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

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Abstract

The spatial organisation of the genome is believed to play an important role in the regulation of gene expression. But could gene expression conversely regulate genome organisation? Here we review recent studies that assessed the requirement of transcription, and/or the transcriptional machinery, for the establishment or maintenance of genome topology. The results reveal different requirements at different scales. The process of transcription is generally not required for higher level compartmentalisation, has only moderate effects on domain organisation, and is not sufficient to create a new domain boundary. However, at a finer scale transcripts or transcription does seem to play a role in sub-compartmentalisation and sub-TAD connections and in stabilising enhancer-promoter interactions. Recent evidence suggests a dynamic, reciprocal interplay between fine-scale genome organisation and transcription, with each able to modulate or reinforce the activity of the other.

Introduction

The genome is organised and compartmentalised within a eukaryotic nucleus by 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 inside the nucleus to form distinct compartments (Figure 1A, B). Second, interphase chromosomes are partitioned into topologically associated domains (TADs **[G]**), which are genomic segments that show extensive self-interactions and are spatially separated from neighbouring segments[4–7] (Figure 1C, D).

Although these two aspects of genome organisation are intricately associated with gene expression, what is cause and what is consequence is still not clear. Here we explore these relationships. Is spatial organisation there to regulate and coordinate gene expression, or does the transcription machinery also control genome organisation? We first highlight examples of compartmentalisation of distinct chromatin types, and then discuss whether and how the transcription machinery may contribute to this nuclear compartmentalisation. Next,

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we provide an overview of the interplay between the transcription machinery and architectural proteins in shaping TAD architecture.

Transcription and 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. These 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 organised around rRNA gene repeats. Here, rRNA is produced by RNA Polymerase I (Pol I), and ribosomes are assembled. It is now thought that the compartmentalisation of nucleoli is in part driven by liquid phase separation: rather than being rigid aggregates, nucleoli appear to be fluid, droplet-like structures that separate from the remainder of the nucleoplasm due to their distinct physicochemical properties[8].

At the onset of mitosis, nucleoli are disassembled concomitant with a shutdown of transcription of the rRNA genes. During interphase, inhibition of Pol I also causes partial disruption of the nucleolar architecture (reviewed in [9,10]). Moreover, during embryonic development, nucleoli are not fully formed in zebrafish and *Drosophila* embryos until the onset of zygotic rRNA transcription[11,12], while maternally deposited rRNA is required for correct nucleolus assembly in early *Xenopus* embryos[13]. Together, these data suggest that transcription and/or rRNA is required for nucleolar compartmentalisation. 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 transcriptional machinery is aided by the transcriptional regulator UBF, which binds to specific motifs across rDNA[14,15]. In addition, RNA polymerase II transcripts from intronic Alu elements **[G]** (which are transcribed in the nuclear interior) accumulate in nucleoli and are also important for nucleolar integrity[16]. Thus, the formation of nucleoli as a distinct compartment is at least guided by local transcription, by a sequence-specific DNA-binding factor, and by a specific RNA produced *in trans*. This raises the interesting question 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 compaction, as visible by microscopy[17]. Generally, transcriptionally inactive or repressed regions of the genome are heterochromatic, whereas transcribed regions are euchromatic. Both chromatin types come in multiple flavours, categorized by their associated sets of proteins and histone modifications **[G]**. In particular, heterochromatin tends to be marked by trimethylated H3K27 or di- or trimethylated H3K9[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 *Drosophila* and mammalian cells show hundreds of large (about 10kb-10Mb) regions termed lamina associated domains (LADs **[G]**)[20–22]. Most genes that are present in LADs exhibit low transcriptional activity. Nucleolus associated domains (NADs) have been studied much less, but initial 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 at nucleoli[26,27]. LADs and neighbouring euchromatin tend to be separated by sharp borders. Some of these borders are demarcated by CTCF **[G]** (see below); others by an active promoter that drives transcription away from the LAD[20]. The latter observation suggests that active promoters can form barriers that somehow block the spreading of nuclear lamina interactions. However, this model has not been tested directly.

Euchromatic 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 lysines. Euchromatin is generally located in the nuclear interior, although it can also interact with nuclear pores[28]. In certain cell types the positions 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 **[G]** [30,31] (Figure 1D). These maps show two major classes of self-associating regions, termed compartments A and B. Each compartment is characterized by extensive contacts with multiple domains of the same type (A or B) that can be >10Mb apart. LADs overlap strongly with compartment B, and euchromatic inter-LAD regions with compartment A[32,33].

Heterochromatin aggregation by proteins—Heterochromatin compartmentalisation is driven in part by the self-association of multiple heterochromatin proteins[34]. For example, in budding yeast the Sir protein complex mediates clustering of telomeric heterochromatin (reviewed in [35]). In *Drosophila*, H3K27me3-marked regions separated by tens of megabases come together in so-called PcG bodies in the nuclear interior[36–38]. In mammalian cells, H3K27me3 domains form intra- and inter-chromosomal networks that can either be part of the A-[32,39] or the B-compartment[40], 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[41,42]. HP1 is one of several proteins that can bind H3K9me2/3. It was recently suggested that HP1 acts through a mechanism involving liquid phase separation[43,44], and indeed polymer modelling supports the concept of phase separation of A/B compartments[34]. Some proteins have been identified that tether heterochromatin to the nuclear lamina[45]. More detailed reviews on the role of various proteins in heterochromatin aggregation can be found elsewhere[33,35,46].

Euchromatin and transcription—Likewise, self-aggregation of euchromatin could contribute to compartmentalisation, as has been suggested by computational modeling[47].

Abundant association between components of the transcriptional machinery is a defining feature of euchromatin, leading to the possibility that the machinery itself, or the RNA molecules it produces, may promote self-association of euchromatin. In support of this model, compartment A/B partitioning is largely lost in mitotic chromosomes, which are mostly transcriptionally silent[48]. Hi-C maps also show very little higher-order structure of chromatin in early *Drosophila* embryos, when transcriptional activity is limited[49]. However, a clear A/B compartment structure is observed in mouse sperm[50–52], which is also virtually transcriptionally silent[53]. Moreover, in an earlier study of selected loci, inhibition of Pol II in mouse cells by alpha-amanitin did not disrupt compartmentalisation[54]. Taken together, these observations suggest that ongoing transcription is not essential for *global* compartmentalisation of heterochromatin and euchromatin. However, as will be discussed below, various regulators of transcription, as well as transcription itself, do contribute to the spatial organisation of the genome at a locus-specific level.

Heterochromatin and specific transcripts—Paradoxically, even though heterochromatin tends to be transcriptionally inactive, specific RNA molecules can enable the formation of a particular type of heterochromatin compartment. A well-studied example is the inactivation of one of the two X chromosomes in mammals (reviewed in [55]), in which expression *in cis* of the non-coding RNA *Xist* plays a key role. How the Xist mRNA initiates silencing of most genes on Xi remains largely unresolved, but an intriguing observation is that it can interact with Lamin B Receptor (LBR), an integral component of the inner nuclear membrane[56].

In genomes of most species, centromeres are flanked by large blocks of repetitive DNA elements packaged into H3K9me3-marked heterochromatin. In mouse this pericentric heterochromatin forms distinct round nuclear compartments that are easily visible when stained with a simple DNA-binding dye. Often centromeres from multiple chromosomes come together in such “chromocenters”. In the early mouse embryo, chromocenter formation is preceded by a burst of transcription of major satellite repeats, which is thought to be necessary for the establishment of chromocenters[57]. How the repeat transcripts promote the aggregation of centromeric heterochromatin into chromocenters is still poorly understood, but recent evidence indicates that these transcripts help to recruit Suv39h methyltransferases that deposit the H3K9me3 mark[58]. This may be akin to observations in fission yeast and Arabidopsis, where transcripts derived from pericentric sequences are locally processed by the RNAi machinery, which in turn locally promote heterochromatin assembly (reviewed in [59]). In *Drosophila* embryos, heterochromatin formation on one particular type of satellite DNA appears to be enhanced by maternally deposited RNA derived from the same sequence[60]. Thus, in this case the repeat-derived RNA can act *in trans*. Together, 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.

Compartment switching of genes—Euchromatin–heterochromatin partitioning is dynamic. When cells differentiate, hundreds of genes are repositioned from the nuclear lamina to the nuclear interior or vice versa[2,22,61]. For about two-thirds of the genes,

detachment from the nuclear lamina coincides temporally with their transcriptional activation. This relocalisation can be important for the activity of the gene. For example, localization of the *Drosophila hunchback* gene to the nuclear lamina during development limits its expression, which is important for the control of neuroblast formation[62]. Despite such striking anecdotes, for most genes it is not clear whether relocalisation relative to the nuclear lamina is cause or consequence of the gene's change in expression.

More than a decade ago, it was found that tethering of the viral VP16 transcriptional activator to a LacO repeat positioned near the nuclear lamina caused a striking relocation of the locus to the interior of the nucleus, often over multiple micrometers[63,64] (Figure 2A, B). This occurred within 1-2 hours and showed features of directed movement, suggesting an active mechanism. Indeed, the relocalisation appeared to involve nuclear actin and myosin. Interestingly, the movement could not be blocked by inhibitors of transcriptional elongation (reviewed in [65]) DRB and alpha-amanitin[63,64], indicating that transcription, at least beyond the pre-initiation complex is not a requirement for relocalisation. Remarkably, the relocalisation could also be triggered by tethering of an artificial peptide that was fortuitously found to decondense chromatin but not to activate transcription[64] (Figure 2C). Very similar results were obtained more recently for individual promoters of genes at the nuclear lamina[66]. 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 large non-coding RNA (lncRNA) is required to change the compartmentalisation of an entire locus[67]. In developing mouse T cell progenitors, the *Bcl11b* gene is activated by a distal enhancer region that includes a ~50 kb lncRNA named *ThymoD*. Expression of this lncRNA coincides with a transition of the entire *ThymoD/Bcl11b* locus from compartment B to A as observed by Hi-C, and 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 relocalisation, and blocked activation of the *Bcl11b* gene[67]. These results suggest that either full-length *ThymoD* RNA or the transcription elongation process itself drives the relocalisation of the entire locus (Figure 2D). A similar compartment B to A transition was recently observed for loci at which read-through transcription was triggered by the influenza A virus NS1 protein[68]. 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 relocation of genomic loci from peripheral heterochromatin to internal euchromatin: one that is transcription-dependent, and one that may be driven by a poorly understood chromatin alteration. Possibly, these mechanisms are partially overlapping.

Relocation within euchromatin—Also, *within* the euchromatic compartment, directed movements of genes have been observed. The *HSP70* gene shows heat-shock induced movement towards so-called nuclear speckles, which are large, dynamic ribonucleoprotein aggregates of unknown function. Heat-shock frequently induces long-range movements of the *HSP70* gene over several micrometers towards a speckle. Again, nuclear actin appears to

be involved in this directed motion[69]. 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 rather a specific protein complex that binds to the *HSP70* promoter that mediates this relocalisation. Association with speckles appeared to facilitate activation of HSP70 transgenes[70]. A new genome-wide mapping approach identified dozens of other genomic regions (termed Speckle-associated domains, SpADs) that show close and reproducible association with speckles (in the absence of heat shock) in human cultured cells[71]. SpADs 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 relocate to nuclear pore complexes (NPCs) upon activation. This has been extensively studied in budding yeast. Again, this does not require active transcription, but instead it is mediated by specific transcription factors that recognize a “zip-code” in the promoter sequence (reviewed in [72]).

Functionally related genes can also co-localize within the euchromatic nuclear interior, possibly forming functionally distinct sub-compartments. When immediate early genes are induced in mouse B lymphocytes, for example, *Myc* moves to the same nuclear foci as the transcriptionally active *Igh* genes[73]. While this was originally observed by microscopy[74], Hi-C and related mapping efforts have produced more evidence for non-random associations of active genes. For example, recent studies found that histone genes preferentially cluster[75], and that multiple “super-enhancers” **[G]** can simultaneously be in proximity of highly active genes[76]. In mouse embryonic stem cells, genomic clusters of pluripotency factor binding sites tend to associate with one another in nuclear space[39,77]. At least in part, these interactions are dependent on the factors Nanog and Oct4, and artificially tethered Nanog could induce some of such interactions[77]. Similarly, paralogous genes are in spatial proximity to each other during early *Drosophila* embryogenesis and are co-expressed both temporally and spatially, although the regulatory mechanism is not known[78].

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

TADs and gene regulation

At the sub-megabase scale, the genome is organised into so-called topologically associated domains (TADs), which are genomic segments that have a higher frequency of interactions within them compared to interactions with neighbouring regions[4–7]. Unlike the compartments discussed above, the definition of TADs is strictly based on Hi-C and related mapping technologies (although they have been confirmed by imaging[79,80]), and it does not take the chromatin composition in terms of associated proteins and histone marks into account. Nevertheless, TADs and compartments are related. For example, LADs and TADs show a nonrandom degree of overlap, although the overlap is far from perfect[33]. In part, this is because TADs are often nested structures (i.e., TAD consist of sub-TADs, and even sub-

sub-TADs), which makes it difficult to define their borders unequivocally. Furthermore, as will be discussed below, TADs and compartments are shaped by different forces.

TADs are thought to influence transcription by at least three mechanisms. First, they may insulate promoters from the action of enhancers located in a neighbouring TAD[81,82]. Loss of a TAD boundary could thereby lead to the mis-expression of genes in a neighbouring TAD due to inappropriate enhancer-promoter interactions, as seen at some loci[81,82]. Second, confinement in a TAD could reduce the effective search space of enhancers and promoters to find each other[83]. Third, TAD boundaries might act as a barrier to the spread of euchromatin into neighbouring heterochromatin[84]. 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 (e.g.[85–88]). But might transcription also affect TAD formation? Before we address this, we must discuss the role of cohesin and the insulator protein CTCF.

TAD organisation, cohesin and CTCF

A major player in TAD organisation is cohesin, a large ring-shaped protein complex. An attractive model, reviewed elsewhere[3,89], is 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[90], and importantly also by depletion of cohesin[91,92] or its chromatin loading/unloading factors [93–95], 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[5,6,96–99]. It is thought that oriented CTCF sites at these borders act as partial or complete roadblocks for cohesin[90], thereby confining the loop extrusion process (Figure 3A). Indeed, deleting or changing the orientation of CTCF sites can alter chromatin conformation and lead to increased cross-talk between two neighbouring TADs[84,100,101]. Furthermore, depletion of the CTCF protein in mouse ES cells leads to a global decrease in TAD insulation, particularly affecting TADs that contain CTCF binding sites[102].

Interestingly, depletion of CTCF has little effect on A/B compartment partitioning, and loss of cohesin even reinforces it[92–94,102]. CTCF and cohesin are therefore required for the organisation of the majority of TADs in vertebrates, but generally not for the spatial segregation of heterochromatin and euchromatin compartments.

TAD-like organisation without CTCF—Despite its essential role in vertebrates, CTCF does not seem to be required for TAD formation in many other species. *Drosophila* embryos develop through embryogenesis without CTCF[103], while other species don’t even have a CTCF ortholog, yet their genomes show a TAD-like spatial organisation. This indicates that other, 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 regions, for example promoters of housekeeping genes or tRNAs. High-resolution Hi-C mapping in budding yeast (*Saccharomyces cerevisiae*) revealed a TAD-like pattern of self-associating domains of 2-10kb in size that typically included 1-5 genes[104]. These domains

are much smaller than in mammalian genomes, but because genes and intergenic regions in yeast are also much shorter, the average number of genes per domain is roughly similar. Similar to metazoa, the boundaries of the yeast TAD-like domains often formed at highly active promoters[104]. Perhaps this is related to earlier observations that a tRNA gene can prevent the spreading of heterochromatin into neighbouring euchromatin, and this boundary function could be mimicked by tethering of histone acetyltransferases to a promoter[105]. Thus, it is possible that active promoters, or chromatin features linked to them, may demarcate TADs (Figure 3B, Figure 4). In keeping with this, TFIIIc, a Pol III TF that transcribes tRNAs, is located at the boundaries of a subset of TADs that contain highly expressed gene clusters[106].

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[107,108] (Figure 4). Treating the bacteria with rifampicin (blocking transcriptional elongation) severely disrupted TAD boundaries, resulting in a general lack of chromosomal domains while the overall shape of the chromosome remained unperturbed. Conversely, relocating a highly expressed gene (*rsaA*) that normally resides at a TAD boundary to a poorly expressed region (the *vanA* locus), was sufficient to create a new TAD boundary[107]. 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[109].

In the plant *A. thaliana*, Hi-C maps do not show detectable TAD-like organisation; rather, single genes (particularly active genes) appear to form mini-compartments[110], which is somewhat similar to the results in budding yeast. In *C. elegans*, a partial TAD-like organisation has been observed, which is more pronounced on the hermaphrodite X chromosome where it is specifically controlled by the dosage compensation complex, which is a specialized condensin complex[111]. 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 TADs.

In summary, organisms that lack CTCF frequently show TAD-like domains that vary in size and are generally linked to the transcription status of the genes that are inside or flanking the TADs.

CTCF and transcription both affect TADs—The transcriptional 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 CTCF sites[5] (Figure 4). In *Drosophila*, it is even the majority of the TAD borders that coincide with active promoters rather than CTCF sites[112] (Figure 4), and active transcription predicts TAD boundaries much more accurately than the occupancy of CTCF and other insulator proteins[113]. Also in mouse ES cells TAD boundaries often coincide with active promoters[114].

The strong correlation between transcription and TAD boundaries raises the possibility that the transcription machinery or an open transcriptional bubble could lead to TAD boundary formation. A number of natural contexts provide interesting models to dissect this

relationship. In *Drosophila*, high temperature 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[115]. However, various insulator proteins also relocated from TAD borders to inside TADs. Whether heat-stress induced re-shaping of TADs is due to changes in transcription or due to changes in insulator binding (or both) is therefore not clear.

Another example of a massive shutdown of gene activity is X chromosome inactivation in differentiating female mammalian cells. This process is accompanied by a chromosome-wide loss of TAD structure and the formation of two very large mega-domains[116,117]. Xist evicts cohesin[118], which may lead to the loss of TAD formation, in keeping with the loss of open chromatin regions containing CTCF sites[116]. TADs remain present at a few gene clusters on the inactive X that escape inactivation. Transcription, together with the binding of factors to open chromatin, may enable the formation or maintenance of these TADs[116].

Surprisingly, mouse sperm, which is also largely transcriptionally silent[53], exhibits a similar TAD organisation as diploid mammalian cells[50–52]. Sperm also contain CTCF and cohesin bound to similar sites as in ESCs, suggesting that these architectural factors may be sufficient to establish chromatin topology in the absence of transcription[52] (Figure 4). However, many promoters in sperm cells are marked by histones with posttranslational modifications that are typically linked to transcriptional activity, so it is also possible that these marks contribute to TAD organisation in the absence of actual transcription (Figure 4).

Does the initial establishment of TADs require transcription? The early embryo provides an excellent system to examine this. The fertilized egg is transcriptional silent, with proteins and RNA being maternally loaded in the oocyte. At the mid blastula transition, the zygote's genome becomes transcriptionally active. Hi-C experiments in *Drosophila* embryos before and right after this zygotic genome activation revealed that the majority of TADs are formed at the onset of the major wave of transcription[49]. The emergence of TAD boundaries is also highly correlated with Pol II occupancy within a 20kb window, although it is difficult to draw mechanistic insights from this given the compact nature of the *Drosophila* genome (with an annotated gene every 6-7 kb). In early mouse embryos TADs are also initially largely absent and gradually appear after zygotic transcription activation[51,119]. In contrast, zebrafish embryos show a more dynamic picture. Here, TADs are already present prior to the zygotic activation, but this organisation is temporarily lost soon after, and then re-established when development proceeds[120]. Hence, TADs in zebrafish early development show a more complex pattern, and 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 inhibition of transcription (commonly used inhibitors are reviewed in [65]). In one study, treatment of *Drosophila* embryos with alpha-amanitin or triptolide, severely reducing Pol II elongation, had little effect on TAD formation[49]. However, the concentration of triptolide used had little effect on Pol II occupancy at the promoter, as seen by the presence of the serine 5 phosphorylated form of Pol II[49]. It is therefore possible that the formation of the pre-initiation complex, or

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transcriptional initiation without elongation, may be sufficient to create TAD boundaries. Using triptolide (targeting initiation) and flavopiridol (blocking Pol II elongation) another study found modest TAD rearrangements upon transcriptional inhibition: TAD border strength was reduced, while inter-TAD interactions increased, suggesting that the absence of transcription may account for at least some of the topology changes[115]. Finally, experiments in a *Drosophila* cell line using a high dosage of triptolide led to over 2-fold reduction of almost 70% of Pol II peaks, and resulted in more dramatic changes in TAD structure[121] (see below). On balance, these studies indicate that active transcription helps to maintain TAD organisation in *Drosophila*.

Inhibition of zygotic gene activation in early mouse embryos did not prevent the formation of TADs; instead, inhibition of replication did block TAD formation[119]. This may reflect interspecies differences between *Drosophila* 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 mouse ES cells to NPCs, new TAD boundaries were formed at the promoters of genes that become activated in their expression (e.g. *Zfp608* and *Sox4*), and in some cases this was in the absence of CTCF binding[114]. However, forced activation of these promoters earlier, in ES cells, by tethering of a strong activator was not sufficient to recapitulate this TAD boundary, even though the level of gene activation was similar to that in NPCs[114]. This is reminiscent of findings on the 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[116]. The results-to-date therefore indicate that transcriptional activation of a single gene in mammalian cells is not sufficient to create a TAD boundary. We cannot exclude that the transcription of multiple genes in linear proximity may be able to generate a new TAD boundary, but it most likely will also requires specific architectural proteins. Possibly, transcription may play a more important role at TAD boundaries in species where CTCF is less prominently involved.

Transcription and sub-TAD structure

With increased mapping resolution, finer domain patterns can be observed in TADs. By ultra-high resolution Hi-C and Hi-ChIP (sequential chromatin immunoprecipitation and Hi-C) in *Drosophila* Kc167 cells, very fine-scale domains (~10kb) were identified that are much smaller than conventional TADs[121]. These domains tend to segregate spatially into transcriptionally active and inactive compartments, which is similar to the large A- and B-type compartments discussed above, but at a much finer scale (Figure 5).

Less than a third of the borders of these fine domains (dubbed “compartmental domains”) contain CTCF binding sites. At the active compartmental domains, transcription occurs internally, rather than at domain borders, and is associated with the elongating form of Pol II along gene bodies. Importantly, triptolide treatment resulted in a decrease in 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[121]. These data indicate that transcription plays a prominent role in the formation of small compartmental domains, and thus in the fine architecture within TADs.

Fine-scale transcriptional state is also a major predictor of TAD structure in mammals and *C. elegans* [121], 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[114]. This is not necessarily in contradiction; the different interpretation may be related to the algorithm used to define domain borders. Compartmental domains were suggested to be a foundation for chromatin organisation in all species, with architectural proteins such as CTCF and cohesin forming an additional layer of organisation[121]. 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 *Drosophila* [113,121,122], whereas in mammals the model's prediction was improved using information on both transcription and CTCF interactions[121].

In order to function, enhancers must relay regulatory information to the basal transcriptional machinery at their target genes' promoter, which are generally thought to occur within the same TAD (see recent reviews[123–126]). Forcing a loop between an enhancer (LCR) and promoter is sufficient to activate transcription at the b-globin locus[127,128], suggesting a causal link between proximity (topology) and transcription. However, at other loci, enhancers and promoters seem to be present in pre-formed topologies prior to gene expression[78,129–131]. Placing an insulator and promoter sites at a distal position from the *Drosophila eve* enhancers was sufficient to form an enhancer-promoter topology in the absence of transcription[132]. However, in cells where transcription is activated the topology becomes more compact, and transcription in turn acts to further stabilise the dynamics of the enhancer-promoter interaction. Although the mechanism is unknown, this suggests that transcription feeds back to stablise chromatin topology, at least at this local (~150kb) scale.

In other cases, transcription may destabilise topology - transcriptional elongation can lead to chromatin remodelling and eventual eviction of CTCF[68,133]. Similarly, transcription also appears to translocate cohesin along the DNA, in both yeast and mammals[134,135]. 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 co-localise at a sub-set (~10%) of CTCF sites, including intergenic and intronic regions[136]. 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[136]. Furthermore, in *Drosophila*, Hi-ChIP with antibodies directed against CP190 (a protein that directly binds to CTCF), as well as some other insulator proteins, is very similar to Hi-ChIP against the elongating form of Pol II (serine 2 phosphorylated)[121]. CTCF occupancy is also correlated with sites of alternative splicing[137]; it was proposed that CTCF mediates promoter-intronic 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[114]. Taken together, these data raise the possibility that part of the regulation of genome organisation by CTCF may be through local effects on transcription.

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 organisation. In mammalian cells, CTCF and cohesin play a major role in TAD formation, but a subset of TAD borders may be set by transcriptionally active genes. In *Drosophila*, the role of transcription appears to be more pronounced, while 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.

Also within TADs active transcription appears to impose a fine structure. The self-association observed for small compartmental domains that sub-divide TADs may be driven by the same mechanisms that segregate heterochromatin from euchromatin (compartment B from A; LADs from inter-LAD regions) at much larger scales. Here, a diversity of mechanisms are involved. 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[138,139], may be responsible for the formation of the euchromatin compartment. Small nuclear speckles of PolII, TFs and cofactors, known for decades[140,141], have recently been proposed to be the result of phase separation[139,142–144]. 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 heterochromatic parts of the genome.

It thus appears that the transcription machinery and its regulators are not only guided by genome organisation, but also contribute to it. How this exactly works is not yet clear; possible mechanisms may involve components of the transcription pre-initiation complex, 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. It will be exciting to further dissect the precise mechanisms.

Glossary

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

CTCF

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Originally known as CCCTC-binding factor; a DNA binding protein that often marks borders of LADs, TADs and DNA loops, and can act as an insulator

Hi-C
A method to systematically identify genomic sequences that are in close proximity of one another inside cell nuclei [31]

Histone modifications/marks
Generic term for a wide range of post-translational modifications present at many positions on histones. Have a variety of functions, including the packaging of chromatin and regulation of transcription

LADs
Lamina-associated domains. Large (about 10 kb - 10 Mb) genomic regions that interact with the nuclear lamina. Typically identified by means of the DamID technology[20]

Nuclear lamina
Layer of proteins that coats the inner nuclear membrane inside the nucleus. Thought to form a large contact surface for LADs

TADs
Topologically associated domains. Defined by Hi-C data as genomic regions with abundant self-self contacts and fewer contacts with surrounding regions

super-enhancers
Somewhat arbitrary definition for genomic regions (regulatory landscapes) that contain a high density of active enhancers[145]

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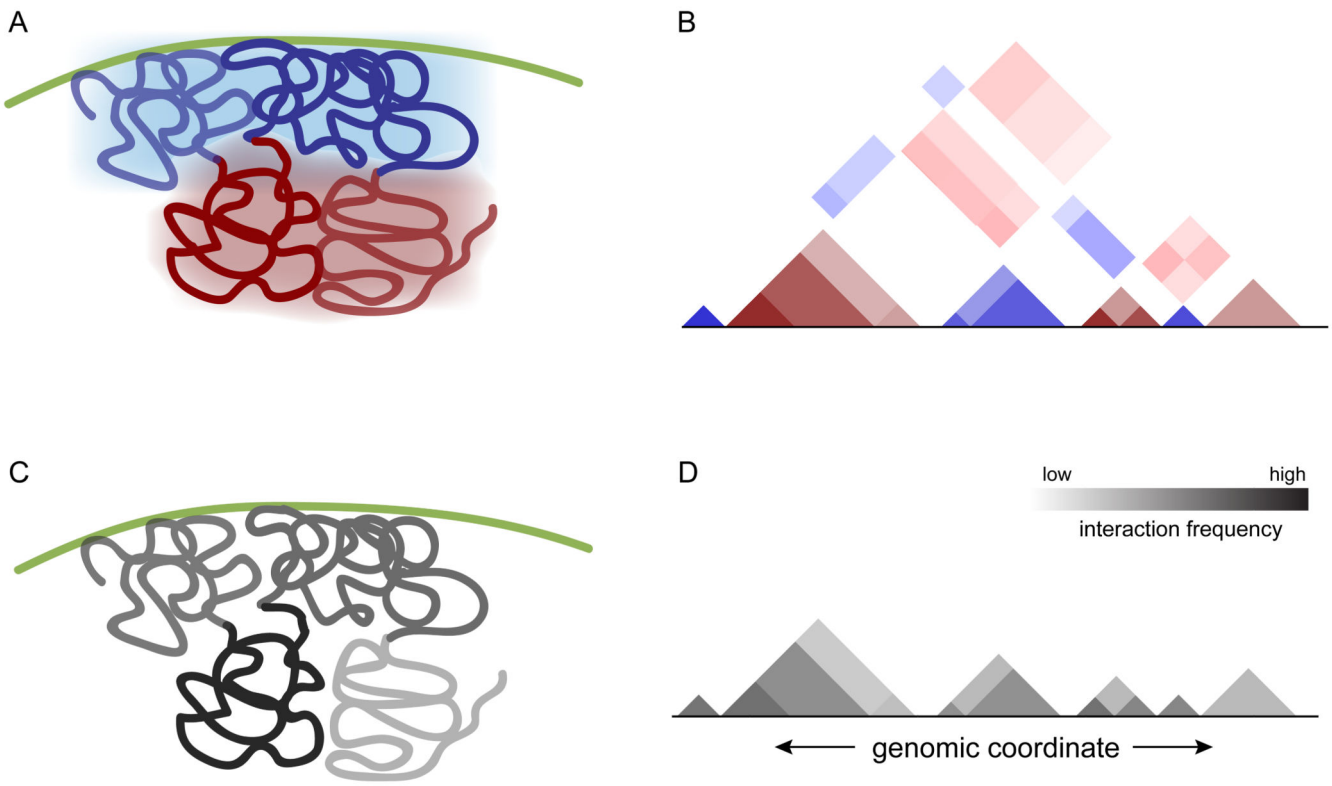

Figure 1. Two main principles of chromosome organisation.

(A) Compartments are formed by aggregation of multiple domains with similar biochemical or functional properties. The two most prominent compartments are heterochromatin (blue, often positioned near the nuclear lamina) and euchromatin (red). **(B)** Self-association of heterochromatin and euchromatin domains is detectable as long-range contacts in Hi-C maps. **(C)** Cartoon illustrating the partitioning of the genome into TADs (different shades of grey), which have primarily intradomain contacts and fewer inter-domain contacts. **(D)** Cartoon representation of part of a Hi-C map, with intra-TAD contacts depicted as a grey scale. TADs are often nested structures.

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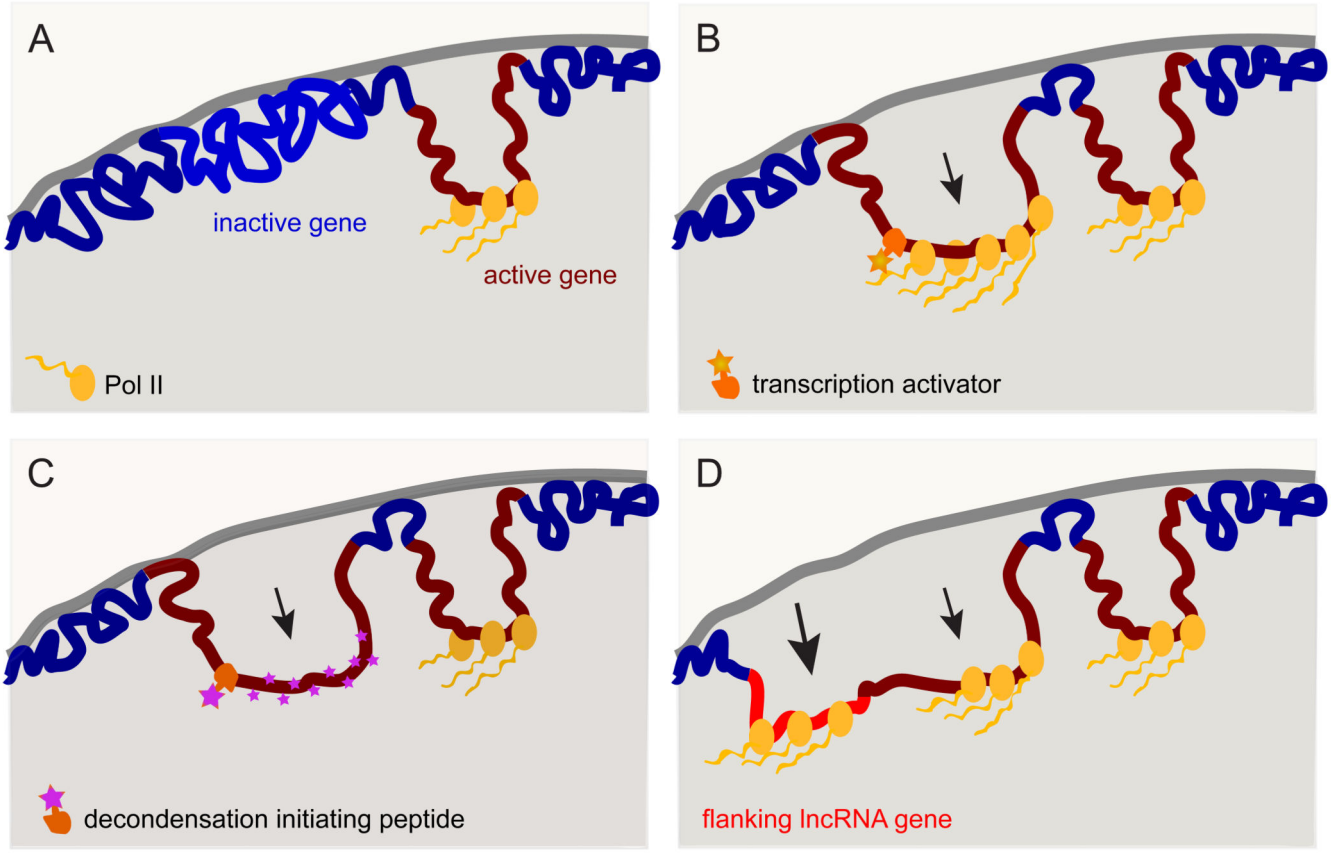

Figure 2. Gene relocation from peripheral heterochromatin to internal euchromatin.

(A) Active genes are typically located in the nuclear interior, while a subset of inactive genes is located in the heterochromatin layer at the nuclear lamina. **(B)** Binding of a strong transcription activator can relocate a gene to the nuclear interior. **(C)** Tethering of a peptide with chromatin decondensing activity can relocate a gene to the nuclear interior without transcription activation. **(D)** Activation of a nearby lncRNA gene can relocate a flanking coding gene to the nuclear interior.

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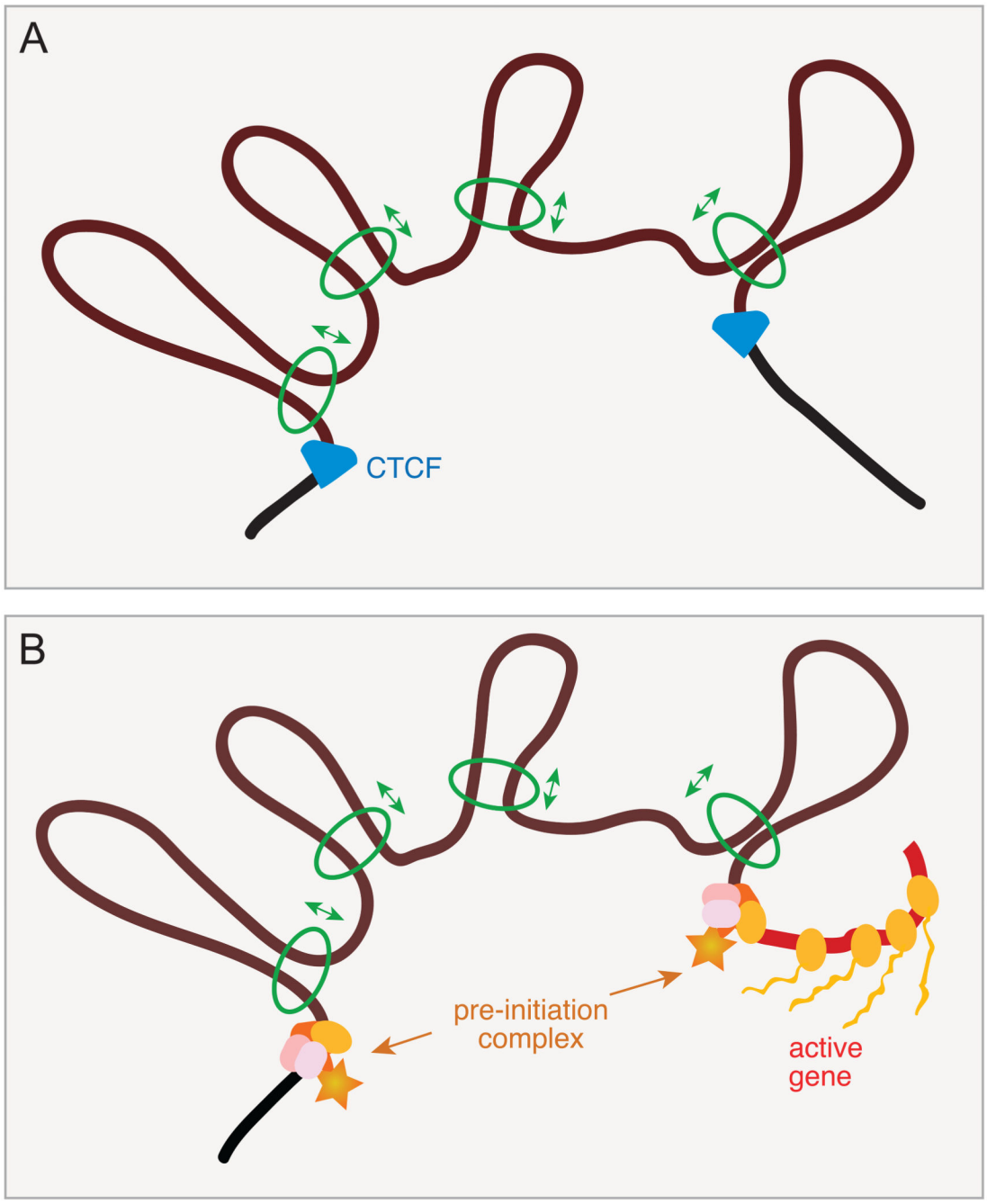

Figure 3. Alternative mechanisms of TAD boundary formation.

(A) TADs may be the result of loop extrusion by cohesin complexes (green rings). One TAD may consist of multiple loops that are dynamically formed and resolved. CTCF, when bound in the correct orientation, could act as a “road block” that stops progression of loop extrusion, thereby creating a TAD border. **(B)** Similarly, active genes, the transcription pre-initiation complex, or a chromatin mark associated with it, could block loop extrusion.

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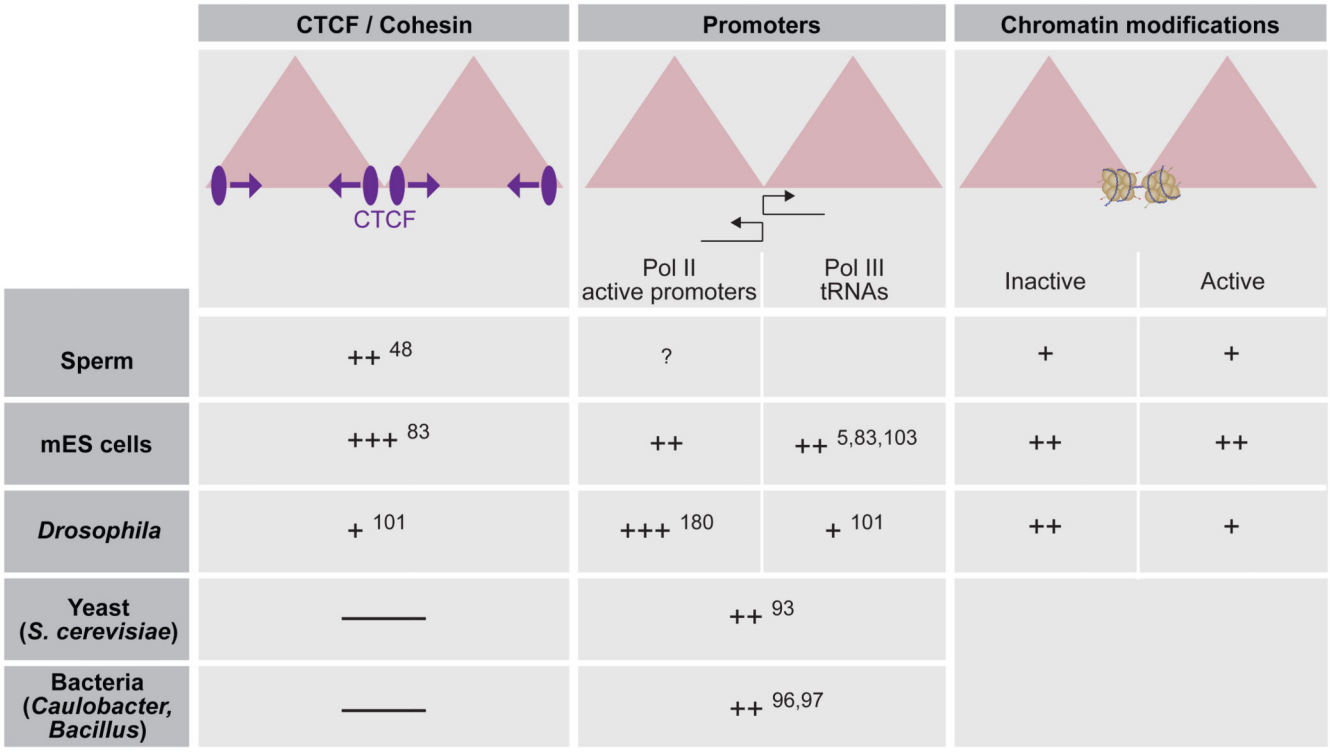

Figure 4. Properties of TAD borders in different cell types and species.

CTCF, active promoters, and associated chromatin marks are found at TAD borders to varying degrees. + denotes low level (<25%), ++ high level (~60%), +++ the vast majority, or - not present (0%). The references are indicated.

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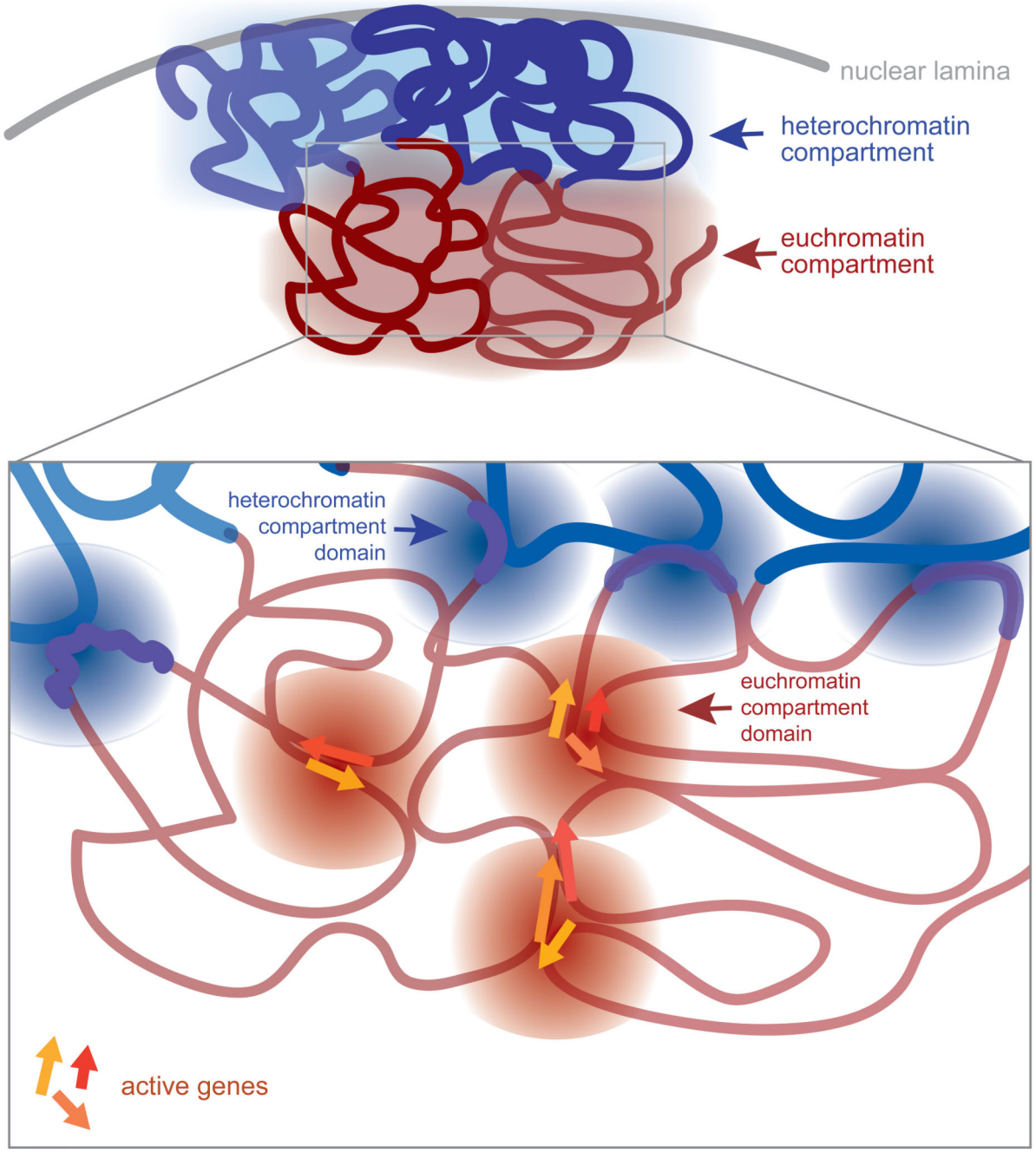

Figure 5. Compartmentalization of active and inactive chromatin.

Cartoon illustrating self-association, which may occur at multiple scales: both at the level of large domains (top panel), and at the level of individual genes (bottom panel). Red shades: compartment A (euchromatin); blue shades: compartment B (heterochromatin, LADs).