.
[110] Li HB, Muller M, Bahechar IA, Kyrchanova O, Ohno K, Georgiev P, et al. Insulators, not Polycomb response elements, are required for long-range interactions between Polycomb targets in Drosophila melanogaster. Mol Cell Biol 2011;31:616–25.
[111] Tiwari VK, Cope L, McGarvey KM, Ohm JE, Baylin SB. A novel 6C assay uncovers Polycomb-mediated higher order chromatin conformations. Genome Res 2008;18:1171–9.
[112] Grimaud C, Bantignies F, Pal-Bhadra M, Ghana P, Bhadra U, Cavalli G. RNAi components are required for nuclear clustering of Polycomb group response elements. Cell 2006;124:957–71.
[113] Cheutin T, Cavalli G. Progressive polycomb assembly on H3K27me3 compartments generates polycomb bodies with developmentally regulated motion. PLoS Genet 2012;8:e1002465.
[114] Cheutin T, Cavalli G. Polycomb silencing: from linear chromatin domains to 3D chromosome folding. Curr Opin Genet Dev 2014;25C:30–7.
[115] Saurin AJ, Shiels C, Williamson J, Satijn DP, Otte AP, Sheer D, et al. The human polycomb group complex associates with pericentromeric heterochromatin to form a novel nuclear domain. J Cell Biol 1998;142:887–98.
[116] Denholtz M, Bonora G, Chronis C, Splinter E, de Laat W, Ernst J, et al. Long-range chromatin contacts in embryonic stem cells reveal a role for pluripotency factors and polycomb proteins in genome organization. Cell Stem Cell 2013;13:602–16.
[117] van Bemmel JG, Filion GJ, Rosado A, Talhout W, de Haas M, van Welsem T, et al. A network model of the molecular organization of chromatin in Drosophila. Mol Cell 2013;49:759–71.
[118] Brown DT. Histone H1 and the dynamic regulation of chromatin function. Biochem Cell Biol 2003;81:221–7.
[119] Hou C, Li L, Qin ZS, Corces VG. Gene density, transcription, and insulators contribute to the partition of the Drosophila genome into physical domains. Mol Cell 2012;48:471–84.
[120] Pascual-Garcia P, Capelson M. Nuclear pores as versatile platforms for gene regulation. Curr Opin Genet Dev 2014;25C:110–7.
[121] Mattout-Drubezki A, Gruenbaum Y. Dynamic interactions of nuclear lamina proteins with chromatin and transcriptional machinery. Cell Mol Life Sci 2003;60:2053–63.
[122] Munoz-Alarcon A, Pavlovic M, Wismar J, Schmitt B, Eriksson M, Kylsten P, et al. Characterization of lamin mutation phenotypes in Drosophila and comparison to human laminopathies. PLoS One 2007;2:e532.
[123] Worman HJ. Nuclear lamins and laminopathies. J Pathol 2012;226:316–25.
[124] Prokocimer M, Barkan R, Gruenbaum Y. Hutchinson-Gilford progeria syndrome through the lens of transcription. Aging Cell 2013;12:533–43.
[125] van Bemmel JG, Pagie L, Braunschweig U, Brugman W, Meuleman W, Kerkhoven RM, et al. The insulator protein SU(HW) fine-tunes nuclear lamina interactions of the Drosophila genome. PLoS One 2010;5:e15013.
[126] Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, et al. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 2008;453:948–51.

[127] Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, et al. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol Cell 2010;38:603–13.
[128] 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;38:1005–14.
[129] Kind J, Pagie L, Ortabozkoyun H, Boyle S, de Vries SS, Janssen H, et al. Single-cell dynamics of genome-nuclear lamina interactions. Cell 2013;153:178–92.
[130] Kind J, van Steensel B. Genome-nuclear lamina interactions and gene regulation. Curr Opin Cell Biol 2010;22:320–5.
[131] Shevelyov YY, Lavrov SA, Mikhaylova LM, Nurminsky ID, Kulathinal RJ, Egorova KS, et al. The B-type lamin is required for somatic repression of testis-specific gene clusters. Proc Natl Acad Sci U S A 2009;106:3282–7.
[132] Kohwi M, Lupton JR, Lai SL, Miller MR, Doe CQ. Developmentally regulated subnuclear genome reorganization restricts neural progenitor competence in Drosophila. Cell 2013;152:97–108.
[133] Meister P, Towbin BD, Pike BL, Ponti A, Gasser SM. The spatial dynamics of tissue-specific promoters during C. elegans development. Genes Dev 2010;24:766–82.
[134] Meuleman W, Peric-Hupkes D, Kind J, Beaudry JB, Pagie L, Kellis M, et al. Constitutive nuclear lamina-genome interactions are highly conserved and associated with A/T-rich sequence. Genome Res 2013;23:270–80.
[135] Milon BC, Cheng H, Tselebrovsky MV, Lavrov SA, Nenasheva VV, Mikhaleva EA, et al. Role of histone deacetylases in gene regulation at nuclear lamina. PLoS One 2012;7:e49692.
[136] Somech R, Shaklai S, Geller O, Amariglio N, Simon AJ, Rechavi G, et al. The nuclear-envelope protein and transcriptional repressor LAP2beta interacts with HDAC3 at the nuclear periphery, and induces histone H4 deacetylation. J Cell Sci 2005;118:4017–25.
[137] Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB, Spooner CJ, et al. DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. Cell 2012;149:1474–87.
[138] Demmerle J, Koch AJ, Holaska JM. The nuclear envelope protein emerin binds directly to histone deacetylase 3 (HDAC3) and activates HDAC3 activity. J Biol Chem 2012;287:22080–8.
[139] Bhaskara S, Chyla BJ, Amann JM, Knutson SK, Cortez D, Sun ZW, et al. Deletion of histone deacetylase 3 reveals critical roles in S phase progression and DNA damage control. Mol Cell 2008;30:61–72.
[140] Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. Nat Genet 2009;41:246–50.
[141] Towbin BD, Gonzalez-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, et al. Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. Cell 2012;150:934–47.
[142] Nemeth A, Conesa A, Santoyo-Lopez J, Medina I, Montaner D, Peterfia B, et al. Initial genomics of the human nucleolus. PLoS Genet 2010;6:e1000889.
[143] van Koningsbruggen S, Gierlinski M, Schofield P, Martin D, Barton GJ, Ariyurek Y, et al. High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. Mol Biol Cell 2010;21:3735–48.
[144] Clowney EJ, LeGros MA, Mosley CP, Clowney FG, Markenskoff-Papadimitriou EC, Myllys M, et al. Nuclear aggregation of olfactory receptor genes governs their monogenic expression. Cell 2012;151:724–37.
[145] Padeken J, Heun P. Nucleolus and nuclear periphery: Velcro for heterochromatin. Curr Opin Cell Biol 2014;28C:54–60.
[146] Passarge E. Emil Heitz and the concept of heterochromatin: longitudinal chromosome differentiation was recognized fifty years ago. Am J Hum Genet 1979;31:106–15.
[147] Brown SW. Heterochromatin. Science 1966;151:417–25.
[148] Elgin SC, Reuter G. Position-effect variegation, heterochromatin formation, and gene silencing in Drosophila. Cold Spring Harb Perspect Biol 2013;5:a017780.
[149] Dimitri P, Caizzi R, Giordano E, Carmela Accardo M, Lattanzi G, Biamonti G. Constitutive heterochromatin: a surprising variety of expressed sequences. Chromosoma 2009;118:419–35.
[150] Moritz KB, Roth GE. Complexity of germline and somatic DNA in Ascaris. Nature 1976;259:55–7.
[151] Smith CD, Shu S, Mungall CJ, Karpen GH. The Release 5.1 annotation of Drosophila melanogaster heterochromatin. Science 2007;316:1586–91.
[152] Wakimoto BT, Hearn MG. The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of Drosophila melanogaster. Genetics 1990;125:141–54.
[153] Riddle NC, Minoda A, Kharchenko PV, Alekseyenko AA, Schwartz YB, Tolstorukov MY, et al. Plasticity in patterns of histone modifications and chromosomal proteins in Drosophila heterochromatin. Genome Res 2011;21:147–63.
[154] Aravin AA, Hannon GJ. Small RNA silencing pathways in germ and stem cells. Cold Spring Harb Symp Quant Biol 2008;73:283–90.
[155] Lu J, Gilbert DM. Proliferation-dependent and cell cycle regulated transcription of mouse pericentric heterochromatin. J Cell Biol 2007;179:411–21.
[156] Schoeftner S, Blasco MA. Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. Nat Cell Biol 2008;10:228–36.
[157] Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001;410:116–20.
[158] Gatti M, Pimpinelli S. Functional elements in Drosophila melanogaster heterochromatin. Annu Rev Genet 1992;26:239–75.
[159] Greil F, van der Kraan I, Delrow J, Smothers JF, de Wit E, Bussemaker HJ, et al. Distinct HP1 and Su(var)3-9 complexes bind to sets of developmentally coexpressed genes depending on chromosomal location. Genes Dev 2003;17:2825–38.
[160] de Wit E, Greil F, van Steensel B. High-resolution mapping reveals links of HP1 with active and inactive chromatin components. PLoS Genet 2007;3:e38.
[161] Girton JR, Johansen KM. Chromatin structure and the regulation of gene expression: the lessons of PEV in Drosophila. Adv Genet 2008;61:1–43.
[162] Finelli P, Sirchia SM, Masciadri M, Crippa M, Recalcati MP, Rusconi D, et al. Juxtaposition of heterochromatic and euchromatic regions by chromosomal translocation mediates a heterochromatic long-range position effect associated with a severe neurological phenotype. Mol Cytogenet 2012;5:16.

[163] Talbert PB, Henikoff S. A reexamination of spreading of position-effect variegation in the white-roughest region of Drosophila melanogaster. Genetics 2000;154:259–72.
[164] Wines DR, Talbert PB, Clark DV, Henikoff S. Introduction of a DNA methyltransferase into Drosophila to probe chromatin structure in vivo. Chromosoma 1996;104:332–40.
[165] Wallrath LL, Elgin SC. Position effect variegation in Drosophila is associated with an altered chromatin structure. Genes Dev 1995;9:1263–77.
[166] Cryderman DE, Morris EJ, Biessmann H, Elgin SC, Wallrath LL. Silencing at Drosophila telomeres: nuclear organization and chromatin structure play critical roles. EMBO J 1999;18:3724–35.
[167] Sun FL, Cuaycong MH, Elgin SC. Long-range nucleosome ordering is associated with gene silencing in Drosophila melanogaster pericentric heterochromatin. Mol Cell Biol 2001;21:2867–79.
[168] Bulut-Karslioglu A, Perrera V, Scaranaro M, de la Rosa-Velazquez IA, van de Nobelen S, Shukeir N, et al. A transcription factor-based mechanism for mouse heterochromatin formation. Nat Struct Mol Biol 2012;19:1023–30.
[169] Padeken J, Mendiburo MJ, Chlamydas S, Schwarz HJ, Kremmer E, Heun P. The nucleoplasmin homolog NLP mediates centromere clustering and anchoring to the nucleolus. Mol Cell 2013;50:236–49.
[170] Mayer R, Brero A, von Hase J, Schroeder T, Cremer T, Dietzel S. Common themes and cell type specific variations of higher order chromatin arrangements in the mouse. BMC Cell Biol 2005;6:44.
[171] Weierich C, Brero A, Stein S, von Hase J, Cremer C, Cremer T, et al. Three-dimensional arrangements of centromeres and telomeres in nuclei of human and murine lymphocytes. Chromosome Res 2003;11:485–502.
[172] Brero A, Easwaran HP, Nowak D, Grunewald I, Cremer T, Leonhardt H, et al. Methyl CpG-binding proteins induce large-scale chromatin reorganization during terminal differentiation. J Cell Biol 2005;169:733–43.
[173] Manuelidis L. Indications of centromere movement during interphase and differentiation. Ann N Y Acad Sci 1985;450:205–21.
[174] Martou G, De Boni U. Nuclear topology of murine, cerebellar Purkinje neurons: changes as a function of development. Exp Cell Res 2000;256:131–9.
[175] Solovei I, Schermelleh L, During K, Engelhardt A, Stein S, Cremer C, et al. Differences in centromere positioning of cycling and postmitotic human cell types. Chromosoma 2004;112:410–23.
[176] Henikoff S, Dreesen TD. Trans-inactivation of the Drosophila brown gene: evidence for transcriptional repression and somatic pairing dependence. Proc Natl Acad Sci U S A 1989;86:6704–8.
[177] Slatis HM. Position Effects at the Brown Locus in Drosophila Melanogaster. Genetics 1955;40:5–23.
[178] Csink AK, Henikoff S. Genetic modification of heterochromatic association and nuclear organization in Drosophila. Nature 1996;381:529–31.
[179] Belyaeva ES, Koryakov DE, Pokholkova GV, Demakova OV, Zhimulev IF. Cytological study of the brown dominant position effect. Chromosoma 1997;106:124–32.
[180] Sass GL, Henikoff S. Pairing-dependent mislocalization of a Drosophila brown gene reporter to a heterochromatic environment. Genetics 1999;152:595–604.
[181] Dorer DR, Henikoff S. Transgene repeat arrays interact with distant heterochromatin and cause silencing in cis and trans. Genetics 1997;147:1181–90.
[182] Martin-Morris LE, Csink AK, Dorer DR, Talbert PB, Henikoff S. Heterochromatic trans-inactivation of Drosophila white transgenes. Genetics 1997;147:671–7.
[183] Dimitri P. Fluorescent in situ hybridization with transposable element probes to mitotic chromosomal heterochromatin of Drosophila. Methods Mol Biol 2004;260:29–39.
[184] Sun X, Le HD, Wahlstrom JM, Karpen GH. Sequence analysis of a functional Drosophila centromere. Genome Res 2003;13:182–94.
[185] Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG. Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. Mol Cell 1999;3:207–17.
[186] Trinh LA, Ferrini R, Cobb BS, Weinmann AS, Hahm K, Ernst P, et al. Down-regulation of TDT transcription in CD4(+) CD8(+) thymocytes by Ikaros proteins in direct competition with an Ets activator. Genes Dev 2001;15:1817–32.
[187] Su RC, Brown KE, Saaber S, Fisher AG, Merkenschlager M, Smale ST. Dynamic assembly of silent chromatin during thymocyte maturation. Nat Genet 2004;36:502–6.
[188] Groth A. Replicating chromatin: a tale of histones. Biochem Cell Biol 2009;87:51–63.
[189] Radman-Livaja M, Verzijlbergen KF, Weiner A, van Welsem T, Friedman N, Rando OJ, et al. Patterns and mechanisms of ancestral histone protein inheritance in budding yeast. PLoS Biol 2011;9:e1001075.
[190] Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA, et al. Organization of the mitotic chromosome. Science 2013;342:948–53.
[191] Thomson I, Gilchrist S, Bickmore WA, Chubb JR. The radial positioning of chromatin is not inherited through mitosis but is established de novo in early G1. Curr Biol 2004;14:166–72.