The role of transcription in shaping the spatial organization of the genome

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Abstract | The spatial organization of the genome into compartments and topologically associated domains can have an important role in the regulation of gene expression. But could gene expression conversely regulate genome organization? Here, we review recent studies that assessed the requirement of transcription and/or the transcription machinery for the establishment or maintenance of genome topology. The results reveal different requirements at different genomic scales. Transcription is generally not required for higher-level genome compartmentalization, has only moderate effects on domain organization and is not sufficient to create new domain boundaries. However, on a finer scale, transcripts or transcription does seem to have a role in the formation of subcompartments and subdomains and in stabilizing enhancer–promoter interactions. Recent evidence suggests a dynamic, reciprocal interplay between fine-scale genome organization and transcription, in which each is able to modulate or reinforce the activity of the other.

The genome is organized and compartmentalized within a eukaryotic nucleus according to two major principles[1–3]. First, chromosomal regions with similar biochemical and functional properties (located either on the same or on different chromosomes) often cluster together inside the nucleus and form distinct compartments (FIG. 1a,b). Second, interphase chromosomes are partitioned into topologically associated domains (TADs), which are genomic regions with extensive internal chromatin interactions and fewer contacts with neighbouring regions[4–7] (FIG. 1c,d).

Although these two types of genome organization are intricately associated with gene expression, it is still unclear what is cause and what is consequence. In this Review, we discuss the relationships between genome organization and gene transcription. Does spatial organization regulate and coordinate gene expression, or does the transcription machinery also control genome organization? We first highlight examples of compartmentalization of distinct chromatin types and then discuss whether and how the transcription machinery may contribute to chromatin type formation. We then discuss the interplay between the transcription machinery and architectural proteins in shaping TADs.

Chromatin compartments

Chromatin compartments are spatially separated genomic regions within the nucleus with distinct biochemical and functional properties. Most have been identified and extensively studied by microscopy. Compartments typically harbour long stretches of DNA that are often referred to as domains. We highlight some of the most conspicuous compartments and their links with transcription.

Nucleoli as archetype compartments

The archetype of a nuclear compartment is the nucleolus, which is organized around gene repeats that produce ribosomal RNA (rRNA). Here, rRNA is produced by RNA polymerase I (Pol I) and is then used for ribosome assembly. Compartmentalization of nucleoli is thought to be driven in part by liquid–liquid phase separation: rather than being rigid aggregates, nucleoli appear to be fluid droplet-like structures that separate from the remainder of the nucleoplasm owing to their distinct physicochemical properties[8].

At the onset of mitosis, nucleoli are disassembled concomitant with a shutdown of transcription of rRNA genes. During interphase, inhibition of Pol I also causes partial disruption of the nucleolar architecture (reviewed elsewhere[9,10]). Moreover, during embryonic development, nucleoli are not fully formed in zebrafish and *Drosophila melanogaster* embryos until the onset of zygotic rRNA transcription[11,12], and maternally deposited rRNA is required for correct nucleolus assembly in early *Xenopus laevis* embryos[13]. These data suggest that transcription and/or rRNA is required for the formation of the nucleolar compartment. This is supported by recent studies suggesting that a local high concentration of rRNA contributes to the assembly of nucleoli[11]. The recruitment of Pol I and other components of the transcription machinery is aided by the transcription

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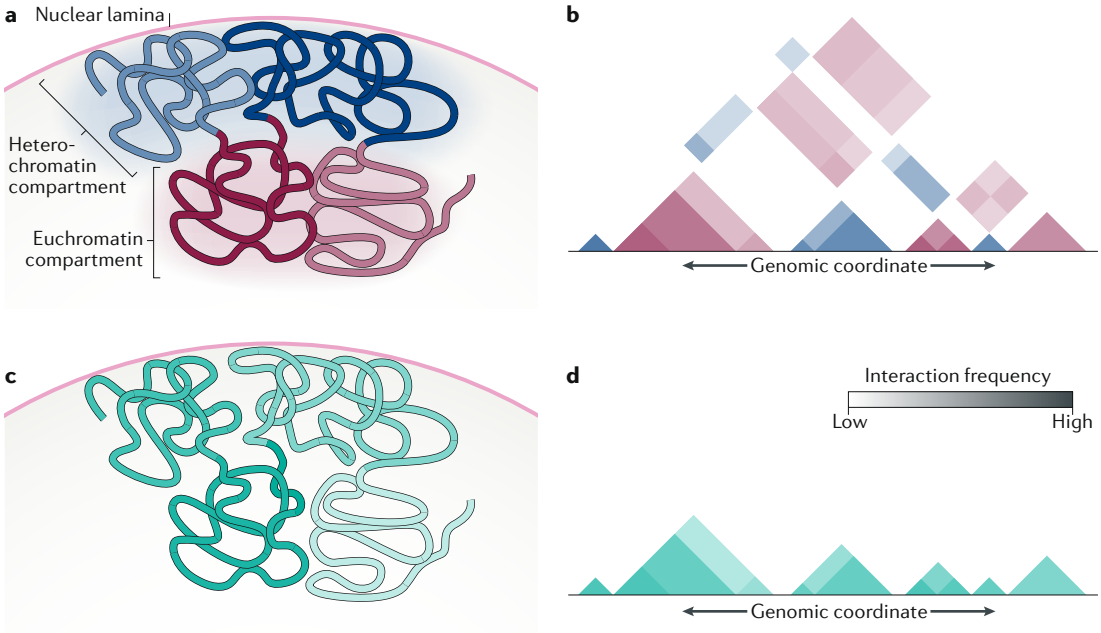


Fig. 1 | **Two main principles of chromosome organization. a** | Compartments are formed by aggregation of multiple domains with similar biochemical or functional properties. The two most prominent compartments are heterochromatin (often positioned near the nuclear lamina) and euchromatin. **b** | The self-association of heterochromatin and euchromatin domains is detectable as long-range chromatin contacts in Hi-C maps. **c** | The cartoon illustrates the partitioning of the genome into topologically associated domains (TADs; different shades of green), which have primarily intradomain contacts and fewer interdomain contacts. **d** | The cartoon represents part of a Hi-C map, with intra-TAD contacts depicted in shades of green. TADs are often nested structures.

Alu elements
A type of short and highly abundant transposable element found throughout primate genomes.

Histone modifications
A generic term for a wide range of post-translational modifications of histone residues. Histone modifications have a variety of functions, including in the packaging of chromatin and regulation of transcription.

Nuclear lamina
A layer of proteins coating the inner nuclear membrane and thought to form a large contact surface for lamina-associated domains.

CCCTC-binding factor
(CTCF). A DNA-binding protein that often marks borders of lamina-associated domains, topologically associated domains and chromatin loops and can act as a transcriptional insulator.

Hi-C
A chromosome conformation capture method that systematically identifies genomic sequences that are in close proximity to one another inside cell nuclei.

factor UBF (also known as nucleolar transcription factor 1), which binds to specific motifs across ribosomal DNA (rDNA)[14,15]. In addition, Pol II transcripts derived from intronic Alu elements (which are transcribed in the nuclear interior) accumulate in nucleoli and are also important for nucleolar integrity[16]. Thus, the formation of the nucleolus as a distinct compartment is at least guided by local transcription, a sequence-specific DNA-binding factor and a specific RNA produced in trans. This raises the interesting question of whether similar principles apply to other chromatin compartments.

Heterochromatin and euchromatin

The other prominent nuclear compartments are heterochromatin and euchromatin, which were originally defined based on differences in apparent chromatin compaction, as visible by microscopy[17]. Generally, transcriptionally inactive or repressed genomic regions are heterochromatic, whereas transcribed regions are euchromatic. Both compartments come in multiple chromatin flavours, which are categorized by their associated sets of proteins and histone modifications. In particular, heterochromatin tends to be marked by trimethylated histone H3 Lys27 (H3K27me3) or by H3K9me3 and H3K9me2 (REFS[18,19]).

In metazoan cells, heterochromatin marked by H3K9me2 and H3K9me3 is typically concentrated at the nuclear lamina and, to a lesser extent, around nucleoli. Genome-wide maps of chromatin in contact with the nuclear lamina in *D. melanogaster* and mammalian cells show hundreds of large (~10 kb to 10 Mb) regions, termed lamina-associated domains (LADs)[20–22]. Most genes localized in LADs exhibit low transcriptional activity. Nucleolus-associated domains have been studied much less than LADs, but initial chromatin contact maps indicate that they overlap substantially with LADs[23–25]. It appears that a subset of LADs is stochastically positioned at either the nuclear lamina or the nucleoli[26,27]. LADs and neighbouring euchromatin tend to be separated by sharp (clearly defined) borders. Some of these borders are demarcated by CCCTC-binding factor (CTCF) (see below), whereas others comprise an active promoter that drives transcription away from the LAD[20]. The latter type of LAD border suggests that active promoters can form barriers that somehow block the spreading of nuclear lamina interactions. However, this model has not been tested directly.

Euchromatin regions are densely populated by active genes and enhancer elements and are typically marked by a multitude of histone modifications such as methylation of H3K4 and acetylation of various histone lysine residues. Euchromatin is generally located in the nuclear interior, although it can also interact with nuclear pores[28]. In certain cell types, the relative positions of heterochromatin and euchromatin are reversed, with heterochromatin being located in the nuclear interior and euchromatin at the periphery[29], but these are exceptions.

Partitioning of euchromatin and heterochromatin is also visible in chromosomal contact maps generated by chromosome conformation capture technologies such as 4C and Hi-C[30,31] (FIG. 1d). These maps show two major classes of self-associating compartment, termed

compartment A and compartment B. Each compartment is characterized by extensive contacts with multiple domains of the same type (A or B), which can be >10 Mb apart. LADs overlap strongly with compartment B, and euchromatic inter-LAD regions overlap with compartment A[32,33]. In part, the self-association of heterochromatin in compartment B is mediated by a variety of proteins (BOX 1).

Heterochromatin compartmentalization by specific transcripts. Paradoxically, even though heterochromatin tends to be transcriptionally inactive, specific RNA molecules can enable the formation of particular types of heterochromatin compartment. A well-studied example is the inactivation of one of the two X chromosomes in mammals (reviewed elsewhere[34]), in which expression in cis of the non-coding RNA X-inactive specific transcript (Xist) has a key role. How Xist initiates silencing of most genes on the inactive X chromosome remains largely unresolved, but an intriguing observation is that Xist can interact with the lamin B receptor (LBR), which is an integral component of the inner nuclear membrane[35].

In genomes of most species, centromeres are flanked by large blocks of repetitive DNA elements known as 'satellite repeats', which are packaged into H3K9me3-marked heterochromatin. In mouse, this pericentric heterochromatin forms distinct, round nuclear compartments called 'chromocentres', which are easily visible when stained with a simple DNA-binding dye. Often centromeres from multiple chromosomes come together in chromocentres. During early mouse embryogenesis, chromocentre formation is preceded by a burst of transcription of major satellite repeats, which is thought to be necessary for the establishment of chromocentres[36]. How the repeat transcripts promote the assembly of centromeric heterochromatin into chromocentres is still poorly understood, but recent evidence indicates that these transcripts help to recruit SUV39H methyltransferases that catalyse the trimethylation of H3K9 (REF.[37]). This may be akin to observations in fission yeast and *Arabidopsis thaliana*, in which transcripts derived from pericentric sequences are locally processed by the RNAi machinery, which in turn locally promotes heterochromatin formation (reviewed elsewhere[38]). In *D. melanogaster* embryos, heterochromatin formation on one particular type of satellite DNA appears to be enhanced by maternally deposited RNA derived from the same sequence[39]. Thus, in this case, the repeat-derived RNA can act in trans. These examples illustrate that it is not simply the act of transcription but rather specific transcripts that help to locally establish H3K9me3-marked heterochromatin compartments.

Euchromatin compartmentalization and transcription. Similarly to heterochromatin, self-association of euchromatin regions could contribute to compartmentalization, as has been suggested by computational modelling[40]. Abundant associations between components of the transcription machinery are a defining feature of euchromatin, suggesting that the machinery itself, or the RNA molecules it produces, promotes the self-association of euchromatin. In support of this model, partitioning into compartments A and B is largely lost in mitotic chromosomes, which are mostly transcriptionally silent[41]. Hi-C maps also show very little higher-order structure of chromatin in early *D. melanogaster* embryos, when transcriptional activity is limited[42]. However, a clear distinction between A and B compartments is observed in mouse sperm[43–45], which is also virtually transcriptionally silent[46]. Moreover, in an earlier study of selected loci, inhibition of Pol II in mouse cells by α-amanitin did not disrupt compartmentalization[47]. Taken together, these observations suggest that ongoing transcription is not essential for global compartmentalization of heterochromatin and euchromatin. However, as discussed below, various regulators of transcription, as well as transcription itself, do contribute to the spatial organization of the genome at a locus-specific level.

Genes switching compartments. Euchromatin–heterochromatin partitioning is dynamic. When cells differentiate, hundreds of genes are repositioned from the nuclear lamina to the nuclear interior or vice versa[2,22,48]. For approximately two-thirds of the genes, detachment from the nuclear lamina coincides temporally with their transcriptional activation. This relocalization can be important for the activity of the gene. For example, localization of the *D. melanogaster* hunchback (*hb*) gene to the nuclear lamina during development limits its expression, which controls neuroblast formation[49]. Despite such striking anecdotes, for most genes it is not clear whether relocalization relative to the nuclear lamina is the cause or the consequence of the change in expression of the gene.

More than a decade ago, tethering of the viral VP16 transcription activator to a LacO repeat positioned near the nuclear lamina was found to cause a striking relocalization of the locus to the interior of the nucleus, often across several micrometres[50,51] (FIG. 2a,b). This occurred within 1–2 hours of tethering and showed features of directed movement, suggesting the involvement of an active relocalization mechanism. Indeed, the relocalization appeared to involve nuclear actin and myosin. Interestingly,

Box 1 | **Spatial clustering of heterochromatin regions by proteins**

Heterochromatin compartmentalization is driven in part by the self-association of various heterochromatin proteins[135]. For example, in budding yeast, the Sir proteins complex mediates clustering of telomeric heterochromatin (reviewed elsewhere[136]). Trimethylation of H3K27 is catalysed by Polycomb group (PcG) proteins, and in *Drosophila melanogaster*, H3K27me3-marked heterochromatic regions separated by tens of megabases come together in so-called PcG bodies in the nuclear interior[137–139]. In mammalian cells, H3K27me3 domains form intrachromosomal and interchromosomal contacts that can be part of either the A compartment[32,64] or the B compartment[140], depending on the cell type.

Early studies demonstrated that heterochromatin protein 1 (HP1) can anchor two loci together, even when they are multiple megabases apart on a linear chromosome[141,142]. HP1 is one of several proteins that can bind H3K9me2 and H3K9me3 and was recently suggested to function through a mechanism involving liquid–liquid phase separation[143,144]. Indeed, polymer modelling supports the concept of phase separation of A and B compartments[135]. Some proteins have been identified that tether heterochromatin to the nuclear lamina[145]. Interestingly, it was recently found that nuclear pore complexes can counteract peripheral localization of heterochromatin, particularly in senescent cells[146]. More-detailed reviews on the role of various proteins in the spatial assembly of heterochromatin in the nucleus can be found elsewhere[33,136,147].

the movement could not be blocked by inhibitors of transcription elongation (reviewed elsewhere[52]) such as dichlororibofuranosylbenzimidazole (DRB) and α-amanitin[50,51], indicating that transcription, at least beyond the stage of pre-initiation complex (PIC) function, is not a requirement for relocalization. Remarkably, the relocalization could also be triggered by tethering of an artificial peptide that was fortuitously found to decondense chromatin but not to activate transcription[51] (FIG. 2c). Very similar results were obtained more recently for individual promoters of genes at the nuclear lamina[53]. Finally, global tethering of VP16 to LADs caused loosening of LAD–nuclear lamina interactions. Here, most genes in LADs were not activated, but the level of H3K9me2 at LADs was reduced[26]. Again, this indicates that a change in chromatin state rather than transcription itself can lead to detachment from the nuclear lamina.

In another context, transcription of a long non-coding RNA (lncRNA) is required to change the compartmentalization of an entire locus[54]. In developing mouse T cell progenitors, the *Bcl11b* gene is activated by a distal enhancer region that includes an ~50 kb lncRNA named ThymoD. Expression of this lncRNA coincides with a transition of the entire ThymoD/*Bcl11b* locus from compartment B to compartment A, as observed by Hi-C, and with its movement away from the nuclear lamina. Disruption of ThymoD transcription by insertion of a termination site near the 5′ end of the gene prevented this relocalization and blocked activation of the *Bcl11b* gene[54]. These results suggest that either full-length ThymoD RNA or the transcription elongation process itself drives the relocalization of the entire locus (FIG. 2d). A similar compartment B to compartment A transition was recently observed for loci at which read-through transcription was triggered by the influenza A virus NS1 protein[55]. This transition could be inhibited by the transcription elongation inhibitor flavopiridol.

Together, these studies indicate that there may be at least two mechanisms that can trigger relocalization of genomic loci from peripheral heterochromatin to internal euchromatin: one that is transcription dependent and one that may be driven by poorly understood changes in chromatin structure. Possibly, these mechanisms partially overlap.

Relocalization within euchromatin. Directed movements of genes have been observed also within the euchromatic compartment. Heat shock frequently induces movements over several micrometres of the heat shock protein 70 (*HSP70*) gene towards nuclear speckles, which are large, dynamic ribonucleoprotein aggregates of unknown function. Here, too, nuclear actin appears to be involved in this directed motion[56]. This heat-shock-induced movement to speckles could be recapitulated by integrated reporters driven by the *HSP70* promoter but not by other promoters. It seems therefore likely that it is not transcriptional activity per se but instead a specific protein complex that binds to the *HSP70* promoter that mediates this relocalization. Association with speckles appeared to facilitate activation of HSP70 transgenes[57]. A new genome-wide mapping approach identified dozens of other genomic regions (termed speckle-associated domains) with close and reproducible association with speckles (in the absence of heat shock) in human cultured cells[58]. Speckle-associated domains tend to be gene dense and transcriptionally highly active. Whether their positioning is driven by their promoters, as is the case for *HSP70*, or by other features, remains to be elucidated.

Some genes relocalize to nuclear pore complexes (NPCs) upon activation. This has been extensively studied in budding yeast. Again, it does not require active transcription but instead is mediated by specific transcription factors that recognize a 'zip code' in the promoter sequence (reviewed elsewhere[59]).

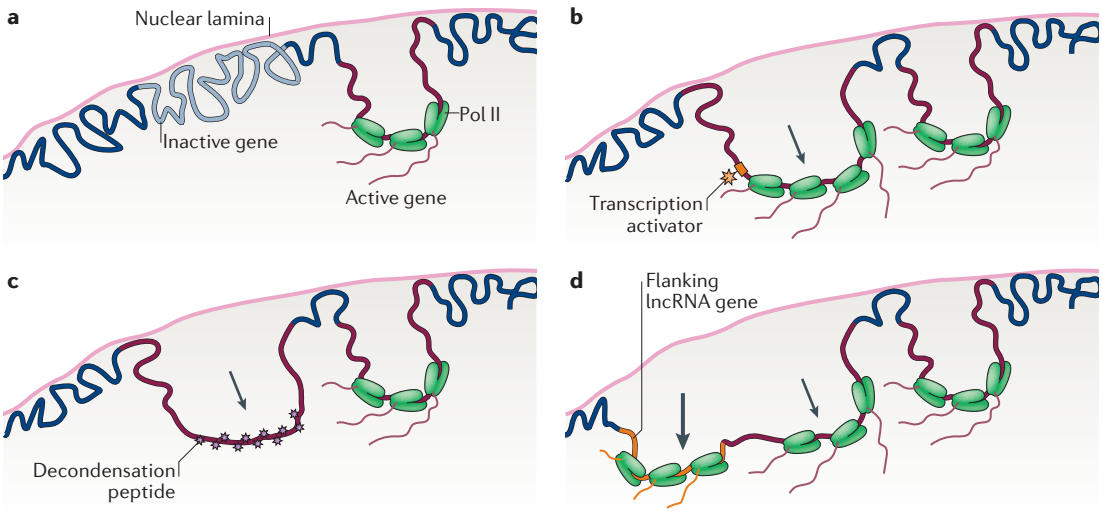


Fig. 2 | **Gene relocalization from peripheral heterochromatin to internal euchromatin. a** | Active genes are typically located in the nuclear interior, whereas a subset of inactive genes is located in heterochromatin at the nuclear lamina. **b** | Tethering a strong transcription activator can relocalize a gene to the nuclear interior. **c** | Tethering of a peptide with chromatin decondensation activity can relocalize a gene to the nuclear interior without transcription activation. **d** | Activation of a long non-coding RNA (lncRNA) gene can relocalize a flanking protein-coding gene to the nuclear interior. Pol II, RNA polymerase II.

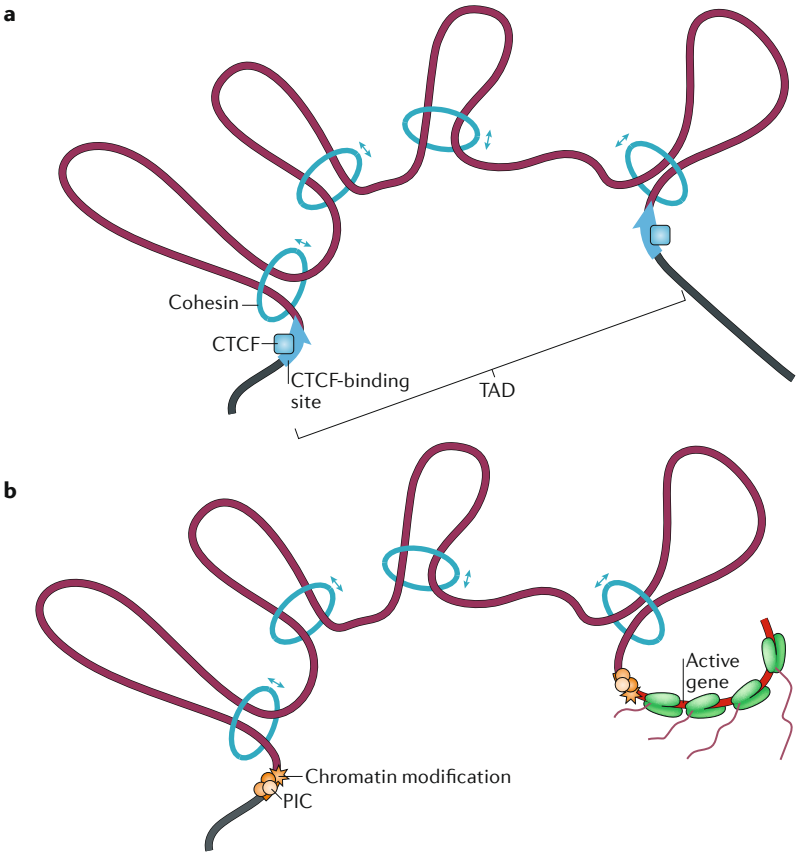


Fig. 3 | **Alternative mechanisms of TAD boundary formation. a** | A topologically associated domain (TAD) may form through loop extrusion by cohesin complexes. One TAD may consist of multiple loops that are dynamically formed and resolved. The CCCTC-binding factor (CTCF), when bound in the correct orientation, could act as a 'roadblock' that stops loop extrusion, thereby creating TAD borders. **b** | Similarly, at active genes, the transcription pre-initiation complex (PIC) or a chromatin modification associated with the PIC could demarcate TAD boundaries.

Functionally related genes can also colocalize within the euchromatic nuclear interior, possibly to form functionally distinct subcompartments. For example, as part of the immediate early gene response in mouse B lymphocytes, *Myc* moves to the same nuclear foci as the transcriptionally active immunoglobulin heavy chain genes[60]. Such non-random associations of functionally related active genes were originally observed by microscopy[61] and have since been confirmed by Hi-C and related mapping efforts. For example, recent studies indicate that histone genes preferentially cluster[62] and that multiple super enhancers can simultaneously be in the proximity of highly active genes[63]. In mouse embryonic stem cells (mESCs), genomic clusters of pluripotency-factor-binding sites tend to associate with one another in nuclear space[64,65]. At least in part, these interactions are dependent on the factors Nanog and OCT4: artificially tethered Nanog could induce some of these interactions[65]. Similarly, some paralogous genes are in spatial proximity to each other during early *D. melanogaster* embryogenesis and are co-expressed both temporally and spatially, although the regulatory mechanism of this coordination is unknown[66].

These data illustrate that genes can move between subcompartments within euchromatin. So far, DNA-binding factors rather than transcription itself appear to mediate these transitions. In most cases, the functional importance of this gene repositioning is still unclear.

Super enhancers
A somewhat arbitrary definition of genomic regions that contain a high density of active enhancers.

TADs and gene regulation

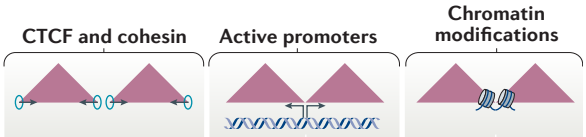
At the sub-megabase scale, the genome is organized into so-called TADs, which are genomic segments that have a higher frequency of interactions within them than they have with neighbouring regions[4–7]. Unlike that of the compartments discussed above, the definition of TADs is based strictly on Hi-C and related mapping technologies (although they have been confirmed by imaging[67,68]) and does not take chromatin composition into account in terms of associated proteins and histone modifications. Nevertheless, TADs and compartments are related. For example, LADs and TADs show a non-random degree of overlap, although the overlap is far from perfect[33]. In part, this is because TADs are often nested structures that consist of sub-TADs and even sub-sub-TADs, which makes it difficult to define their borders unequivocally. Furthermore, as we discuss below, TADs and compartments are shaped by different forces.

TADs are thought to influence transcription by at least three mechanisms. First, they may insulate a promoter from the action of an enhancer located in a neighbouring TAD[69,70]. Loss of a TAD boundary could thereby lead to the misexpression of genes in a neighbouring TAD owing to inappropriate enhancer–promoter interactions, as observed at some loci[69,70]. Second, confinement in a TAD could reduce the effective search space of enhancers and promoters to find each other[71]. Third, TAD boundaries might act as barriers for the spreading of euchromatin into neighbouring heterochromatin or vice versa[72]. Although more experimental evidence is needed to support these mechanistic models, it seems reasonable to assume that TADs can influence gene expression, as discussed in recent reviews[73–76]. However, might transcription also affect TAD formation? Before we address this question, we must discuss the role of cohesin and the insulator protein CTCF in TAD organization.

TAD organization by cohesin and CTCF

Cohesin is a large ring-shaped protein complex. An attractive genome organization model, reviewed elsewhere[3,77], posits that cohesin dynamically forms large chromatin loops by an extrusion mechanism. Most likely, TADs are the result of multiple dynamic loops formed by cohesin. This model is supported by computational modelling[78] and, importantly, also by the depletion of cohesin[79,80] or its chromatin loading and unloading factors[81–83], which result in loss of the majority of TADs.

CTCF is a DNA-binding protein that recognizes a specific sequence motif. In mammals, CTCF-binding sites are enriched at TAD borders[5,6,84–87]. It is thought that CTCF sites in a head-to-head orientation at these borders act as partial or complete roadblocks for extrusion by cohesin[78], thereby confining the loop extrusion process and defining the TAD borders (FIG. 3a). Indeed, deleting or changing the orientation of CTCF sites can alter chromatin conformation and lead to increased crosstalk between two neighbouring TADs[72,88,89]. Furthermore,



	CTCF and cohesin	Active promoters		Chromatin modifications	
		Pol II	Pol III–TFIIIC (tRNAs)	Inactive	Active
Mouse sperm	++[45]	NA		+	+
mESCs	+++[5,86,87]	++[102]	++[94,84]	++	++
Drosophila melanogaster	+[6,85,100]	+++[6]	+[85,100]	++	+[100]
Saccharomyces cerevisiae	–	++[92]		–	
Bacteria (*Caulobacter crescentus*, *Bacillus subtilis*)	–	++[95,96]		–	

Fig. 4 | **Properties of TAD borders in different cell types and species.** CCCTC-binding factor (CTCF), active promoters of RNA polymerase II (Pol II) and of Pol III and associated chromatin modifications are found at topologically associated domain (TAD) borders to varying degrees. + denotes low proportion (<25%) of TAD borders; ++, high proportion of TAD borders (~60%); +++, the great majority of TAD borders; –, not present in TAD borders (0%). mESCs, mouse embryonic stem cells; NA, not applicable or no information available; TFIIIC, transcription factor IIIC.

depletion of the CTCF protein in mESCs leads to a global decrease in TAD insulation, particularly affecting TADs that contain CTCF-binding sites[90].

Interestingly, depletion of CTCF has little effect on A–B compartment partitioning, and loss of cohesin even reinforces it[80–82,90]. CTCF and cohesin are therefore required for the organization of the majority of TADs in vertebrates but generally not for the spatial segregation of heterochromatin and euchromatin compartments.

TAD-like organization without CTCF

Despite its essential role in vertebrates, in some species, CTCF does not seem to be required for TAD formation. *D. melanogaster* embryos undergo embryogenesis without CTCF[91]; other species do not even have a CTCF orthologue, yet their genomes show a TAD-like spatial organization. This indicates that CTCF-independent mechanisms can also generate TAD domains. The common feature at the boundaries of these domains appears to be the presence of actively transcribed DNA, for example, promoters of housekeeping genes or tRNAs. High-resolution Hi-C mapping in the budding yeast *Saccharomyces cerevisiae* revealed a TAD-like pattern of self-associating domains of 2–10 kb in size that typically included 1–5 genes[92]. These domains are much smaller than TADs in mammalian genomes, but because genes and intergenic regions in yeast are also much shorter, the average number of genes per domain is similar. Similar to those of Metazoa, the boundaries of the yeast TAD-like domains often form at highly active promoters[92]. Perhaps this is related to earlier observations that a tRNA gene can prevent the spreading of heterochromatin into neighbouring euchromatin and that this boundary function could be mimicked by tethering of histone acetyltransferases to a promoter[93]. Thus, it is possible that active promoters, or chromatin features linked to them, may demarcate TADs (FIGS 3b,4). In keeping with this, transcription factor IIIC, which recruits Pol III to tRNA genes, is located at the boundaries of a subset of TADs that contain highly expressed gene clusters[94].

Similar observations have been made in bacteria. Chromosomes of *Caulobacter crescentus* and *Bacillus subtilis* also form TAD-like structures (here for simplicity referred to as TADs) whose boundaries are enriched in highly expressed genes[95,96] (FIG. 4). Blocking transcription elongation in bacteria severely disrupted TAD boundaries, resulting in a general lack of chromosomal domains, while the overall shape of the chromosome remained unperturbed. Conversely, changing the genomic position of a highly expressed gene from a TAD boundary to a poorly expressed region was sufficient to create a new TAD boundary[95]. Further analyses indicated that long active genes are more effective as boundary elements, possibly because they adopt an extended conformation that physically separates neighbouring chromosomal domains[97].

In the plant *A. thaliana*, Hi-C maps do not show detectable TAD-like organization; rather, single genes (particularly active genes) appear to form minicompartments[98], which are somewhat similar to TAD-like structures in budding yeast. In *Caenorhabditis elegans*, a partial TAD-like organization has been observed, which is more pronounced on the hermaphrodite X chromosome, where it is specifically controlled by the specialized, dosage compensation condensin complex[99]. However, deletion of a key subunit of this complex leads to only a partial loss of TADs on the X chromosomes, indicating that an additional mechanism helps to shape these TADs.

In summary, organisms that lack CTCF frequently have TAD-like domains that vary in size and are generally linked to transcribed genes situated inside or flanking the TADs.

Both CTCF and transcription affect TADs

The transcription machinery appears to help shape TADs even in organisms that do have CTCF. In mammals, for example, a subset of TAD borders overlap with actively transcribed genes but not with CTCF sites[5]. In *D. melanogaster*, the majority of the TAD borders coincide with active promoters rather than with CTCF sites[100] (FIG. 4), and active transcription predicts TAD boundaries much more accurately than does the occupancy of CTCF and other insulator proteins[101]. Furthermore, in mESCs, TAD boundaries often coincide with active promoters[102] (FIG. 4).

The strong correlation between transcription and TAD boundaries raises the possibility that the transcription machinery or an open transcription bubble could participate in TAD boundary formation. In *D. melanogaster*, heat stress results in the rapid activation of heat shock genes and the simultaneous repression of almost all other genes. These dramatic changes in expression are accompanied by a rearrangement of TAD borders[103]. However, in addition to transcriptional changes, various insulator proteins also relocalize from TAD borders to inside TADs. Whether heat-stress-induced reshaping of TADs is due to changes in transcription or changes in insulator binding (or both) is therefore not clear.

Another example of a massive shutdown of gene activity is mammalian X chromosome inactivation in

differentiating female cells. This process is accompanied by a chromosome-wide loss of TADs and the formation of two chromosomal megadomains[104,105]. During X chromosome inactivation, Xist evicts cohesin[106], which may lead to the loss of TADs, in keeping with the concurrent loss of regions of open chromatin containing CTCF sites[104]. TADs remain on the inactive X chromosome at a few gene clusters that escape inactivation. Transcription and the binding of transcription factors to open chromatin may enable the formation or maintenance of these TADs[104].

Surprisingly, mouse sperm, which is also largely transcriptionally silent[46], exhibits a TAD organization similar to that of diploid mammalian cells[43–45]. Sperm also contain CTCF and cohesin bound to sites that are similar to those in mESCs, suggesting that these architectural factors may be sufficient to establish chromatin topology in the absence of transcription[45]. However, many promoters in sperm cells include histones with post-translational modifications that are typically associated with transcriptional activity; hence, it is also possible that these modifications contribute to TAD organization in the absence of actual transcription (FIG. 4).

Does the initial establishment of TADs require transcription? The early metazoan embryo provides an excellent system to examine this question. The fertilized egg is transcriptionally silent and relies on maternally (oocyte)-provided proteins and RNA. At the midblastula transition, the zygotic genome becomes transcriptionally active. Hi-C experiments in *D. melanogaster* embryos before and right after this zygotic genome activation (ZGA) revealed that the majority of TADs are formed at the onset of the major wave of transcription[42]. The emergence of TAD boundaries is also highly correlated with Pol II occupancy within a 20 kb window, although it is difficult to draw mechanistic insights from this observation given the compact nature of the *D. melanogaster* genome, which has an annotated gene every 6–7 kb. In early mouse embryos, TADs are also initially largely absent and gradually appear after ZGA[44,107]. By contrast, in zebrafish, TADs are already present before ZGA, but this organization is temporarily lost soon after ZGA and then re-established later in development[108]. Hence, in zebrafish early development, the establishment of TADs does not correlate with the emergence of transcription.

Transcription perturbation studies

A more direct test of whether transcription dictates TAD structure is to employ chemical inhibitors of transcription (commonly used inhibitors are reviewed elsewhere[52]). In one study, the use of α-amanitin or triptolide to reduce transcription elongation by Pol II in *D. melanogaster* embryos had little effect on TAD formation[42]. However, the concentration of triptolide used had little effect on Pol II occupancy at promoters[42], and it is therefore possible that the formation of the transcription PIC or transcription initiation without elongation may be sufficient to create TAD boundaries. In another study, triptolide was used to inhibit transcription initiation and flavopiridol to block Pol II elongation, which resulted in modest TAD rearrangements: TAD border strength was reduced, whereas inter-TAD interactions increased, suggesting that the absence of transcription may account for at least some of the topology changes[103]. Finally, the use of a high dose of triptolide in a *D. melanogaster* cell line reduced Pol II binding to chromatin by more than twofold at almost 70% of its binding sites and resulted in more dramatic changes in TAD structure[109] (see below). On balance, these studies indicate that active transcription helps to maintain TAD organization in *D. melanogaster*.

Inhibition of ZGA in early mouse embryos did not prevent the formation of TADs; instead, inhibition of replication blocked TAD formation[107]. This may reflect interspecies differences between *D. melanogaster* and mammals or technical differences, as many studies (even within the same species) use different drugs or different concentrations of the same drug. During the differentiation of mESCs to NPCs, new TAD boundaries were formed at the promoters of genes that became activated, and in some cases, this happened in the absence of CTCF binding[102]. However, forced activation of these promoters earlier in mESCs by the tethering of a strong transcription activator was not sufficient to recapitulate the formation of a TAD boundary, even though the level of gene activation was similar to that in NPCs[102]. This is reminiscent of findings on the inactive X chromosome, where the spontaneous activation of eight separate genes in a mutant clone was not sufficient to create new TADs or compartments at those loci[104]. The results to date therefore indicate that transcription activation of a single gene in mammalian cells is not sufficient to create a TAD boundary. We cannot exclude the possibility that transcription of multiple genes in linear proximity could generate a new TAD boundary, but most likely it will also require specific architectural proteins. Transcription may play a more important role at TAD boundaries in species where CTCF is less prominently involved in TAD organization.

Transcription and sub-TAD structures

With increased Hi-C mapping resolution, finer domain patterns can be observed inside TADs. Using ultra-high-resolution Hi-C and sequential chromatin immunoprecipitation and Hi-C (Hi-ChIP) in *D. melanogaster* Kc167 cells, genomic domains of ~10 kb were identified, which are much smaller than conventional TADs[109]. These domains tend to segregate spatially into transcriptionally active and inactive compartments. This is reminiscent of the large A and B compartments discussed above but on a much finer scale; thus, these smaller domains are dubbed 'compartmental domains' (FIG. 5).

Fewer than a third of the borders of compartmental domains contain CTCF-binding sites. At the active compartmental domains, transcription occurs at internal regions (rather than at domain borders), where gene bodies are associated with the elongating form of Pol II. Importantly, triptolide treatment resulted in a decrease in chromatin interaction frequency within and between active compartmental domains; the regions where Pol II occupancy was the most strongly depleted by triptolide had the strongest reduction in Hi-C interaction frequencies[109]. These data indicate that transcription has a prominent role in the formation of small compartmental domains and thus in the chromatin architecture within TADs.

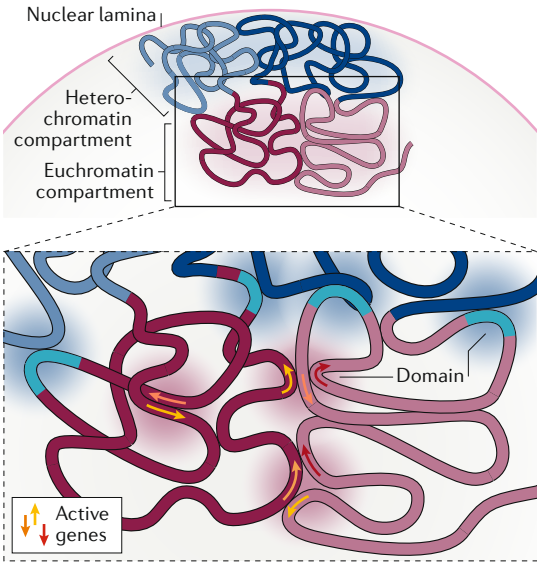


Fig. 5 | **Compartmentalization of active and inactive chromatin.** The cartoon illustrates self-association, which may occur at multiple scales: both at the level of large domains (top panel) and at the level of individual genes, which may form subcompartments at a much finer scale (bottom panel, red and blue haloes). Red shades, compartment A (euchromatin); blue shades, compartment B (heterochromatin, lamina-associated domains (not shown)).

The fine-scale transcriptional state is also a major predictor of TAD structure in mammals and *C. elegans*[109], suggesting that it is a conserved property. However, a recent study of Hi-C maps at extremely high resolution in mouse cells did not report this feature; instead, it indicated that the boundaries of TADs are predominantly demarcated by promoters of active genes[102]. This is not necessarily a contradiction, as the different interpretations may be related to the algorithm used to define domain borders. Compartmental domains were suggested to be a foundation for chromatin organization in all species, with architectural proteins such as CTCF and cohesin forming an additional layer of organization[109]. The balance between the two levels of organization may depend on the species, as transcriptional state alone was sufficient to predict global Hi-C patterns in *D. melanogaster*[101,109,110], whereas in mammals, the model's prediction of TAD boundaries was improved using information on both transcription and CTCF interactions[109].

In order to function, enhancers must relay regulatory information to the basal transcriptional machinery at the target gene promoters; enhancer–promoter interactions are generally thought to occur within the same TAD (recently reviewed elsewhere[111–114]). Forcing loop formation between an enhancer of the β-globin locus, known as the locus control region, and a promoter is sufficient to activate transcription at this locus[115,116], suggesting a causal link between proximity (topology) and transcription. However, at other loci, enhancers and promoters seem to be present in pre-formed topologies before gene expression[66,117–119], and in some instances, transcriptional output does not appear to be linked to increased enhancer–promoter proximity[120,121]. Placing an insulator and promoter element at a distal position from the *D. melanogaster eve* enhancers was sufficient to form enhancer–promoter looping in the absence of transcription[122]. However, in cells in which transcription is activated, this topology becomes more compact, and in turn, transcription acts to further stabilize the dynamics of the enhancer–promoter interaction. Although the mechanism is unknown, this suggests that transcription feeds back to stabilize chromatin interactions, at least at such a 'local' scale (~150 kb).

In other cases, transcription may destabilize topology. Transcription elongation can lead to chromatin remodelling and eventual eviction of CTCF[55,123]. Similarly, transcription also appears to translocate cohesin along the DNA in both yeast and mammals[124,125]. Potentially, this could modulate loop extrusion by cohesin, but it is not known whether this has any impact on finer structures at a sub-TAD scale.

A dual role for CTCF?

Besides its role in chromatin looping, CTCF has also been proposed to have a more direct role in different steps of transcription, and it is interesting to speculate that part of its function at TAD boundaries may involve modulating Pol II activity. CTCF can, for example, directly bind to the large subunit of Pol II, and the two colocalize at a subset (~10%) of CTCF sites, including at intergenic and intronic regions[126]. A CTCF motif (but not a mutated form) is sufficient to activate transcription from a promoter-less target gene, presumably through the recruitment of Pol II via the CTCF site[126]. Furthermore, in *D. melanogaster*, Hi-ChIP maps produced with antibodies directed against CP190, which is a protein that directly binds to CTCF, as well as against other insulator proteins are very similar to Hi-ChIP maps produced using antibodies against the elongating form (Ser2 phosphorylated) of Pol II[109]. CTCF occupancy also correlates with sites of alternative splicing[127], and it was proposed that CTCF mediates the formation of promoter–intron loops that promote exon inclusion. This may explain the observed clustering of long-range interactions between exons of different active genes, which scales with the number of exons and splicing events[102]. Taken together, these data raise the possibility that part of the regulation of genome organization by CTCF may be through local effects on transcription and co-transcriptional processes.

Conclusions

The studies reviewed here have uncovered an intricate interplay between the transcription machinery, chromatin components, DNA-binding factors and CTCF and cohesin in the control of genome organization. In mammalian cells, CTCF and cohesin have a major role in TAD formation, but a subset of TAD borders may be set by transcriptionally active genes. In *D. melanogaster*, the role of transcription in TAD organization appears to be more pronounced, whereas the role of CTCF may be less important. It is poorly understood how active genes affect TADs and TAD borders; possibly, contributions are made by multiple features, such as a component of active promoters or the decondensed conformation of the transcribed gene.

Furthermore, within TADs, active transcription appears to impose a chromatin structure. The self-association of small compartmental domains that subdivide TADs may be driven by the same mechanisms that segregate heterochromatin from euchromatin (or compartment B from compartment A and LADs from inter-LAD regions) at much larger scales. A diversity of mechanisms are involved in the formation and function of these fine compartmental domains. Specific transcription factors tend to be responsible for the clustering of functionally related genes and for the association of particular genes with nuclear landmarks such as nuclear pores and nuclear speckles. The collective action of these factors, perhaps together with a general 'stickiness' or phase separation that may be an intrinsic property of the transcription machinery[128,129], may be responsible for the organization and function of the euchromatin compartment. Small nuclear speckles comprising Pol II, transcription factors and cofactors, which have been known for decades[130,131], have recently been proposed to be formed through liquid–liquid phase separation[129,132–134]. It is tempting to speculate that such 'condensates' of multiple proteins might act as hubs for multiple genomic loci, but direct evidence to support this model is still lacking. Much more established is the role of heterochromatin proteins, in some cases guided by specific locally produced transcripts, in mediating the self-association of the heterochromatic parts of the genome.

It thus appears that the transcription machinery and its regulators are not only guided by genome organization but also contribute to it. Exactly how this works is not yet clear; possible mechanisms may involve components of the transcription PIC, changes in protein composition that mediate phase separation, changes in histone modifications, activities of specific chromatin-remodelling complexes and modulation of loop extrusion, to name a few. Further dissecting the precise mechanisms will be both a challenging and exciting endeavour.

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